BMJ Open

The slighter decrease of platelet counts after treatments is associated with improved outcomes in sepsis patients: a retrospective observational study based on the MIMIC-IV database

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-069027
Article Type:	Original research
Date Submitted by the Author:	08-Oct-2022
Complete List of Authors:	liu, xing; Sichuan University West China Hospital, Department of Critical Care Medicine Yin, Wanhong; Sichuan University West China Hospital, Department of Critical Care Medicine Li, Yi; Sichuan University West China Hospital, Department of Critical Care Medicine Qin, Yiwei; Chengdu Medical College The First Affiliated Hospital, Department of Intensive Medicine Zou, Tongjuan; Sichuan University West China Hospital, Department of Critical Care Medicine
Keywords:	INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The slighter decrease of platelet counts after treatments is associated with improved outcomes in sepsis patients: a retrospective observational study based on the MIMIC-IV database

Xing Liu[1], MS; Wanhong Yin[1], MD; Yi Li[1], MD; Yiwei Qin[2], MS; Tongjuan Zou[1], MS

[1]Department of Critical Care Medicine, West China Hospital, Sichuan University, No. 37 Guo Xue Road, Chengdu Sichuan 610041, China

[2]Department of Intensive Medicine, The First Affiliated Hospital of Chengdu Medical College, Chengdu, Sichuan 610500, China

Correspondence author: Wanhong Yin, MD

E-mail address: yinwanhong@wchscu.cn

Abstract

Objective Recent studies have revealed that platelets are also central inflammatory cells in sepsis. However, nowadays there is scant research on whether platelets could act as an indicator of evaluating the effects of sepsis treatments. This study aimed to investigate the association between platelet variations on day 4 and the mortality.

Design A retrospective cohort study.

Setting Medical Information Mart for Intensive Care (MIMIC-IV) is a public critical database.

Participants 7981 patients admitted to the Beth Israel Deaconess Medical Center (BIDMC) were analyzed according to Sepsis-3 criteria from MIMIC-IV between 2008 and 2019.

Primary and secondary outcome measures The primary outcome was the mortality within 30 days. The secondary outcomes were ICU length of stay and the length of hospitalization.

Results The difference was significant in 30-day mortality between the two groups (14.1% vs. 23.5%, p < 0.001, Kaplan-Meier analysis, p < 0.001). In a multivariable analysis, the mortality within 30 days declined with a decreasing proportion of platelet counts of ≤10% on day 4 of ICU admission (OR = 0.73, 95% CI = 0.64 to 0.82, p < 0.001). In the secondary outcomes, these patients had a shorter ICU stay than those of >10% (6.8 vs. 7.5, p < 0.001). In addition, in restricted cubic spline curves based on logistic models, the mortality dropped along with the platelet change ratio increased.

Conclusions A ≤10% decrease in platelets among sepsis patients after treatments

could be independently associated with a decrease in mortality within 30 days. The percentage of platelets after treatments could serve as a reference for the therapeutic effects of sepsis.

Strengths and limitations of this study

The retrospective study data derived from the high-quality public Medical Information Mart for Intensive Care (MIMIC-IV) database.

The restricted cubic spline curves based on logistic models were used to visualize the trend of platelet change ratio.

There were small amounts of missing data, which were multiple-imputed by applying classification and regression trees.

Due to missing the long-term follow-up data in this database, the difference in the long-term outcomes between groups could be not determined.

Introduction

Sepsis is a dangerous illness that requires acute treatment and is a primary global health concern because of its high incidence, high mortality, and poor prognosis. In America, sepsis is one of the most expensive illnesses in total hospital expenditures as well as a prevalent death-related illness among hospitalised patients [1, 2]. According to research from some high-income nations, an estimated 50.9 million sepsis patients and 5.3 million people died yearly from sepsis-related conditions [3].

International experts agree that sepsis is characterized by multiple organ failures endangering life due to dysregulated individuals' defenses to infection [4]. Sepsis is a complicated pathophysiological process in which a pathogen triggers a person's inflammatory-immune solid response. This leads the body to either activate or repress several facets, including endothelium, coagulopathy, immunological, and hormonal functions. Endothelial damage, inflammatory pathways, and coagulation work together to activate platelets in sepsis, which are crucial for pathogenic defense [5-7]. Platelets could be effectively bactericidal by releasing platelet antimicrobial peptides [8]. Nevertheless, platelet hyperreactivity could contribute to sepsis complications [9]. Moreover, recent research has discovered that sepsis induces platelet transcription and translation, and circulating platelets have higher levels of integrin subunit α IIb (ITGA2B), which is linked to higher mortality [10].

Platelet counts have been included in the sequential organ failure assessment (i.e., Sofa Score) as one of the critical markers of patients with sepsis and reflects the patient's prognosis [11]. Numerous studies [12-14] have demonstrated a correlation between

thrombocytopenia and an adverse prognosis. According to Mavrommatis AC et al. [15] , a lower platelet count was associated with a more severe incident of sepsis. Consequently, researchers proposed that thrombocytopenia patients could benefit from medications to improve the sepsis prognosis by stimulating platelet counts [16]. By using recombinant human thrombopoietin (rhTPO), sepsis with thrombocytopenia could raise platelet counts in patients effectively, resulting in a shorter stay in the intensive care unit [17]. On the other hand, other trials have targeted sepsis with antiplatelet drugs to reduce undesirable thrombosis, inflammatory host responses and organ damage [18, 19]. However, nowadays there is scant research on whether platelets could act as an indicator of evaluating the effects of sepsis treatments.

This study aimed to retrospectively analyze the association between the rate of platelet change after treatments and the prognosis of patients with sepsis. Therefore, this study investigated the relationship between the percentage of platelet counts on day 4 after the ICU admission and the prognosis of patients with sepsis.

Materials and Methods

Source of data

This study was reported according to the Strengthening the Reporting of Observational studies in Epidemiology guidelines[20]. Data were retrieved from the Medical Information Mart for Intensive Care (MIMIC-IV version 1.0) [21]. It comprises some patients admitted to the Beth Israel Deaconess Medical Center (BIDMC) between 2008 and 2019. Patient records were fully anonymized, and data collection was performed with both BIDMC and Massachusetts Institute of Technology Institutional Review

Board (IRB) approval. After completing the training program and passing the test, we are eligible to receive free access to the database and conduct the related research, according to the rules. The author passed the certification for the Collaborative Institutional Training Initiative (Certification Number 48605954 for author Xing Liu).

Study population

The inclusion criteria were as follows: 1) individuals with sepsis, according to Sepsis-3 standard [4] ; 2) adults (age≥18 years); 3) length of ICU stay≥ 72 h. and 4) in some patients with records of multiple ICU stays and admissions, only data involving the first ICU stay and admission were included. Before being admitted to the ICU, patients diagnosed with cirrhosis, lymphoma, and taking clopidogrel, aspirin, rivaroxaban, and warfarin were excluded. Patients with prednisone were not taken into consideration while they were transferred to the ICU. Finally, samples with missing data for day 1 and day 4 platelet counts were eliminated.

Variables

Platelet counts on day 1 and 4 following admission to the ICU were extracted from MIMIC-IV because these variables could represent the proportion of platelet counts on day 4 compared with day 1 in the observational analysis. During the data extraction process, the variables from day 1 of ICU admissions (such as age, gender, weight, ethnicity, chronic diseases, sofa score, apsiii, sapsii, pt, aptt, and white blood cell counts) were taken into consideration. These characteristics served as possible confounders in this study. PostgreSQL was used to extracted general variables from the MIMIC-IV database.

Outcomes

The primary endpoint was the mortality within 30 days. The length of hospitalization and ICU stay were considered secondary outcomes.

Missing values

All variables in this study had less than 11% missing values (online supplemental material 1). By applying classification and regression trees [22, 23], the missing values were multiple-imputed, including weight, pt, aptt, and white blood cell counts.

Statistical analysis

The ratio of platelet counts on day 4 following ICU admission was compared with the baseline. The cut-off of the platelet-count percentage was calculated using the receiver operating characteristic curve (ROC), which was employed to categorize patients in the baseline characteristics table. For computational simplicity, the threshold for the ROC was minus 9.5%, nearly equivalent to minus 10%. Statistical significance was defined as 2-sided p-values <0.05. A multivariate logistic regression analysis was employed to assess further the possible relationship between the proportion of platelet counts and the mortality within 30 days.

The variables listed in Table 1 were applied to identify potential confounding variables for logistic regression. The multivariate analysis included these factors as adjusting variables with a p-value of less than 0.05 in the univariate regression analysis. The Kaplan-Meier (KM) approach was employed to visualize the survival curves and compare various changes in platelet counts over 30 days. Survival was assessed via the log-rank test. Based on logistic models, restricted cubic spline curves were used to

evaluate

Table 1: Baseline of patient characteristics stratified by proportion of platelet counts on day 4 of ICU admission

Characteristic	Overall	declining proportion of platelet counts		*p* value
		platelet>10%	platelet≤10%	
Number	7981	3802	4179	
Gender, M (%)	4594 (57.6)	2180 (57.3)	2414 (57.8)	0.717
Median age (IQR), yr	65.8[53.6 77.3]	66.2 [54.6 77.4]	65.3[52.6 77.3]	0.012
Median weight (IQR), kg	80.0[67.0 96.5]	79.8[66.8 96.5]	80.0[67.0 96.4]	0.586
Ethnicity, n (%)				0.671
American Indian	20 (0.3)	8 (0.2)	12 (0.3)	
Asian	205 (2.6)	100 (2.6)	105 (2.5)	
Black	707 (8.9)	325 (8.5)	382 (9.1)	
White	5025 (63.0)	2382 (62.7)	2643 (63.2)	
Hispanic	258 (3.2)	132 (3.5)	126 (3.0)	
Others	1766 (22.1)	855 (22.5)	911 (21.8)	
Select comorbidities,[a] *n* (%)				
Cardiovascular disease	2826 (35.4)	1473 (38.7)	1353 (32.4)	<0.001
Chronic pulmonary disease	1945 (24.4)	941 (24.8)	1004 (24.0)	0.467
Liver disease	1466 (18.4)	816 (21.5)	650 (15.6)	<0.001
Renal disease	1582 (19.8)	796 (20.9)	786 (18.8)	0.019
diabetes	2256 (28.3)	1109 (29.2)	1147 (27.4)	0.093
Vascular disease	2359 (29.6)	1082 (28.5)	1277 (30.6)	0.043
Cancer	1189 (14.9)	620 (16.3)	569 (13.6)	0.001
Aids	56 (0.7)	19 (0.5)	37 (0.9)	0.054
Others	1507 (18.9)	705 (18.5)	802 (19.2)	0.477
Status at admission (median [IQR])				
Sofa score	7.0 [5.0 11.0]	8.0[5.0 12.0]	6.0 [4.0 9.0]	<0.001
Apsiii	61.0[44.0 81.0]	66.0[49.0 88.8]	56.0[42.0 74.0]	<0.001
Sapsii	40.0[32.0 51.0]	44.0[35.0 54.0]	38.0[30.0 47.0]	<0.001
Laboratory test (median [IQR])				
White blood cell counts, k/ul	12.0 [8.6 16.3]	13.0 [9.3 17.8]	11.3 [8.3 15.1]	<0.001
pt (s)	14.3[12.7 17.3]	14.6[12.8 18.2]	14.1 [12.6 16.5]	<0.001
aptt(s)	31.7 [27.5 40.7]	33.0[28.1 44.6]	30.8[27.2 37.7]	<0.001
ICU outcome				
30-day mortality, n (%)	1483 (18.6)	892 (23.5)	591 (14.1)	<0.001
Median hospital LOS(IQR), d	13.9 [9.0 22.0]	14.1 [9.0 22.3]	13.7 [9.0 21.8]	0.122
Median ICU LOS(IQR), d	7.1 [4.8 12.1]	7.5 [4.9 12.9]	6.8 [4.7 11.3]	<0.001

IQR, interquartile range; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; Sapsii, simplified acute physiology score; pt, prothrombin time; aptt, activated partial thromboplastin time; LOS, length of stay.
[a]Comorbidities are defined by the Charlson comorbidity index, cancer includes

malignant cancer and metastatic solid tumor.

the relationship between the proportion of platelet counts and the principal endpoint.

Data with a normal distribution were shown as mean and standard deviation, whereas data with a non-normal distribution were presented as median (interquartile range). Normally distributed continuous variables were compared between groups using analysis of variance, while non-normally distributed continuous variables were subjected to the Kruskal-Wallis test. R software was employed for all data analysis (R version 4.2.1).

The variables were subclassified for subgroup analysis, including age, gender, sofa score, pt, aptt, and white blood cell counts. The association between the platelet percentage and mortality was shown in a forest plot.

Patient and public involvement

No patient or members of the public were involved in any part of this study.

Results

There were 35010 sepsis patients in the research. After deleting samples with missing platelet counts on day 1 and 4 of ICU admission and applying inclusion and exclusion criteria, the final research population consisted of 7981 adult patients diagnosed with sepsis using Sepsis-3 criteria (Figure 1).

All the research populations' initial characteristics were listed in Table 1. There were two groups made up of the whole population. According to the descending proportion of platelet counts on day 4, 3802 sepsis patients had a platelet>10%, and 4179 patients

had a platelet≤10%. Participants in the group with platelet>10 % were older (age 66.2 vs. 65.3, p = 0.012) and had more severe coagulation dysfunction (pt 14.6 vs. 14.1, p<0.001 and aptt 33.0 vs. 30.8, p<0.001). Meanwhile, higher white blood cell counts (13.0 vs. 11.3, p<0.001), as well as a higher sofa score (8 vs. 6, p<0.001), were obviously detected in the patients with platelet>10%. Similar performance might be indicated in other organ dysfunction rating systems.

Patients with platelet≤10% showed lower mortality within 30 days than those with platelet>10% (14.1% [591] vs. 23.5% [892], p<0.001) and ICU stays tended to be shorter (6.8 vs. 7.5, p<0.001). However, as opposed to patients with platelet>10%, there was no discernible difference in the length of hospital stays across the groups (13.7 vs. 14.1, p = 0.122). For the mortality within 30 days, patients with platelet ≤ 10% existed two days longer than those with platelet>10% (mean survival time 26.4 vs. 24.4 days) by Kaplan-Meier analysis (p<0.0001) (Figure 2). In the multivariable logistic regression model, a platelet ≤10% was independently linked to a reduction in the mortality within 30 days (odds ratio [OR], 0.73; 95% confidence interval [CI], 0.64 to 0.82; p-value [p]<0.001). Age, the sapsii score, the sofa score, the apsiii score and white blood cell counts were all considered when the analysis was modified (Table 2). Based on logistic models, the restricted cubic spline (RCS) predicted that the lower the decreasing proportion of platelet counts on day 4, the lower the likelihood of the mortality within 30 days would be. Furthermore, the sepsis patients had better results, as identified by the increasing platelet percentage (Figure 3).

In subgroup analysis, age, gender, organ dysfunction status, infection level, and the

coagulation functional condition were stratified. Similar trends in subgroups were observed in the drop of the mortality within 30 days with platelet≤10% (Figure 4).

Table 2: Univariate and multivariate analysis for assessing the mortality within 30 days

	Univariate analysis			Multivariate analysis		
Variables	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.41	1.26-1.58	<0.001	1.07	0.94-1.23	0.307
Liver disease	1.94	1.70-2.21	<0.001	1.61	1.37-1.89	<0.001
Renal disease	1.48	1.30-1.69	<0.001	0.97	0.83-1.12	0.646
Vascular disease	1.31	1.16-1.48	<0.001	1.56	1.36-1.78	<0.001
Cancer	1.75	1.52-2.02	<0.001	1.91	1.62-2.25	<0.001
sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-0.99	<0.001
Sofa score	1.15	1.13-1.16	<0.001	1.01	0.99-1.03	0.469
apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.03	<0.001
White blood cell counts	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.080
pt (s)	1.03	1.02-1.04	<0.001	1.01	1.01-1.02	<0.001
aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.051
[a]platelet≤10%	0.54	0.48-0.60	<0.001	0.73	0.64-0.82	<0.001

CI, confidence interval; OR, odds ratio; pt, prothrombin time; aptt, activated partial thromboplastin time.
[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Discussion

In this analysis of 7981 patients with sepsis, a platelet≤10% on day 4 after ICU admission was related to a decrease of mortality within 30 days. Subgroup findings indicated consistent results. The mortality within 30 days decreased in the subsequent research as the extent of platelet decreasing lessened. These findings could give an effect assessment measure in sepsis treatments.

The mature megakaryocytes in the bone marrow are the source of platelets, which are anucleate cells. According to previous studies, platelet activity aids in hemostasis. As a result, platelets perform various other tasks, such as host defence against infection,

including phagocytosis of bacteria and viruses, superoxide production, and platelet-derived micro-bactericidal proteins [24]. A series of surface receptors and adhesion molecules on platelets allow them to interact with leukocytes and pathogens in the bloodstream, which is critical for the pro-inflammatory and chemotactic processes [6]. Platelets are activated and interact with leukocytes directly in the blood in conditions of infection, such as sepsis [25-27]. Circulating leukocytes may be able to complete their task due to the engagement. Neutrophils may locate infection sites because of their interaction with platelets[28]. Activated neutrophils produce and release neutrophil extracellular traps (NETs) to capture and destroy infections [29].

Recent investigations have demonstrated that thrombocytopenia is associated with an unfavourable prognosis. In both medical and surgical intensive care units, Moreau D et al. found that a 30% fall in platelet counts was an independent predictor of death [12]. According to several researchers, a slow or absent increase in platelet counts among surgical ICU patients was connected with a higher death rate. Within 10 days after ICU admission, the author calculated the platelet percentage. The value was more than five times higher in survivors compared with non-survivors ($30 \pm 46 \cdot 10^3/mm^3/day$ vs. $6 \pm 28 \cdot 10^3/mm^3/day$, $p<0.001$) [30]. However, in a retrospective analysis of sepsis patients with leukocytosis, the hospital mortality rose by 6.9% in the thrombocytosis group, which was classified as having > 500,000 platelets/L [31]. There is minimal research that assessed the correlation between platelet change rate after treatments and sepsis outcome. Furthermore, the evidence is lacking for platelet as a therapeutic evaluation indicator.

Platelets are often applied as clinical monitoring indices and play a role in anti-infective responses [26]. If a correlation between the proportion of platelet counts and the effects of sepsis after treatments could be demonstrated, the foundation will be established for employing platelets as one of the indicators to judge the effectiveness of treatments. This study examined the relationship between the result of sepsis and the proportion of platelet counts on day 4, which is a usual time node for monitoring the effects after treatments. This research has illustrated that slighter decreasing platelets are linked to decreased mortality and proportion of platelet counts could serve as a clinical reference for treatments changes. Additionally, thrombocytosis could be a standalone indicator of a favourable prognosis in ICU patients. Specifically, 21.7% of patients with general and trauma ICU platelet counts of more than $450 \cdot 10^9$/litre were associated with lower ICU morality (P = 0.003) [32]. This outcome supports our further research.

Furthermore, there are several limitations of this study. Firstly, this observational research did not aim to identify the underlying mechanism of how platelet change might affect sepsis outcomes. Therefore, it is challenging to assume the physio-pathological reasons whether platelet change is connected to a positive result or not. Secondly, the results might lack a causal association because we only examined data from an extensive public retrospective database. High-quality trials are needed to compare the evaluation of different platelets change threshold on the therapeutic effect of sepsis in the future. Thirdly, this observational study did not demonstrate the association between treatments and platelet levels. However, by taking advantage of the large

sample size of the public database, this study provides a reference for further prospective studies in sepsis.

Conclusions

To summarise, a ≤10% decrease in platelets among sepsis patients after treatments could be independently associated with an improvement in mortality within 30 days. Meanwhile, this study insists that the extent of platelet decreasing lessen while that of platelet increasing enlarge, and the mortality within 30 days in sepsis patients presents a downward trend. Furthermore, these findings could provide a fresh perspective on evaluating the effects of sepsis treatments.

Contributors WHY and XL designed the study. YWQ and XL conducted data collection and data analysis. XL wrote the manuscript. WHY, YL and TJZ analysed and interpreted the results. All authors have reviewed this manuscript.

Competing interests None declared.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement Data are available upon reasonable request. The data used in this study can be obtained by the corresponding author upon request.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Ethics approval This study was in accordance with the ethical standards of the

Declaration of Helsinki and was approved by the ethics review boards of Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center (researcher certification number 48605954). MIMIC-IV was retrospective with lack of patient intervention, and all patients' data were de-identified; thus individual patient informed consent was not required.

