BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022896
Article Type:	Research
Date Submitted by the Author:	12-Mar-2018
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Keywords:	sepsis, PLR, mortality, MIMIC III

Title page

Platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: Interaction effect with disease severity. A retrospective study.

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Word count: 3316.

Abstract

Objective: The role of platelet-to-lymphocyte ratio (PLR) as an indicator of inflammation has been receiving research attention. We aimed to investigate the predictive value of PLR for sepsis.

Design: A retrospective cohort study.

Setting and Participants: Data were extracted from the Multi-parameter Intelligent Monitoring in Intensive Care III database. Data on 5,537 sepsis patients were analyzed.

Methods: Logistic regression was used to explore the association between PLR and hospital mortality. Subgroup analyses were performed based on vasopressor use, acute kidney injury (AKI), and a Sequential Organ Failure Assessment (SOFA) score > 10.

Results: In the logistic model with linear spline function, a PLR > 200 was significantly (odds ratio [OR], 1.0002; 95% confidence interval [CI], 1.0001 – 1.0004) associated with mortality; the association was insignificant for PLRs ≤ 200 (OR, 0.997; 95% CI, 1.19 – 1.67). In the logistic model using the PLR as a design variable, only high PLRs were significantly associated with mortality (OR, 1.29; 95% CI, 1.09 – 1.53); the association with low PLRs was insignificant (OR, 1.15; 95% CI, 0.96 – 1.38). In the subgroups with vasopressor use, AKI and a SOFA score > 10, the association between high PLR and mortality was insignificant; this remained significant in the subgroups without vasopressor use (OR, 1.39; 95% CI, 1.08 – 1.77) and AKI (OR, 1.54; 95% CI, 1.20 – 1.99), and with a SOFA score ≤ 10 (OR, 1.51; 95% CI, 1.17 – 1.94).

Conclusions: High PLRs at admission were associated with an increased risk of mortality. In patients with vasopressor use, AKI or a SOFA score > 10, this association was insignificant.

Keywords: sepsis, PLR, mortality, MIMIC III.

Strengths and limitations of this study:

The large sample size facilitated a robust conclusion.

Subgroup analysis was performed to investigate the interaction between disease severity and PLR.

High PLRs was associated with an increased risk of mortality in sepsis patients without vasopressor use, AKI or a SOFA score > 10.

In sepsis patients with severe condition (vasopressor use, AKI or a SOFA score > 10), the association between PLRs and mortality was insignificant.

INTRODUCTION

Sepsis is a major cause of morbidity and mortality, worldwide, and it results from a dysregulation of the systemic inflammatory response to infection [1 2]. Despite significant advances in the pathophysiology and therapeutic strategies for sepsis, the mortality remains high [3], at 300 deaths per 100,000 people [4]. An extremely complex systemic expression of inflammatory and anti-inflammatory response plays a critical role in the pathophysiological process of sepsis, which is strongly associated with an increased risk of mortality [5]. Identifying patients who are at a high risk of poor outcomes, in the early stage of sepsis, is vital for timely and adequate intervention [6]. While a significant amount of effort has been put into investigating promising biomarkers, the challenge of identifying these at-risk patients remains [7].

In recent years, studies have reported that platelets and lymphocytes play critical roles in the inflammatory process. Therefore, the platelet-to-lymphocyte ratio (PLR)--a novel inflammatory factor--has received research attention in recent times, as it may act as an indicator of inflammation [8] in a wide spectrum of diseases, such as myocardial infarction [9], acute kidney injury (AKI) [10], hepatocellular carcinoma [11], and non-small cell lung cancer [12].

Based on the findings of previous studies, it is reasonable to speculate the presence of a potential relationship between PLR and mortality for sepsis. However, no investigation has been conducted.

MATERIALS AND METHODS

Database introduction

All the data in the current study were extracted from an online international database--"Multi-parameter Intelligent Monitoring in Intensive Care III (MIMIC III)"—that was published by the Massachusetts Institute of Technology, with approval from the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. All the patients in the database were de-identified for privacy protection. This database included more than 58,000 patients who were admitted to the intensive care unit (ICU) of the Beth Israel Deaconess Medical Center from 2001 to 2008. Author Y Shen obtained access to this database (certification number: 1564657), and was responsible for data extraction.

Inclusion and exclusion criteria

Adult patients meeting the criteria for sepsis were initially screened. The definition of sepsis was adapted from the recommendation in the Surviving Sepsis Campaign 2016 [13]. Accordingly, sepsis was defined as the presence of a Sequential Organ Failure Assessment (SOFA) score ≥ 2 within 24 hours after ICU admission, accompanied by at least one infection site. The following criteria were used to exclude patients from this analysis: 1. Age lower than 18 years; 2. Having spent less than 48 hours in the ICU; and 3. Absence of data on the serum platelet and lymphocyte counts within 24 hours after ICU admission. For patients who were admitted to the ICU more than once, only the first ICU stay was considered in this study.

Data extraction

Data on the demographic characteristics, laboratory outcomes, infection sites, vasopressor use, and disease severity score were extracted from the database. Only patients with data on the serum platelet and lymphocyte counts within the first 24 hours after ICU admission were included. The first blood sample after ICU admission was used to calculate the PLR, which was defined as the ratio of the absolute platelet count and absolute lymphocyte count. Septic shock was considered as a special subgroup of sepsis. However, it was difficult to identify patients with septic shock in this database due to a lack of relevant information. Thus, data on vasopressor use were extracted for the subgroup analysis. Vasopressor use was defined as the use of any vasopressor agent, including norepinephrine, epinephrine, dobutamine, dopamine or vasopressin, within 48 hours after ICU admission.

Outcome definition

The primary endpoint was hospital mortality, which was defined as death during hospitalization. The presence of AKI was defined according to the Creatinine-based Kidney Disease Improving Global Outcome criteria without urine output [14 15]. A 1.5-fold increase in the serum creatinine (SCr) level during the ICU stay, relative to the level at the baseline, was considered as the presence of AKI. In the present cohort, data on the baseline SCr values were missing in 20.3% of the cases. For patients without previous SCr data, the estimated baseline SCr was calculated using the following formula[16]: SCr = 0.74 - 0.2 (if female) + 0.08 (if black) + 0.0039 * age (in years).

Management of missing data

Variables with missing data are common in the MIMIC III database, as it comprises more than 58,000 admissions. Variables with more than 20% of missing values were excluded from our analysis; these included serum albumin and lactate. For variables with less than 5% of missing values, such as age and fluid balance, we replaced the missing values with the mean values, instead of using the multiple imputation technique. For dichotomous variables with less than 5% of missing values, the missing values were not filled.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or median (interquartile range), as appropriate. A Student's t test, analysis of variance, Wilcoxon rank-sum test, or Kruskal–Wallis test was used, as appropriate. Categorical data were expressed as proportions, and compared using the $\chi 2$ test. A knot of PLR (at a level of around 200) was detected using the Lowess smoother technique; thus, the linear spline function was initially used in the multivariate logistic regression. Thereafter, all the patients were further divided into three levels: those with a PLR ≤ 150 (level 1), 150 < PLR ≤ 250 (level 2), and PLR > 250 (level 3). Variables including demographic characteristics, infection sites, disease severity score, and laboratory measures potentially associated with mortality, or those that had a p value < 0.20 in the univariate analyses were included in the multivariate logistic regression analyses. An extended model approach was used for covariate adjustment: Model 1 = adjusted for age, admitted ICU type. Model 2 = Model 1+ (fluid balance at 48 hours after ICU admission). Model 3 = Model 2 + (infection sites). Model 4 = Model 3 + (Maximum

SOFA score during the ICU stay). As we detected a U-shaped association between PLR and mortality, we did not introduce interaction items (such as PLR multiply other variables) in the logistic models. Instead, subgroup analyses were performed, according to the presence of AKI and vasopressor use and the median SOFA score. Multi-collinearity was tested using the variance inflation factor (VIF) method, with a VIF ≥ 5 indicating the presence of multi-collinearity. All the logistic regression models underwent a goodness of fit test. A two-tailed test was performed, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA 11.2 (College Station, TX, USA).

RESULTS

Baseline characteristics

Data on a total of 5,537 sepsis patients were included in this analysis. The overall mortality observed was 25.1%. Data on the comparisons of the baseline characteristics between the three PLR levels are listed in Table 1. The mean age at admission was 64.9 years, and 44.9% of the participants were male. The rate of vasopressor use (701/1780 vs. 482/1380, p=0.01), and a maximum SOFA score (10 (7–14) vs. 9 (7 – 12), p<0.001) were significantly higher in PLR level 1 than level 2; the presence of these variables was insignificant in level 3. The mortality was significantly higher both in those in level 1 (475/1780 vs. 291/1380, p<0.001) and level 3 (621/2377 vs. 291/1380, p=0.001).

Association between PLR and hospital mortality

The PLR was initially used as a continuous variable in the logistic model, using linear spline function, as shown in Table 2. We observed that, for PLRs ≤ 200, the odds ratio (OR) of mortality was insignificant (OR, 0.997; 95% confidence interval [CI], 1.19 – 1.67), while the OR for PLRs > 200 was significant (OR, 1.0002; 95% CI, 1.0001 – 1.0004), after adjustment for covariates including the SOFA score. In the extended multiple logistic regression analysis (Table 3), both low and the high PLR levels were significantly associated with increased hospital mortality, in model 1 (OR, 1.41; 95% CI, 1.19 – 1.67 and OR, 1.28; 95% CI, 1.09 – 1.51, respectively), model 2 (OR, 1.34; 95% CI, 1.13 – 1.59 and OR, 1.23; 95% CI, 1.05 – 1.45, respectively) and model 3 (OR, 1.35; 95% CI, 1.14 – 1.61 and OR, 1.21; 95% CI, 1.03 – 1.43, respectively). However, after adjustment for the maximum SOFA score in model 4, the OR for low PLR levels became insignificant (OR, 1.15; 95% CI, 0.96 – 1.38, p=0.123), while that for high PLR levels remained significant (OR, 1.29; 95% CI, 1.09 – 1.53, p=0.003). The ORs of the covariates in model 4 are listed in Table S1.

Subgroup analysis

As the association between PLR and mortality was largely confounded by the SOFA score (Table 3), we suspected that there was an interaction effect between disease severity and PLR level. Thus, we performed a subgroup analysis according to the existence of vasopressor use and AKI, and the median SOFA score (> 10 points), as shown in Figure 1. Unlike previous findings, the association between high PLRs and mortality became insignificant in the subgroups with vasopressor use (OR, 1.20; 95% CI, 0.95 – 1.53), AKI (OR, 1.07; 95% CI, 0.85 – 1.36), and a SOFA score > 10 (OR,

1.14; 95% CI, 0.90 – 1.44), and remained significant in the subgroups without vasopressor use (OR, 1.39; 95% CI, 1.08 – 1.77) and AKI (OR, 1.54; 95% CI, 1.20 – 1.99), and with a SOFA score ≤ 10 (OR, 1.51; 95% CI, 1.17 – 1.94). In the case of lower PLRs, the OR of mortality was insignificant in all the subgroups, after adjustment, except for the subgroup with AKI. Data on the comparisons of the characteristics between these subgroups are listed in Table S2.

DISCUSSION

In this study, we observed a crude U-shaped association between the PLR and hospital mortality in patients with sepsis. However, after adjustment for the disease severity score, only high PLRs remained significantly associated with increased mortality; the association with low PLRs became insignificant. Furthermore, in the subgroup analysis, a significant association between high PLRs and mortality only existed in the subgroups without vasopressor use and AKI, or those with a SOFA score ≤ 10.