References

1 Liu V, Escobar GJ, Greene JD, *et al*. Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA* 2014;312:90-2. https://doi.org/10.1001/jama.2014.5804

2 Rudd KE, Johnson SC, Agesa KM, *et al*. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020;395:200-11. https://doi.org/10.1016/s0140-6736(19)32989-7

3 Fleischmann C, Scherag A, Adhikari NK, *et al*. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med* 2016;193:259-72. https://doi.org/10.1164/rccm.201504-0781OC

4 Singer M, Deutschman CS, Seymour CW, *et al*. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;315:801-10. https://doi.org/10.1001/jama.2016.0287

5 Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* 2010;67:525-44. https://doi.org/10.1007/s00018-009-0210-4

6 Semple JW, Italiano JE, Jr.,Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* 2011;11:264-74. https://doi.org/10.1038/nri2956

7 Schouten M, Wiersinga WJ, Levi M, *et al*. Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol* 2008;83:536-45. https://doi.org/10.1189/jlb.0607373

8 de Stoppelaar SF, van 't Veer C,van der Poll T. The role of platelets in sepsis. *Thromb Haemost* 2014;112:666-77. https://doi.org/10.1160/th14-02-0126

9 Greco E, Lupia E, Bosco O, *et al*. Platelets and Multi-Organ Failure in Sepsis. *Int J Mol Sci* 2017;18:2200. https://doi.org/10.3390/ijms18102200

10 Middleton EA, Rowley JW, Campbell RA, *et al*. Sepsis alters the transcriptional and translational landscape of human and murine platelets. *Blood* 2019;134:911-23. https://doi.org/10.1182/blood.2019000067

11 Vincent JL, Moreno R, Takala J, *et al*. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707-10. https://doi.org/10.1007/bf01709751

12 Moreau D, Timsit JF, Vesin A, *et al*. Platelet count decline: an early prognostic marker in critically ill patients with prolonged ICU stays. *Chest* 2007;131:1735-41. https://doi.org/10.1378/chest.06-2233

13 Akca S, Haji-Michael P, de Mendonça A, *et al*. Time course of platelet counts in critically ill patients. *Crit Care Med* 2002;30:753-6. https://doi.org/10.1097/00003246-200204000-00005

14 Jacoby RC, Owings JT, Holmes J, *et al*. Platelet activation and function after trauma. *J Trauma* 2001;51:639-47. https://doi.org/10.1097/00005373-200110000-00003

15 Mavrommatis AC, Theodoridis T, Orfanidou A, *et al*. Coagulation system and platelets are fully activated in uncomplicated sepsis. *Crit Care Med* 2000;28:451-7. https://doi.org/10.1097/00003246-200002000-00027

16 Zhou Z, Feng T, Xie Y, *et al*. The effect of recombinant human thrombopoietin (rhTPO) on sepsis patients with acute severe thrombocytopenia: a study protocol for a multicentre randomised controlled trial (RESCUE trial). *BMC Infect Dis* 2019;19:780. https://doi.org/10.1186/s12879-019-4388-2

17 Liu Y, Jin G, Sun J, *et al*. Recombinant human thrombopoietin in critically ill patients with sepsis-associated thrombocytopenia: A clinical study. *Int J Infect Dis* 2020;98:144-9. https://doi.org/10.1016/j.ijid.2020.06.045

18 Pu Q, Wiel E, Corseaux D, *et al*. Beneficial effect of glycoprotein IIb/IIIa inhibitor (AZ-1) on endothelium in Escherichia coli endotoxin-induced shock. *Crit Care Med* 2001;29:1181-8. https://doi.org/10.1097/00003246-200106000-00019

19 Valerio-Rojas JC, Jaffer IJ, Kor DJ, *et al*. Outcomes of severe sepsis and septic shock patients on chronic antiplatelet treatment: a historical cohort study. *Crit Care Res Pract* 2013;2013:1-9. https://doi.org/10.1155/2013/782573

20 von Elm E, Altman DG, Egger M, *et al*. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg* 2014;12:1495-9. https://doi.org/10.1016/j.ijsu.2014.07.013

21 Johnson A, Bulgarelli L, Pollard T, *et al*. *MIMIC-IV (version 1.0)*, 2021.

22 Zhang Z. Missing data imputation: focusing on single imputation. *Ann Transl Med* 2016;4:9. https://doi.org/10.3978/j.issn.2305-5839.2015.12.38

23 Speybroeck N. Classification and regression trees. *Int J Public Health* 2012;57:243-6. https://doi.org/10.1007/s00038-011-0315-z

24 van der Poll T, Parker RI. Platelet Activation and Endothelial Cell Dysfunction. *Crit Care Clin* 2020;36:233-53. https://doi.org/10.1016/j.ccc.2019.11.002

25 McDonald B, Urrutia R, Yipp BG, *et al*. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 2012;12:324-

33. https://doi.org/10.1016/j.chom.2012.06.011

26 Clark SR, Ma AC, Tavener SA, *et al*. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13:463-9. https://doi.org/10.1038/nm1565

27 Zarbock A, Singbartl K,Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006;116:3211-9. https://doi.org/10.1172/jci29499

28 Mine S, Fujisaki T, Suematsu M, *et al*. Activated platelets and endothelial cell interaction with neutrophils under flow conditions. *Intern Med* 2001;40:1085-92. https://doi.org/10.2169/internalmedicine.40.1085

29 Brinkmann V, Reichard U, Goosmann C, *et al*. Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532-5. https://doi.org/10.1126/science.1092385

30 Nijsten MW, ten Duis HJ, Zijlstra JG, *et al*. Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Crit Care Med* 2000;28:3843-6. https://doi.org/10.1097/00003246-200012000-00017

31 Bakey S, Karamanos E, Louwers L, *et al*. 1047: Thrombocytosis versus Thrombocytopenia as Risk Factor for Increased Mortality in Sepsis. Abstr. *Critical care Medicine* 2013;41:A263. https://doi.org/10.1097/01.ccm.0000440283.47246.33

32 Gurung AM, Carr B,Smith I. Thrombocytosis in intensive care. *Br J Anaesth* 2001;87:926-8. https://doi.org/10.1093/bja/87.6.926

Figure Legends

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

Figure 3. The relationship between the proportion of platelet counts (PPC) and the mortality within 30 days in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subset. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at a odds ratio of 1.0. Violet density curves show the fraction of population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, whom with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, sapsii, apsiii and white blood cell counts at baseline.

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

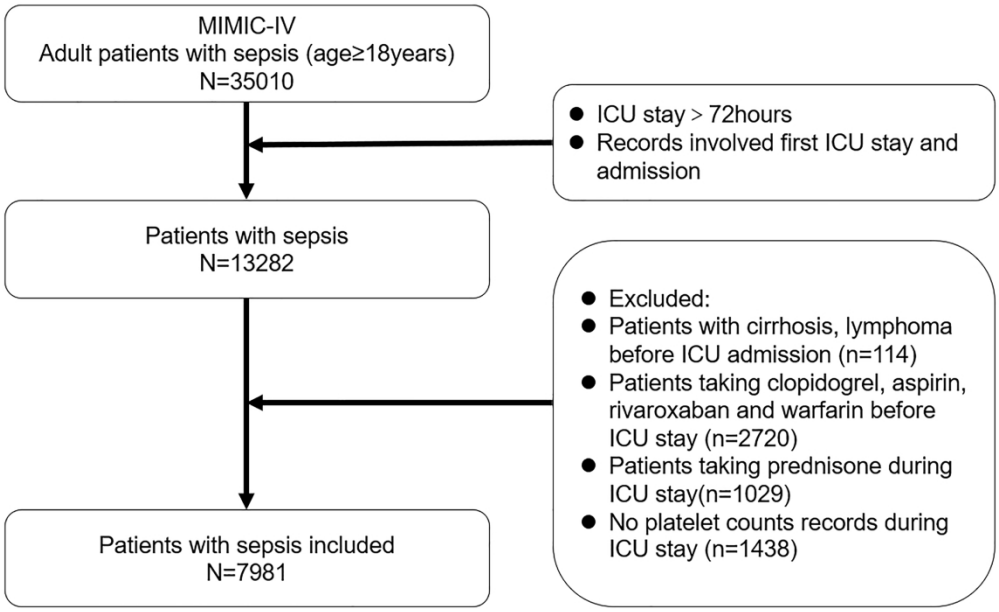

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

232x140mm (300 x 300 DPI)

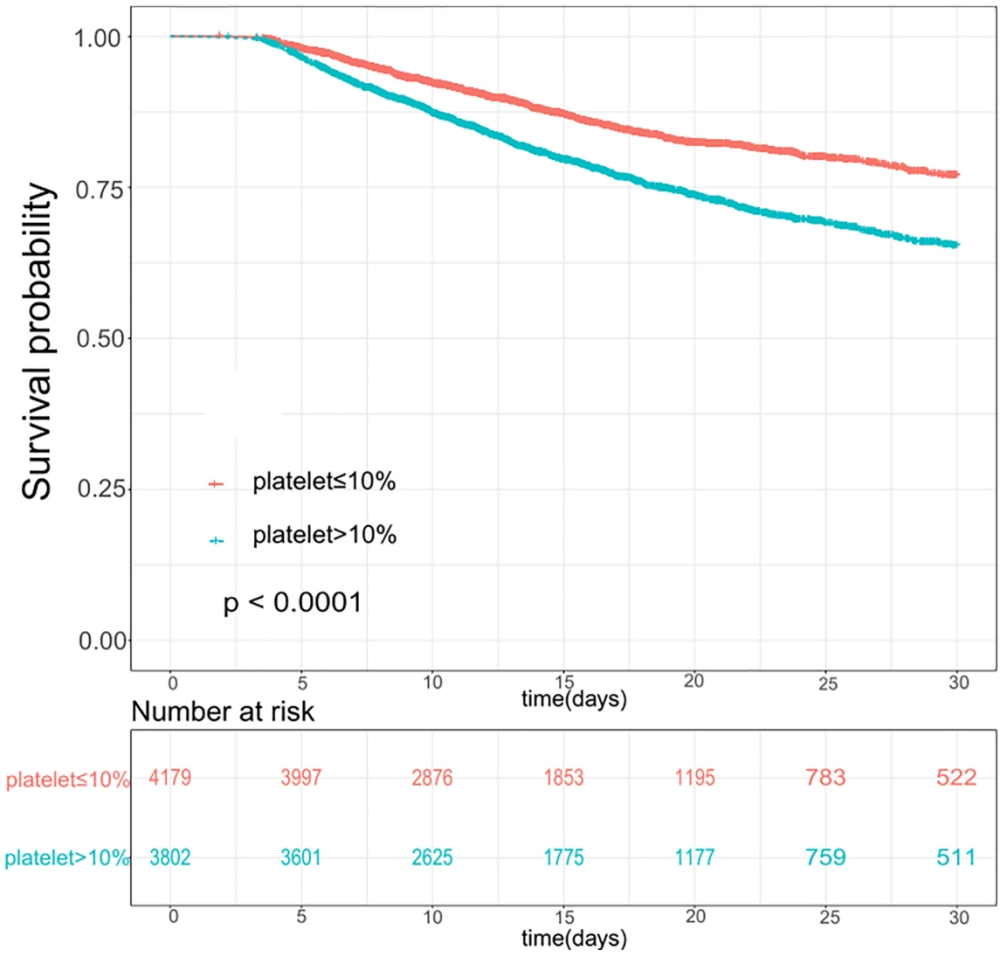

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

162x155mm (300 x 300 DPI)

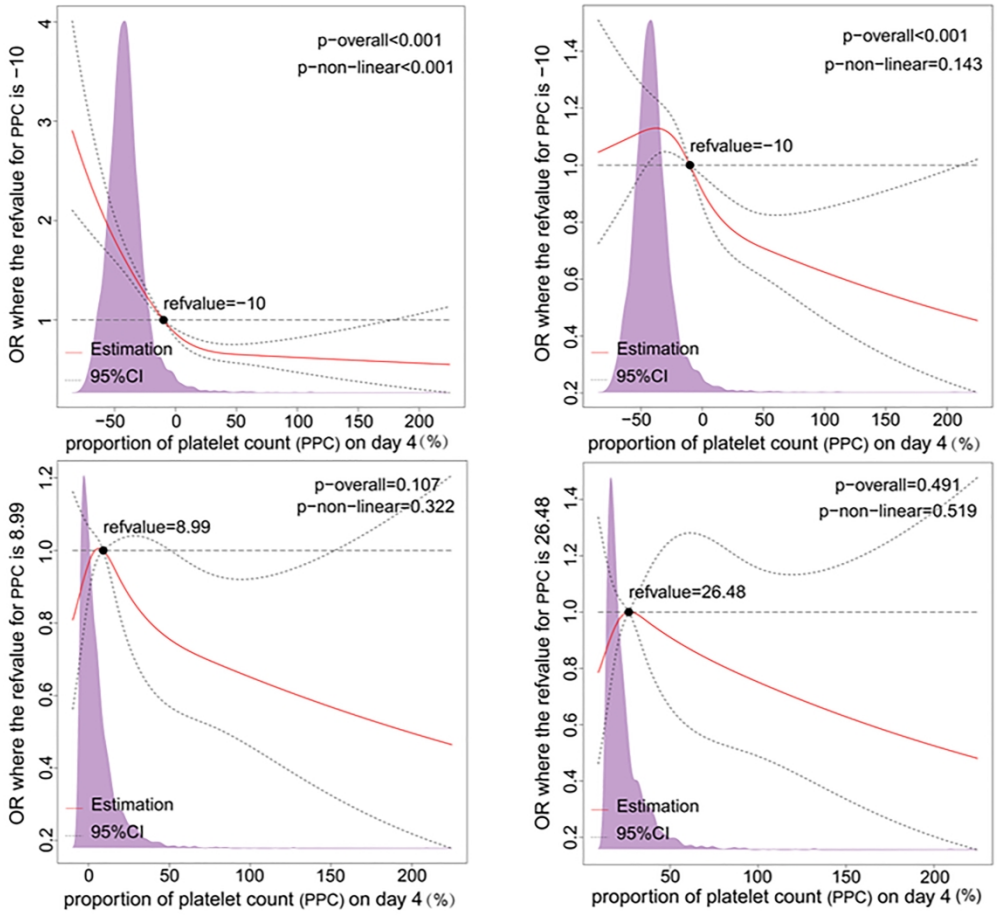

Figure 3. The relationship between the proportion of platelet counts (PPC) and the mortality within 30 days in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subset. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at a odds ratio of 1.0. Violet density curves show the fraction of population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjust-ed model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, whom with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, sapsii, apsiii and white blood cell counts at baseline.

202x186mm (300 x 300 DPI)

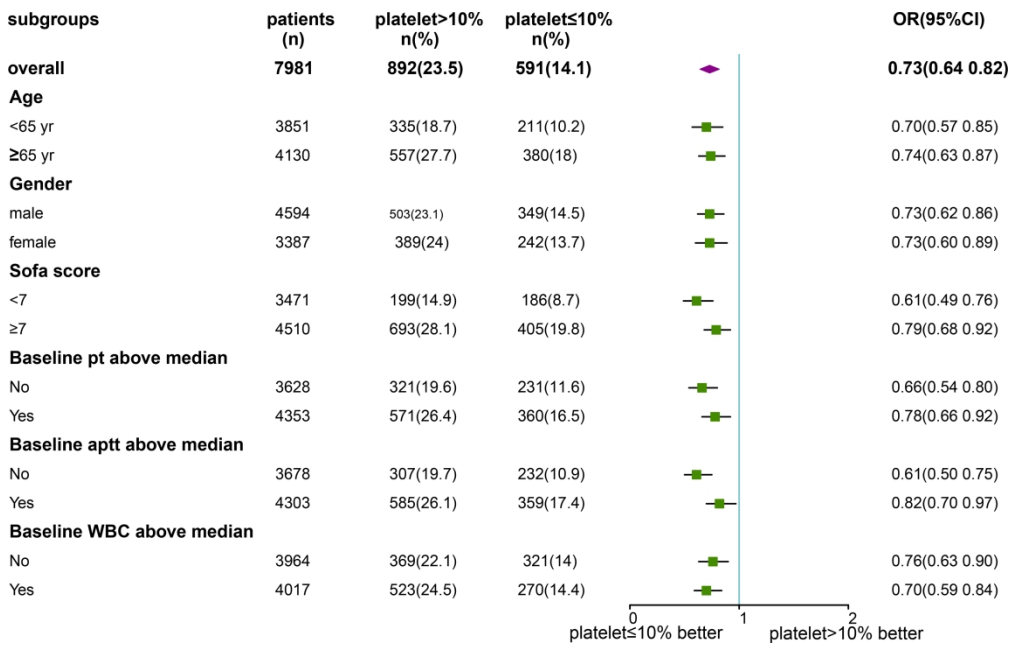

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

336x212mm (300 x 300 DPI)

Supplemental Material 1

All Missing values of data from MIMIC-IV

variables	Missing number n (%)
age	
gender	
weight	38 (0.47)
ethnicity	
myocardial_infarct	
congestive_heart_failure	
peripheral_vascular_disease	
cerebrovascular_disease	
dementia	
chronic_pulmonary_disease	
rheumatic_disease	
peptic_ulcer_disease	
mild_liver_disease	
diabetes_without_cc	
diabetes_with_cc	
paraplegia	
renal_disease	
malignant_cancer	
severe_liver_disease	
metastatic_solid_tumor	
aids	
sofa score	
apsiii	
sapsii	
pt	760 (9.52)
aptt	805 (10.08)
white_blood_cell_counts	3 (0.03)
platelet_counts0[a]	
platelet_counts3[b]	
LOS_hospital	
hospital_expire_flag[c]	
LOS_ICU	

LOS=length of stay

[a]platelete_counts0 are regarded as platelet counts on day 1 of ICU admission

[b]platelete_counts3 are regarded as platelet counts on day 4 of ICU admission

[c] hospital_expire_flag is regarded as in-hospital death

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4，5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5，6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6，7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7，8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7，8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	/
		(*e*) Describe any sensitivity analyses	8，9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	/
		(b) Give reasons for non-participation at each stage	/
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8，9
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	/
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	/
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8，9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11，12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11，12
Generalisability	21	Discuss the generalisability (external validity) of the study results	/
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between minimal decrease of platelet counts and outcomes in septic patients: a retrospective observational study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-069027.R1
Article Type:	Original research
Date Submitted by the Author:	22-Feb-2023
Complete List of Authors:	liu, xing; Sichuan University West China Hospital, Department of Critical Care Medicine Yin, Wanhong; Sichuan University West China Hospital, Department of Critical Care Medicine Li, Yi; Sichuan University West China Hospital, Department of Critical Care Medicine Qin, Yiwei; Chengdu Medical College The First Affiliated Hospital, Department of Intensive Medicine Zou, Tongjuan; Sichuan University West China Hospital, Department of Critical Care Medicine
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Infectious diseases
Keywords:	INTENSIVE & CRITICAL CARE, Adult intensive & critical care < INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association between minimal decrease of platelet counts and outcomes in septic patients: a retrospective observational study

Xing Liu[1], MS; Wanhong Yin[1], MD; Yi Li[1], MD; Yiwei Qin[2], MS; Tongjuan Zou[1], MS

[1]Department of Critical Care Medicine, West China Hospital, Sichuan University, No. 37 Guo Xue Road, Chengdu Sichuan 610041, China

[2]Department of Intensive Medicine, The First Affiliated Hospital of Chengdu Medical College, Chengdu, Sichuan 610500, China

Correspondence author: Wanhong Yin, MD

E-mail address: yinwanhong@wchscu.cn

Abstract

Objective Recent studies have revealed that platelets are also central inflammatory cells in sepsis. However, scant research has analyzed platelets as an indicator for evaluating the effects of sepsis treatments. This study aimed to investigate the association between the percentage change in platelet count on day four compared to day one and the mortality to determine whether platelets could be used to assess the effectiveness of sepsis therapy.

Design A retrospective cohort study.

Setting Medical Information Mart for Intensive Care (MIMIC-IV) is a public database in critical care from the Beth Israel Deaconess Medical Center in Boston, Massachusetts, USA.

Participants 7981 patients admitted to the Beth Israel Deaconess Medical Center (BIDMC) were analyzed according to Sepsis-3 criteria from MIMIC-IV between 2008 and 2019.

Primary and secondary outcome measures The primary outcome was 30-day mortality after admission. The secondary outcomes included ICU length of stay and length of hospitalization.

Results The difference was significant for 30-day mortality between the two groups (14.1% vs. 23.5%, $p < 0.001$, Kaplan-Meier analysis, $p < 0.0001$). In a multivariable analysis, 30-day mortality declined with a group of ≤ 10% decreasing proportion of platelet counts on day four of ICU admission (OR = 0.73, 95% CI = 0.64 to 0.82, $p < 0.001$). For the secondary outcomes, the patients with ≤ 10% decreasing proportion of

platelet counts had a shorter ICU stay than those with >10% (6.8 vs. 7.5, p < 0.001). In addition, the restricted cubic spline curves based on logistic models showed that mortality decreased as the platelet change proportion increased.

Conclusions A decrease of≤10% in platelets among sepsis patients after treatments could be independently associated with improved 30-day mortality. The percentage of platelets after treatments could serve as a reference for assessing the therapeutic effects of sepsis.

Strengths and limitations of this study

The large sample size data used in the retrospective study were derived from the high-quality MIMIC-IV database to increase the credibility of the research.

The restricted cubic spline curve model was employed to further confirm the association between the trend of platelet change rate and 30-day mortality in septic patients.

There was a small amount of missing data, which was handled by multiple-imputation using classification and regression trees.

Due to lack of the long-term follow-up data in this database, the difference in the long-term outcomes between groups could not be determined.

Introduction

Sepsis is a dangerous illness that requires acute treatment and is a primary global health concern because of its high incidence, high mortality, and poor prognosis. In America, sepsis is not only one of the most expensive illnesses in total hospital expenditures, but also a prevalent death-related illness among hospitalised patients [1, 2]. According to research conducted in some high-income nations, an estimated 50.9 million patients develop sepsis every year, and 5.3 million people die annually due to sepsis-related conditions [3].

International experts agree that sepsis is characterized by multiple organ failures endangering life due to dysregulated host response to infection [4]. Sepsis is a complicated pathophysiological process in which a pathogen triggers a person's inflammatory-immune solid response leading to the activation or repression of various facets, including endothelium, coagulopathy, immunological, and hormonal functions. Endothelial damage, inflammatory pathways, and coagulation work together to activate platelets in sepsis, which are crucial for pathogenic defense. Platelets possess unambiguous structures and functions of host defense effector cells, including the expression of toll-like receptors that detect hallmark signals of bacterial infection, an array of microbicidal peptides, and other host defense molecules and functions [5-7]. Platelets could be effectively bactericidal by releasing platelet antimicrobial peptides [8]. Nevertheless, platelet hyperreactivity could contribute to sepsis complications, such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), acute kidney injury (AKI), and septic cardiomyopathy [9]. Moreover, recent

research has discovered that sepsis induces platelet transcription and translation, and circulating platelets have higher levels of integrin subunit αIIb (ITGA2B), which is linked to higher mortality [10].

Platelet counts have been included in the sequential organ failure assessment (i.e., Sofa Score) as one of the critical markers of patients with sepsis and reflect their prognosis [11]. Numerous studies [12-14] have demonstrated a correlation between thrombocytopenia and an adverse prognosis. According to Mavrommatis et al. [15] , a lower platelet count was associated with a more severe incident of sepsis. Consequently, researchers proposed that thrombocytopenia patients could benefit from medications that stimulate platelet counts to improve the sepsis prognosis [16]. Using recombinant human thrombopoietin (rhTPO), sepsis with thrombocytopenia could raise platelet counts in patients effectively, resulting in a shorter stay in the intensive care unit [17]. On the other hand, other trials have targeted sepsis with antiplatelet drugs to reduce undesirable thrombosis, inflammatory host responses and organ damage [18, 19]. However, little research has been conducted to determine whether platelets could be used as an indicator for evaluating the effects of sepsis treatments.

This study aimed to retrospectively analyze the association between the rate of platelet change after treatments and the outcomes of patients with sepsis.

Materials and Methods

Source of data

This study was reported according to the Strengthening the Reporting of Observational studies in Epidemiology guidelines[20]. Data were retrieved from the Medical

Information Mart for Intensive Care (MIMIC-IV version 1.0) [21], which collected clinical data from a custom hospital-wide electronic health record (EHR) and an ICU-specific clinical information system where more than 380,000 patients were admitted to the Beth Israel Deaconess Medical Center (BIDMC) in Boston, Massachusetts, USA, between 2008 and 2019. The database includes detailed patient demographics, laboratory tests, medication use, vital signs, disease diagnosis, etc. It contains records for patients admitted to the BIDMC emergency department or the intensive care units, and data standards are clearly defined. Patient records were fully anonymized, and data collection was carried out with approval from both BIDMC and Massachusetts Institute of Technology Institutional Review Board (IRB). After completing the training program and passing the test, we are eligible to receive free access to the database and conduct the related research in accordance to the rules. The author passed the certification for the Collaborative Institutional Training Initiative (Certification Number 48605954 for author Xing Liu).

Study population

The inclusion criteria were as follows: 1) individuals with sepsis, according to Sepsis-3 standard [4] ; 2) adults (age≥18 years); 3) length of ICU stay≥ 72 h; and 4) for some patients with records of multiple ICU stays and admissions, only data involving the first ICU stay and admission were included. Before being admitted to the ICU, patients diagnosed with cirrhosis, lymphoma, and taking clopidogrel, aspirin, rivaroxaban, and warfarin were excluded. Patients with prednisone were not taken into consideration while they were transferred to the ICU. Finally, samples with missing data for day one

and day four platelet counts were eliminated.