Growing evidence indicates that immune dysregulation (especially cellular immunity), including pro-inflammatory or anti-inflammatory responses during different stages, is common in cases of sepsis [17]. In recent times, studies have reported that platelets play an important role in both the immunomodulatory and inflammatory process [18 19], by inducing the release of inflammatory cytokines [20] and interacting with different kinds of bacterias and immune cells, including neutrophils, T-lymphocytes, NK-cells and macrophages, which contribute to the initiation or

exacerbation of the inflammatory process [21]. Low lymphocyte counts, which to a certain degree represent a suppressed immune and inflammatory response [22 23], have also been reported to be associated with inflammatory diseases, such as cardiovascular disease [24] and type II diabetes [25].

Based on these findings, the PLR was suggested as being a novel systematic inflammatory indicator [26], and its use was initially reported in the prognostic prediction of neoplastic disorders, such as hepatocellular carcinoma and breast cancer. Accumulating evidence suggests that elevated PLRs are strongly associated with increased systemic inflammation, which may contribute to the progression and prognoses of many disorders, such as atherosclerosis [27] and diabetes mellitus [28].

In contrast to our findings, Zheng et al. [10] reported that both high and low PLRs are associated with increased mortality, among critically ill patients with AKI, after adjustment for the disease severity score in the Cox proportional hazards models. In that study, unlike in ours, a significant association was also observed in patients with vasopressin use. Several factors may contribute to this inconsistency between the findings, such as the use of different cohorts, PLR knots, and definitions of vasopressor use. It is worth noting that, as the association between PLRs and outcomes varies greatly between different cohorts, the inter-heterogeneity within critically ill patients may also lead to a biased conclusion.

Akbas et al. indicated that a high PLR was positively associated with increased epicardial adipose tissue deposition in diabetes patients [29]; this may be caused by higher inflammation rates. Wang et al. [30] reviewed 134 patients with lung

adenosquamous cancer, and reported that high PLRs (> 150) were independently associated with shorter disease-free days and lower overall survival rates. Another study [31], including 270 patients with hepatocellular carcinoma, found that elevated PLRs (above 220) were predictors of poor prognoses, while low PLRs (< 248.0) were associated with a low tumor, node and metastasis stage, and low surgery incidence, in 695 patients with lung cancer [32]. Despite the fact that the study cohorts used in those studies were quite different from those used in ours, the reported PLR knots were quite similar to ours. However, the small sample sizes in those studies limited the statistical power for further stratification and subgroup analysis of low PLR. In the current study, we noticed that high PLRs (> 250) were associated with increased hospital mortality. As higher platelet levels, to a certain extent, are predictive of inflammation of a higher severity and low lymphocyte counts may represent a suppressed immune and inflammatory response [22 23], an increase in the PLR may reflect the degree of the inflammatory and immune response to the infection, which related to a poor prognosis.

We also detected an insignificant association between low PLRs and mortality, in the case of sepsis. The association between low PLRs and outcomes was also reported in several studies. In a retrospective study [33] including 899 cases of laryngeal cancer, patients were divided into three PLR categories (low (≤ 119.55), moderate (> 119.55 and ≤ 193.55), and high (> 193.55)), and only patients with high PLRs experienced poor outcomes, including malnutrition and more advanced cancer stage; the association between outcomes and PLR levels were insignificant for those

with low PLRs. Despite the cohort of that study being different from ours, the conclusion was consistent with that of our study. In the case of sepsis, a low platelet count is potentially associated with poor outcomes. In a large study including 931 patients with sepsis, Claushuis et al. reported that patients with a low platelet count at ICU admission had a higher disease severity score and increased mortality risk [34]. Furthermore, thrombocytopenia--one of the most common hemostatic disorders in the case of sepsis--which is related with platelet consumption, was also associated with higher mortality [35]. However, in the present study, a significant association between low PLR and mortality was not detected. Further studies are needed to validate this conclusion.

Furthermore, according to the subgroup analysis, the association between high PLR and mortality became insignificant in the subgroups with vasopressor use, AKI or a SOFA score > 10; this association remained significant in the other subgroups. This finding further supported our speculation that there may be an interaction between PLR and disease severity. To the best of our knowledge, ours is the first study to report this interaction. However, the underlying mechanism of this interaction remains largely unknown. A critical characteristic of sepsis is fluid resuscitation, and, in the current study, patients with vasopressor use, AKI or a SOFA score > 10, to a certain degree, represented patients with inflammation of a higher severity, and they may have a stronger need for fluid resuscitation. We also noticed that the fluid balance within 48 hours after ICU admission was significantly larger in these subgroups. It needs to be further investigated if fluid resuscitation affects the

predictive value of the PLR.

One of the strengths of our study is the large sample size, which enabled us to adjust for confounding factors and perform subgroup analyses. However, there are also several limitations to our study. First, the MIMIC III database comprises data on patients from 2001; since then, the guidelines for sepsis have changed significantly. The most recent definition of Sepsis 3.0 was used in the current study, and this may have introduced selection bias despite the fact that most of the basic interventions (use of fluids, vasopressors and antimicrobial agents) remained the same. Furthermore, as a decrease in the platelet count was a part of the SOFA score, using the definition of sepsis 3.0, to a certain degree, may lead to a relatively low mean platelet count and potential multi-collinearity. This bias cannot be fully avoided. However, the potential multi-collinearity was verified in all the logistic models. Second, septic shock is a special subgroup of sepsis. However, patients with septic shock could not be distinguished in this study. Thus, patients were divided into subgroups, according to the existence of vasopressor use, AKI or a SOFA score >10, which, to a certain extent, indicates the presence of an inflammatory response of a higher severity. Third, one of the main hypotheses of our study was the interaction effect between disease severity and PLR; yet, this interaction term was not introduced in the logistic model due to the U-shaped association between PLR and mortality. Further prospective studies are needed to verify our hypothesis. Finally, as high PLRs are associated with poor outcomes in various disorders while low PLRs are not, it is not clear if interventions aimed at changing the PLR value may improve

outcomes.

Conclusion

In patients with sepsis, a high PLR was significantly associated with poor survival, while the association was insignificant for those with a low PLR. However, the former association became insignificant in patients with more severe conditions, including those with vasopressor use, AKI or a SOFA score > 10. Future studies are needed to verify our hypothesis.

Financial support and sponsorship: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors**.**

Acknowledgments: None.

Conflicts of interest: None.

Authors' contributions:

Yanfei Shen.: Responsible for data extraction and writing of the manuscript.

Xinmei Huang.: Responsible for data analysis.

Weimin Zhang.: Responsible for data validation.

Data sharing statement: Full data set available from the corresponding author at snow.shen@hotmail.com. However, reanalysis of the full data need to be approved by MIMIC III Institute.

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Table 1 Comparison of baseline characteristics within three PLR levels

Variable	PLR ≤ 150 (n = 1780)	150< PLR ≤ 250 (n = 1380)	PLR > 250 (n = 2377)	p
Age (years)	63.0 ± 16.6	65.0 ± 16.6	66.1 ± 15.5	< 0.001
Gender (male) [n (%)]	805 (45.2)	590 (42.7)	1096 (46.1)	0.133
BMI (Kg/m^2)	30.8 ± 8.9	34.1 ±13.5	35.2 ± 39.5	0.001
Ethnicity				
White [n (%)]	1202 (67.5)	987 (71.5)	1761 (74.0)	0.754
Black [n (%)]	180 (10.1)	101 (7.3)	146 (6.1)	< 0.001
Asian [n (%)]	39 (2.2)	30 (2.1)	71 (2.9)	0.169
Emergency [n (%)]	1641 (92.1)	1284 (93.0)	2229 (93.7)	0.138
ICU type				
MICU [n (%)]	953 (53.5)	727 (52.6)	1362 (57.2)	0.008
CCU/CSRU [n (%)]	413 (23.2)	323 (23.4)	453 (19.0)	0.001
TSICU/SICU [n (%)]	414 (23.2)	330 (23.9)	562 (23.6)	0.908
Vasopressors				
Norepinephrine [n (%)]	566 (31.7)	374 (27.1)	711 (29.9)	0.016
Dopamine [n (%)]	198 (11.1)	151 (10.9)	256 (10.7)	0.013
Epinephrine [n (%)]	67 (3.7)	28 (2.0)	37 (1.5)	< 0.001
Vasopressin [n (%)]	156 (8.7)	88 (6.3)	172 (7.2)	0.033
Overall vasopressor use	701 (39.3)	482 (34.9)	858 (36.1)	0.022
Fluid input/output				
Fluid intake (ml/kg/48hr)	99.9 ± 60.9	90.7 ± 57.6	97.2 ± 61.2	< 0.001
Urine output (ml/kg/48hr)	42.0 ± 32.0	42.9 ± 30.3	41.9 ± 29.5	0.5659
Fluid balance (ml/kg/48hr)	46.7 ± 59.4	38.3 ± 55.1	46.0 ± 60.4	< 0.001
Infection site				
Respiratory infection	1048 (58.8)	929 (67.3)	1580 (66.4)	< 0.001
Blood infection	768 (43.1)	509 (36.8)	998 (41.9)	0.001
Urinary infection	549 (30.8)	409 (29.6)	682 (28.6)	0.323
Abdominal infection	245 (13.7)	159 (11.5)	334 (14.0)	0.072
Cerebral infection	153 (8.5)	106 (7.6)	169 (7.1)	0.206
Disease severity scores				
SOFA on ICU admission median (IQR)	6 (4–9)	5 (4–8)	5 (3–7)	< 0.001
Maximum SOFA during ICU stay median (IQR)	10 (7–14)	9 (7 – 12)	9 (7 – 12)	< 0.001
Laboratory outcomes				
Maximum serum creatinine (mg/L)	2.5 ± 2.7	2.2 ± 2.1	2.1 ± 1.9	< 0.001
Minimum hemoglobin level (g/dl)	8.3 ± 1.7	8.69 ± 1.7	8.4 ± 1.6	< 0.001
Maximum serum sodium (mmol/L)	145.1 ± 5.4	145.0 ± 5.2	144.6 ± 5.1	0.009
Maximum serum lactate (mmol/L)	4.1 ± 3.8 (n=1536)	3.4 ± 3.1 (n=1174)	3.1 ± 3.0 (n=2112)	< 0.001
Platelet count (10^9/L)	146.7 ± 88.0	225.1 ± 107.2	297.5 ± 163.4	< 0.001
Lymphocyte count (10^9/L)	2.1 ± 5.7	1.1 ± 0.5	0.68 ± 0.4	< 0.001

PLR	91.8 ± 37.1	195.8 ± 28.6	557.5 ± 484.8	< 0.001
Clinical outcomes				
ICU LOS	9.9 ± 10.1	9.3 ± 8.7	10.1 ± 9.9	0.071
Hospital LOS	17.7 ± 15.1	16.6 ± 13.5	17.2 ± 13.7	0.082
AKI [n (%)]	861 (48.3)	601 (43.5)	1080 (45.4)	0.022
Hospital mortality [n (%)]	475 (26.6)	291 (21.0)	621 (26.1)	< 0.001

Abbreviations:

PLR: platelet to lymphocyte ratio; BMI body mass index; MICU, multiple intensive care unit; CCU, coronary care unit; CSRU, cardiac surgery care unit; TSICU, traumatic surgical intensive care unit; SICU, surgical intensive care unit; SOFA, sequential organ failure assessment; SAPS II, Simplified Acute Physiology Score II; IQR, interquartile range; LOS length of stay; AKI, acute kidney injury.

Table 2 multivariable logistic regressions of PLR using linear spline function

Variables	Crude Odds ratio	95% CI	p	Adjusted Odds ratio	95% CI	p
PLR (≤ 200)	0.997	0.996 – 0.998	< 0.001	0.9993	0.9980 – 1.0006	0.319
PLR (> 200)	1.0002	1.0001 – 1.0004	0.001	1.0002	1.0000 – 1.0003	0.025
Age (> 65)	1.77	1.56 – 2.11	< 0.001	2.32	1.99 – 2.64	< 0.001
Maximum SOFA	1.20	1.18 – 1.22	< 0.001	1.18	1.16 – 1.20	< 0.001
Urinary infection	0.66	0.57 – 0.76	< 0.001	0.65	0.56 – 0.76	< 0.001
Respiratory infection	1.29	1.13 – 1.47	< 0.001	1.25	1.09 – 1.45	0.002
Blood infection	2.14	1.89 – 2.42	< 0.001	1.49	1.29 – 1.71	< 0.001
Fluid balance (ml/kg/48hrs)	1.006	1.005 – 1.007	< 0.001	1.002	1.0008 – 1.0031	0.001
MICU	1.34	1.15 – 1.56	< 0.001	1.15	0.97 – 1.37	0.089
CCU/CSRU	1.22	1.01 – 1.47	0.032	1.03	0.84 – 1.26	0.752

Note: The mean variance inflation factor was 2.89 and p value of goodness of fit was 0.632.

Abbreviation: PLR platelet to lymphocyte ratio; CI confidence interval; SOFA sequential organ failure assessment; MICU multiple intensive care unit; CCU coronary care unit; CSRU cardiac surgery care unit.

Table 3 Association between three PLR levels and hospital mortality

	PLR ≤ 150		150 < PLR ≤ 250		PLR > 250	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Model 1	1.41 (1.19 – 1.67)	< 0.001	Ref.	–	1.28 (1.09 – 1.51)	0.002
Model 2	1.34 (1.13 – 1.59)	0.001	Ref.	–	1.23 (1.05 – 1.45)	0.009
Model 3	1.35 (1.14 – 1.61)	0.001	Ref.	–	1.21 (1.03 – 1.43)	0.018
Model 4	1.15 (0.96 – 1.38)	0.123	Ref.	–	1.29 (1.09 – 1.53)	0.003

Adjusted covariates: Model 1 = age, admitted ICU type. Model 2 = Model 1+ (fluid balance at 48 hours after ICU admission). Model 3= Model 2 + (infection sites). Model 4 = Model 3+ (Maximum SOFA score during ICU stay).

Abbreviations: PLR platelet to lymphocyte ratio; OR = odds ratio; CI= confidence interval; Ref reference category.

Figure legend:

Figure 1: The crude and adjusted odds ratios in the subgroup analysis. PLR level 2 was used as the reference level in all the logistic models.

Abbreviations: PLR, platelet-to-lymphocyte ratio; AKI, acute kidney injury; SOFA, Sequential Organ Failure Assessment; CI, confidence interval

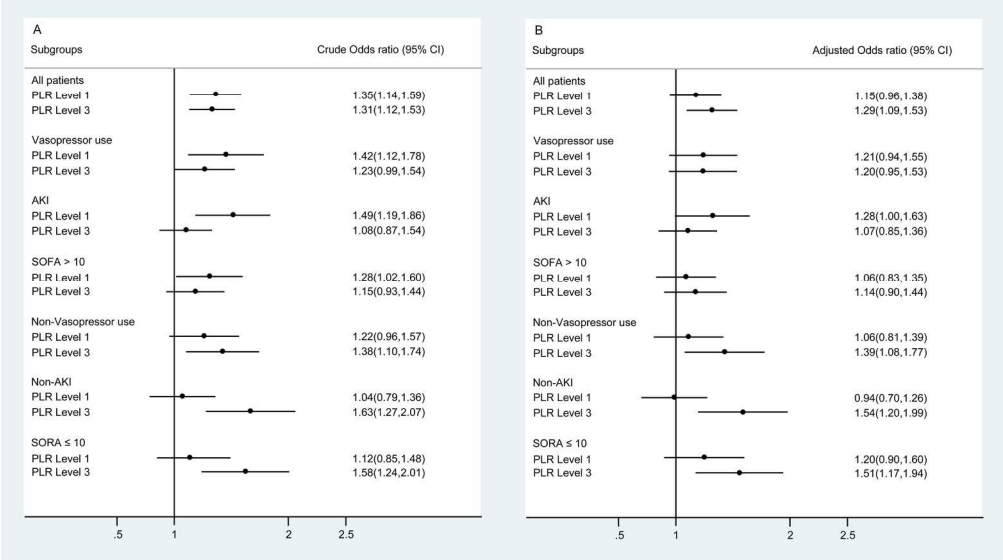


Figure 1: The crude and adjusted odds ratios in the subgroup analysis. PLR level 2 was used as the reference level in all the logistic models.

182x102mm (300 x 300 DPI)

Table S1 Adjusted odds ratio of hospital mortality using PLR as design variable in multivariable logistic regression

Variables	Odds ratio	95% CI	p
PLR Level 1 (≤ 150)	1.15	0.96 – 1.38	0.123
PLR Level 2 (151 ~ 250)	Ref.	–	–
PLR Level 3 (> 250)	1.29	1.09 – 1.53	0.003
Age (> 65)	2.27	1.97 – 2.62	< 0.001
Maximum SOFA	1.19	1.16 – 1.21	< 0.001
Urinary infection	0.65	0.56 – 0.76	< 0.001
Respiratory infection	1.25	1.08 – 1.44	0.002
Blood infection	1.49	1.29 – 1.71	< 0.001
Fluid balance (ml/kg/48hrs)	1.002	1.0008 – 1.0031	0.001
MICU	1.16	0.98 – 1.37	0.082
CCU/CSRU	1.04	0.84 – 1.27	0.700

Note: The mean variance inflation factor (VIF) was 2.53 and p value of goodness of fit was 0.665.

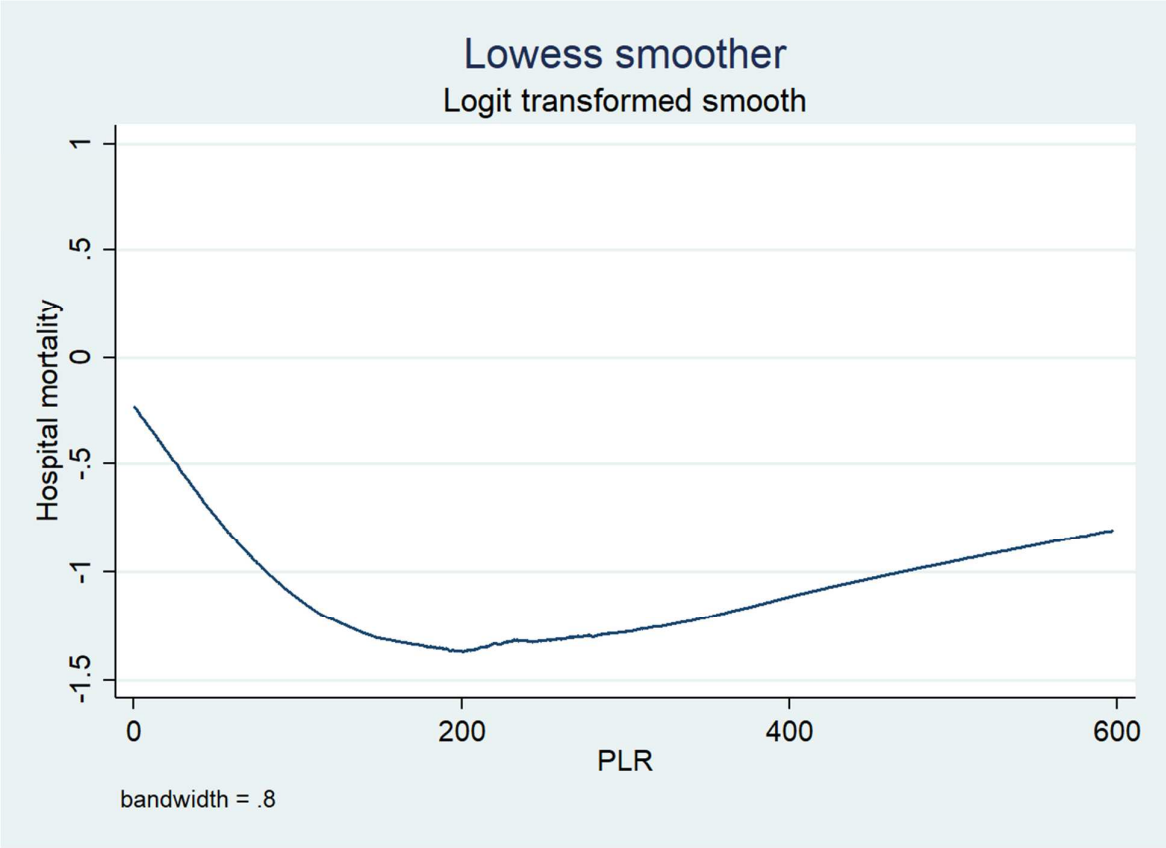
Abbreviation: PLR platelet to lymphocyte ratio; CI confidence interval; SOFA sequential organ failure assessment; MICU multiple intensive care unit; CCU coronary care unit; CSRU cardiac surgery care unit.

Table S2 Comparisons of subgroups according to the existence of vasopressor use, AKI and SOFA score

Variables	Vasopressor-use (n = 2554)	Non-Vasopressor-use (n = 2983)	p	AKI (n = 2542)	Non-AKI (n = 2995)	p	SOFA > 10 (n = 2390)	SOFA <= 10 (n = 2147)	p
age	66.3 ± 15.2	63.6 ± 16.8	< 0.001	65.1 ± 15.4	64.6 ± 16.8	0.211	64.0 ± 15.7	65.5 ± 16.4	< 0.001
Vasopressor-use [n (%)]	-	-	-	1321	1233	< 0.001	1554	1000	< 0.001
Fluid intake (ml/kg/48hr)	114.4 ± 661.	81.1 ± 50.0	< 0.001	94.3 ± 62.0	98.3 ± 58.8	0.013	110.7 ± 65.5	85.6 ±53.6	< 0.001
Fluid balance (ml/kg/48hr)	63.2 ± 64.1	28.1 ± 48.5	< 0.001	48.4 ± 60.9	40.8 ± 56.9	< 0.001	62.0 ± 64.1	30.8 ± 50.6	< 0.001
Maximum SOFA median (IQR)	12 (9 – 14)	8 (6 – 11)	< 0.001	11 (8 – 14)	9 (7 – 11)	< 0.001	13 (12 – 15)	7 (6 – 9)	< 0.001
Platelet count (10^9/L)	225.0 ± 240.1	236.0 ± 148.7	0.005	219.2 ± 143.4	240.9 ± 145.5	< 0.001	208.4 ± 147.1	248.1 ± 140.8	< 0.001
Lymphocyte count (10^9/L)	1.26 ± 3.32	1.28 ± 3.32	0.890	1.21 ± 2.21	1.32 ± 4.03	0.246	1.24 ± 3.42	1.29 ± 3.24	0.529
Hospital LOS	17.8 ± 14.3	16.7 ± 14.1	0.002	19.7 ± 15.6	15.1 ± 12.3	< 0.001	19.6 ± 14.6	15.5 ± 13.4	< 0.001
AKI [n (%)]	1321	1221	< 0.001	-	-	-	1360	1182	< 0.001
Hospital mortality [n (%)]	777	612	< 0.001	875	514	< 0.001	884	505	< 0.001

Abbreviation: SOFA sequential organ failure assessment; IQR interquartile range; LOS length of stay; AKI acute kidney injury.