Variables

Platelet counts on day one and day four following admission to the ICU were extracted from MIMIC-IV to analyze the variation in platelet counts. The variation was calculated using the formula: (platelet $counts_{day4}$ - platelet $counts_{day1}$) / platelet $counts_{day1}$ ×100%. During the data extraction process, the variables from day one of ICU admissions (such as age, gender, weight, ethnicity, chronic diseases, sofa score, acute physiology score (aps iii), simplified acute physiology score (saps ii), prothrombin time (pt), activated partial thromboplastin time (aptt) and white blood cell counts) were taken into consideration. These characteristics served as possible confounders in this study. PostgreSQL was used to extract general variables from the MIMIC-IV database.

Patient and public involvement

No patient or members of the public were involved in any part of this study.

Outcomes

The primary endpoint was 30-day mortality after admission. The length of hospitalization and ICU stay were considered as secondary outcomes.

Missing values

All variables in this study had less than 11% missing values (Table S1). By applying classification and regression trees [22, 23], the missing values for variables including weight, pt, aptt, and white blood cell counts were multiple-imputed.

Statistical analysis

The percentage change of platelet counts was on day four following ICU admission

compared with the baseline. The cut-off of the platelet-count percentage was calculated using the receiver operating characteristic curve (ROC), which was employed to categorize patients in the baseline characteristics table. For computational simplicity, the threshold for the ROC was minus 9.5%, nearly equivalent to minus 10%. Statistical significance was defined as 2-sided p-values <0.05. A multivariate logistic regression analysis was further employed to assess the association between the proportion of platelet counts and 30-day mortality.

The variables listed in Table 1 were applied to identify potential confounding variables for logistic regression. The multivariate analysis included these factors as adjusting variables with a p-value of less than 0.05 in the univariate regression analysis. The Kaplan-Meier (KM) approach was employed to visualize the survival curves and compare changes in platelet counts over 30 days. Survival was assessed via the log-rank test. Based on the logistic models, restricted cubic spline curves were used to evaluate the relationship between the proportion of platelet counts and the principal endpoint.

Data with a normal distribution were summarized using mean and standard deviation, whereas data with a non-normal distribution were presented as median and interquartile range. Normal distributed continuous variables were compared between groups using analysis of variance, while non-normal distributed continuous variables were applied to the Kruskal-Wallis test. R software was employed for all data analysis (R version 4.2.1).

The variables were subclassified for subgroup analysis, including age, gender, sofa

score, pt, aptt, and white blood cell counts. The association between the platelet percentage and mortality was shown in a forest plot.

Table 1: Baseline of patient characteristics stratified by proportion of platelet counts on day 4 of ICU admission

Characteristic	Overall	declining proportion of platelet counts		*p* value
		platelet>10%	platelet≤10%	
Number	7981	3802	4179	
Gender, M (%)	4594 (57.6)	2180 (57.3)	2414 (57.8)	0.717
Median age (IQR), yr	65.8[53.6 77.3]	66.2 [54.6 77.4]	65.3[52.6 77.3]	0.012
Median weight (IQR), kg	80.0[67.0 96.5]	79.8[66.8 96.5]	80.0[67.0 96.4]	0.586
Ethnicity, n (%)				0.671
American Indian	20 (0.3)	8 (0.2)	12 (0.3)	
Asian	205 (2.6)	100 (2.6)	105 (2.5)	
Black	707 (8.9)	325 (8.5)	382 (9.1)	
White	5025 (63.0)	2382 (62.7)	2643 (63.2)	
Hispanic	258 (3.2)	132 (3.5)	126 (3.0)	
Others	1766 (22.1)	855 (22.5)	911 (21.8)	
Select comorbidities,[a] n (%)				
Cardiovascular disease	2826 (35.4)	1473 (38.7)	1353 (32.4)	<0.001
Chronic pulmonary disease	1945 (24.4)	941 (24.8)	1004 (24.0)	0.467
Liver disease	1466 (18.4)	816 (21.5)	650 (15.6)	<0.001
Renal disease	1582 (19.8)	796 (20.9)	786 (18.8)	0.019
diabetes	2256 (28.3)	1109 (29.2)	1147 (27.4)	0.093
Vascular disease	2359 (29.6)	1082 (28.5)	1277 (30.6)	0.043
Cancer[b]	1189 (14.9)	620 (16.3)	569 (13.6)	0.001
Aids	56 (0.7)	19 (0.5)	37 (0.9)	0.054
Others[c]	1507 (18.9)	705 (18.5)	802 (19.2)	0.477
Status at admission (median [IQR])				
Sofa score	7.0 [5.0 11.0]	8.0[5.0 12.0]	6.0 [4.0 9.0]	<0.001
Apsiii	61.0[44.0 81.0]	66.0[49.0 88.8]	56.0[42.0 74.0]	<0.001
Sapsii	40.0[32.0 51.0]	44.0[35.0 54.0]	38.0[30.0 47.0]	<0.001
Laboratory test (median [IQR])				
White blood cell counts, k/ul	12.0 [8.6 16.3]	13.0 [9.3 17.8]	11.3 [8.3 15.1]	<0.001
Pt (s)	14.3[12.7 17.3]	14.6[12.8 18.2]	14.1 [12.6 16.5]	<0.001
Aptt(s)	31.7 [27.5 40.7]	33.0[28.1 44.6]	30.8[27.2 37.7]	<0.001
ICU outcome				
30-day mortality, n (%)	1483 (18.6)	892 (23.5)	591 (14.1)	<0.001
Median hospital LOS(IQR), d	13.9 [9.0 22.0]	14.1 [9.0 22.3]	13.7 [9.0 21.8]	0.122
Median ICU LOS(IQR), d	7.1 [4.8 12.1]	7.5 [4.9 12.9]	6.8 [4.7 11.3]	<0.001

IQR, interquartile range; Sofa, sequential organ failure assessment; Apsiii, acute

physiology score; Sapsii, simplified acute physiology score; Pt, prothrombin time; Aptt, activated partial thromboplastin time; LOS, length of stay.
[a]Comorbidities are defined by the Charlson comorbidity index
[b]Cancer includes malignant cancer and metastatic solid tumor
[c]Others includes dementia, rheumatic disease, peptic ulcer disease and paraplegia

Results

There were 35010 adult sepsis patients in the database. After deleting samples with missing platelet counts on day one and day four of ICU admission and applying inclusion and exclusion criteria, the final research population consisted of 7981 adult patients diagnosed with sepsis using Sepsis-3 criteria (Figure 1).

All the research populations' initial characteristics were listed in Table 1. There were two groups that made up of the whole population. According to the descending proportion of platelet counts on day four, 3802 sepsis patients had a platelet>10%, and 4179 patients had a platelet≤10%. Participants in the group with platelet>10 % were older (age 66.2 vs. 65.3, p = 0.012) and had more severe coagulation dysfunction (pt 14.6 vs. 14.1, p<0.001 and aptt 33.0 vs. 30.8, p<0.001). Meanwhile, higher white blood cell counts (13.0 vs. 11.3, p<0.001), as well as a higher sofa score (8 vs. 6, p<0.001), were obviously detected in the patients with platelet>10%. Similar performance might be indicated in other organ dysfunction rating systems.

Patients with platelet≤10% showed lower mortality within 30 days than those with platelet>10% (14.1% [591] vs. 23.5% [892], p<0.001) and their ICU stays tended to be shorter (6.8 vs. 7.5, p<0.001). However, compared to patients with platelet>10%, there was no discernible difference in the length of hospital stays across the groups (13.7 vs. 14.1, p = 0.122). For the mortality within 30 days, patients with platelet ≤ 10% survived

on average of two days longer than those with platelet>10% (mean survival time 26.4 vs. 24.4 days) by Kaplan-Meier analysis (p<0.0001) (Figure 2). In the multivariable logistic regression model, a platelet ≤10% was independently linked to a reduction in the mortality within 30 days (odds ratio [OR], 0.73; 95% confidence interval [CI], 0.64 to 0.82; p-value [p]<0.001). Age, sapsii score, sofa score, apsiii score and white blood cell counts were all considered when the analysis was modified (Table 2). Based on logistic models, the restricted cubic spline (RCS) predicted that the lower the decreasing proportion of platelet counts on day four, the lower the likelihood of mortality within 30 days. Additionally, the model showed improved 30-day mortality in septic patients with increasing proportions of platelet counts in the different patient subsets, including those with just a decrease of≤10% and those with elevated platelet count changes (Figure 3). Our findings were supported by different sub-analysis that excluded patients with no decline or even an increase in platelet counts on day four and with platelet counts < 100K/ul on day one at the time of study inclusion (Table S2,Table S3, Figure S1, Figure S2).

In the subgroup analysis, age, gender, organ dysfunction status, infection level, and the coagulation functional condition were stratified. Similar trends in subgroups were observed in the drop of mortality within 30 days with platelet≤10% (Figure 4).

Table 2: Univariate and multivariate analysis for assessing the mortality within 30 days

	Univariate analysis			Multivariate analysis		
Variables	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.41	1.26-1.58	<0.001	1.07	0.94-1.23	0.307
Liver disease	1.94	1.70-2.21	<0.001	1.61	1.37-1.89	<0.001
Renal disease	1.48	1.30-1.69	<0.001	0.97	0.83-1.12	0.646
Vascular disease	1.31	1.16-1.48	<0.001	1.56	1.36-1.78	<0.001
Cancer	1.75	1.52-2.02	<0.001	1.91	1.62-2.25	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-0.99	<0.001
Sofa score	1.15	1.13-1.16	<0.001	1.01	0.99-1.03	0.469
Apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.03	<0.001
WBC (k/ul)	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.080
Pt (s)	1.03	1.02-1.04	<0.001	1.01	1.01-1.02	<0.001
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.051
[a]platelet≤10%	0.54	0.48-0.60	<0.001	0.73	0.64-0.82	<0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.
[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Discussion

In this analysis of 7981 patients with sepsis, a platelet≤10% on day four after ICU admission was related to a decrease of mortality within 30 days. Subgroup analysis established consistent results. The mortality within 30 days decreased in the subsequent research as the proportion of platelet decreasing lessened. These findings could give an effect assessment measure in sepsis treatments.

The mature megakaryocytes in the bone marrow are the source of platelets, which are anucleate cells. According to previous studies, platelet activity aids in hemostasis.

As a result, platelets perform various other tasks, such as host defence against infection, including phagocytosis of bacteria and viruses, superoxide production, and platelet-derived micro-bactericidal proteins [24]. A series of surface receptors and adhesion molecules on platelets allow them to interact with leukocytes and pathogens in the bloodstream, which is critical for the pro-inflammatory and chemotactic processes [6]. In conditions of infection, such as sepsis, platelets are activated and interact with leukocytes directly in the blood [25-27]. Circulating leukocytes may be able to complete their task due to the engagement. Neutrophils may locate infection sites because of their interaction with platelets[28]. Activated neutrophils produce and release neutrophil extracellular traps (NETs) to capture and destroy infections [29].

Recent investigations have demonstrated that thrombocytopenia is associated with an unfavourable prognosis. In both medical and surgical intensive care units, Moreau D et al. found that a 30% fall in platelet counts was an independent predictor of death [12]. According to several researchers, a slow or absent increase in platelet counts among surgical ICU patients was related to a higher death rate. Within 10 days after ICU admission, the author calculated the platelet percentage. The value was more than five times higher in survivors compared with non-survivors ($30 \pm 46 \cdot 10^3/mm^3/day$ vs. $6 \pm 28 \cdot 10^3/mm^3/day$, $p<0.001$) [30]. However, in a retrospective analysis of sepsis patients with leukocytosis, the hospital mortality rose by 6.9% in the thrombocytosis group, which was classified as having > 500,000 platelets/L [31]. Minimal research has assessed the correlation between platelet change rate after treatments and sepsis outcomes. Furthermore, the evidence is lacking for platelet as a therapeutic evaluation indicator.

Platelets are often applied as clinical monitoring indices and play a role in anti-infective responses [26]. Nevertheless, few studies use platelets as a new inflammatory cell for clinical evaluation of the inflammatory response process in sepsis. The host response is the core of sepsis, and different pathogens causing sepsis may require various monitoring of the inflammatory response, such as procalcitonin and 1,3-β-d-glucan (BDG) testing. Currently, platelets, as an inflammatory mediator, essentially respond to the sepsis host response process [8] . Our results showed that septic patients with the proportion of platelet decrease lessened on day four compared to day one had improved 30-day mortality. Platelets could become a new indicator that directly responds to inflammatory changes in sepsis. Meanwhile, the day four is a time window for assessing the effect of sepsis treatment, so changes in platelet count on day four are expected to be a reference to help clinicians evaluate treatment effectiveness and optimize treatment regimens. Additionally, thrombocytosis could be a standalone indicator of a favourable prognosis in ICU patients. Specifically, 21.7% of patients with general and trauma ICU platelet counts of more than $450 \cdot 10^9$/litre were associated with lower ICU morality (P = 0.003) [32]. This outcome supports our further research.

Furthermore, there are several limitations of this study. Firstly, this observational research did not aim to identify the underlying mechanism of how platelet change might affect sepsis outcomes. Therefore, assuming the physio-pathological reasons for whether platelet change is connected to a positive result is challenging. Our findings may have limitations in generalizability to all critical care patients in the ICU because the eligible population was limited to septic patients. Secondly, the results might lack

a causal association because we only examined data from an extensive public retrospective database. Future larger clinical trials are needed to compare the different percentages of platelets in evaluating the therapeutic effect of sepsis in the future. Thirdly, this observational study did not demonstrate the association between treatments and platelet levels. However, by taking advantage of the large sample size of the public database, this study provides a reference for further prospective studies in sepsis.

Conclusions

To summarise, a decrease of ≤10% in platelets among sepsis patients after treatments could be independently associated with an improvement in mortality within 30 days. Meanwhile, this study found that as the proportion of platelet decrease lessens and the proportion of platelet increase enlarges, there is a downward trend in mortality within 30 days in sepsis patients. Furthermore, these findings could provide a fresh perspective on evaluating the effects of sepsis treatments.

Contributors WHY and XL designed this study. YWQ and XL conducted data collection and data analysis. XL wrote the manuscript. WHY, YL and TJZ analyzed and interpreted the results. All authors have reviewed this manuscript.

Competing interests None declared.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement Data are available upon reasonable request. The data used in this study can be obtained by the corresponding author upon request.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Ethics approval This study was in accordance with the ethical standards of the Declaration of Helsinki and was approved by the ethics review boards of Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center (researcher certification number 48605954). MIMIC-IV was retrospective with lack of patient intervention, and all patients' data were de-identified; thus individual patient informed consent was not required.

References

1 Liu V, Escobar GJ, Greene JD, *et al.* Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA* 2014;312:90-2. https://doi.org/10.1001/jama.2014.5804

2 Rudd KE, Johnson SC, Agesa KM, *et al.* Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020;395:200-11. https://doi.org/10.1016/s0140-6736(19)32989-7

3 Fleischmann C, Scherag A, Adhikari NK, *et al.* Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med* 2016;193:259-72. https://doi.org/10.1164/rccm.201504-0781OC

4 Singer M, Deutschman CS, Seymour CW, *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;315:801-10. https://doi.org/10.1001/jama.2016.0287

5 Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* 2010;67:525-44. https://doi.org/10.1007/s00018-009-0210-4

6 Semple JW, Italiano JE, Jr.,Freedman J Platelets and the immune continuum. *Nat Rev Immunol* 2011;11:264-74. https://doi.org/10.1038/nri2956

7 Schouten M, Wiersinga WJ, Levi M, *et al.* Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol* 2008;83:536-45. https://doi.org/10.1189/jlb.0607373

8 de Stoppelaar SF, van 't Veer C,van der Poll T The role of platelets in sepsis. *Thromb Haemost* 2014;112:666-77. https://doi.org/10.1160/th14-02-0126

9 Greco E, Lupia E, Bosco O, *et al.* Platelets and Multi-Organ Failure in Sepsis. *Int J Mol Sci* 2017;18:2200. https://doi.org/10.3390/ijms18102200

10 Middleton EA, Rowley JW, Campbell RA*, et al.* Sepsis alters the transcriptional and translational landscape of human and murine platelets. *Blood* 2019;134:911-23. https://doi.org/10.1182/blood.2019000067

11 Vincent JL, Moreno R, Takala J*, et al.* The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707-10. https://doi.org/10.1007/bf01709751

12 Moreau D, Timsit JF, Vesin A*, et al.* Platelet count decline: an early prognostic marker in critically ill patients with prolonged ICU stays. *Chest* 2007;131:1735-41. https://doi.org/10.1378/chest.06-2233

13 Akca S, Haji-Michael P, de Mendonça A*, et al.* Time course of platelet counts in critically ill patients. *Crit Care Med* 2002;30:753-6. https://doi.org/10.1097/00003246-200204000-00005

14 Jacoby RC, Owings JT, Holmes J*, et al.* Platelet activation and function after trauma. *J Trauma* 2001;51:639-47. https://doi.org/10.1097/00005373-200110000-00003

15 Mavrommatis AC, Theodoridis T, Orfanidou A*, et al.* Coagulation system and platelets are fully activated in uncomplicated sepsis. *Crit Care Med* 2000;28:451-7. https://doi.org/10.1097/00003246-200002000-00027

16 Zhou Z, Feng T, Xie Y*, et al.* The effect of recombinant human thrombopoietin (rhTPO) on sepsis patients with acute severe thrombocytopenia: a study protocol for a multicentre randomised controlled trial (RESCUE trial). *BMC Infect Dis* 2019;19:780. https://doi.org/10.1186/s12879-019-4388-2

17 Liu Y, Jin G, Sun J, *et al.* Recombinant human thrombopoietin in critically ill patients with sepsis-associated thrombocytopenia: A clinical study. *Int J Infect Dis* 2020;98:144-9. https://doi.org/10.1016/j.ijid.2020.06.045

18 Pu Q, Wiel E, Corseaux D, *et al.* Beneficial effect of glycoprotein IIb/IIIa inhibitor (AZ-1) on endothelium in Escherichia coli endotoxin-induced shock. *Crit Care Med* 2001;29:1181-8. https://doi.org/10.1097/00003246-200106000-00019

19 Valerio-Rojas JC, Jaffer IJ, Kor DJ, *et al.* Outcomes of severe sepsis and septic shock patients on chronic antiplatelet treatment: a historical cohort study. *Crit Care Res Pract* 2013;2013:1-9. https://doi.org/10.1155/2013/782573

20 von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg* 2014;12:1495-9. https://doi.org/10.1016/j.ijsu.2014.07.013

21 Johnson A, Bulgarelli L, Pollard T, *et al. MIMIC-IV (version 1.0)*, 2021

22 Zhang Z Missing data imputation: focusing on single imputation. *Ann Transl Med* 2016;4:9. https://doi.org/10.3978/j.issn.2305-5839.2015.12.38

23 Speybroeck N Classification and regression trees. *Int J Public Health* 2012;57:243-6. https://doi.org/10.1007/s00038-011-0315-z

24 van der Poll T, Parker RI Platelet Activation and Endothelial Cell Dysfunction. *Crit Care Clin* 2020;36:233-53. https://doi.org/10.1016/j.ccc.2019.11.002

25 McDonald B, Urrutia R, Yipp BG, *et al.* Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 2012;12:324-

33. https://doi.org/10.1016/j.chom.2012.06.011

26 Clark SR, Ma AC, Tavener SA, *et al.* Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13:463-9. https://doi.org/10.1038/nm1565

27 Zarbock A, Singbartl K,Ley K Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006;116:3211-9. https://doi.org/10.1172/jci29499

28 Mine S, Fujisaki T, Suematsu M, *et al.* Activated platelets and endothelial cell interaction with neutrophils under flow conditions. *Intern Med* 2001;40:1085-92. https://doi.org/10.2169/internalmedicine.40.1085

29 Brinkmann V, Reichard U, Goosmann C, *et al.* Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532-5. https://doi.org/10.1126/science.1092385

30 Nijsten MW, ten Duis HJ, Zijlstra JG, *et al.* Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Crit Care Med* 2000;28:3843-6. https://doi.org/10.1097/00003246-200012000-00017

31 Bakey S, Karamanos E, Louwers L, *et al.* 1047: Thrombocytosis versus Thrombocytopenia as Risk Factor for Increased Mortality in Sepsis. Abstr. *Critical care Medicine* 2013;41:A263. https://doi.org/10.1097/01.ccm.0000440283.47246.33

32 Gurung AM, Carr B,Smith I Thrombocytosis in intensive care. *Br J Anaesth* 2001;87:926-8. https://doi.org/10.1093/bja/87.6.926

Figure Legends

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

Figure 3. The association between the proportion of platelet counts (PPC) and the mortality within 30 days was shown in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subsets. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at an odds ratio of 1.0. Violet density curves show the fraction of the population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, saps ii, aps iii, pt, aptt and white blood cell counts at baseline.