Figure S1 Crude relationship between hospital mortality and PLR



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
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*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
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	*4*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
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	7-8
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A.
		(*e*) Describe any sensitivity analyses	N/A.
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	8
		(b) Give reasons for non-participation at each stage	N/A.
		(c) Consider use of a flow diagram	N/A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	N/A.
Outcome data	15*	Report numbers of outcome events or summary measures over time	9
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	7
		(*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	N/A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A.
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	15

*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

A retrospective study of platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: interaction effect with disease severity

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022896.R1
Article Type:	Research
Date Submitted by the Author:	11-May-2018
Complete List of Authors:	Shen, Yanfei; Intensive Care Unit Huang, Xinmei; Jinhua TCM hospital, Department of otolaryngological Zhang, Weimin; Department of Intensive Care Unit, Dongyang People's Hospital
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Intensive care, Infectious diseases
Keywords:	sepsis, PLR, mortality, MIMIC III

A retrospective study of platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: interaction effect with disease severity

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Word count: 2955

Abstract

Objective: The role of platelet-to-lymphocyte ratio (PLR) as an indicator of inflammation has received recent scientific attention. We aimed to investigate the prognostic value of PLR for sepsis.

Design: Retrospective cohort study.

Setting and Participants: Data were extracted from the Medical Information Mart for Intensive Care III database. Data of 5,537 patients with sepsis were analyzed.

Methods: Logistic regression was used to explore the association between PLR and hospital mortality. Subgroup analyses were performed based on vasopressor use, acute kidney injury (AKI), and a Sequential Organ Failure Assessment (SOFA) score > 10.

Results: In the logistic model with linear spline function, a PLR > 200 was significantly associated with mortality (odds ratio [OR], 1.0002; 95% confidence interval [CI], 1.0001 – 1.0003); the association was non-significant for PLRs ≤ 200 (OR, 0.999; 95% CI, 0.998 – 1.001). In the logistic model using the PLR as a design variable, only high PLRs were significantly associated with mortality (OR, 1.29; 95% CI, 1.09 – 1.53). The association between mortality and low PLRs was non-significant (OR, 1.15; 95% CI, 0.96 – 1.38). In the subgroups with vasopressor use, AKI, and a SOFA score > 10, the association between high PLR and mortality was non-significant; this remained significant in the subgroups without vasopressor use (OR, 1.39; 95% CI, 1.08 – 1.77) or AKI (OR, 1.54; 95% CI, 1.20 – 1.99), and with a SOFA score ≤ 10 (OR, 1.51; 95% CI, 1.17 – 1.94).

Conclusions: High PLRs at admission were associated with an increased risk of mortality. In patients with vasopressor use, AKI, or a SOFA score > 10, this association was non-significant.

Keywords: Sepsis, PLR, mortality, MIMIC III

Strengths and limitations of this study:

- The large sample size facilitated a robust conclusion.
- Subgroup analysis was performed to investigate the interaction between disease severity and platelet-to-lymphocyte ratio.
- Pre-ICU data were not available in this database, which may lead to bias.
- Patients with septic shock could not be identified in this database.

INTRODUCTION

Sepsis is a major cause of morbidity and mortality worldwide, and it results from a dysregulation of the systemic inflammatory response to infection.[1 2] Despite significant advances in the pathophysiology and therapeutic strategies for sepsis, the mortality remains high,[3] at 300 deaths per 100,000 people.[4] An extremely complex systemic expression of inflammatory and anti-inflammatory response plays a critical role in the pathophysiological process of sepsis, which is strongly associated with an increased risk of mortality.[5] Identifying patients in the early stage of sepsis who are at a high risk of poor outcomes is vital for timely and adequate intervention.[6] While a significant amount of effort has been put into investigating promising biomarkers, the challenge of identifying these at-risk patients remains.[7]

In recent years, studies have reported that platelets and lymphocytes play critical roles in the inflammatory process. Therefore, the platelet-to-lymphocyte ratio (PLR)—a novel inflammatory factor—has received recent research attention, as it may act as an indicator of inflammation[8] in a wide spectrum of diseases, such as myocardial infarction,[9] acute kidney injury (AKI),[10] hepatocellular carcinoma,[11] and non-small cell lung cancer.[12]

Based on the findings of previous studies, it is reasonable to speculate the presence of a potential relationship between PLR and mortality for sepsis. However, no investigation has been conducted.

MATERIALS AND METHODS

Database introduction

All data in the current study were extracted from an online international database, "Medical Information Mart for Intensive Care III (MIMIC III)," published by the Massachusetts Institute of Technology, with approval from the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. All patient information in the database was de-identified for privacy protection, and the need for informed consent was waived. This database included more than 58,000 patients who were admitted to the intensive care unit (ICU) of the Beth Israel Deaconess Medical Center from 2001 to 2012. The corresponding author obtained access to this database (certification number: 1564657), and was responsible for data extraction.

Inclusion and exclusion criteria

Adult patients meeting the criteria for sepsis were initially screened. The definition of sepsis was adapted from the recommendation in the Surviving Sepsis Campaign 2016.[13] Accordingly, sepsis was defined as the presence of a Sequential Organ Failure Assessment (SOFA) score ≥ 2 within 24 hours after ICU admission, accompanied by at least one infection site. The following criteria were used to exclude patients from this analysis: 1) age lower than 18 years; 2) having spent less than 48 hours in the ICU, and 3) absence of data on the serum platelet and lymphocyte counts within 24 hours after ICU admission. For patients who were admitted to the ICU more than once, only the first ICU stay was considered in this study.

Data extraction

Data on patient demographic characteristics, laboratory outcomes, infection sites, vasopressor use, and disease severity score were extracted from the database. Only patients with data on the serum platelet and lymphocyte counts within the first 24 hours after ICU admission were included. The first blood sample after ICU admission was used to calculate the PLR, which was defined as the ratio of the absolute platelet count and absolute lymphocyte count. Septic shock was considered as a special subgroup of sepsis. However, it was difficult to identify patients with septic shock in this database due to a lack of relevant information. Thus, data on vasopressor use were extracted for the subgroup analysis. Vasopressor use was defined as the use of any vasopressor agent, including norepinephrine, epinephrine, dobutamine, dopamine, or vasopressin, within 48 hours after ICU admission.

Outcome definition

The primary endpoint was hospital mortality, which was defined as death during hospitalization. The presence of AKI was defined according to the Creatinine-based Kidney Disease Improving Global Outcome criteria without urine output.[14 15] A 1.5-fold increase in the serum creatinine (SCr) level during the ICU stay, relative to the level at the baseline, was considered to indicate the presence of AKI. In the present cohort, data on the baseline SCr values were missing in 20.3% of the cases. For patients without previous SCr data, the estimated baseline SCr was calculated using the following formula:[16] SCr = 0.74 - 0.2 (if female) + 0.08 (if black) + 0.0039 * age (in years).

Management of missing data

Variables with missing data are common in the MIMIC III database, as it comprises more than 58,000 admissions. Variables with more than 20% of missing values were excluded from our analysis; these included serum albumin and lactate. For non-normal distribution variables with less than 5% of missing values, such as age and fluid balance, we replaced the missing values with the mean values, and for non-normal distribution parameters, missing values were replaced by the respective median, instead of using the multiple imputation technique. For dichotomous variables with less than 5% of missing values, the missing values were not imputed.

Patient and public involvement

No patient was involved in any part of this study.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or median (interquartile range), as appropriate. A Student's t test, analysis of variance, Wilcoxon rank-sum test, or Kruskal–Wallis test was used, as appropriate. Categorical data were expressed as proportions, and compared using the χ^2 test. A knot of PLR (at a level of around 200, Supplementary Figure S1) was detected using the LOWESS smoother technique; thus, the linear spline function was initially used in the multivariate logistic regression. Thereafter, all patients were further divided into three levels: those with a PLR ≤ 150 (level 1), 150 < PLR ≤ 250 (level 2), and PLR > 250 (level 3). Variables including demographic characteristics, infection sites, disease severity score, and laboratory measures potentially associated with mortality,

or those that had a p value < 0.20 in the univariate analyses, were included in the multivariate logistic regression analyses. An extended model approach was used for covariate adjustment: Model 1 = adjusted for age, admitted ICU type. Model 2 = Model 1 + (fluid balance at 48 hours after ICU admission). Model 3 = Model 2 + (infection sites). Model 4 = Model 3 + (Maximum SOFA score during the ICU stay). As we detected a U-shaped association between PLR and mortality, we did not introduce interaction items (such as PLR multiplied with other variables) in the logistic models. Instead, subgroup analyses were performed, according to the presence of AKI and vasopressor use and the median SOFA score. Multi-collinearity was tested using the variance inflation factor (VIF) method, with a VIF ≥ 5 indicating the presence of multi-collinearity. All logistic regression models underwent a goodness of fit test. A two-tailed test was performed, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA 11.2 (StataCorp, LLC, College Station, TX, USA).

RESULTS

Baseline characteristics

Data on a total of 5,537 sepsis patients were included in this analysis. The overall mortality observed was 25.1%. Data on the comparisons of the baseline characteristics between the three PLR levels are listed in Table 1. The mean age at admission was 64.9 years, and 44.9% of the participants were male. The rate of vasopressor use (701/1780 vs. 482/1380, p = 0.01), and a maximum SOFA score

(10 (7–14) vs. 9 (7 – 12), p < 0.001) were significantly higher in PLR level 1 than in level 2; the presence of these variables was non-significant in level 3. The mortality was significantly higher both in the level 1 group (475/1780 vs. 291/1380, p < 0.001) and the level 3 group (621/2377 vs. 291/1380, p = 0.001).

Association between PLR and hospital mortality

The PLR was initially used as a continuous variable in the logistic model, using linear spline function, as shown in Table 2. We observed that, for PLRs ≤ 200, the odds ratio (OR) of mortality was non-significant (OR, 0.999; 95% confidence interval [CI], 0.998 – 1.001), while the OR for PLRs > 200 was significant after adjustment for covariates, including the SOFA score (OR, 1.0002; 95% CI, 1.0001 – 1.0003). In the extended multiple logistic regression analysis (Table 3), both low and high PLR levels were significantly associated with increased hospital mortality, in model 1 (OR, 1.41; 95% CI, 1.19 – 1.67 and OR, 1.28; 95% CI, 1.09 – 1.51, respectively), model 2 (OR, 1.34; 95% CI, 1.13 – 1.59 and OR, 1.23; 95% CI, 1.05 – 1.45, respectively) and model 3 (OR, 1.35; 95% CI, 1.14 – 1.61 and OR, 1.21; 95% CI, 1.03 – 1.43, respectively). However, after adjustment for the maximum SOFA score in model 4, the OR for low PLR levels became non-significant (OR, 1.15; 95% CI, 0.96 – 1.38, p = 0.123), while the OR for high PLR levels remained significant (OR, 1.29; 95% CI, 1.09 – 1.53, p = 0.003). The univariate results are presented in Supplementary Table S1 and the ORs of the covariates in model 4 are listed in Supplementary Table S2.

Subgroup analysis

As the association between PLR and mortality was largely confounded by the SOFA

score (Table 3), we suspected that there was an interaction effect between disease severity and PLR level. Thus, we performed a subgroup analysis according to the existence of vasopressor use and AKI, and the median SOFA score (> 10 points), as shown in Figure 1. Unlike previous findings, the association between high PLRs and mortality became non-significant in the subgroups with vasopressor use (OR, 1.20; 95% CI, 0.95 – 1.53), AKI (OR, 1.07; 95% CI, 0.85 – 1.36), and a SOFA score > 10 (OR, 1.14; 95% CI, 0.90 – 1.44), and remained significant in the subgroups without vasopressor use (OR, 1.39; 95% CI, 1.08 – 1.77) and AKI (OR, 1.54; 95% CI, 1.20 – 1.99), and with a SOFA score ≤ 10 (OR, 1.51; 95% CI, 1.17 – 1.94). In the case of lower PLRs, the OR of mortality was non-significant in all subgroups, after adjustment, except for the subgroup with AKI. Data on the comparisons of the characteristics between these subgroups are listed in Supplementary Table S3.

DISCUSSION

In this study, we observed a crude U-shaped association between the PLR and hospital mortality in patients with sepsis. However, after adjustment for the disease severity score, only high PLRs remained significantly associated with increased mortality; the association with low PLRs became non-significant. Furthermore, in the subgroup analysis, a significant association between high PLRs and mortality only existed in the subgroups without vasopressor use and AKI, or those with a SOFA score ≤ 10.

Growing evidence indicates that immune dysregulation (especially cellular

immunity), including pro-inflammatory or anti-inflammatory responses during different stages, is common in cases of sepsis.[17] Recent studies have reported that platelets play an important role in both the immunomodulatory and inflammatory process,[18 19] by inducing the release of inflammatory cytokines[20] and interacting with different kinds of bacteria and immune cells, including neutrophils, T-lymphocytes, NK-cells, and macrophages, which contribute to the initiation or exacerbation of the inflammatory process.[21] Low lymphocyte counts, which to a certain degree represent a suppressed immune and inflammatory response,[22 23] have also been reported to be associated with inflammatory diseases, such as cardiovascular disease[24] and type II diabetes.[25]

Based on these findings, the PLR was suggested as being a novel systematic inflammatory indicator,[26] and its use was initially reported in the prognostic prediction of neoplastic disorders, such as hepatocellular carcinoma and breast cancer. Accumulating evidence suggests that elevated PLRs are strongly associated with increased systemic inflammation, which may contribute to the progression and prognoses of many disorders, such as atherosclerosis[27] and diabetes mellitus.[28]

In contrast to our findings, Zheng et al.[10] reported that both high and low PLRs are associated with increased mortality among critically ill patients with AKI, after adjustment for the disease severity score in the Cox proportional hazards models. In that study, unlike in ours, a significant association was also observed in patients with vasopressin use. Several factors may contribute to this inconsistency between the findings, such as the use of different cohorts, PLR knots, and definitions of

vasopressor use. It is worth noting that, as the association between PLRs and outcomes varies greatly between different cohorts, the inter-heterogeneity within critically ill patients may also lead to a biased conclusion.

Akbas et al. indicated that a high PLR was positively associated with increased epicardial adipose tissue deposition in diabetes patients;[29] this may be caused by higher inflammation rates. Wang et al.[30] reviewed 134 patients with lung adenosquamous cancer, and reported that high PLRs (> 150) were independently associated with shorter disease-free days and lower overall survival rates. Another study,[31] including 270 patients with hepatocellular carcinoma, found that elevated PLRs (> 220) were predictors of poor prognoses, while low PLRs (< 248) were associated with a low tumor, node and metastasis stage, and low surgery incidence in 695 patients with lung cancer.[32] Despite the fact that the study cohorts used in those studies were quite different from those used in ours, the reported PLR knots were quite similar to ours. However, the small sample sizes in those studies limited the statistical power for further stratification and subgroup analysis of low PLR. In the current study, we noticed that high PLRs (> 250) were associated with increased hospital mortality. As higher platelet levels, to a certain extent, are prognostic of inflammation of a higher severity, and low lymphocyte counts may represent a suppressed immune and inflammatory response,[22 23] an increase in the PLR may reflect the degree of the inflammatory and immune response to the infection, which relates to a poor prognosis.

We also detected a non-significant association between low PLRs and mortality

from sepsis. This association between low PLRs and outcomes was also reported in several studies. In a retrospective study[33] including 899 cases of laryngeal cancer, patients were divided into three PLR categories (low (≤ 119.55), moderate (> 119.55 and ≤ 193.55), and high (> 193.55)), and only patients with high PLRs experienced poor outcomes, including malnutrition and more advanced cancer stage; the association between outcomes and PLR levels were non-significant for those with low PLRs. Despite the cohort of that study being different from ours, the conclusion was consistent with that of our study. In the case of sepsis, a low platelet count is potentially associated with poor outcomes. In a large study including 931 patients with sepsis, Claushuis et al. reported that patients with a low platelet count at ICU admission had a higher disease severity score and increased mortality risk.[34] Furthermore, thrombocytopenia, one of the most common hemostatic disorders in the case of sepsis and which is related with platelet consumption, was also associated with higher mortality.[35] However, in the present study, a significant association between low PLR and mortality was not detected. Further studies are needed to validate this conclusion.

Furthermore, according to the subgroup analysis, the association between high PLR and mortality became non-significant in the subgroups with vasopressor use, AKI, or a SOFA score > 10; this association remained significant in the other subgroups. This finding further supported our speculation that there may be an interaction between PLR and disease severity. To the best of our knowledge, ours is the first study to report this interaction. However, the underlying mechanism of this

interaction remains largely unknown. A critical characteristic of sepsis is fluid resuscitation, and, in the current study, patients with vasopressor use, AKI, or a SOFA score > 10, to a certain degree, represented patients with inflammation of a higher severity, and thus they may have had a stronger need for fluid resuscitation. We also noticed that the fluid balance within 48 hours after ICU admission was significantly larger in these subgroups. Whether fluid resuscitation affects the prognostic value of the PLR needs further investigation.

One of the strengths of our study is the large sample size, which enabled us to adjust for confounding factors and perform subgroup analyses. However, there are also several limitations to our study. First, the MIMIC III database comprises data on patients from 2001; since then, the guidelines for sepsis have changed significantly. The most recent definition of Sepsis 3.0 was used in the current study, and this may have introduced selection bias despite the fact that most of the basic interventions (use of fluids, vasopressors, and antimicrobial agents) remained the same. Furthermore, as a decrease in the platelet count was a part of the SOFA score, using the definition of sepsis 3.0 might lead to a relatively low mean platelet count and potential multi-collinearity. This bias cannot be fully avoided. However, the potential multi-collinearity was verified in all the logistic models. Second, the platelet count can be affected by many confounders, such as types of malignities, immunological factors, and types of drugs. However, due to the retrospective nature of this study, these situations could not be identified in this database. Third, septic shock is a special subgroup of sepsis. However, patients with septic shock could not be

distinguished in this study. Thus, patients were divided into subgroups, according to the existence of vasopressor use, AKI, or a SOFA score >10, as these may indicate the presence of a more severe inflammatory response. Fourth, one of the main hypotheses of our study was the interaction effect between disease severity and PLR; however, this interaction term was not introduced in the logistic model due to the U-shaped association between PLR and mortality. Further prospective studies are needed to verify our hypothesis. Finally, as high PLRs are associated with poor outcomes in various disorders while low PLRs are not, it is not clear if interventions aimed at changing the PLR value may improve outcomes.

Conclusion

In patients with sepsis, a high PLR was significantly associated with poor survival, while the association was non-significant for those with a low PLR. However, the former association became non-significant in patients with more severe conditions, including those with vasopressor use, AKI, or a SOFA score > 10. Future studies are needed to verify our hypothesis.

Financial support and sponsorship: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors**.**

Ethical approval: The use of this database was approved by the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. All the patient information in the database has been de-identified for privacy protection and the need for informed consent was waived.

Acknowledgments: None.

Conflicts of interest: None.

Authors' contributions:

Yanfei Shen: Responsible for data extraction and writing of the manuscript.

Xinmei Huang: Responsible for data analysis.

Weimin Zhang: Responsible for data validation.

Data sharing statement: The full data set used in this study is available from the corresponding author at snow.shen@hotmail.com. However, reanalysis of the full data for other use requires approval by the MIMIC III Institute.

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Table 1 Comparison of baseline characteristics within three PLR levels

Variable	PLR ≤ 150 (n = 1780)	150< PLR ≤ 250 (n = 1380)	PLR > 250 (n = 2377)	p
Age (years)	63.0 ± 16.6	65.0 ± 16.6	66.1 ± 15.5	< 0.001
Gender (male) [n (%)]	805 (45.2)	590 (42.7)	1096 (46.1)	0.133
BMI (Kg/m^2)	30.8 ± 8.9	34.1 ±13.5	35.2 ± 39.5	0.001
Ethnicity				
White [n (%)]	1202 (67.5)	987 (71.5)	1761 (74.0)	0.754
Black [n (%)]	180 (10.1)	101 (7.3)	146 (6.1)	< 0.001
Asian [n (%)]	39 (2.2)	30 (2.1)	71 (2.9)	0.169
Emergency [n (%)]	1641 (92.1)	1284 (93.0)	2229 (93.7)	0.138
ICU type				
MICU [n (%)]	953 (53.5)	727 (52.6)	1362 (57.2)	0.008
CCU/CSRU [n (%)]	413 (23.2)	323 (23.4)	453 (19.0)	0.001
TSICU/SICU [n (%)]	414 (23.2)	330 (23.9)	562 (23.6)	0.908
Vasopressors				
Norepinephrine [n (%)]	566 (31.7)	374 (27.1)	711 (29.9)	0.016
Dopamine [n (%)]	198 (11.1)	151 (10.9)	256 (10.7)	0.013
Epinephrine [n (%)]	67 (3.7)	28 (2.0)	37 (1.5)	< 0.001
Vasopressin [n (%)]	156 (8.7)	88 (6.3)	172 (7.2)	0.033
Overall vasopressor use	701 (39.3)	482 (34.9)	858 (36.1)	0.022
Fluid input/output				
Fluid intake (ml/kg/48hr)	99.9 ± 60.9	90.7 ± 57.6	97.2 ± 61.2	< 0.001
Urine output (ml/kg/48hr)	42.0 ± 32.0	42.9 ± 30.3	41.9 ± 29.5	0.5659
Fluid balance (ml/kg/48hr)	46.7 ± 59.4	38.3 ± 55.1	46.0 ± 60.4	< 0.001
Infection site				
Respiratory infection	1048 (58.8)	929 (67.3)	1580 (66.4)	< 0.001
Blood infection	768 (43.1)	509 (36.8)	998 (41.9)	0.001
Urinary infection	549 (30.8)	409 (29.6)	682 (28.6)	0.323
Abdominal infection	245 (13.7)	159 (11.5)	334 (14.0)	0.072
Cerebral infection	153 (8.5)	106 (7.6)	169 (7.1)	0.206
Disease severity scores				
SOFA on ICU admission median (IQR)	6 (4–9)	5 (4–8)	5 (3–7)	< 0.001
Maximum SOFA during ICU stay median (IQR)	10 (7–14)	9 (7 – 12)	9 (7 – 12)	< 0.001
Laboratory outcomes				
Maximum serum creatinine (mg/L)	2.5 ± 2.7	2.2 ± 2.1	2.1 ± 1.9	< 0.001
Minimum hemoglobin level (g/dl)	8.3 ± 1.7	8.69 ± 1.7	8.4 ± 1.6	< 0.001
Maximum serum sodium (mmol/L)	145.1 ± 5.4	145.0 ± 5.2	144.6 ± 5.1	0.009
Maximum serum lactate (mmol/L)	4.1 ± 3.8 (n=1536)	3.4 ± 3.1 (n=1174)	3.1 ± 3.0 (n=2112)	< 0.001
Platelet count (10^9/L)	146.7 ± 88.0	225.1 ± 107.2	197.5 ± 163.4	< 0.001
Lymphocyte count (10^9/L)	2.1 ± 5.7	1.1 ± 0.5	0.68 ± 0.4	< 0.001

PLR	91.8 ± 37.1	195.8 ± 28.6	557.5 ± 484.8	< 0.001
Clinical outcomes				
ICU LOS	9.9 ± 10.1	9.3 ± 8.7	10.1 ± 9.9	0.071
Hospital LOS	17.7 ± 15.1	16.6 ± 13.5	17.2 ± 13.7	0.082
AKI [n (%)]	861 (48.3)	601 (43.5)	1080 (45.4)	0.022
Hospital mortality [n (%)]	475 (26.6)	291 (21.0)	621 (26.1)	< 0.001

Abbreviations:

PLR: platelet to lymphocyte ratio; BMI body mass index; MICU, multiple intensive care unit; CCU, coronary care unit; CSRU, cardiac surgery care unit; TSICU, traumatic surgical intensive care unit; SICU, surgical intensive care unit; SOFA, sequential organ failure assessment; SAPS II, Simplified Acute Physiology Score II; IQR, interquartile range; LOS length of stay; AKI, acute kidney injury.