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

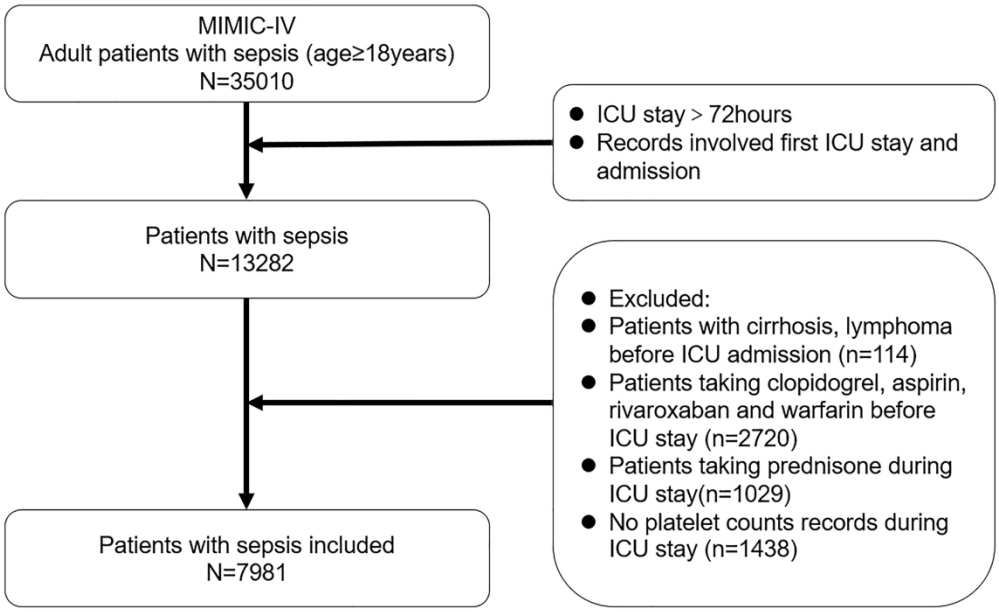

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

232x140mm (300 x 300 DPI)

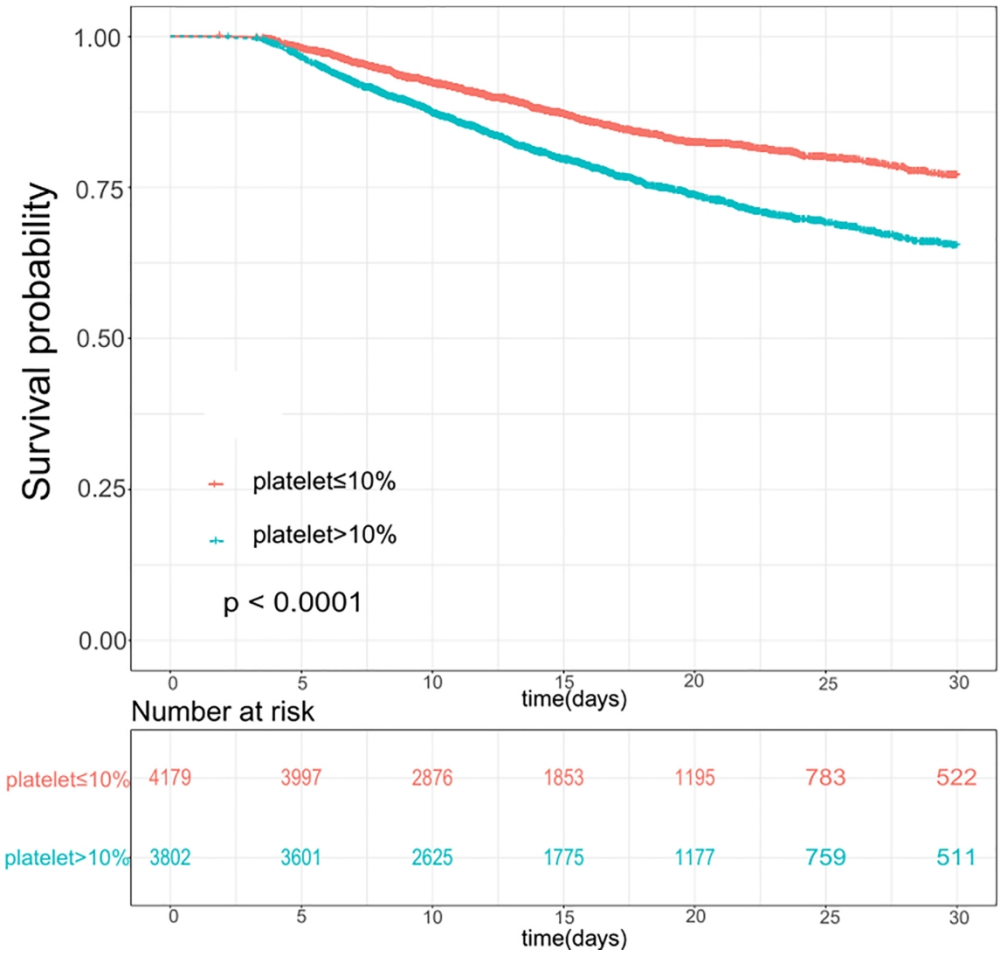

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

162x155mm (300 x 300 DPI)

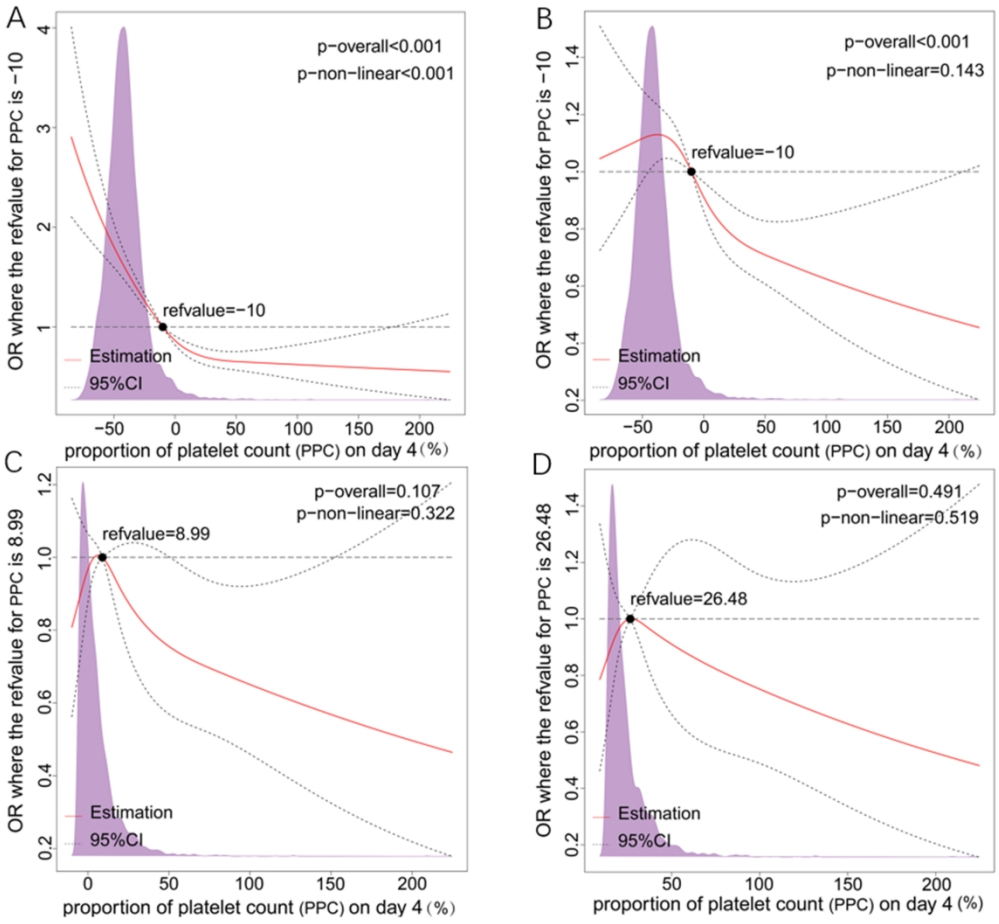

Figure 3. The association between the proportion of platelet counts (PPC) and the mortality within 30 days was shown in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subsets. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at an odds ratio of 1.0. Violet density curves show the fraction of the population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, saps ii, aps iii, pt, aptt and white blood cell counts at baseline.

188x172mm (300 x 300 DPI)

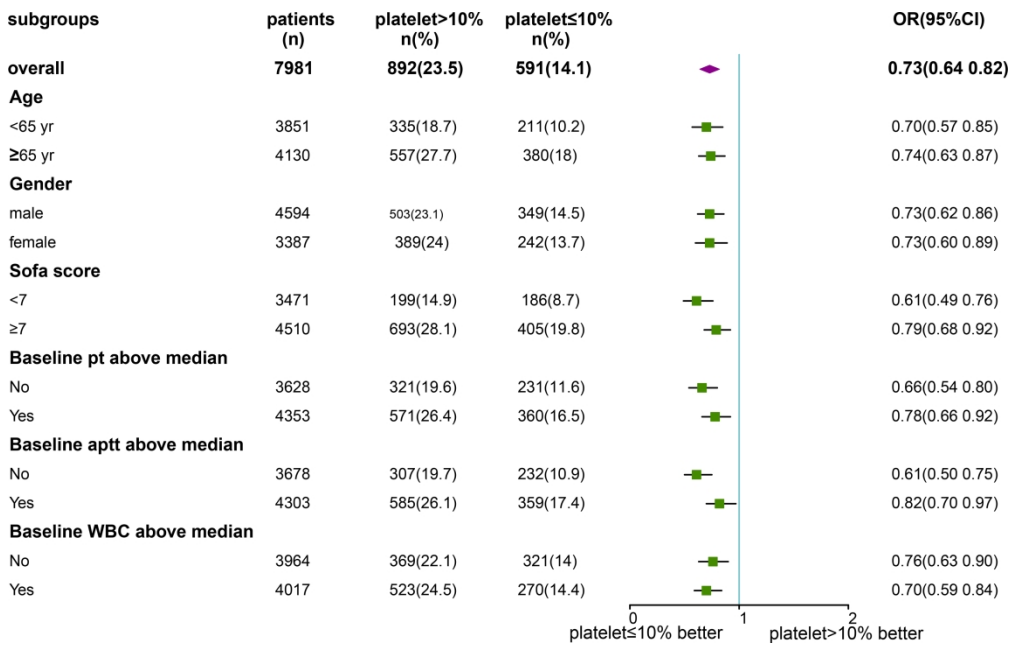

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

336x212mm (300 x 300 DPI)

Table S1. All Missing values of data from MIMIC-IV

variables	Missing number n (%)
age	
gender	
weight	38 (0.47)
ethnicity	
myocardial_infarct	
congestive_heart_failure	
peripheral_vascular_disease	
cerebrovascular_disease	
dementia	
chronic_pulmonary_disease	
rheumatic_disease	
peptic_ulcer_disease	
mild_liver_disease	
diabetes_without_cc	
diabetes_with_cc	
paraplegia	
renal_disease	
malignant_cancer	
severe_liver_disease	
metastatic_solid_tumor	
aids	
sofa score	
apsiii	
sapsii	
pt	760 (9.52)
aptt	805 (10.08)
white_blood_cell_counts	3 (0.03)
platelet_counts0[a]	
platelet_counts3[b]	
LOS_hospital	
hospital_expire_flag[c]	
LOS_ICU	

LOS=length of stay

[a]platelete_counts0 are regarded as platelet counts on day 1 of ICU admission

[b]platelete_counts3 are regarded as platelet counts on day 4 of ICU admission

[c] hospital_expire_flag is regarded as in-hospital death

Table S2. Univariate and multivariate analyses were performed to assess mortality within 30 days, excluding patients who had no decline or even an increase in platelet counts on day four at the time of study inclusion

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.01-1.02	<0.001	1.03	1.02-1.03	<0.001
Cardiovascular disease	1.32	1.15-1.52	<0.001	1.09	0.93-1.28	0.294
Liver disease	1.94	1.66-2.26	<0.001	1.57	1.30-1.89	<0.001
Renal disease	1.33	1.13-1.56	0.001	0.92	0.76-1.10	0.347
Vascular disease	1.22	1.06-1.41	0.007	1.56	1.32-1.83	<0.001
Cancer	1.69	1.42-2.00	<0.001	1.83	1.50-2.22	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-1.00	0.003
Sofa	1.14	1.12-1.16	<0.001	1.00	0.97-1.03	0.885
Apsiii	1.03	1.02-1.03	<0.001	1.03	1.03-1.03	<0.001
WBC (k/ul)	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.166
Pt (s)	1.03	1.03-1.04	<0.001	1.02	1.01-1.03	<0.001
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.139
[a]platelet≤10%	0.55	0.46-0.65	<0.001	0.69	0.57-0.84	0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Figure S1. Kaplan-Meier survival curves

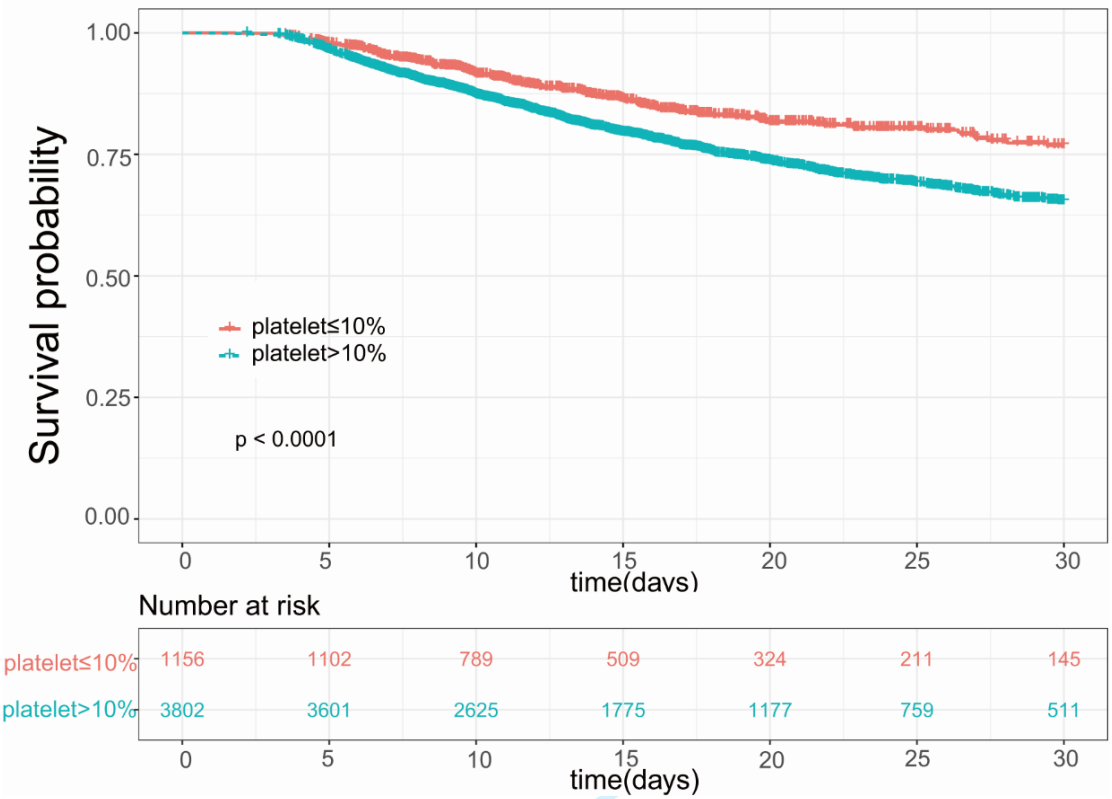

Figure S1. Kaplan-Meier survival curves were used to analyze the association between the percentage change in platelet count on day four compared to day one and the mortality within 30 days, excluding patients with no decline or even an increase in platelet counts on day four at the time of inclusion into the study

Table S3. Univariate and multivariate analyses were performed to assess mortality within 30 days, excluding patients with platelet counts < 100 K/ul on day one at the time of inclusion

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.03	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.49	1.31-1.69	<0.001	1.10	0.95-1.28	0.210
Liver disease	1.63	1.37-1.92	<0.001	1.40	1.15-1.70	0.001
Renal disease	1.50	1.30-1.74	<0.001	0.94	0.80-1.11	0.484
Vascular disease	1.40	1.22-1.59	<0.001	1.59	1.37-1.83	<0.001
Cancer	1.70	1.44-2.00	<0.001	1.90	1.57-2.29	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-1.00	0.001
Sofa	1.14	1.12-1.16	<0.001	0.99	0.97-1.02	0.576
Apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.04	<0.001
WBC (k/ul)	1.02	1.01-1.03	<0.001	1.01	1.00-1.01	0.143
Pt (s)	1.02	1.02-1.03	<0.001	1.01	1.00-1.02	0.016
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.125
[a]platelet≤10%	0.54	0.47-0.61	<0.001	0.72	0.63-0.83	<0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Figure S2. Kaplan-Meier survival curves

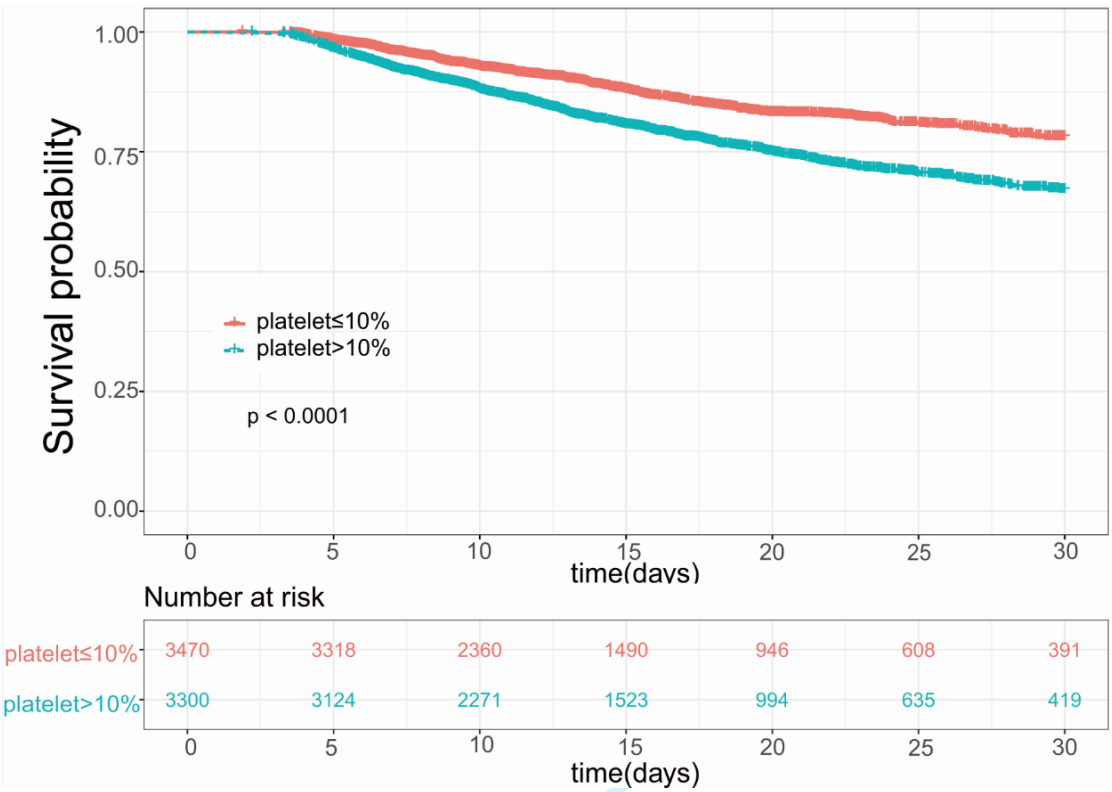

Figure S2. Kaplan-Meier survival curves were used to analyze the association between the percentage change in platelet count on day four compared to day one and the mortality within 30 days, excluding patients with platelet counts < 100 K/ul on day one at the time of inclusion into the study.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4，5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5，6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6，7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7，8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7，8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	/
		(*e*) Describe any sensitivity analyses	8，9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	/
		(b) Give reasons for non-participation at each stage	/
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8，9
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	/
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	/
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8，9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11，12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11，12
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between minimal decrease of platelet counts and outcomes in septic patients: a retrospective observational study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-069027.R2
Article Type:	Original research
Date Submitted by the Author:	23-Mar-2023
Complete List of Authors:	liu, xing; Sichuan University West China Hospital, Department of Critical Care Medicine Yin, Wanhong; Sichuan University West China Hospital, Department of Critical Care Medicine Li, Yi; Sichuan University West China Hospital, Department of Critical Care Medicine Qin, Yiwei; Chengdu Medical College The First Affiliated Hospital, Department of Intensive Medicine Zou, Tongjuan; Sichuan University West China Hospital, Department of Critical Care Medicine
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Infectious diseases
Keywords:	INTENSIVE & CRITICAL CARE, Adult intensive & critical care < INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association between minimal decrease of platelet counts and outcomes in septic patients: a retrospective observational study

Xing Liu[1], Wanhong Yin[1], Yi Li[1], Yiwei Qin[2], Tongjuan Zou[1]

[1]Department of Critical Care Medicine, West China Hospital, Sichuan University, No. 37 Guo Xue Road, Chengdu Sichuan 610041, China

[2]Department of Intensive Medicine, The First Affiliated Hospital of Chengdu Medical College, Chengdu, Sichuan 610500, China

Correspondence to: Dr Wanhong Yin; **yinwanhong@wchscu.cn**

Abstract

Objectives Recent studies have revealed that platelets are also central inflammatory cells in sepsis. However, scant research has analysed platelets as an indicator for evaluating the effects of sepsis treatments. This study aimed to investigate the association between the percentage change in platelet count on day four compared to day one and the mortality to determine whether platelets could be used to assess the effectiveness of sepsis therapy.

Design A retrospective cohort study.

Setting Medical Information Mart for Intensive Care (MIMIC-IV) is a public database in critical care from the Beth Israel Deaconess Medical Center in Boston, Massachusetts, USA.

Participants 7981 patients admitted to the Beth Israel Deaconess Medical Center (BIDMC) were analysed according to Sepsis-3 criteria from MIMIC-IV between 2008 and 2019.

Primary and secondary outcome measures The primary outcome was 30-day mortality after admission. The secondary outcomes included ICU length of stay and length of hospitalization.

Results In the patients with ≤ 10% reduced platelet count, we found lower 30-day mortality (14.1% vs. 23.5%, p<0.001, Kaplan-Meier analysis, p<0.0001). In a multivariable analysis, 30-day mortality declined with a group of ≤ 10% decreasing proportion of platelet counts on day four of ICU admission (OR = 0.73, 95% CI = 0.64 to 0.82, p < 0.001). For the secondary outcomes, the patients with ≤ 10% decreasing

proportion of platelet counts had a shorter ICU stay than those with >10% (6.8 vs. 7.5, $p < 0.001$). In addition, the restricted cubic spline curves based on logistic models showed that mortality decreased as the platelet change proportion increased.

Conclusions A decrease of≤10% in platelets among sepsis patients after treatments could be independently associated with improved 30-day mortality. The percentage of platelets after treatments could serve as a reference for assessing the therapeutic effects of sepsis.

Strengths and limitations of this study

- The large sample size data used in the retrospective study were derived from the high-quality MIMIC-IV database to increase the credibility of the research.
- The restricted cubic spline curve model was employed to confirm further the association between the trend of platelet change rate and 30-day mortality in septic patients.
- There was a small amount of missing data, which was handled by multiple-imputation using classification and regression trees.
- Due to the lack of long-term follow-up data in this database, the difference in the long-term outcomes between groups could not be determined.

Introduction

Sepsis is a dangerous illness that requires acute treatment and is a primary global health concern because of its high incidence, high mortality, and poor prognosis. In America, sepsis is not only one of the most expensive illnesses in total hospital expenditures, but also a prevalent death-related illness among hospitalised patients [1, 2]. According to research conducted in some high-income nations, an estimated 50.9 million patients develop sepsis every year, and 5.3 million people die annually due to sepsis-related conditions [3].

International experts agree that sepsis is characterized by multiple organ failures endangering life due to dysregulated host response to infection [4]. Sepsis is a complicated pathophysiological process in which a pathogen triggers a person's inflammatory-immune solid response leading to the activation or repression of various facets, including endothelium, coagulopathy, immunological, and hormonal functions. Endothelial damage, inflammatory pathways, and coagulation work together to activate platelets in sepsis, which are crucial for pathogenic defence. Platelets possess unambiguous structures and functions of host defence effector cells, including the expression of toll-like receptors that detect hallmark signals of bacterial infection, an array of microbicidal peptides, and other host defence molecules and functions [5-7]. Platelets could be effectively bactericidal by releasing platelet antimicrobial peptides [8]. Nevertheless, platelet hyperreactivity could contribute to sepsis complications, such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), acute kidney injury (AKI), and septic cardiomyopathy [9]. Moreover, recent

research has discovered that sepsis induces platelet transcription and translation, and circulating platelets have higher levels of integrin subunit αIIb (ITGA2B), which is linked to higher mortality [10].

Platelet counts have been included in the sequential organ failure assessment (i.e., Sofa Score) as one of the critical markers of patients with sepsis and reflect their prognosis [11]. Numerous studies [12-14] have demonstrated a correlation between thrombocytopenia and an adverse prognosis. Mavrommatis et al found that a lower platelet count was associated with a more severe incident of sepsis [15]. Consequently, researchers proposed that thrombocytopenia patients could benefit from medications that stimulate platelet counts to improve the sepsis prognosis [16]. Using recombinant human thrombopoietin (rhTPO), sepsis with thrombocytopenia could raise platelet counts in patients effectively, resulting in a shorter stay in the intensive care unit [17]. On the other hand, other trials have targeted sepsis with antiplatelet drugs to reduce undesirable thrombosis, inflammatory host responses and organ damage [18, 19]. However, little research has been conducted to determine whether platelets could be used as an indicator for evaluating the effects of sepsis treatments.