Table 2 multivariable logistic regressions of PLR using linear spline function

Variables	Crude Odds ratio	95% CI	p	Adjusted Odds ratio	95% CI	p
PLR (≤ 200)	0.997	0.996 – 0.998	< 0.001	0.9993	0.9980 – 1.0006	0.319
PLR (> 200)	1.0002	1.0001 – 1.0004	0.001	1.0002	1.0000 – 1.0003	0.025
Age (> 65)	1.77	1.56 – 2.11	< 0.001	2.32	1.99 – 2.64	< 0.001
Maximum SOFA	1.20	1.18 – 1.22	< 0.001	1.18	1.16 – 1.20	< 0.001
Urinary infection	0.66	0.57 – 0.76	< 0.001	0.65	0.56 – 0.76	< 0.001
Respiratory infection	1.29	1.13 – 1.47	< 0.001	1.25	1.09 – 1.45	0.002
Blood infection	2.14	1.89 – 2.42	< 0.001	1.49	1.29 – 1.71	< 0.001
Fluid balance (ml/kg/48hrs)	1.006	1.005 – 1.007	< 0.001	1.002	1.0008 – 1.0031	0.001
MICU	1.34	1.15 – 1.56	< 0.001	1.15	0.97 – 1.37	0.089
CCU/CSRU	1.22	1.01 – 1.47	0.032	1.03	0.84 – 1.26	0.752

Note: The mean variance inflation factor was 2.89 and p value of goodness of fit was 0.632.

Abbreviation: PLR platelet to lymphocyte ratio; CI confidence interval; SOFA sequential organ failure assessment; MICU multiple intensive care unit; CCU coronary care unit; CSRU cardiac surgery care unit.

Table 3 Association between three PLR levels and hospital mortality

	PLR ≤ 150		150 < PLR ≤ 250		PLR > 250	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Model 1	1.41 (1.19 – 1.67)	< 0.001	Ref.	–	1.28 (1.09 – 1.51)	0.002
Model 2	1.34 (1.13 – 1.59)	0.001	Ref.	–	1.23 (1.05 – 1.45)	0.009
Model 3	1.35 (1.14 – 1.61)	0.001	Ref.	–	1.21 (1.03 – 1.43)	0.018
Model 4	1.15 (0.96 – 1.38)	0.123	Ref.	–	1.29 (1.09 – 1.53)	0.003

Adjusted covariates: Model 1 = age, admitted ICU type. Model 2 = Model 1+ (fluid balance at 48 hours after ICU admission). Model 3= Model 2 + (infection sites). Model 4 = Model 3+ (Maximum SOFA score during ICU stay).

Abbreviations: PLR platelet to lymphocyte ratio; OR = odds ratio; CI= confidence interval; Ref reference category.

Figure legend:

Figure 1: The crude and adjusted odds ratios in the subgroup analysis. PLR level 2 was used as the reference level in all of the logistic models.

Abbreviations: PLR, platelet-to-lymphocyte ratio; AKI, acute kidney injury; SOFA, Sequential Organ Failure Assessment; CI, confidence interval

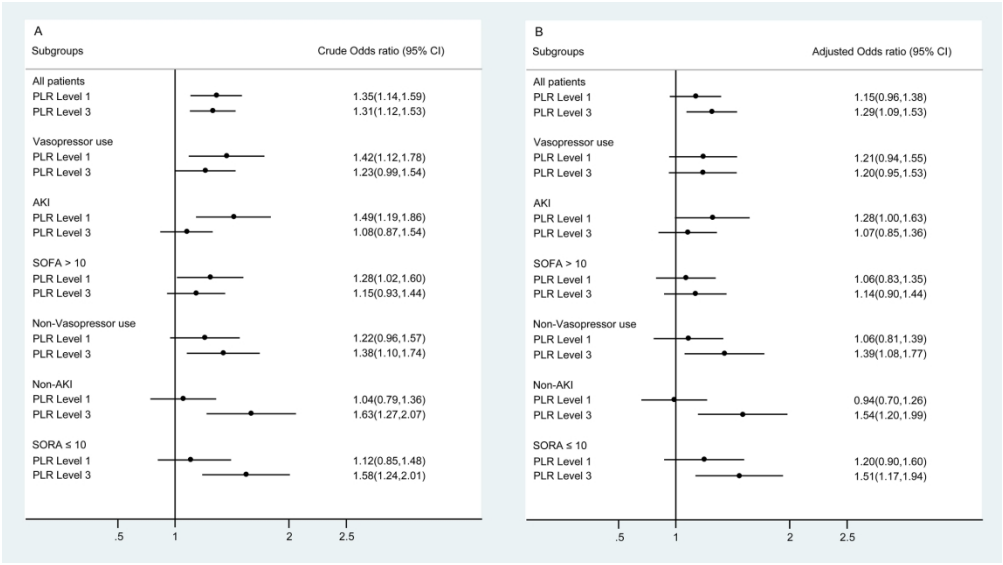

Figure 1: The crude and adjusted odds ratios in the subgroup analysis. PLR level 2 was used as the reference level in all of the logistic models.
Abbreviations: PLR, platelet-to-lymphocyte ratio; AKI, acute kidney injury; SOFA, Sequential Organ Failure Assessment; CI, confidence interval

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Table S1 Risk factors associated with in-hospital mortality

Variables	Odds ratio	95% CI	p
Age (> 65)	1.77	1.56 – 2.01	< 0.001
Gender (male)	0.91	0.81 – 1.03	0.173
BMI (kg/m^2)	1.00	0.99 – 1.00	0.485
TSICU/SICU	Ref.	–	–
MICU	1.34	1.15 – 1.56	< 0.001
CCU/CSRU	1.22	1.01 – 1.47	0.032
Fluid balance (ml/kg/48hrs)	1.006	1.005 – 1.007	< 0.001
Urine output (ml/kg/48hrs)	0.98	0.98 – 0.98	< 0.001
Respiratory infection	1.29	1.13 – 1.47	< 0.001
Blood infection	2.14	1.89 – 2.42	< 0.001
Urinary infection	0.66	0.57 – 0.76	< 0.001
Abdominal infection	0.93	0.77 – 1.11	0.458
Cerebral infection	0.66	0.51 – 0.85	0.002
SOFA on ICU admission	1.15	1.13 – 1.17	< 0.001
Maximum SOFA	1.20	1.18 – 1.22	< 0.001
Maximum serum creatinine (mg/L)	1.15	1.12 – 1.19	< 0.001
Minimum hemoglobin level (g/dl)	0.84	0.81 – 0.88	< 0.001
Maximum serum sodium (mmol/L)	1.02	1.01 – 1.02	< 0.001
Platelet count (10^9/L)	0.998	0.997 – 0.998	< 0.001
Lymphocyte count (10^9/L)	0.972	0.934 – 1.01	0.163

Abbreviations: BMI, body mass index; MICU, multiple intensive care unit; CCU, coronary care unit; CSRU, cardiac surgery care unit; TSICU, traumatic surgical intensive care unit; SICU, surgical intensive care unit; SOFA, sequential organ failure assessment;

Table S2 Adjusted odds ratio of hospital mortality using PLR as design variable in multivariable logistic regression

Variables	Odds ratio	95% CI	p
PLR Level 1 (≤ 150)	1.15	0.96 – 1.38	0.123
PLR Level 2 (151 ~ 250)	Ref.	–	–
PLR Level 3 (> 250)	1.29	1.09 – 1.53	0.003
Age (> 65)	2.27	1.97 – 2.62	< 0.001
Maximum SOFA	1.19	1.16 – 1.21	< 0.001
Urinary infection	0.65	0.56 – 0.76	< 0.001
Respiratory infection	1.25	1.08 – 1.44	0.002
Blood infection	1.49	1.29 – 1.71	< 0.001
Fluid balance (ml/kg/48hrs)	1.002	1.0008 – 1.0031	0.001
MICU	1.16	0.98 – 1.37	0.082
CCU/CSRU	1.04	0.84 – 1.27	0.700

Note: The mean variance inflation factor (VIF) was 2.53 and p value of goodness of fit was 0.665.

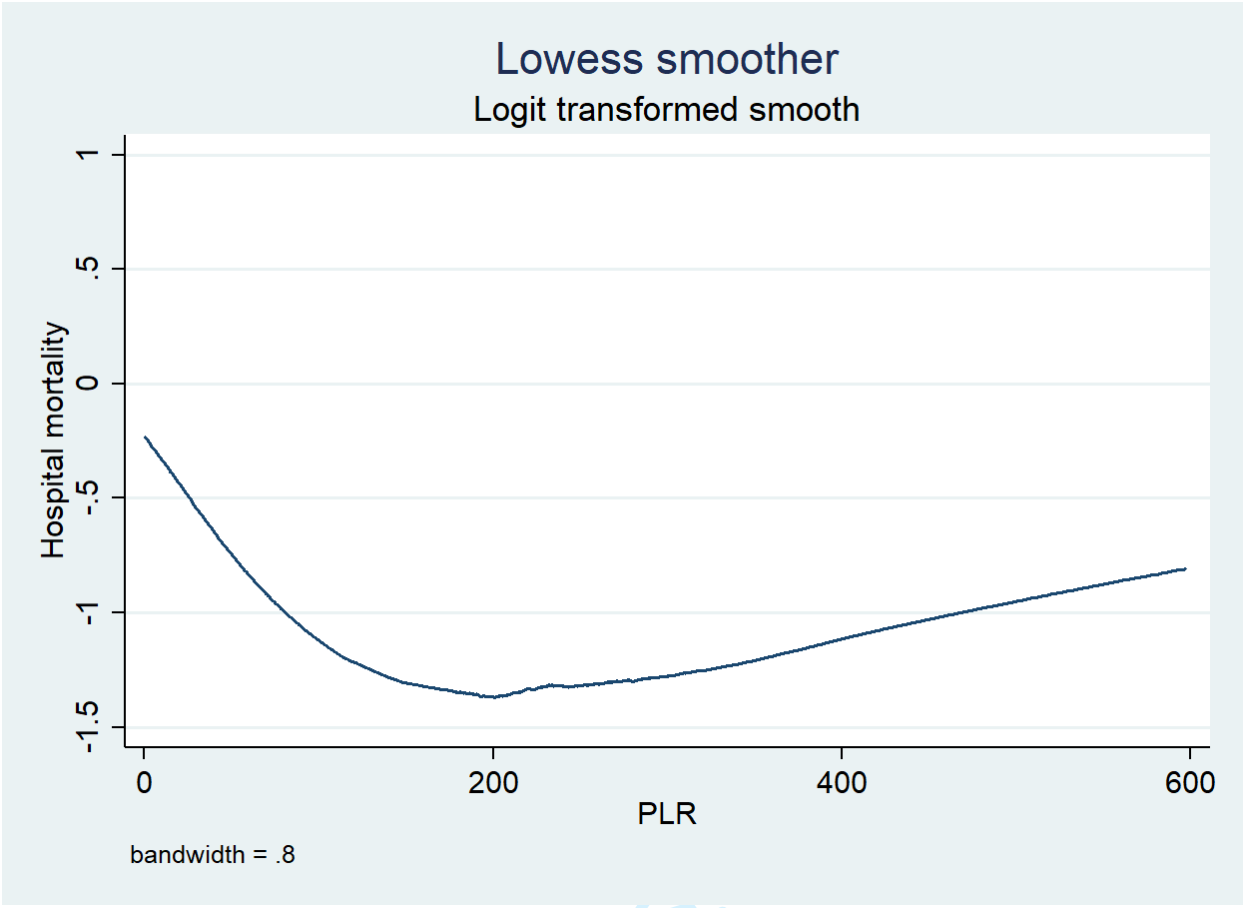
Abbreviation: PLR, platelet to lymphocyte ratio; CI, confidence interval; SOFA, sequential organ failure assessment; MICU, multiple intensive care unit; CCU, coronary care unit; CSRU, cardiac surgery care unit.

Table S3 Comparisons of subgroups according to the existence of vasopressor use, AKI and SOFA score

Variables	Vasopressor-use (n = 2554)	Non-Vasopressor-use (n = 2983)	p	AKI (n = 2542)	Non-AKI (n = 2995)	p	SOFA > 10 (n = 2390)	SOFA ≤ 10 (n = 2147)	p
Age	66.3 ± 15.2	63.6 ± 16.8	< 0.001	65.1 ± 15.4	64.6 ± 16.8	0.211	64.0 ± 15.7	65.5 ± 16.4	< 0.001
Vasopressor-use [n (%)]	-	-	-	1321	1233	< 0.001	1554	1000	< 0.001
Fluid intake (ml/kg/48hr)	114.4 ± 661.	81.1 ± 50.0	< 0.001	94.3 ± 62.0	98.3 ± 58.8	0.013	110.7 ± 65.5	85.6 ±53.6	< 0.001
Fluid balance (ml/kg/48hr)	63.2 ± 64.1	28.1 ± 48.5	< 0.001	48.4 ± 60.9	40.8 ± 56.9	< 0.001	62.0 ± 64.1	30.8 ± 50.6	< 0.001
Maximum SOFA median (IQR)	12 (9 – 14)	8 (6 – 11)	< 0.001	11 (8 – 14)	9 (7 – 11)	< 0.001	13 (12 – 15)	7 (6 – 9)	< 0.001
Platelet count (10^9/L)	225.0 ± 240.1	236.0 ± 148.7	0.005	219.2 ± 143.4	240.9 ± 145.5	< 0.001	208.4 ± 147.1	248.1 ± 140.8	< 0.001
Lymphocyte count (10^9/L)	1.26 ± 3.32	1.28 ± 3.32	0.890	1.21 ± 2.21	1.32 ± 4.03	0.246	1.24 ± 3.42	1.29 ± 3.24	0.529
Hospital LOS	17.8 ± 14.3	16.7 ± 14.1	0.002	19.7 ± 15.6	15.1 ± 12.3	< 0.001	19.6 ± 14.6	15.5 ± 13.4	< 0.001
AKI [n (%)]	1321	1221	< 0.001	-	-	-	1360	1182	< 0.001
Hospital mortality [n (%)]	777	612	< 0.001	875	514	< 0.001	884	505	< 0.001

Abbreviation: SOFA, sequential organ failure assessment; IQR, interquartile range; LOS, length of stay; AKI, acute kidney injury.

Figure S1 Crude relationship between hospital mortality and PLR



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
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*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
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	*5*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
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	8
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A.
		(*e*) Describe any sensitivity analyses	N/A.
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	8
		(b) Give reasons for non-participation at each stage	N/A.
		(c) Consider use of a flow diagram	N/A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	N/A.
Outcome data	15*	Report numbers of outcome events or summary measures over time	9
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	7
		(*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	N/A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A.
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	15

*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

Platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: Interaction effect with disease severity. A retrospective study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022896.R2
Article Type:	Research
Date Submitted by the Author:	23-Jul-2018
Complete List of Authors:	Shen, Yanfei; Zhejiang Hospital, Intensive care unit Huang, Xinmei; Jinhua TCM hospital, Department of otolaryngological Zhang, Weimin; Department of Intensive Care Unit, Dongyang People's Hospital
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Intensive care, Infectious diseases
Keywords:	sepsis, PLR, mortality, MIMIC III

Title page

Platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: Interaction effect with disease severity. A retrospective study

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# Author Xinmei Huang contributed equally to this study and was listed as co-first author.

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Word count: 3542.

Abstract

Objective: The role of platelet-to-lymphocyte ratio (PLR) as an indicator of inflammation has been the focus of research recently. We aimed to investigate the prognostic value of PLR for sepsis.

Design: A retrospective cohort study.

Setting and Participants: Data were extracted from the Multi-parameter Intelligent Monitoring in Intensive Care III database. Data on 5,537 sepsis patients were analyzed.

Methods: Logistic regression was used to explore the association between PLR and hospital mortality. Subgroup analyses were performed based on vasopressor use, acute kidney injury (AKI), and a Sequential Organ Failure Assessment (SOFA) score > 10.

Results: In the logistic model with linear spline function, a PLR > 200 was significantly (odds ratio [OR], 1.0002; 95% confidence interval [CI], 1.0001 – 1.0004) associated with mortality; the association was non-significant for PLRs ≤ 200 (OR, 0.997; 95% CI, 1.19 – 1.67). In the logistic model using the PLR as a design variable, only high PLRs were significantly associated with mortality (OR, 1.29; 95% CI, 1.09 – 1.53); the association with low PLRs was non-significant (OR, 1.15; 95% CI, 0.96 – 1.38). In the subgroups with vasopressor use, AKI, and a SOFA score > 10, the association between high PLR and mortality was non-significant; this remained significant in the subgroups without vasopressor use (OR, 1.39; 95% CI, 1.08 – 1.77) and AKI (OR, 1.54; 95% CI, 1.20 – 1.99), and with a SOFA score ≤ 10 (OR, 1.51; 95% CI, 1.17 – 1.94).

Conclusions: High PLRs at admission were associated with an increased risk of mortality. In patients with vasopressor use, AKI, or a SOFA score > 10, this association was non-significant.

Keywords: sepsis, PLR, mortality, MIMIC III.

Strengths and limitations of this study:

The large sample size facilitated a robust conclusion.

Subgroup analysis was performed to investigate the interaction between disease severity and PLR.

Pre-ICU data were not available in this database which may lead to bias.

Patients with septic shock could not be identified in this database.

INTRODUCTION

Sepsis is a major cause of morbidity and mortality, worldwide, and it results from a dysregulation of the systemic inflammatory response to infection [1 2]. Despite significant advances in the pathophysiology and therapeutic strategies for sepsis, the mortality remains high [3], at 300 deaths per 100,000 people [4]. An extremely complex systemic expression of inflammatory and anti-inflammatory response plays a critical role in the pathophysiological process of sepsis, which is strongly associated with an increased risk of mortality [5]. Identifying patients who are at a high risk of poor outcomes, in the early stage of sepsis, is vital for timely and adequate intervention [6]. While a significant amount of effort has been put into investigating promising biomarkers, the challenge of identifying these at-risk patients remains [7].

In recent years, studies have reported that platelets and lymphocytes play critical roles in the inflammatory process. Therefore, the platelet-to-lymphocyte ratio (PLR)--a novel inflammatory factor--has received research attention recently, as it may act as an indicator of inflammation [8] in a wide spectrum of diseases, such as myocardial infarction [9], acute kidney injury (AKI) [10], hepatocellular carcinoma [11], and non-small cell lung cancer [12].

Based on the findings of previous studies, it is reasonable to speculate the presence of a potential relationship between PLR and mortality for sepsis. However, no investigation has been conducted. Therefore, in this study we aimed to investigate the prognostic value of PLR for sepsis.

MATERIALS AND METHODS

Database introduction

All the data in the current study were extracted from an online international database—Multi-parameter Intelligent Monitoring in Intensive Care III (MIMIC III)—that was published by the Massachusetts Institute of Technology, with approval from the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. All the patients in the database were de-identified for privacy protection and the need for informed consent was waived. This database included more than 58,000 patients who were admitted to the intensive care unit (ICU) of the Beth Israel Deaconess Medical Center from 2001 to 2008. Author Y Shen obtained access to this database (certification number: 1564657), and was responsible for data extraction.

Inclusion and exclusion criteria

Adult patients meeting the criteria for sepsis were initially screened. The definition of sepsis was adapted from the recommendation in the Surviving Sepsis Campaign 2016 [13]. Accordingly, sepsis was defined as the presence of a Sequential Organ Failure Assessment (SOFA) score ≥ 2 within 24 hours after ICU admission, accompanied by at least one infection site. The following criteria were used to exclude patients from this analysis: 1. Age lower than 18 years; 2. Having spent less than 48 hours in the ICU; and 3. Absence of data on the serum platelet and lymphocyte counts within 24 hours after ICU admission. For patients who were admitted to the ICU more than once, only the first ICU stay was considered in this study.

Data extraction

Data on the demographic characteristics, laboratory outcomes, infection sites, vasopressor use, and disease severity score were extracted from the database. Only patients with data on the serum platelet and lymphocyte counts within the first 24 hours after ICU admission were included. The first blood sample after ICU admission was used to calculate the PLR, which was defined as the ratio of the absolute platelet count and absolute lymphocyte count. Septic shock was considered as a special subgroup of sepsis. However, it was difficult to identify patients with septic shock in this database due to a lack of relevant information. Thus, data on vasopressor use were extracted for the subgroup analysis. Vasopressor use was defined as the use of any vasopressor agent, including norepinephrine, epinephrine, dobutamine, dopamine or vasopressin, within 48 hours after ICU admission.

Outcome definition

The primary endpoint was hospital mortality, which was defined as death during hospitalization. The presence of AKI was defined according to the Creatinine-based Kidney Disease Improving Global Outcome criteria without urine output [14 15]. A 1.5-fold increase in the serum creatinine (SCr) level during the ICU stay, relative to the level at the baseline, was considered as the presence of AKI. In the present cohort, data on the baseline SCr values were missing in 20.3% of the cases. As AKI was not the primary outcome, we used a reported estimation equation[16] (reported median absolute error was 0.1–0.2 mg/dL) to calculate the missing values for patients without previous SCr data: SCr = 0.74 - 0.2 (if female) + 0.08 (if black) + 0.0039 * age (in years).

Management of missing data

Variables with missing data are common in the MIMIC III database, as it comprises more than 58,000 admissions. The percentage of missing values of serum lactate and albumin was 12.9% and 26.3%, respectively. For serum lactate, the crude comparison within three PLR levels is presented in Table 1, but was not included in the logistic models. The serum albumin was completely excluded from this study. For the rest of the variables included in the current study, the percentage of missing values was less than 5%. For normal distribution variables, such as age and fluid balance, we replaced the missing values with their mean values; For non-normal distribution parameters, missing values were replaced by the respective median, instead of using the multiple imputation technique. For dichotomous variables with less than 5% of missing values, the missing values were not filled.

Patient and Public Involvement:

No patient was involved in any part of this study.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or median (interquartile range), as appropriate. A Student's t test, analysis of variance, Wilcoxon rank-sum test, or Kruskal–Wallis test was used, as appropriate. Categorical data were expressed as proportions, and compared using the $\chi 2$ test. A knot of PLR (at a level of around 200) was detected using the Lowess smoother technique; thus, the linear spline function was initially used in the multivariate logistic regression. Thereafter, all the patients were further divided into three levels: those with a PLR ≤ 150 (level 1), 150 < PLR ≤ 250 (level 2), and PLR > 250 (level 3). Variables including demographic

characteristics, infection sites, disease severity score, and laboratory measures potentially associated with mortality, or those that had a p value < 0.20 in the univariate analyses were included in the multivariate logistic regression analyses[17 18]. An extended model approach was used for covariate adjustment: Model 1 = adjusted for age, admitted ICU type. Model 2 = Model 1+ (fluid balance at 48 hours after ICU admission). Model 3 = Model 2 + (infection sites). Model 4 = Model 3 + (Maximum SOFA score during the ICU stay). As we detected a U-shaped association between PLR and mortality, we did not introduce interaction items (such as PLR multiply other variables) in the logistic models. Instead, subgroup analyses were performed, according to the presence of AKI and vasopressor use and the median SOFA score. Multi-collinearity was tested using the variance inflation factor (VIF) method, with a VIF ≥ 5 indicating the presence of multi-collinearity. All the logistic regression models underwent a goodness of fit test. A two-tailed test was performed, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA 11.2 (College Station, TX, USA).