This study aimed to retrospectively analyse the association between the rate of platelet change after treatments and the outcomes of patients with sepsis.

Materials and Methods

Source of data

This study was reported according to the STrengthening the Reporting of OBservational studies in Epidemiology guidelines[20]. Data were retrieved from the

Medical Information Mart for Intensive Care (MIMIC-IV version 1.0) [21], which collected clinical data from a custom hospital-wide electronic health record (EHR) and an ICU-specific clinical information system where more than 380,000 patients were admitted to the Beth Israel Deaconess Medical Center (BIDMC) in Boston, Massachusetts, USA, between 2008 and 2019. The database includes detailed patient demographics, laboratory tests, medication use, vital signs, disease diagnosis, etc. It contains records for patients admitted to the BIDMC emergency department or the intensive care units, and data standards are clearly defined. Patient records were fully anonymized, and data collection was carried out with approval from both BIDMC and Massachusetts Institute of Technology Institutional Review Board (IRB). After completing the training program and passing the test, we are eligible to receive free access to the database and conduct the related research in accordance to the rules. The author passed the certification for the Collaborative Institutional Training Initiative (Certification Number 48605954 for author Xing Liu).

Patient and public involvement

No patient or members of the public were involved in any part of this study.

Study population

The inclusion criteria were as follows: 1) individuals with sepsis, according to Sepsis-3 standard [4] ; 2) adults (age≥18 years); 3) length of ICU stay≥ 72 h; and 4) for some patients with records of multiple ICU stays and admissions, only data involving the first ICU stay and admission were included. Before being admitted to the ICU, patients diagnosed with cirrhosis, lymphoma, and taking clopidogrel, aspirin, rivaroxaban, and

warfarin were excluded. Patients with prednisone were not taken into consideration while they were transferred to the ICU. Finally, samples with missing data for day one and day four platelet counts were eliminated.

Variables extraction

Platelet counts on day one and day four following admission to the ICU were extracted from MIMIC-IV to analyse the variation in platelet counts. The variation was calculated using the formula: (platelet $counts_{day4}$ - platelet $counts_{day1}$) / platelet $counts_{day1}$ ×100%. During the data extraction process, the variables from day one of ICU admissions (such as age, gender, weight, ethnicity, chronic diseases, sofa score, acute physiology score (aps iii), simplified acute physiology score (saps ii), prothrombin time (pt), activated partial thromboplastin time (aptt) and white blood cell counts) were taken into consideration. These characteristics served as possible confounders in this study. PostgreSQL was used to extract general variables from the MIMIC-IV database.

Outcomes

The primary endpoint was 30-day mortality after admission. The length of hospitalization and ICU stay were considered as secondary outcomes.

Missing values

All variables in this study had less than 11% missing values (Table S1). By applying classification and regression trees [22, 23], the missing values for variables including weight, pt, aptt, and white blood cell counts were multiple-imputed.

Statistical analysis

The percentage change of platelet counts was on day four following ICU admission

compared with the baseline. The cut-off of the platelet-count percentage was calculated using the receiver operating characteristic curve (ROC), which was employed to categorize patients in the baseline characteristics table. For computational simplicity, the threshold for the ROC was minus 9.5%, nearly equivalent to minus 10%. Statistical significance was defined as 2-sided p-values <0.05. A multivariate logistic regression analysis was further employed to assess the association between the proportion of platelet counts and 30-day mortality.

The variables listed in Table S2 were applied to identify potential confounding variables for logistic regression. The multivariate analysis included these factors as adjusting variables with a p-value of less than 0.05 in the univariate regression analysis. The Kaplan-Meier (KM) approach was employed to visualize the survival curves and compare changes in platelet counts over 30 days. Survival was assessed via the log-rank test. Based on the logistic models, restricted cubic spline curves were used to evaluate the relationship between the proportion of platelet counts and the principal endpoint.

Data with a normal distribution were summarized using mean and standard deviation, whereas data with a non-normal distribution were presented as median and interquartile range. Normal distributed continuous variables were compared between groups using analysis of variance, while non-normal distributed continuous variables were applied to the Kruskal-Wallis test. R software was employed for all data analysis (R version 4.2.1).

The variables were subclassified for subgroup analysis, including age, gender, sofa

score, pt, aptt, and white blood cell counts. The association between the platelet percentage and mortality was shown in a forest plot.

Results

There were 35010 adult sepsis patients in the database. After deleting samples with missing platelet counts on day one and day four of ICU admission and applying inclusion and exclusion criteria, the final research population consisted of 7981 adult patients diagnosed with sepsis using Sepsis-3 criteria (Figure 1).

All the research populations' initial characteristics were listed in Table S2. There were two groups that made up of the whole population. According to the descending proportion of platelet counts on day four, 3802 sepsis patients had a platelet>10%, and 4179 patients had a platelet≤10%. Participants in the group with platelet>10 % were older (age 66.2 vs. 65.3, p = 0.012) and had more severe coagulation dysfunction (pt 14.6 vs. 14.1, p<0.001 and aptt 33.0 vs. 30.8, p<0.001). Meanwhile, higher white blood cell counts (13.0 vs. 11.3, p<0.001), as well as a higher sofa score (8 vs. 6, p<0.001), were obviously detected in the patients with platelet>10%. Similar performance might be indicated in other organ dysfunction rating systems.

Patients with platelet≤10% showed lower mortality within 30 days than those with platelet>10% (14.1% [591] vs. 23.5% [892], p<0.001) and their ICU stays tended to be shorter (6.8 vs. 7.5, p<0.001). However, compared to patients with platelet>10%, there was no discernible difference in the length of hospital stays across the groups (13.7 vs. 14.1, p = 0.122). For the mortality within 30 days, patients with platelet ≤ 10% survived on average of two days longer than those with platelet>10% (mean survival time 26.4

vs. 24.4 days) by Kaplan-Meier analysis (p<0.0001) (Figure 2). In the multivariable logistic regression model, a platelet ≤10% was independently linked to a reduction in the mortality within 30 days (odds ratio [OR], 0.73; 95% confidence interval [CI], 0.64 to 0.82; p-value [p]<0.001). Age, sapsii score, sofa score, apsiii score and white blood cell counts were all considered when the analysis was modified (Table S3). Based on logistic models, the restricted cubic spline (RCS) predicted that the lower the decreasing proportion of platelet counts on day four, the lower the likelihood of mortality within 30 days. Additionally, the model showed improved 30-day mortality in septic patients with increasing proportions of platelet counts in the different patient subsets, including those with just a decrease of≤10% and those with elevated platelet count changes (Figure 3). Our findings were supported by different sub-analysis that excluded patients with no decline or even an increase in platelet counts on day four and with platelet counts < 100K/ul on day one at the time of study inclusion (Table S4, Table S5, Figure S1, Figure S2).

In the subgroup analysis, age, gender, organ dysfunction status, infection level, and coagulation functional condition were stratified. Similar trends in subgroups were observed in the drop of mortality within 30 days with platelet≤10% (Figure 4).

Discussion

In this analysis of 7981 patients with sepsis, a platelet≤10% on day four after ICU admission was related to a decrease in mortality within 30 days. Subgroup analysis established consistent results. The mortality within 30 days decreased in the subsequent research as the proportion of platelet decreasing lessened. These findings could give an

effect assessment measure in sepsis treatments.

The mature megakaryocytes in the bone marrow are the source of platelets, which are anucleate cells. According to previous studies, platelet activity aids in hemostasis. As a result, platelets perform various other tasks, such as host defence against infection, including phagocytosis of bacteria and viruses, superoxide production, and platelet-derived micro-bactericidal proteins [24]. A series of surface receptors and adhesion molecules on platelets allow them to interact with leukocytes and pathogens in the bloodstream, which is critical for the pro-inflammatory and chemotactic processes [6]. In conditions of infection, such as sepsis, platelets are activated and interact with leukocytes directly in the blood [25-27]. Circulating leukocytes may be able to complete their task due to the engagement. Neutrophils may locate infection sites because of their interaction with platelets[28]. Activated neutrophils produce and release neutrophil extracellular traps (NETs) to capture and destroy infections [29].

Recent investigations have demonstrated that thrombocytopenia is associated with an unfavourable prognosis. In both medical and surgical intensive care units, Moreau et al showed that a 30% fall in platelet counts was an independent predictor of death [12]. According to several researchers, a slow or absent increase in platelet counts among surgical ICU patients was related to a higher death rate. Within ten days after ICU admission, the author calculated the platelet percentage. The value was more than five times higher in survivors compared with non-survivors ($30 \pm 46 \cdot 10^3/mm^3/day$ vs. $6 \pm 28 \cdot 10^3/mm^3/day$, $p<0.001$) [30]. However, in a retrospective analysis of sepsis patients with leukocytosis, the hospital mortality rose by 6.9% in the thrombocytosis group,

which was classified as having > 500,000 platelets/L [31]. Minimal research has assessed the correlation between platelet change rate after treatments and sepsis outcomes. Furthermore, the evidence is lacking for *platelet* as a therapeutic evaluation indicator.

Platelets are often applied as clinical monitoring indices and play a role in anti-infective responses [26]. Nevertheless, few studies use platelets as a new inflammatory cell for clinical evaluation of the inflammatory response process in sepsis. The host response is the core of sepsis, and different pathogens causing sepsis may require various monitoring of the inflammatory response, such as procalcitonin and 1,3-β-d-glucan (BDG) testing. Currently, platelets, as an inflammatory mediator, essentially respond to the sepsis host response process [8] . Our results showed that septic patients with the proportion of platelet decrease lessened on day four compared to day one had improved 30-day mortality. Platelets could become a new indicator that directly responds to inflammatory changes in sepsis. Meanwhile, day four is a time window for assessing the effect of sepsis treatment, so changes in platelet count on day four are expected to be a reference to help clinicians evaluate treatment effectiveness and optimize treatment regimens. Additionally, thrombocytosis could be a standalone indicator of a favourable prognosis in ICU patients. Specifically, 21.7% of patients with general and trauma ICU platelet counts of more than $450 \cdot 10^9$/litre were associated with lower ICU morality (P = 0.003) [32]. This outcome supports our further research.

Furthermore, there are several limitations of this study. Firstly, this observational research did not aim to identify the underlying mechanism of how platelet change might affect sepsis outcomes. Therefore, assuming the physio-pathological reasons for

whether platelet change is connected to a positive result is challenging. Secondly, our findings may not generalise to all critical care patients in the ICU because the eligible population was limited to septic patients. Thirdly, the results might lack a causal association because we only examined data from an extensive public retrospective database. Future larger clinical trials are needed to compare the different percentages of platelets in evaluating the therapeutic effect of sepsis in the future. Fourthly, this observational study did not demonstrate the association between treatments and platelet levels. However, by taking advantage of the large sample size of the public database, this study provides a reference for further prospective studies in sepsis.

Conclusions

To summarise, a decrease of ≤10% in platelets among sepsis patients after treatments could be independently associated with an improvement in mortality within 30 days. Meanwhile, this study found that as the proportion of platelet decrease lessens and the proportion of platelet increase enlarges, there is a downward trend in mortality within 30 days in sepsis patients. Furthermore, these findings could provide a fresh perspective on evaluating the effects of sepsis treatments.

Contributors YWH and LX designed this study. QYW and LX conducted data collection and data analysis. LX wrote the manuscript. YWH, LY and ZTJ analysed and interpreted the results. All authors have reviewed and approved this manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was in accordance with the ethical standards of the Declaration of Helsinki and was approved by the ethics review boards of Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center (researcher certification number 48605954). MIMIC-IV was retrospective with lack of patient intervention, and all patients' data were de-identified; thus individual patient informed consent was not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The data used in this study can be obtained by the corresponding author upon request.

References

1 Liu V, Escobar GJ, Greene JD, *et al.* Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA* 2014;312:90-2. https://doi.org/10.1001/jama.2014.5804

2 Rudd KE, Johnson SC, Agesa KM, *et al.* Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020;395:200-11. https://doi.org/10.1016/s0140-6736(19)32989-7

3 Fleischmann C, Scherag A, Adhikari NK, *et al.* Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med* 2016;193:259-72. https://doi.org/10.1164/rccm.201504-0781OC

4 Singer M, Deutschman CS, Seymour CW, *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;315:801-10. https://doi.org/10.1001/jama.2016.0287

5 Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* 2010;67:525-44. https://doi.org/10.1007/s00018-009-0210-4

6 Semple JW, Italiano JE, Jr.,Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* 2011;11:264-74. https://doi.org/10.1038/nri2956

7 Schouten M, Wiersinga WJ, Levi M, *et al.* Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol* 2008;83:536-45. https://doi.org/10.1189/jlb.0607373

8 de Stoppelaar SF, van 't Veer C,van der Poll T. The role of platelets in sepsis. *Thromb Haemost* 2014;112:666-77. https://doi.org/10.1160/th14-02-0126

9 Greco E, Lupia E, Bosco O, *et al.* Platelets and Multi-Organ Failure in Sepsis. *Int J Mol Sci* 2017;18:2200. https://doi.org/10.3390/ijms18102200

10 Middleton EA, Rowley JW, Campbell RA*, et al.* Sepsis alters the transcriptional and translational landscape of human and murine platelets. *Blood* 2019;134:911-23. https://doi.org/10.1182/blood.2019000067

11 Vincent JL, Moreno R, Takala J*, et al.* The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707-10. https://doi.org/10.1007/bf01709751

12 Moreau D, Timsit JF, Vesin A*, et al.* Platelet count decline: an early prognostic marker in critically ill patients with prolonged ICU stays. *Chest* 2007;131:1735-41. https://doi.org/10.1378/chest.06-2233

13 Akca S, Haji-Michael P, de Mendonça A*, et al.* Time course of platelet counts in critically ill patients. *Crit Care Med* 2002;30:753-6. https://doi.org/10.1097/00003246-200204000-00005

14 Jacoby RC, Owings JT, Holmes J*, et al.* Platelet activation and function after trauma. *J Trauma* 2001;51:639-47. https://doi.org/10.1097/00005373-200110000-00003

15 Mavrommatis AC, Theodoridis T, Orfanidou A*, et al.* Coagulation system and platelets are fully activated in uncomplicated sepsis. *Crit Care Med* 2000;28:451-7. https://doi.org/10.1097/00003246-200002000-00027

16 Zhou Z, Feng T, Xie Y*, et al.* The effect of recombinant human thrombopoietin (rhTPO) on sepsis patients with acute severe thrombocytopenia: a study protocol for a multicentre randomised controlled trial (RESCUE trial). *BMC Infect Dis* 2019;19:780. https://doi.org/10.1186/s12879-019-4388-2

17 Liu Y, Jin G, Sun J, *et al.* Recombinant human thrombopoietin in critically ill patients with sepsis-associated thrombocytopenia: A clinical study. *Int J Infect Dis* 2020;98:144-9. https://doi.org/10.1016/j.ijid.2020.06.045

18 Pu Q, Wiel E, Corseaux D, *et al.* Beneficial effect of glycoprotein IIb/IIIa inhibitor (AZ-1) on endothelium in Escherichia coli endotoxin-induced shock. *Crit Care Med* 2001;29:1181-8. https://doi.org/10.1097/00003246-200106000-00019

19 Valerio-Rojas JC, Jaffer IJ, Kor DJ, *et al.* Outcomes of severe sepsis and septic shock patients on chronic antiplatelet treatment: a historical cohort study. *Crit Care Res Pract* 2013;2013:1-9. https://doi.org/10.1155/2013/782573

20 von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg* 2014;12:1495-9. https://doi.org/10.1016/j.ijsu.2014.07.013

21 Johnson A, Bulgarelli L, Pollard T, *et al. MIMIC-IV (version 1.0)*, 2021

22 Zhang Z. Missing data imputation: focusing on single imputation. *Ann Transl Med* 2016;4:9. https://doi.org/10.3978/j.issn.2305-5839.2015.12.38

23 Speybroeck N. Classification and regression trees. *Int J Public Health* 2012;57:243-6. https://doi.org/10.1007/s00038-011-0315-z

24 van der Poll T, Parker RI. Platelet Activation and Endothelial Cell Dysfunction. *Crit Care Clin* 2020;36:233-53. https://doi.org/10.1016/j.ccc.2019.11.002

25 McDonald B, Urrutia R, Yipp BG, *et al.* Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 2012;12:324-

33. https://doi.org/10.1016/j.chom.2012.06.011

26 Clark SR, Ma AC, Tavener SA, *et al.* Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13:463-9. https://doi.org/10.1038/nm1565

27 Zarbock A, Singbartl K,Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006;116:3211-9. https://doi.org/10.1172/jci29499

28 Mine S, Fujisaki T, Suematsu M, *et al.* Activated platelets and endothelial cell interaction with neutrophils under flow conditions. *Intern Med* 2001;40:1085-92. https://doi.org/10.2169/internalmedicine.40.1085

29 Brinkmann V, Reichard U, Goosmann C, *et al.* Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532-5. https://doi.org/10.1126/science.1092385

30 Nijsten MW, ten Duis HJ, Zijlstra JG, *et al.* Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Crit Care Med* 2000;28:3843-6. https://doi.org/10.1097/00003246-200012000-00017

31 Bakey S, Karamanos E, Louwers L, *et al.* 1047: Thrombocytosis versus Thrombocytopenia as Risk Factor for Increased Mortality in Sepsis. Abstr. *Critical care Medicine* 2013;41:A263. https://doi.org/10.1097/01.ccm.0000440283.47246.33

32 Gurung AM, Carr B,Smith I. Thrombocytosis in intensive care. *Br J Anaesth* 2001;87:926-8. https://doi.org/10.1093/bja/87.6.926

Figure Legends

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

Figure 3. The association between the proportion of platelet counts (PPC) and the mortality within 30 days was shown in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subsets. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at an odds ratio of 1.0. Violet density curves show the fraction of the population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, saps ii, aps iii, pt, aptt and white blood cell counts at baseline.

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

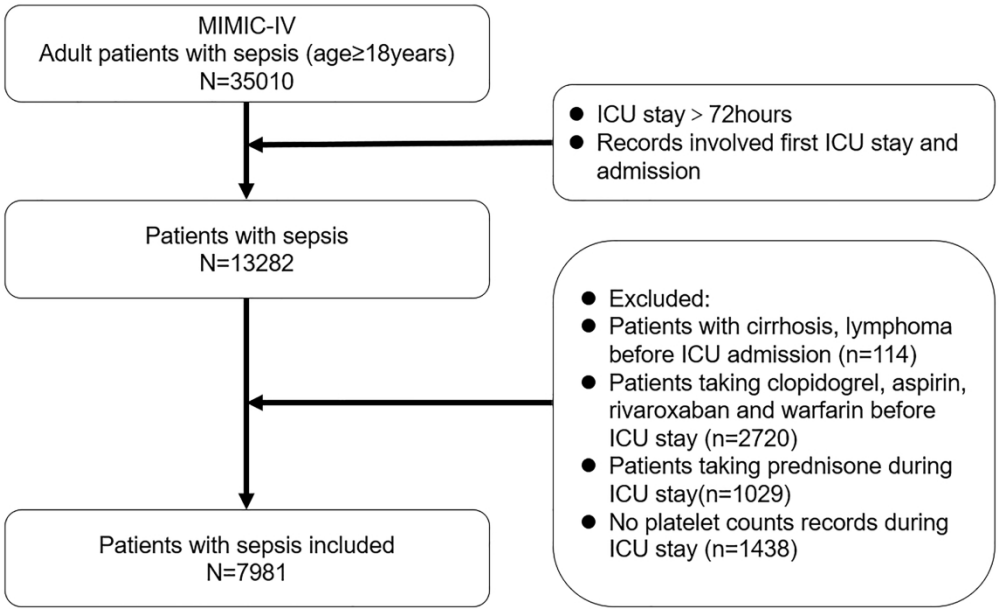

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

232x140mm (300 x 300 DPI)

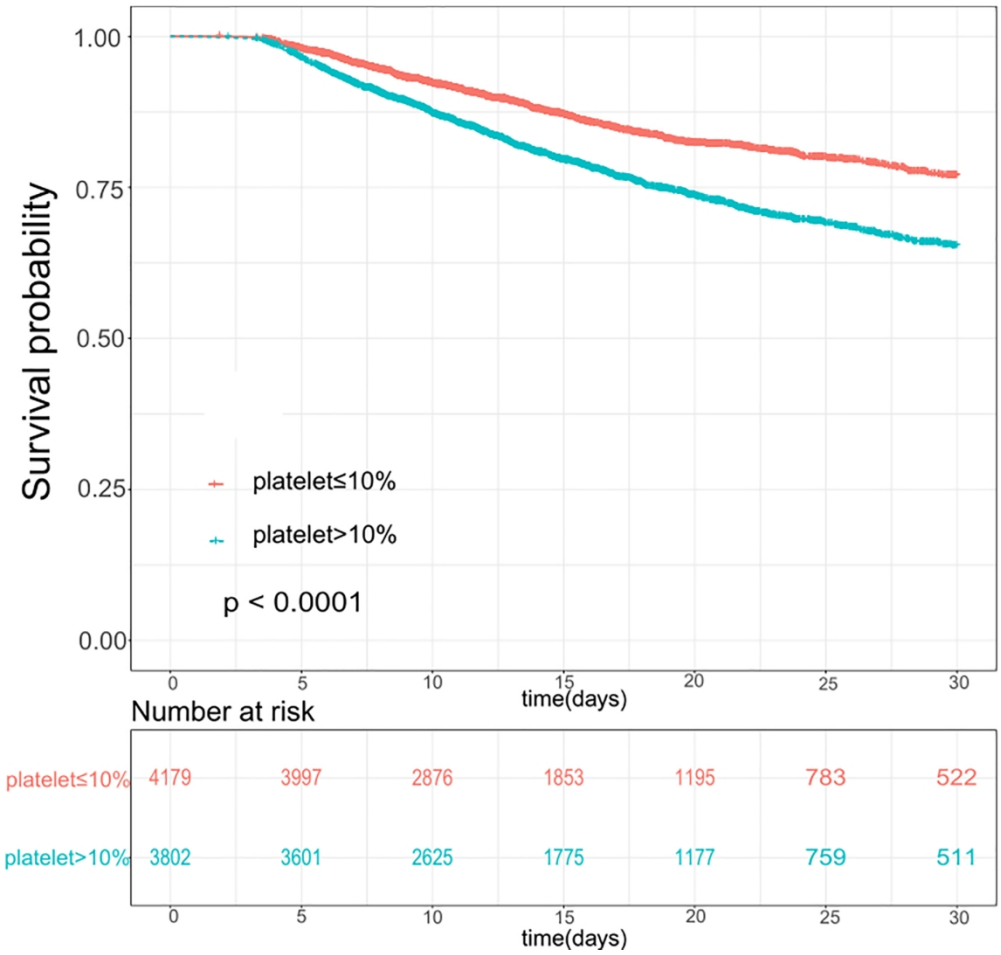

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

162x155mm (300 x 300 DPI)

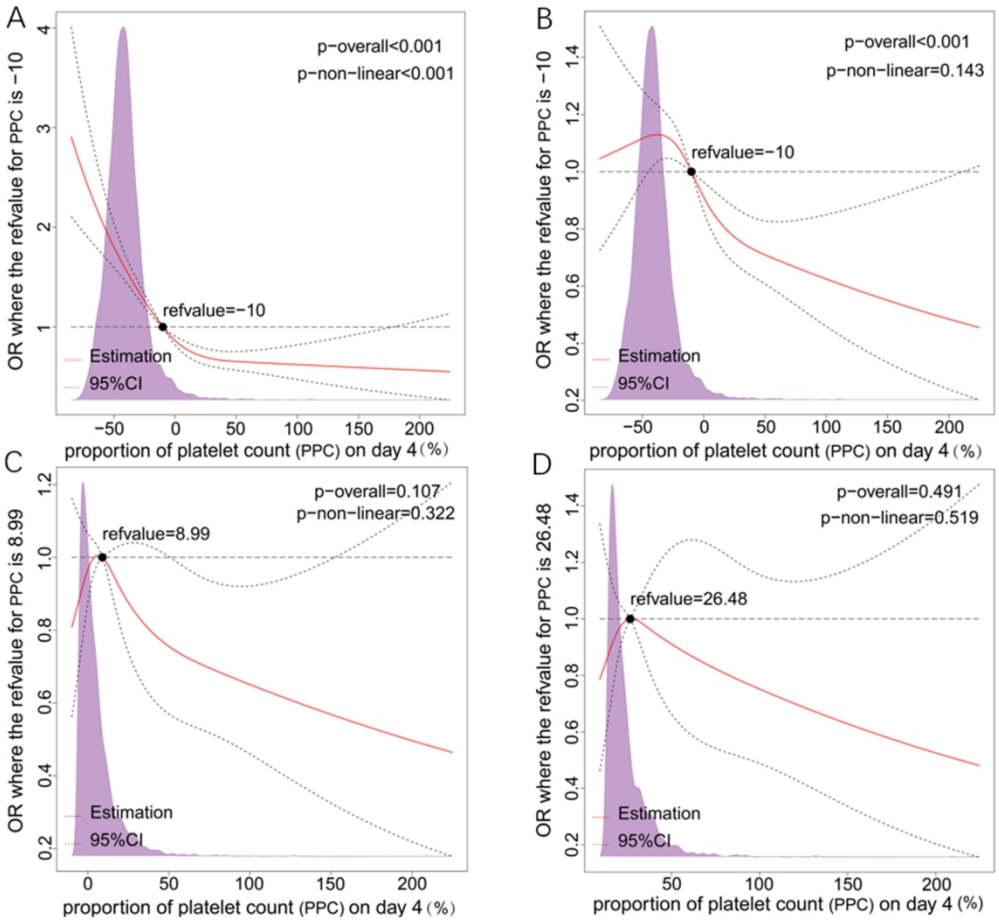

Figure 3. The association between the proportion of platelet counts (PPC) and the mortality within 30 days was shown in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subsets. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at an odds ratio of 1.0. Violet density curves show the fraction of the population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, saps ii, aps iii, pt, aptt and white blood cell counts at baseline.

188x172mm (300 x 300 DPI)

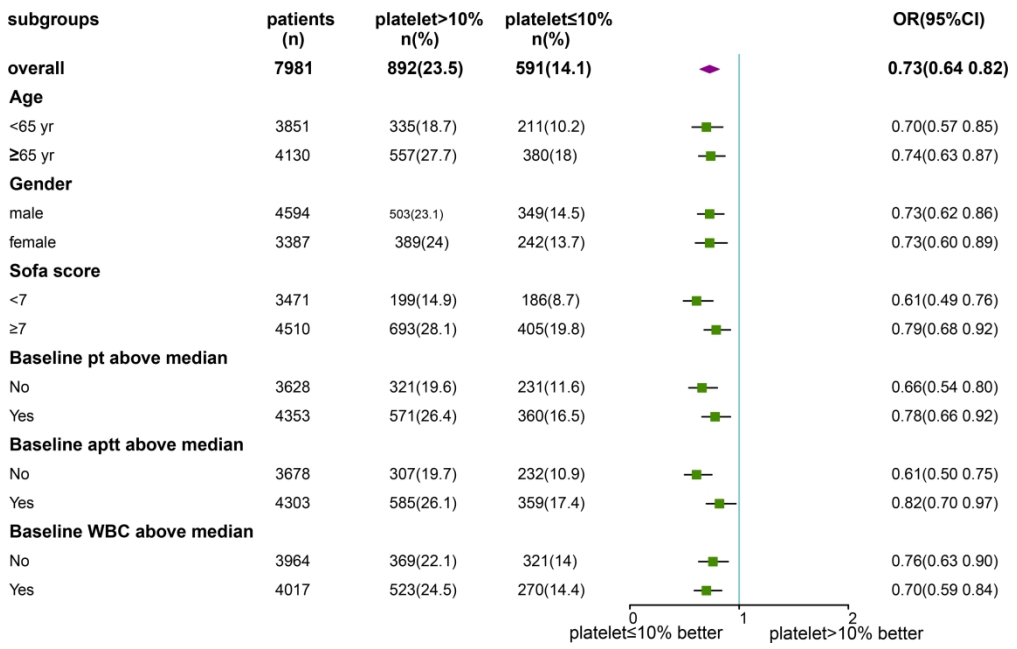

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

336x212mm (300 x 300 DPI)

Table S1. All Missing values of data from MIMIC-IV

variables	Missing number n (%)
age	
gender	
weight	38 (0.47)
ethnicity	
myocardial_infarct	
congestive_heart_failure	
peripheral_vascular_disease	
cerebrovascular_disease	
dementia	
chronic_pulmonary_disease	
rheumatic_disease	
peptic_ulcer_disease	
mild_liver_disease	
diabetes_without_cc	
diabetes_with_cc	
paraplegia	
renal_disease	
malignant_cancer	
severe_liver_disease	
metastatic_solid_tumor	
aids	
sofa score	
apsiii	
sapsii	
pt	760 (9.52)
aptt	805 (10.08)
white_blood_cell_counts	3 (0.03)
platelet_counts0[a]	
platelet_counts3[b]	
LOS_hospital	
hospital_expire_flag[c]	
LOS_ICU	

LOS=length of stay

[a]platelete_counts0 are regarded as platelet counts on day 1 of ICU admission

[b]platelete_counts3 are regarded as platelet counts on day 4 of ICU admission

[c]hospital_expire_flag is regarded as in-hospital death

Table S2: Baseline of patient characteristics stratified by proportion of platelet counts on day four of ICU admission

Characteristic	Overall	declining proportion of platelet counts		*p* value
		platelet>10%	platelet≤10%	
Number	7981	3802	4179	
Gender, M (%)	4594 (57.6)	2180 (57.3)	2414 (57.8)	0.717
Median age (IQR), yr	65.8 [53.6 77.3]	66.2 [54.6 77.4]	65.3 [52.6 77.3]	0.012
Median weight (IQR), kg	80.0 [67.0 96.5]	79.8 [66.8 96.5]	80.0 [67.0 96.4]	0.586
Ethnicity, n (%)				0.671
American Indian	20 (0.3)	8 (0.2)	12 (0.3)	
Asian	205 (2.6)	100 (2.6)	105 (2.5)	
Black	707 (8.9)	325 (8.5)	382 (9.1)	
White	5025 (63.0)	2382 (62.7)	2643 (63.2)	
Hispanic	258 (3.2)	132 (3.5)	126 (3.0)	
Others	1766 (22.1)	855 (22.5)	911 (21.8)	
Select comorbidities[a], n (%)				
Cardiovascular disease	2826 (35.4)	1473 (38.7)	1353 (32.4)	<0.001
Chronic pulmonary disease	1945 (24.4)	941 (24.8)	1004 (24.0)	0.467
Liver disease	1466 (18.4)	816 (21.5)	650 (15.6)	<0.001
Renal disease	1582 (19.8)	796 (20.9)	786 (18.8)	0.019
diabetes	2256 (28.3)	1109 (29.2)	1147 (27.4)	0.093
Vascular disease	2359 (29.6)	1082 (28.5)	1277 (30.6)	0.043
Cancer[b]	1189 (14.9)	620 (16.3)	569 (13.6)	0.001
Aids	56 (0.7)	19 (0.5)	37 (0.9)	0.054
Others[c]	1507 (18.9)	705 (18.5)	802 (19.2)	0.477
Status at admission (median [IQR])				
Sofa score	7.0 [5.0 11.0]	8.0 [5.0 12.0]	6.0 [4.0 9.0]	<0.001
Apsiii	61.0 [44.0 81.0]	66.0 [49.0 88.8]	56.0 [42.0 74.0]	<0.001
Sapsii	40.0 [32.0 51.0]	44.0 [35.0 54.0]	38.0 [30.0 47.0]	<0.001
Laboratory test (median [IQR])				
White blood cell counts, k/ul	12.0 [8.6 16.3]	13.0 [9.3 17.8]	11.3 [8.3 15.1]	<0.001
Pt (s)	14.3 [12.7 17.3]	14.6 [12.8 18.2]	14.1 [12.6 16.5]	<0.001
Aptt (s)	31.7 [27.5 40.7]	33.0 [28.1 44.6]	30.8 [27.2 37.7]	<0.001
ICU outcome				
30-day mortality, n (%)	1483 (18.6)	892 (23.5)	591 (14.1)	<0.001
Median hospital LOS (IQR), d	13.9 [9.0 22.0]	14.1 [9.0 22.3]	13.7 [9.0 21.8]	0.122
Median ICU LOS (IQR), d	7.1 [4.8 12.1]	7.5 [4.9 12.9]	6.8 [4.7 11.3]	<0.001

IQR, interquartile range; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; Sapsii, simplified acute physiology score; Pt, prothrombin time; Aptt, activated partial thromboplastin time; LOS, length of stay.

[a]Comorbidities are defined by the Charlson comorbidity index.

[b]Cancer includes malignant cancer and metastatic solid tumor.

[c]Others includes dementia, rheumatic disease, peptic ulcer disease and paraplegia.

Table S3: Univariate and multivariate analysis for assessing the mortality within 30 days

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.41	1.26-1.58	<0.001	1.07	0.94-1.23	0.307
Liver disease	1.94	1.70-2.21	<0.001	1.61	1.37-1.89	<0.001
Renal disease	1.48	1.30-1.69	<0.001	0.97	0.83-1.12	0.646
Vascular disease	1.31	1.16-1.48	<0.001	1.56	1.36-1.78	<0.001
Cancer	1.75	1.52-2.02	<0.001	1.91	1.62-2.25	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-0.99	<0.001
Sofa score	1.15	1.13-1.16	<0.001	1.01	0.99-1.03	0.469
Apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.03	<0.001
WBC (k/ul)	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.080
Pt (s)	1.03	1.02-1.04	<0.001	1.01	1.01-1.02	<0.001
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.051
[a]platelet≤10%	0.54	0.48-0.60	<0.001	0.73	0.64-0.82	<0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Table S4. Univariate and multivariate analyses were performed to assess mortality within 30 days, excluding patients who had no decline or even an increase in platelet counts on day four at the time of study inclusion

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.01-1.02	<0.001	1.03	1.02-1.03	<0.001
Cardiovascular disease	1.32	1.15-1.52	<0.001	1.09	0.93-1.28	0.294
Liver disease	1.94	1.66-2.26	<0.001	1.57	1.30-1.89	<0.001
Renal disease	1.33	1.13-1.56	0.001	0.92	0.76-1.10	0.347
Vascular disease	1.22	1.06-1.41	0.007	1.56	1.32-1.83	<0.001
Cancer	1.69	1.42-2.00	<0.001	1.83	1.50-2.22	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-1.00	0.003
Sofa	1.14	1.12-1.16	<0.001	1.00	0.97-1.03	0.885
Apsiii	1.03	1.02-1.03	<0.001	1.03	1.03-1.03	<0.001
WBC (k/ul)	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.166
Pt (s)	1.03	1.03-1.04	<0.001	1.02	1.01-1.03	<0.001
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.139
[a]platelet≤10%	0.55	0.46-0.65	<0.001	0.69	0.57-0.84	0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Figure S1. Kaplan-Meier survival curves

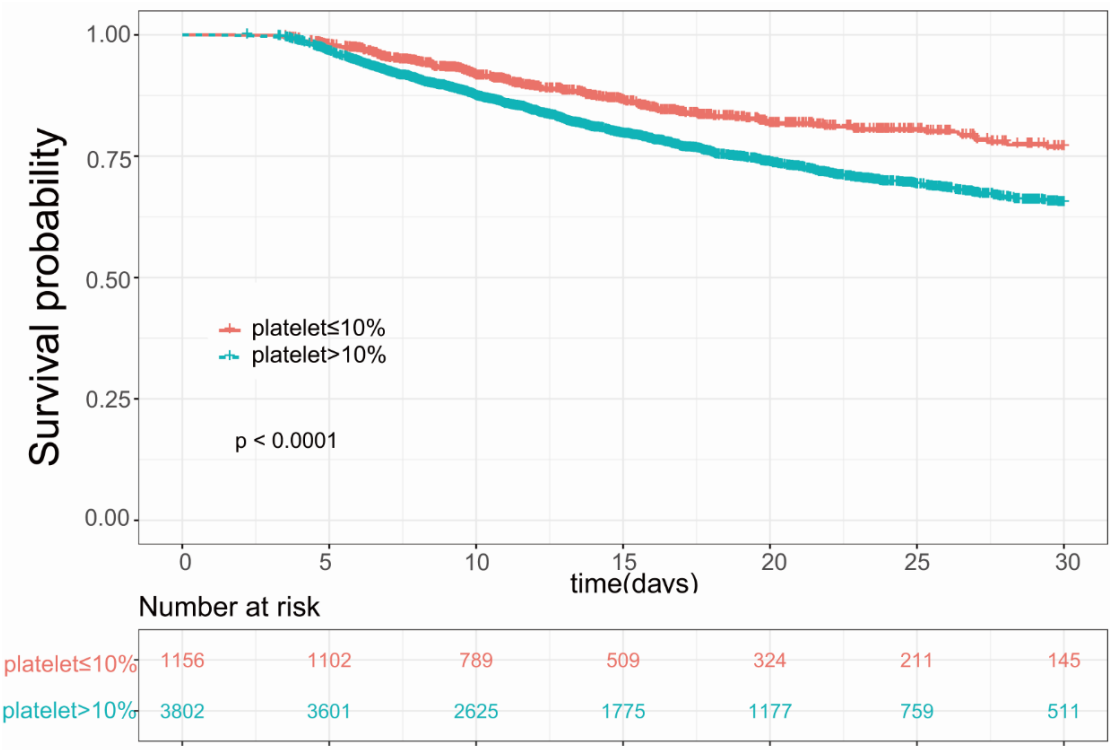

Figure S1. Kaplan-Meier survival curves were used to analyse the association between the percentage change in platelet count on day four compared to day one and the mortality within 30 days, excluding patients with no decline or even an increase in platelet counts on day four at the time of inclusion into the study

Table S5. Univariate and multivariate analyses were performed to assess mortality within 30 days, excluding patients with platelet counts < 100 K/ul on day one at the time of inclusion

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.03	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.49	1.31-1.69	<0.001	1.10	0.95-1.28	0.210
Liver disease	1.63	1.37-1.92	<0.001	1.40	1.15-1.70	0.001
Renal disease	1.50	1.30-1.74	<0.001	0.94	0.80-1.11	0.484
Vascular disease	1.40	1.22-1.59	<0.001	1.59	1.37-1.83	<0.001
Cancer	1.70	1.44-2.00	<0.001	1.90	1.57-2.29	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-1.00	0.001
Sofa	1.14	1.12-1.16	<0.001	0.99	0.97-1.02	0.576
Apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.04	<0.001
WBC (k/ul)	1.02	1.01-1.03	<0.001	1.01	1.00-1.01	0.143
Pt (s)	1.02	1.02-1.03	<0.001	1.01	1.00-1.02	0.016
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.125
[a]platelet≤10%	0.54	0.47-0.61	<0.001	0.72	0.63-0.83	<0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Figure S2. Kaplan-Meier survival curves

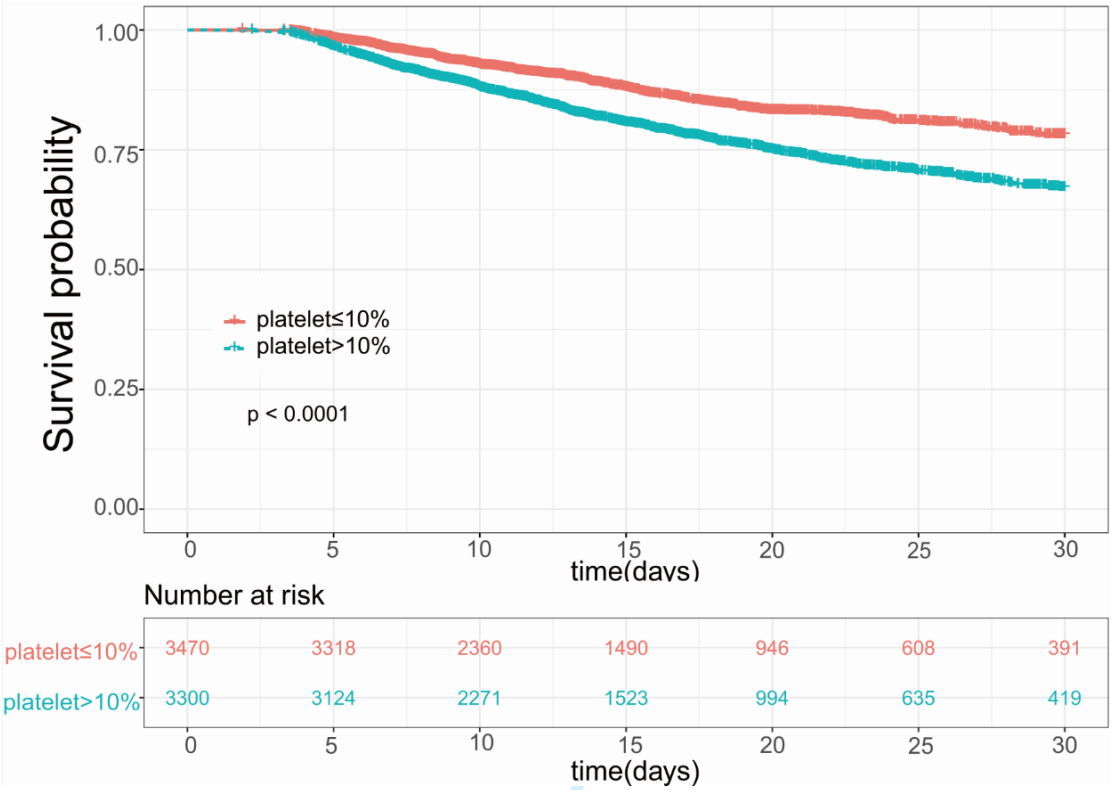

Figure S2. Kaplan-Meier survival curves were used to analyse the association between the percentage change in platelet count on day four compared to day one and the mortality within 30 days, excluding patients with platelet counts < 100 K/ul on day one at the time of inclusion into the study.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4，5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5，6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6，7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7，8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7，8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	/
		(*e*) Describe any sensitivity analyses	8，9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	/
		(b) Give reasons for non-participation at each stage	/
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8，9
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	/
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	/
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8，9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11，12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11，12
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between minimal decrease of platelet counts and outcomes in septic patients: a retrospective observational study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-069027.R3
Article Type:	Original research
Date Submitted by the Author:	09-Apr-2023
Complete List of Authors:	liu, xing; West China Hospital of Sichuan University, Department of Critical Care Medicine Yin, Wanhong; West China Hospital of Sichuan University, Department of Critical Care Medicine Li, Yi; West China Hospital of Sichuan University, Department of Critical Care Medicine Qin, Yiwei; Chengdu Medical College The First Affiliated Hospital, Department of Intensive Medicine Zou, Tongjuan; West China Hospital of Sichuan University, Department of Critical Care Medicine
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Infectious diseases
Keywords:	INTENSIVE & CRITICAL CARE, Adult intensive & critical care < INTENSIVE & CRITICAL CARE, INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association between minimal decrease of platelet counts and outcomes in septic patients: a retrospective observational study

Xing Liu[1], Wanhong Yin[1], Yi Li[1], Yiwei Qin[2], Tongjuan Zou[1]

[1]Department of Critical Care Medicine, West China Hospital, Sichuan University, No. 37 Guo Xue Road, Chengdu Sichuan 610041, China

[2]Department of Intensive Medicine, The First Affiliated Hospital of Chengdu Medical College, Chengdu, Sichuan 610500, China

Correspondence to: Dr Wanhong Yin; **yinwanhong@wchscu.cn**

Abstract

Objectives: Although platelets have been linked to inflammatory development in sepsis, knowledge on their role as an indicator in sepsis treatment is dearth. Here, we investigated the association between time-dependent changes in platelet counts with mortality rates to reveal the role of platelets in sepsis therapy.

Design: A retrospective cohort study.

Setting: We screened the Medical Information Mart for Intensive Care (MIMIC-IV), a public database comprising data from critical care subjects at the Beth Israel Deaconess Medical Center in Boston, Massachusetts, USA.

Participants: A total of 7981 patients, who were admitted to the Beth Israel Deaconess Medical Center (BIDMC) between 2008 and 2019, were analysed based on Sepsis-3 criteria from MIMIC-IV.

Primary and secondary outcome measures: Primary and secondary outcomes included 30-day mortality after admission and length of ICU stay and hospitalization, respectively.

Results: Patients with≤10% reduction in proportion of platelet counts were associated with significantly lower 30-day mortality (14.1% vs. 23.5%, p<0.001, Kaplan-Meier analysis, p<0.0001). Multivariable analysis revealed that decreased platelet-count percentage ≤ 10% on day four after ICU admission was associated with lower probability of 30-day non-survival (OR = 0.73, 95% CI = 0.64 to 0.82, p < 0.001). Patients in the≤10% group had significantly shorter ICU stays than those in the >10% group (6.8 vs. 7.5, p < 0.001). Restricted cubic spline curves revealed that mortality

rates decreased with increase in proportion of platelet counts.

Conclusions: A ≤10% decrease in platelet-count percentage among sepsis patients after treatments is independently associated with decreased 30-day mortality, suggesting that changes in proportion of platelet counts after treatments could be an indicator for assessing the therapeutic effects of sepsis.

Strengths and limitations of this study

- This retrospective study employed a large sample size, from the high-quality MIMIC-IV database, which increases the credibility of the findings.
- We employed the restricted cubic spline curve model to reveal the association between rates of platelet count change and 30-day mortality in septic patients.
- There was a small amount of missing data, which was handled by multiple-imputation using classification and regression trees.
- We did not evaluate differences in long-term outcomes between groups due to a lack of long-term follow-up data in the targeted database.

Introduction

Sepsis is a life-threatening condition that requires acute treatment. The associated high incidence and mortality, as well as poor patient prognosis have made it a primary global health concern. In America, sepsis does not only comprise one of the highest expenses, but also causes numerous deaths among hospitalised patients [1, 2]. Estimates from some high-income nations indicate that about 50.9 million patients develop sepsis every year, of which 5.3 million die annually due to the associated complications [3].

Studies have shown that sepsis is characterized by multiple organ failure, which subsequently endanger life due to dysregulated host response to infections [4]. Sepsis is a complicated pathophysiological process in which a pathogen triggers a person's inflammatory-immune solid response, thereby leading to activation or repression of various facets, including endothelium, coagulopathy, immunological, and hormonal functions. Endothelial damage, inflammatory pathways, and coagulation synergize to activate platelets in sepsis, which are crucial for pathogenic defense. Platelets not only possess unambiguous structures, but also play crucial functions in host defence, including regulating expression of toll-like receptors that detect hallmark signals of bacterial infection, an array of microbicidal peptides, as well as other host defence molecules and functions [5-7]. Some studies have shown that platelets can exert a bactericidal effect by releasing platelet antimicrobial peptides [8], while others have demonstrated that platelet hyperreactivity could contribute to sepsis complications, such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), acute kidney injury (AKI), and septic cardiomyopathy [9]. Moreover,

a recent study revealed that sepsis induces platelet transcription and translation, while circulating platelets exhibited higher levels of integrin subunit αIIb (ITGA2B), which is linked to higher mortality [10].

Platelet counts have been used as critical markers for sequential organ failure assessment (i.e., Sofa Score) in patients with sepsis, and effectively reflect patient prognosis [11]. Numerous studies have demonstrated that thrombocytopenia is correlated with poor prognosis [12-14]. Moreover, Mavrommatis et al.[15] found that a lower platelet count was associated with more severe sepsis incidence. Consequently, researchers have hypothesized that thrombocytopenia patients could benefit from platelet-elevating medications to improve sepsis prognosis [16]. One study targeted recombinant human thrombopoietin (rhTPO) and found that sepsis with thrombocytopenia could effectively promote platelet counts in patients, thereby resulting in shorter stays in the intensive care unit [17]. Other trials investigating the efficacy of antiplatelet drugs in sepsis subjects showed that they reduce undesirable thrombosis, inflammatory host responses and organ damage [18, 19]. To date, however, the potential for platelets as an indicator in evaluation of the effects of sepsis treatments remains unknown. Therefore, this study aimed to retrospectively analyse the relationship between changes in proportion of platelet counts after treatments with clinical outcomes of patients with sepsis.

Materials and methods

Data sources

This study was conducted in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology guidelines[20]. Data were retrieved from the

Medical Information Mart for Intensive Care (MIMIC-IV version 1.0) [21], which comprises clinical data from a custom hospital-wide electronic health record (EHR) and an ICU-specific clinical information system for more than 380,000 patients who were admitted to the Beth Israel Deaconess Medical Center (BIDMC) in Boston, Massachusetts, USA, between 2008 and 2019. The database includes detailed information on patient demographics, laboratory tests, medication use, vital signs, and disease diagnosis, among others. It also contains records for patients admitted to the BIDMC emergency department or the intensive care units, with clearly defined data standards. Patient records were fully anonymized, and data collection was following approval by the BIDMC and Massachusetts Institute of Technology Institutional Review Board (IRB). We first underwent training on the program and passed the Collaborative Institutional Training Initiative test, before we were eligible to receive free access to the database. Thereafter, we conducted the related research in accordance with the rules. The author (Xing Liu) passed certification for the Collaborative Institutional Training Initiative (Certification Number 48605954).

Patient and public involvement

Neither patients nor members of the public were involved in any part of this study.

Selection criteria

Data were included in the study if the patients met the following criteria: 1) were diagnosed with sepsis, according to Sepsis-3 standard [4] ; 2) were adults, aged 18 years and above; and 3) their ICU stay was ≥ 72 h. For patients with records showing multiple ICU stays and admissions, only data involving the first ICU stay and admission were

included. We excluded data for patients diagnosed with cirrhosis, lymphoma, and taking clopidogrel, aspirin, rivaroxaban, and warfarin. In addition, we did not consider patients with prednisone while they were transferred to the ICU, and also excluded datasets with missing data for day one and day four platelet counts.

Data extraction

Data extraction from the MIMIC-IV database was achieved using PostgreSQL. Platelet counts, recorded on the 1st and 4th day after admission to the ICU were extracted from MIMIC-IV. Differences in platelet counts were calculated using the formula: (platelet $counts_{day4}$ - platelet $counts_{day1}$) / platelet $counts_{day1}$ ×100%. The variables at day one of ICU admission included age, gender, weight, ethnicity, chronic diseases, sofa score, acute physiology score (aps iii), simplified acute physiology score (saps ii), prothrombin time (pt), activated partial thromboplastin time (aptt) and white blood cell counts. These characteristics served as possible confounders in this study.

Outcomes

The primary endpoint was 30-day mortality after admission, whereas length of hospitalization and ICU stay were considered secondary outcomes.

Missing values

All variables in this study had less than 11% missing values (Table S1). We employed classification and regression trees [22, 23] for multiple imputation of the missing values for variables, including weight, pt, aptt, and white blood cell counts.

Statistical analysis

The percentage change in platelet counts was recorded on day four following ICU

admission. We generated receiver operating characteristic curve (ROC) to calculate the cut-off of the platelet-count percentage, which was subsequently employed to categorize patients in the baseline characteristics table. For computational simplicity, the threshold for the ROC was minus 9.5% (almost equivalent to minus 10%). Statistical significance was defined as 2-sided p-values <0.05. Further, we used a multivariate logistic regression analysis to assess the relationship between the proportion of platelet counts and 30-day mortality.

Next, we applied the variables listed in Table S2 to identify potential confounding variables for logistic regression. These factors were incorporated into the multivariate regression model, as adjusting variables, at a p-value of less than 0.05. we generated Kaplan-Meier (KM) curves to visualize the survival curves and compare changes in proportion of platelet counts over 30 days, while the log-rank test was used for survival analysis. Restricted cubic spline curves were generated using logistic models and used to evaluate the relationship between proportion of platelet counts and the principal endpoint.

Data with normal distribution were presented as means and respective standard deviations, whereas non-normally distributed variables were presented as medians and interquartile ranges. Normally and non-normally distributed continuous variables were compared between groups using analysis of variance and Kruskal-Wallis test, respectively. For subgroup analysis, variables were subclassified into age, gender, sofa score, pt, aptt, and white blood cell counts categories, then forest plots generated to depict the relationship between proportion of platelet counts and mortality rates. All

statistical analyses were performed using packages implemented in R software, version 4.2.1.

Results

The MIMIC-IV database contains data for 35010 adult sepsis patients. A total of 7981 adult patients diagnosed with sepsis using Sepsis-3 criteria met our inclusion criteria and were included in the study (Figure 1). All participants were classified into two groups, and basic characteristics are outlined in Table S2. Analysis of platelet-count percentages on day four showed that 3802 and 4179 sepsis patients had reduced platelet-count percentage of >10%, and ≤10%, respectively. Patients in the >10 % group were significantly older (age 66.2 vs. 65.3, p = 0.012) and displayed markedly severe coagulation dysfunction (pt 14.6 vs. 14.1, p<0.001 and aptt 33.0 vs. 30.8, p<0.001) than their counterparts in the ≤10% group. Similarly, patients with >10% reduction in platelet-count percentage displayed significantly higher white blood cell counts (13.0 vs. 11.3, p<0.001) and a markedly higher sofa score (8 vs. 6, p<0.001) than those with ≤10%. The higher score in patients with >10% reduction in proportion of platelet counts might also be indicated in other organ dysfunction rating systems, such as aps iii and saps ii.

Patients with ≤10% reduced proportion of platelet counts had significantly lower mortality rates within 30 days (14.1% [591] vs. 23.5% [892], p<0.001) and significantly shorter ICU stays (6.8 vs. 7.5, p<0.001) than their counterparts in the >10% group. However, we found no statistically significant differences between the groups with regards to the length of hospital stays (13.7 vs. 14.1, p = 0.122). Kaplan-Meier curves

showed that patients in the ≤10% group had significantly longer survival times within 30 days than those in the >10% group (mean survival time 26.4 vs. 24.4 days; p<0.0001) (Figure 2). Multivariable logistic regression model showed that reduced platelet-count percentage ≤10% was an independent predictor of reduction in mortality rates within 30 days (odds ratio [OR], 0.73; 95% confidence interval [CI], 0.64 to 0.82; p-value [p]<0.001). In the modified analysis, we adjusted for confounders such as, age, saps ii score, sofa score, aps iii score and white blood cell counts (Table S3). Restricted cubic spline model showed that lower reduction in proportion of platelet counts on day four were associated with lower mortality rates within 30 days. The model also showed that higher proportions of platelet counts were a predictor for improved 30-day mortality in septic patients with one subset excluding reduction in the proportion of platelet counts >10% on day four after ICU admission, while the other having elevated proportion of platelet counts on day four after ICU admission (Figure 3). Our findings were supported by different sub-analysis in which one subset excluded no decline or even an increase in platelet counts on day four compared to day one after ICU admission, while the other subset excluded platelet count <100k/ul on day one after ICU admission (Table S4, Table S5, Figure S1, Figure S2). Subgroup analysis revealed that age, gender, organ dysfunction status, infection level, and coagulation functional condition were all stratified, a trend that was mirrored by a reduction in mortality within 30 days among patients in the ≤10% group (Figure 4).

Discussion

In the present study, we analysed data for 7981 patients with sepsis and found that a ≤

10% decrease in proportion of platelet count on day four after ICU admission was associated with low mortality rates within 30 days. Subgroup analysis results corroborated these findings. Moreover, restricted cubic spline curves revealed that increased proportion of platelet counts was associated with reduced mortality rates within 30 days. These findings showed that change in proportion of platelet counts would be used as a reference to evaluate the effect of sepsis treatments.

Platelets, anucleate cells, originate from mature megakaryocytes in the bone marrow. Previous studies have shown that platelets not only play a role in hemostasis, but also in various other tasks, such as host defence against infection, including phagocytosis of bacteria and viruses, superoxide production, and platelet-derived micro-bactericidal proteins [24]. Notably, platelets have a series of surface receptors and adhesion molecules that allow them to interact with leukocytes and pathogens in the bloodstream, which is critical for the pro-inflammatory and chemotactic processes [6]. When sepsis occurs, due to conditions such as infections, platelets are activated and directly interact with leukocytes in the blood [25-27]. Through the interaction, circulating leukocytes can effectively exert anti-infection effects. Neutrophils may locate infection sites because of their interaction with platelets [28], while activated neutrophils produce and release neutrophil extracellular traps (NETs) that subsequently capture and destroy infections [29].

Recent research evidence has revealed that thrombocytopenia is associated with a poor patient prognosis. For example, Moreau et al.[12] showed that a 30% fall in platelet counts was an independent predictor of mortality in both medical and surgical intensive

care units , while Nijsten et al.[30] demonstrated that a slow or lack of increase in platelet counts among surgical ICU patients was associated with higher mortality rates. The authors calculated platelet proportions ten days after ICU admission, and found that the value was more than five times higher in survivors compared to non-survivors ($30 \pm 46 \cdot 10^3/mm^3/day$ vs. $6 \pm 28 \cdot 10^3/mm^3/day$, $p<0.001$). However, results from a retrospective analysis of sepsis patients with leukocytosis revealed a 6.9% increase in hospital mortality rates among patients in the thrombocytosis group, which was classified as having > 500,000 platelets/L [31]. To date, a handful of studies have evaluated the correlation between platelet counts and sepsis outcomes while the role of platelets as an indicator of therapeutic efficacy remains unknown.

Platelets are not only often applied as clinical monitoring indices, but also play a role in anti-infective responses [26]. Nevertheless, few studies have employed platelets as a new inflammatory cell for clinical evaluation of inflammatory response in sepsis. Host response is the core pathomechanism of sepsis, and different pathogens causing sepsis may require various monitoring of the inflammatory response, such as procalcitonin and 1,3-β-d-glucan (BDG) testing. Currently, platelets, as an inflammatory mediator, essentially respond to the sepsis host response process [8] . Results of the present study showed that septic patients with minimal decrease of platelet counts on day four compared to day one had improved 30-day mortality. Meanwhile, day four is the optimum period for assessing the effect of sepsis treatment, therefore changes in proportion of platelet counts on this day are expected to be a reference for future evaluation of clinical efficacy and optimization of treatment regimens. Additionally,

thrombocytosis has potential as a standalone indicator of a favourable prognosis in ICU patients. A previous study found that patients with general and trauma ICU platelet counts of more than 450·109/litre on at least one occasion were associated with lower ICU mortality (P = 0.003) [32]. This outcome was consistent with our findings of research.

This study had several limitations. Firstly, considering that this was an observational study, we did not elucidate the underlying mechanism of how platelet counts change might affect sepsis outcomes, thus further studies are needed. Secondly, our findings may not generalise to all critical care patients in the ICU because the eligible population was limited to septic patients. Thirdly, our results lack a causal association because we only examined data from an extensive public retrospective database. In future, larger clinical trials are needed to compare changes in proportion of platelet counts and their effect in evaluating the therapeutic effect of sepsis. Lastly, we did not analyse the association between treatments and platelet levels owing to the observational nature of the study. However, by taking advantage of the large sample size of the public database, this study provides a reference for further prospective studies in sepsis.

Conclusions

In summary, reduction in proportion of platelet counts of ≤10% in sepsis patients after treatments are an independent predictor of improved mortality rates within 30 days. Meanwhile, this study found that a downward trend in mortality within 30 days in sepsis patients as the platelet counts increased. Collectively, these findings provide new insights into the role of platelets in evaluating efficacy of sepsis treatments.

Contributors YWH and LX designed this study. QYW and LX conducted data collection and data analysis. LX wrote the manuscript. YWH, LY and ZTJ analysed and interpreted the results. All authors have reviewed and approved this manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was in accordance with the ethical standards of the Declaration of Helsinki and was approved by the ethics review boards of Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center (researcher certification number 48605954). MIMIC-IV was retrospective with lack of patient intervention, and all patients' data were de-identified; thus individual patient informed consent was not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The data used in this study can be obtained by the corresponding author upon request.

References

1 Liu V, Escobar GJ, Greene JD, *et al.* Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA* 2014;312:90-2. https://doi.org/10.1001/jama.2014.5804

2 Rudd KE, Johnson SC, Agesa KM, *et al.* Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020;395:200-11. https://doi.org/10.1016/s0140-6736(19)32989-7

3 Fleischmann C, Scherag A, Adhikari NK, *et al.* Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med* 2016;193:259-72. https://doi.org/10.1164/rccm.201504-0781OC

4 Singer M, Deutschman CS, Seymour CW, *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;315:801-10. https://doi.org/10.1001/jama.2016.0287

5 Yeaman MR Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* 2010;67:525-44. https://doi.org/10.1007/s00018-009-0210-4

6 Semple JW, Italiano JE, Jr.,Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* 2011;11:264-74. https://doi.org/10.1038/nri2956

7 Schouten M, Wiersinga WJ, Levi M, *et al.* Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol* 2008;83:536-45. https://doi.org/10.1189/jlb.0607373

8 de Stoppelaar SF, van 't Veer C,van der Poll T. The role of platelets in sepsis. *Thromb Haemost* 2014;112:666-77. https://doi.org/10.1160/th14-02-0126

9 Greco E, Lupia E, Bosco O, *et al.* Platelets and Multi-Organ Failure in Sepsis. *Int J Mol Sci* 2017;18:2200. https://doi.org/10.3390/ijms18102200

10 Middleton EA, Rowley JW, Campbell RA*, et al.* Sepsis alters the transcriptional and translational landscape of human and murine platelets. *Blood* 2019;134:911-23. https://doi.org/10.1182/blood.2019000067

11 Vincent JL, Moreno R, Takala J*, et al.* The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;22:707-10. https://doi.org/10.1007/bf01709751

12 Moreau D, Timsit JF, Vesin A*, et al.* Platelet count decline: an early prognostic marker in critically ill patients with prolonged ICU stays. *Chest* 2007;131:1735-41. https://doi.org/10.1378/chest.06-2233

13 Akca S, Haji-Michael P, de Mendonça A*, et al.* Time course of platelet counts in critically ill patients. *Crit Care Med* 2002;30:753-6. https://doi.org/10.1097/00003246-200204000-00005

14 Jacoby RC, Owings JT, Holmes J*, et al.* Platelet activation and function after trauma. *J Trauma* 2001;51:639-47. https://doi.org/10.1097/00005373-200110000-00003

15 Mavrommatis AC, Theodoridis T, Orfanidou A*, et al.* Coagulation system and platelets are fully activated in uncomplicated sepsis. *Crit Care Med* 2000;28:451-7. https://doi.org/10.1097/00003246-200002000-00027

16 Zhou Z, Feng T, Xie Y*, et al.* The effect of recombinant human thrombopoietin (rhTPO) on sepsis patients with acute severe thrombocytopenia: a study protocol for a multicentre randomised controlled trial (RESCUE trial). *BMC Infect Dis* 2019;19:780. https://doi.org/10.1186/s12879-019-4388-2

17 Liu Y, Jin G, Sun J, *et al.* Recombinant human thrombopoietin in critically ill patients with sepsis-associated thrombocytopenia: A clinical study. *Int J Infect Dis* 2020;98:144-9. https://doi.org/10.1016/j.ijid.2020.06.045

18 Pu Q, Wiel E, Corseaux D, *et al.* Beneficial effect of glycoprotein IIb/IIIa inhibitor (AZ-1) on endothelium in Escherichia coli endotoxin-induced shock. *Crit Care Med* 2001;29:1181-8. https://doi.org/10.1097/00003246-200106000-00019

19 Valerio-Rojas JC, Jaffer IJ, Kor DJ, *et al.* Outcomes of severe sepsis and septic shock patients on chronic antiplatelet treatment: a historical cohort study. *Crit Care Res Pract* 2013;2013:1-9. https://doi.org/10.1155/2013/782573

20 von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg* 2014;12:1495-9. https://doi.org/10.1016/j.ijsu.2014.07.013

21 Johnson A, Bulgarelli L, Pollard T, *et al. MIMIC-IV (version 1.0)*, 2021

22 Zhang Z. Missing data imputation: focusing on single imputation. *Ann Transl Med* 2016;4:9. https://doi.org/10.3978/j.issn.2305-5839.2015.12.38

23 Speybroeck N Classification and regression trees. *Int J Public Health* 2012;57:243-6. https://doi.org/10.1007/s00038-011-0315-z

24 van der Poll T, Parker RI. Platelet Activation and Endothelial Cell Dysfunction. *Crit Care Clin* 2020;36:233-53. https://doi.org/10.1016/j.ccc.2019.11.002

25 McDonald B, Urrutia R, Yipp BG, *et al.* Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 2012;12:324-

33. https://doi.org/10.1016/j.chom.2012.06.011

26 Clark SR, Ma AC, Tavener SA, *et al.* Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 2007;13:463-9. https://doi.org/10.1038/nm1565

27 Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006;116:3211-9. https://doi.org/10.1172/jci29499

28 Mine S, Fujisaki T, Suematsu M, *et al.* Activated platelets and endothelial cell interaction with neutrophils under flow conditions. *Intern Med* 2001;40:1085-92. https://doi.org/10.2169/internalmedicine.40.1085

29 Brinkmann V, Reichard U, Goosmann C, *et al.* Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532-5. https://doi.org/10.1126/science.1092385

30 Nijsten MW, ten Duis HJ, Zijlstra JG, *et al.* Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Crit Care Med* 2000;28:3843-6. https://doi.org/10.1097/00003246-200012000-00017

31 Bakey S, Karamanos E, Louwers L, *et al.* 1047: Thrombocytosis versus Thrombocytopenia as Risk Factor for Increased Mortality in Sepsis. Abstr. *Critical care Medicine* 2013;41:A263. https://doi.org/10.1097/01.ccm.0000440283.47246.33

32 Gurung AM, Carr B,Smith I. Thrombocytosis in intensive care. *Br J Anaesth* 2001;87:926-8. https://doi.org/10.1093/bja/87.6.926

Figure Legends

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

Figure 3. The association between proportion of platelet counts (PPC) and mortality within 30 days was shown in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subsets. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at an odds ratio of 1.0. Violet density curves show the fraction of the population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, saps ii, aps iii, pt, aptt and white blood cell counts at baseline.

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

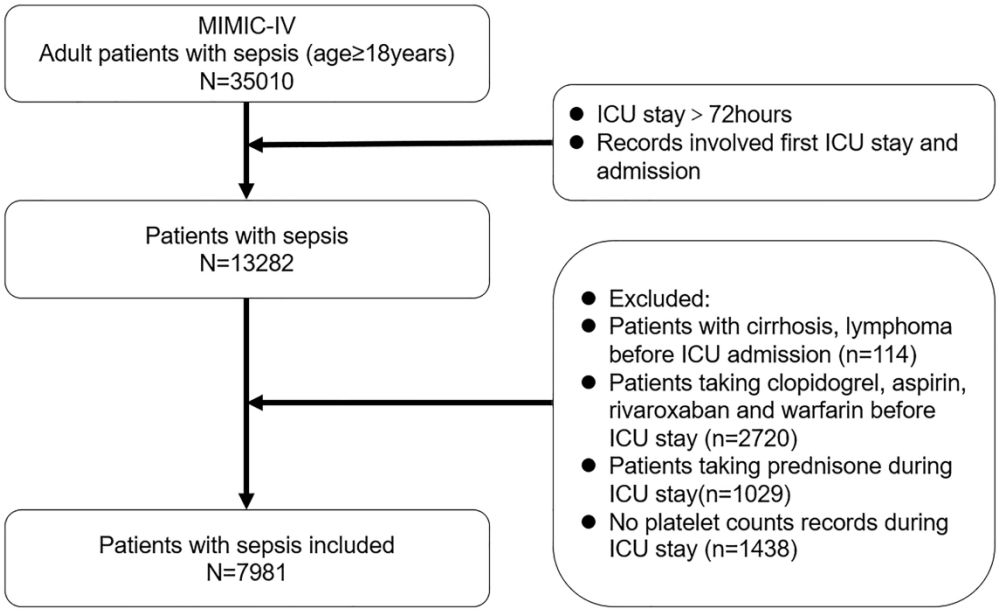

Figure 1. Flowchart showing step-by-step selection on patients included in the study.

232x140mm (300 x 300 DPI)

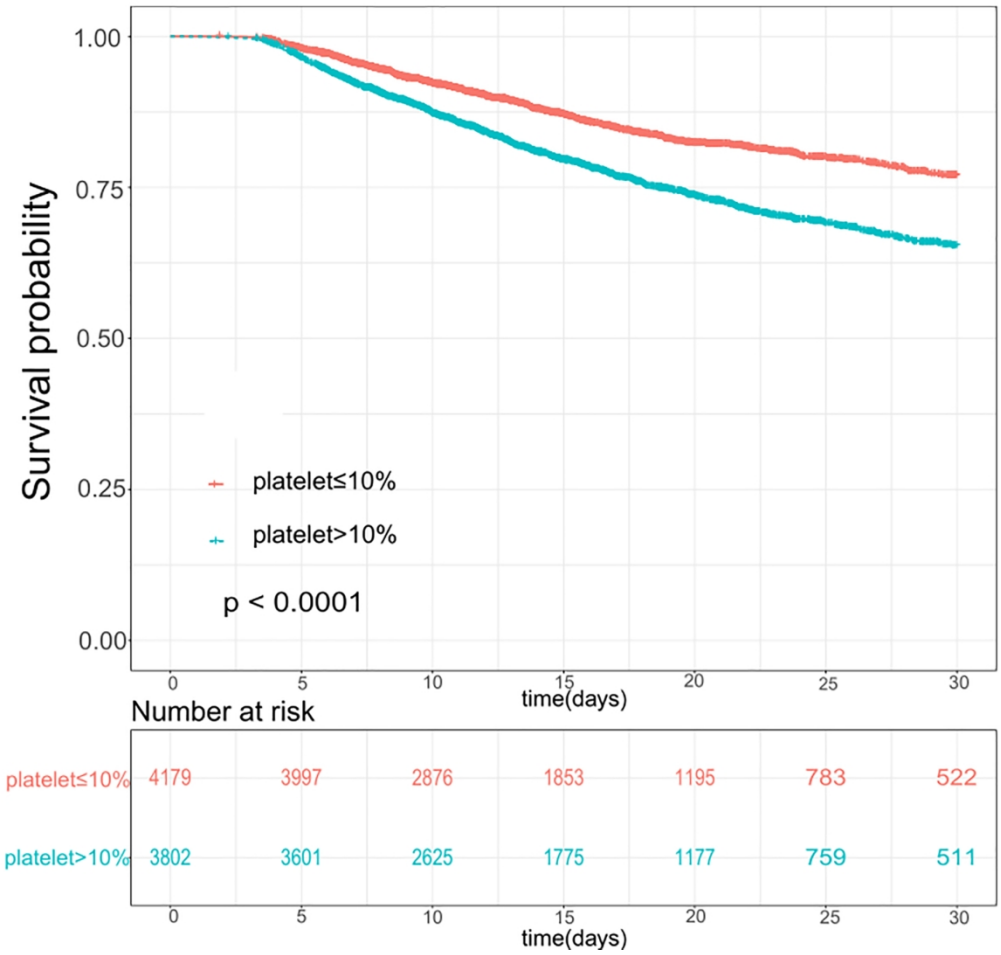

Figure 2. Kaplan-Meier survival curves for the mortality within 30 days.

162x155mm (300 x 300 DPI)

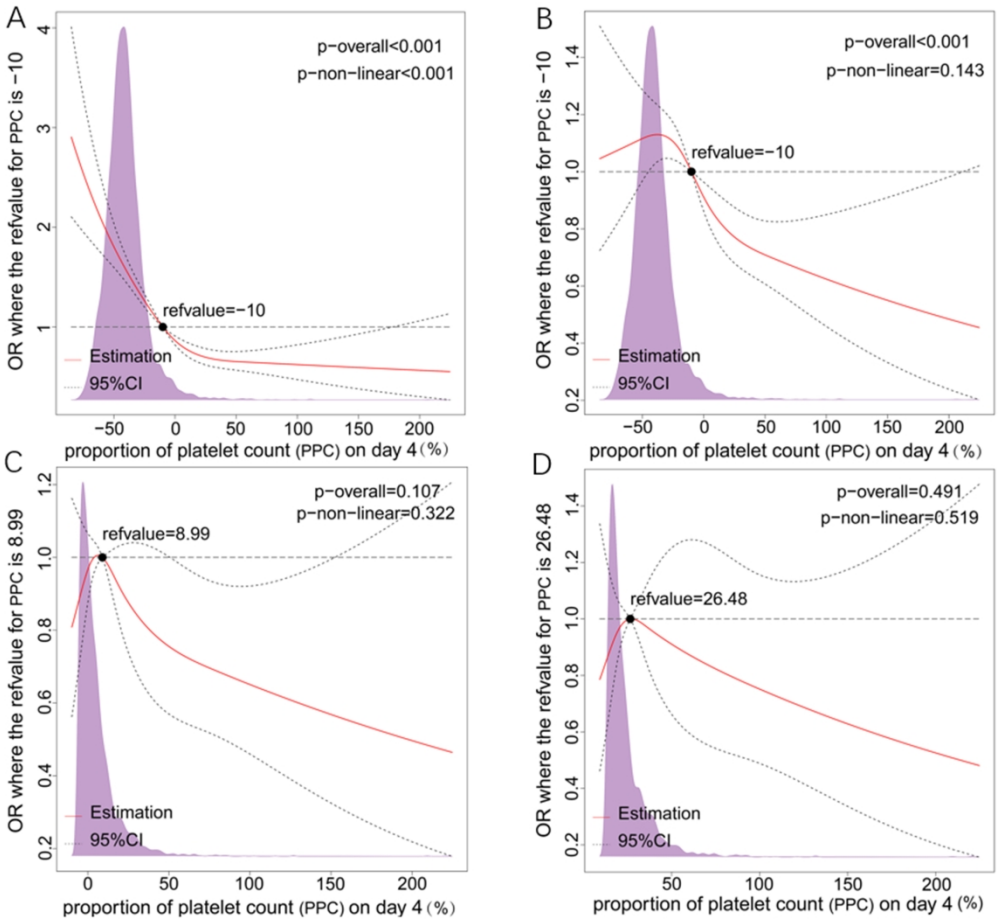

Figure 3. The association between proportion of platelet counts (PPC) and mortality within 30 days was shown in restricted cubic spline curves (RCS) based on logistic models in the whole population and different subsets. Solid red lines are odds ratio, with dashed black lines showing 95% confidence intervals derived from restricted cubic spline regressions with four knots. Reference lines for no association are indicated by the dashed grey lines at an odds ratio of 1.0. Violet density curves show the fraction of the population with different levels of the proportion of platelet counts. Refvalue indicates PPC improves mortality within 30 days. (A) (B)The proportion of platelet counts was modeled as a continuous variable and fitted in an unadjusted and adjusted model using restricted cubic spline analysis in the whole population. (C) (D) The proportion of platelet counts was modeled as a continuous variable and fitted in the adjusted models in the patients, with a decline of the proportion of platelet counts >10% excluded and an increasing proportion of platelet counts≥8.99% included. Analysis was adjusted for age, cardiovascular disease, liver disease, renal disease, vascular disease, cancer, sofa, saps ii, aps iii, pt, aptt and white blood cell counts at baseline.

188x172mm (300 x 300 DPI)

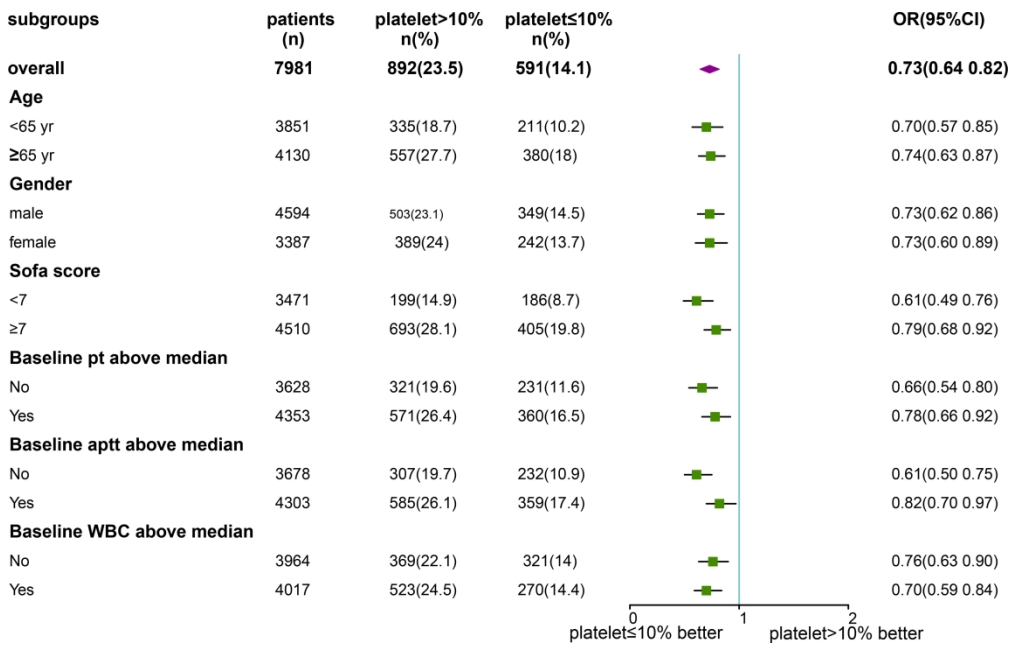

Figure 4. Forest-plot of subgroups. In different subgroups, the continuity variables including age, sofa score, pt, aptt and white blood cell counts (WBC) were stratified by the median.

336x212mm (300 x 300 DPI)

Table S1. All Missing values of data from MIMIC-IV

variables	Missing number n (%)
age	
gender	
weight	38 (0.47)
ethnicity	
myocardial_infarct	
congestive_heart_failure	
peripheral_vascular_disease	
cerebrovascular_disease	
dementia	
chronic_pulmonary_disease	
rheumatic_disease	
peptic_ulcer_disease	
mild_liver_disease	
diabetes_without_cc	
diabetes_with_cc	
paraplegia	
renal_disease	
malignant_cancer	
severe_liver_disease	
metastatic_solid_tumor	
aids	
sofa score	
apsiii	
sapsii	
pt	760 (9.52)
aptt	805 (10.08)
white_blood_cell_counts	3 (0.03)
platelet_counts0[a]	
platelet_counts3[b]	
LOS_hospital	
hospital_expire_flag[c]	
LOS_ICU	

LOS=length of stay

[a]platelete_counts0 are regarded as platelet counts on day 1 of ICU admission

[b]platelete_counts3 are regarded as platelet counts on day 4 of ICU admission

[c]hospital_expire_flag is regarded as in-hospital death

Table S2: Baseline of patient characteristics stratified by proportion of platelet counts on day four of ICU admission

Characteristic	Overall	declining proportion of platelet counts		*p* value
		platelet>10%	platelet≤10%	
Number	7981	3802	4179	
Gender, M (%)	4594 (57.6)	2180 (57.3)	2414 (57.8)	0.717
Median age (IQR), yr	65.8 [53.6 77.3]	66.2 [54.6 77.4]	65.3 [52.6 77.3]	0.012
Median weight (IQR), kg	80.0 [67.0 96.5]	79.8 [66.8 96.5]	80.0 [67.0 96.4]	0.586
Ethnicity, n (%)				0.671
American Indian	20 (0.3)	8 (0.2)	12 (0.3)	
Asian	205 (2.6)	100 (2.6)	105 (2.5)	
Black	707 (8.9)	325 (8.5)	382 (9.1)	
White	5025 (63.0)	2382 (62.7)	2643 (63.2)	
Hispanic	258 (3.2)	132 (3.5)	126 (3.0)	
Others	1766 (22.1)	855 (22.5)	911 (21.8)	
Select comorbidities[a], n (%)				
Cardiovascular disease	2826 (35.4)	1473 (38.7)	1353 (32.4)	<0.001
Chronic pulmonary disease	1945 (24.4)	941 (24.8)	1004 (24.0)	0.467
Liver disease	1466 (18.4)	816 (21.5)	650 (15.6)	<0.001
Renal disease	1582 (19.8)	796 (20.9)	786 (18.8)	0.019
diabetes	2256 (28.3)	1109 (29.2)	1147 (27.4)	0.093
Vascular disease	2359 (29.6)	1082 (28.5)	1277 (30.6)	0.043
Cancer[b]	1189 (14.9)	620 (16.3)	569 (13.6)	0.001
Aids	56 (0.7)	19 (0.5)	37 (0.9)	0.054
Others[c]	1507 (18.9)	705 (18.5)	802 (19.2)	0.477
Status at admission (median [IQR])				
Sofa score	7.0 [5.0 11.0]	8.0 [5.0 12.0]	6.0 [4.0 9.0]	<0.001
Apsiii	61.0 [44.0 81.0]	66.0 [49.0 88.8]	56.0 [42.0 74.0]	<0.001
Sapsii	40.0 [32.0 51.0]	44.0 [35.0 54.0]	38.0 [30.0 47.0]	<0.001
Laboratory test (median [IQR])				
White blood cell counts, k/ul	12.0 [8.6 16.3]	13.0 [9.3 17.8]	11.3 [8.3 15.1]	<0.001
Pt (s)	14.3 [12.7 17.3]	14.6 [12.8 18.2]	14.1 [12.6 16.5]	<0.001
Aptt (s)	31.7 [27.5 40.7]	33.0 [28.1 44.6]	30.8 [27.2 37.7]	<0.001
ICU outcome				
30-day mortality, n (%)	1483 (18.6)	892 (23.5)	591 (14.1)	<0.001
Median hospital LOS (IQR), d	13.9 [9.0 22.0]	14.1 [9.0 22.3]	13.7 [9.0 21.8]	0.122
Median ICU LOS (IQR), d	7.1 [4.8 12.1]	7.5 [4.9 12.9]	6.8 [4.7 11.3]	<0.001

IQR, interquartile range; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; Sapsii, simplified acute physiology score; Pt, prothrombin time; Aptt, activated partial thromboplastin time; LOS, length of stay.

[a]Comorbidities are defined by the Charlson comorbidity index.

[b]Cancer includes malignant cancer and metastatic solid tumor.

[c]Others includes dementia, rheumatic disease, peptic ulcer disease and paraplegia.

Table S3: Univariate and multivariate analysis for assessing the mortality within 30 days

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.41	1.26-1.58	<0.001	1.07	0.94-1.23	0.307
Liver disease	1.94	1.70-2.21	<0.001	1.61	1.37-1.89	<0.001
Renal disease	1.48	1.30-1.69	<0.001	0.97	0.83-1.12	0.646
Vascular disease	1.31	1.16-1.48	<0.001	1.56	1.36-1.78	<0.001
Cancer	1.75	1.52-2.02	<0.001	1.91	1.62-2.25	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-0.99	<0.001
Sofa score	1.15	1.13-1.16	<0.001	1.01	0.99-1.03	0.469
Apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.03	<0.001
WBC (k/ul)	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.080
Pt (s)	1.03	1.02-1.04	<0.001	1.01	1.01-1.02	<0.001
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.051
[a]platelet≤10%	0.54	0.48-0.60	<0.001	0.73	0.64-0.82	<0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Table S4. Univariate and multivariate analyses were performed to assess mortality within 30 days, excluding patients who had no decline or even an increase in platelet counts on day four at the time of study inclusion

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.02	1.01-1.02	<0.001	1.03	1.02-1.03	<0.001
Cardiovascular disease	1.32	1.15-1.52	<0.001	1.09	0.93-1.28	0.294
Liver disease	1.94	1.66-2.26	<0.001	1.57	1.30-1.89	<0.001
Renal disease	1.33	1.13-1.56	0.001	0.92	0.76-1.10	0.347
Vascular disease	1.22	1.06-1.41	0.007	1.56	1.32-1.83	<0.001
Cancer	1.69	1.42-2.00	<0.001	1.83	1.50-2.22	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-1.00	0.003
Sofa	1.14	1.12-1.16	<0.001	1.00	0.97-1.03	0.885
Apsiii	1.03	1.02-1.03	<0.001	1.03	1.03-1.03	<0.001
WBC (k/ul)	1.01	1.01-1.02	<0.001	1.01	1.00-1.01	0.166
Pt (s)	1.03	1.03-1.04	<0.001	1.02	1.01-1.03	<0.001
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.139
[a]platelet≤10%	0.55	0.46-0.65	<0.001	0.69	0.57-0.84	0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Figure S1. Kaplan-Meier survival curves

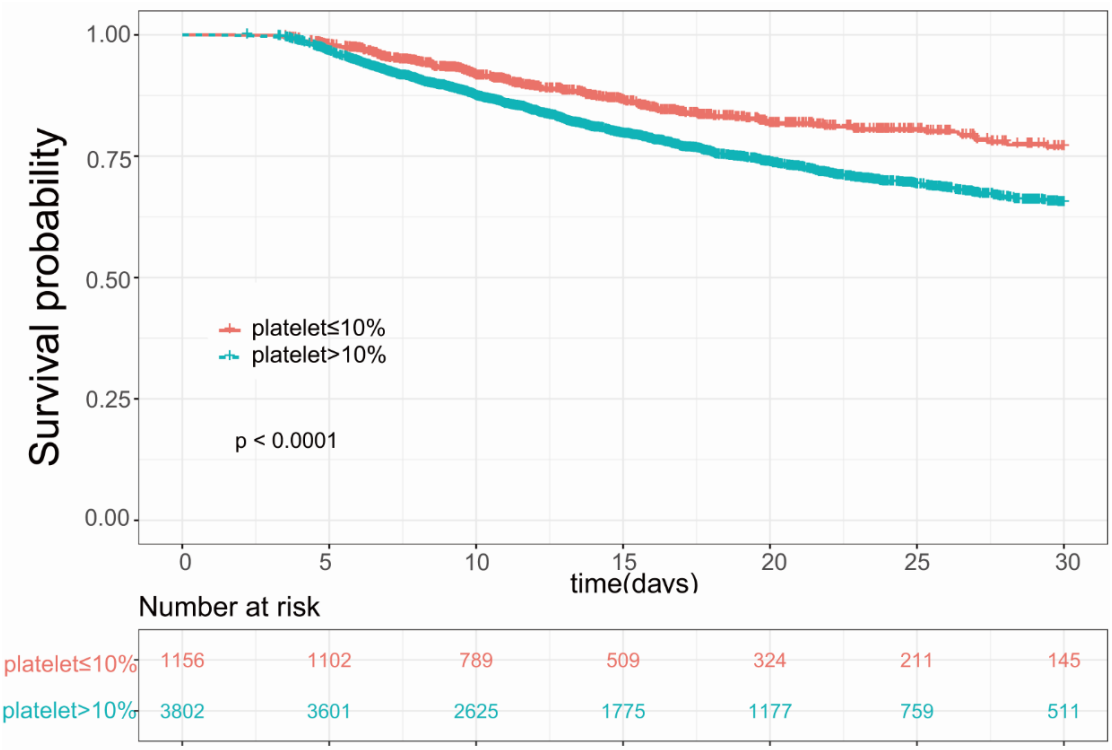

Figure S1. Kaplan-Meier survival curves were used to analyse the association between the percentage change in platelet count on day four compared to day one and the mortality within 30 days, excluding patients with no decline or even an increase in platelet counts on day four at the time of inclusion into the study

Table S5. Univariate and multivariate analyses were performed to assess mortality within 30 days, excluding patients with platelet counts < 100 K/ul on day one at the time of inclusion

Variables	Univariate analysis			Multivariate analysis		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Age	1.03	1.02-1.03	<0.001	1.03	1.03-1.04	<0.001
Cardiovascular disease	1.49	1.31-1.69	<0.001	1.10	0.95-1.28	0.210
Liver disease	1.63	1.37-1.92	<0.001	1.40	1.15-1.70	0.001
Renal disease	1.50	1.30-1.74	<0.001	0.94	0.80-1.11	0.484
Vascular disease	1.40	1.22-1.59	<0.001	1.59	1.37-1.83	<0.001
Cancer	1.70	1.44-2.00	<0.001	1.90	1.57-2.29	<0.001
Sapsii	1.04	1.03-1.04	<0.001	0.99	0.98-1.00	0.001
Sofa	1.14	1.12-1.16	<0.001	0.99	0.97-1.02	0.576
Apsiii	1.03	1.03-1.03	<0.001	1.03	1.03-1.04	<0.001
WBC (k/ul)	1.02	1.01-1.03	<0.001	1.01	1.00-1.01	0.143
Pt (s)	1.02	1.02-1.03	<0.001	1.01	1.00-1.02	0.016
Aptt (s)	1.01	1.01-1.01	<0.001	1.00	1.00-1.01	0.125
[a]platelet≤10%	0.54	0.47-0.61	<0.001	0.72	0.63-0.83	<0.001

CI, confidence interval; OR, odds ratio; Sapsii, simplified acute physiology score; Sofa, sequential organ failure assessment; Apsiii, acute physiology score; WBC, white blood cell counts; Pt, prothrombin time; Aptt, activated partial thromboplastin time.

[a]platelet≤10% is regarded as declining proportion of platelet counts on day 4 of ICU admission.

Figure S2. Kaplan-Meier survival curves

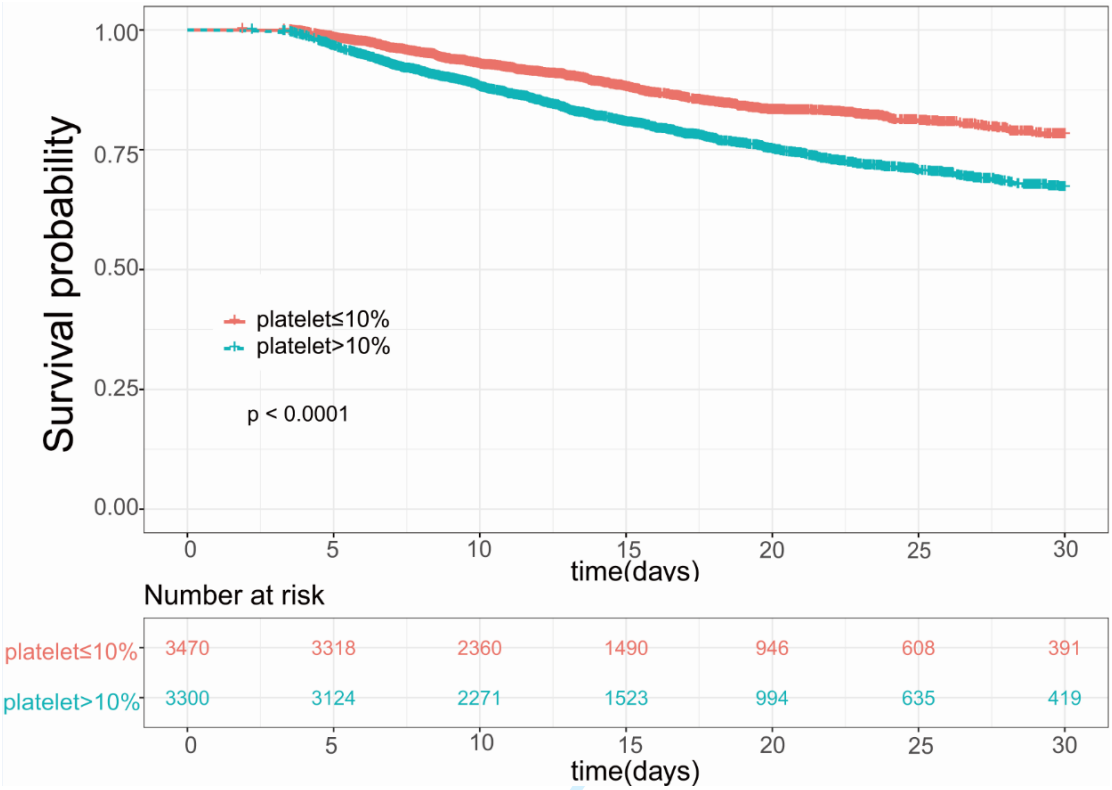

Figure S2. Kaplan-Meier survival curves were used to analyse the association between the percentage change in platelet count on day four compared to day one and the mortality within 30 days, excluding patients with platelet counts < 100 K/ul on day one at the time of inclusion into the study.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4，5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5，6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6，7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	7，8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7，8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	/
		(*e*) Describe any sensitivity analyses	8，9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	/
		(b) Give reasons for non-participation at each stage	/
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8，9
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	/
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	/
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8，9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11，12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11，12
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.