RESULTS

Baseline characteristics

Data on a total of 5,537 sepsis patients were included in this analysis. The overall mortality observed was 25.1%. Data on the comparisons of the baseline characteristics between the three PLR levels are listed in Table 1. The mean age at admission was 64.9 years, and 44.9% of the participants were male. The rate of vasopressor use

(701/1780 vs. 482/1380, p=0.01), and a maximum SOFA score (10 (7–14) vs. 9 (7 – 12), p<0.001) were significantly higher in PLR level 1 than level 2; the presence of these variables was non-significant in level 3. The mortality was significantly higher among those in level 1 (475/1780 vs. 291/1380, p<0.001) and level 3 (621/2377 vs. 291/1380, p=0.001).

Association between PLR and hospital mortality

The PLR was initially used as a continuous variable in the logistic model, using linear spline function, as shown in Table 2. We observed that, for PLRs ≤ 200, the odds ratio (OR) of mortality was non-significant (OR, 0.997; 95% confidence interval [CI], 1.19 – 1.67), while the OR for PLRs > 200 was significant (OR, 1.0002; 95% CI, 1.0001 – 1.0004), after adjustment for covariates including the SOFA score, with a mean VIF of 2.89. The crude association between hospital mortality and PLR was also presented in Figure S1. In the extended multiple logistic regression analysis (Table 3), both low and high PLR levels were significantly associated with increased hospital mortality, in model 1 (OR, 1.41; 95% CI, 1.19 – 1.67 and OR, 1.28; 95% CI, 1.09 – 1.51, respectively), model 2 (OR, 1.34; 95% CI, 1.13 – 1.59 and OR, 1.23; 95% CI, 1.05 – 1.45, respectively) and model 3 (OR, 1.35; 95% CI, 1.14 – 1.61 and OR, 1.21; 95% CI, 1.03 – 1.43, respectively). However, after adjustment for the maximum SOFA score in model 4, the OR for low PLR levels became non-significant (OR, 1.15; 95% CI, 0.96 – 1.38, p=0.123), while that for high PLR levels remained significant (OR, 1.29; 95% CI, 1.09 – 1.53, p=0.003), with a mean VIF of 2.53. The ORs of the covariates in model 4 are listed in Table S1.

Subgroup analysis

As the association between PLR and mortality was largely confounded by the SOFA score (Table 3), we suspected that there was an interaction effect between disease severity and PLR level. Thus, we performed a subgroup analysis according to the existence of vasopressor use and AKI, and the median SOFA score (> 10 points), as shown in Figure 1. Unlike previous findings, the association between high PLRs and mortality became non-significant in the subgroups with vasopressor use (OR, 1.20; 95% CI, 0.95 – 1.53), AKI (OR, 1.07; 95% CI, 0.85 – 1.36), and a SOFA score > 10 (OR, 1.14; 95% CI, 0.90 – 1.44), and remained significant in the subgroups without vasopressor use (OR, 1.39; 95% CI, 1.08 – 1.77) and AKI (OR, 1.54; 95% CI, 1.20 – 1.99), and with a SOFA score ≤ 10 (OR, 1.51; 95% CI, 1.17 – 1.94). In the case of lower PLRs, the OR of mortality was non-significant in all the subgroups, after adjustment, except for the subgroup with AKI. Data on the comparisons of the characteristics between these subgroups are listed in Table S2. Finally, all the potential risk factors associated with in-hospital mortality were listed in Table S3.

DISCUSSION

In this study, we observed a crude U-shaped association between the PLR and hospital mortality in patients with sepsis. However, after adjustment for the disease severity score, only high PLRs remained significantly associated with increased mortality; the association with low PLRs became non-significant. Furthermore, in the subgroup analysis, a significant association between high PLRs and mortality only

existed in the subgroups without vasopressor use and AKI, or those with a SOFA score ≤ 10.

Growing evidence indicates that immune dysregulation (especially cellular immunity), including pro-inflammatory or anti-inflammatory responses during different stages, is common in cases of sepsis [19]. Recently, studies have reported that platelets play an important role in both the immunomodulatory and inflammatory process [20 21], by inducing the release of inflammatory cytokines [22] and interacting with different kinds of bacteria and immune cells, including neutrophils, T-lymphocytes, NK-cells and macrophages, which contribute to the initiation or exacerbation of the inflammatory process [23]. Low lymphocyte counts, which to a certain degree represent a suppressed immune and inflammatory response [24 25], have also been reported to be associated with inflammatory diseases, such as cardiovascular disease [26] and type II diabetes [27].

Based on these findings, the PLR was suggested as being a novel systematic inflammatory indicator [28], and its use was initially reported in the prognostic prediction of neoplastic disorders, such as hepatocellular carcinoma and breast cancer. Accumulating evidence suggests that elevated PLRs are strongly associated with increased systemic inflammation, which may contribute to the progression and prognoses of many disorders, such as atherosclerosis [29] and diabetes mellitus [30].

In contrast to our findings, Zheng et al. [10] reported that both high and low PLRs are associated with increased mortality, among critically ill patients with AKI, after adjustment for the disease severity score in the Cox proportional hazards models. In

that study, unlike in ours, a significant association was also observed in patients with vasopressin use. Several factors may contribute to this inconsistency between the findings, such as the use of different cohorts, PLR knots, and definitions of vasopressor use. It is worth noting that, as the association between PLRs and outcomes varies greatly between different cohorts, the inter-heterogeneity within critically ill patients may also lead to a biased conclusion.

Akbas et al. indicated that a high PLR was positively associated with increased epicardial adipose tissue deposition in diabetes patients [31]; this may be caused by higher inflammation rates. Wang et al. [32] reviewed 134 patients with lung adenosquamous cancer, and reported that high PLRs (> 150) were independently associated with shorter disease-free days and lower overall survival rates. Another study [33], including 270 patients with hepatocellular carcinoma, found that elevated PLRs (above 220) were predictors of poor prognoses, while low PLRs (< 248.0) were associated with a lower tumor, node and metastasis stage, and low surgery incidence, in 695 patients with lung cancer [34]. Despite the fact that the study cohorts used in those studies were quite different from those used in ours, the reported PLR knots were quite similar to ours. However, the small sample sizes in those studies limited the statistical power for further stratification and subgroup analysis of low PLR. In the current study, we noticed that high PLRs (> 250) were associated with increased hospital mortality. As higher platelet levels, to a certain extent, are prognostic of inflammation of a higher severity and low lymphocyte counts may represent a suppressed immune and inflammatory response [24 25], an increase in the PLR may

reflect the degree of the inflammatory and immune response to the infection, which is related to a poor prognosis.

We also detected a non-significant association between low PLRs and mortality, in the case of sepsis. The association between low PLRs and outcomes was also reported in several studies. In a retrospective study [35] including 899 cases of laryngeal cancer, patients were divided into three PLR categories (low (≤ 119.55), moderate (> 119.55 and ≤ 193.55), and high (> 193.55)), and only patients with high PLRs experienced poor outcomes, including malnutrition and more advanced cancer stage; the association between outcomes and PLR levels were non-significant for those with low PLRs. Despite the cohort of that study being different from ours, the conclusion was consistent with that of our study. In the case of sepsis, a low platelet count is potentially associated with poor outcomes. In a large study including 931 patients with sepsis, Claushuis et al. reported that patients with a low platelet count at ICU admission had a higher disease severity score and increased mortality risk [36]. Furthermore, thrombocytopenia--one of the most common hemostatic disorders in the case of sepsis--which is related with platelet consumption, was also associated with higher mortality [37]. However, in the present study, a significant association between low PLR and mortality was not detected. Further studies are needed to validate this conclusion.

Furthermore, according to the subgroup analysis, the association between high PLR and mortality became non-significant in the subgroups with vasopressor use, AKI, or a SOFA score > 10; this association remained significant in the other subgroups. This

finding further supported our speculation that there may be an interaction between PLR and disease severity. To the best of our knowledge, ours is the first study to report this interaction. However, the underlying mechanism of this interaction remains largely unknown. A critical characteristic of sepsis is fluid resuscitation, and, in the current study, patients with vasopressor use, AKI, or a SOFA score > 10, to a certain degree, represented patients with inflammation of a higher severity, and they may have a stronger need for fluid resuscitation. We also noticed that the fluid balance within 48 hours after ICU admission was significantly larger in these subgroups. It needs to be further investigated if fluid resuscitation affects the prognostic value of the PLR.

One of the strengths of our study is the large sample size, which enabled us to adjust for confounding factors and perform subgroup analyses. However, there are also several limitations to our study. First, the MIMIC III database comprises data on patients from 2001; since then, the guidelines for sepsis have changed significantly. The most recent definition of Sepsis 3.0 was used in the current study, and this may have introduced selection bias despite the fact that most of the basic interventions (use of fluids, vasopressors, and antimicrobial agents) remained the same. Furthermore, as a decrease in the platelet count was a part of the SOFA score, using the definition of Sepsis 3.0, to a certain degree, may lead to a relatively low mean platelet count and potential multi-collinearity. This bias cannot be fully avoided. However, the potential multi-collinearity was verified in all the logistic models. Second, the platelet count can be affected by many cofounders, such as kinds of

malignancies, immunological factors and kinds of drugs. However, due to the nature of retrospective study, these situations cannot be identified in this database. In addition, in the logistic model using PLR as a continuous variable (Table 2), the OR was relatively small, despite the wide PLR range. Caution is therefore needed when interpreting these findings. Third, septic shock is a special subgroup of sepsis. However, patients with septic shock could not be distinguished in this study. Thus, patients were divided into subgroups, according to the existence of vasopressor use, AKI, or a SOFA score >10, which, to a certain extent, indicates the presence of an inflammatory response of a higher severity. Fourth, one of the main hypotheses of our study was the interaction effect between disease severity and PLR; yet, this interaction term was not introduced in the logistic model due to the U-shaped association between PLR and mortality. Further prospective studies are needed to verify our hypothesis. Finally, as high PLRs are associated with poor outcomes in various disorders while low PLRs are not, it is not clear if interventions aimed at changing the PLR value may improve outcomes.

Conclusion

In patients with sepsis, a high PLR was significantly associated with poor survival, while the association was non-significant for those with a low PLR. However, the former association became non-significant in patients with more severe conditions, including those with vasopressor use, AKI, or a SOFA score > 10. Future studies are needed to verify our hypothesis.

Financial support and sponsorship: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Ethical approval: All the data presented in this study were extracted from an online database named "MIMIC III," which was approved by the review boards of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center. Thus, requirement for individual patient consent was waived because the study did not impact clinical care and all protected health information was de-identified.

Acknowledgments: None.

Conflicts of interest: None.

Authors' contributions:

Yanfei Shen.: Responsible for data extraction and writing of the manuscript.

Xinmei Huang.: Responsible for data analysis.

Weimin Zhang.: Responsible for data validation.

Data sharing statement: The full data set is available from the corresponding author at snow.shen@hotmail.com. However, reanalysis of the full data need to be approved by MIMIC III Institute.

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Figure legend:

Figure 1: The crude and adjusted odds ratios in the subgroup analysis. PLR level 2 was used as the reference level in all the logistic models.

Abbreviations: PLR, platelet-to-lymphocyte ratio; AKI, acute kidney injury; SOFA, Sequential Organ Failure Assessment; CI, confidence interval

Table 1 Comparison of baseline characteristics within three PLR levels

Variable	PLR ≤ 150 (n = 1780)	150< PLR ≤ 250 (n = 1380)	PLR > 250 (n = 2377)	p
Age (years)	63.0 ± 16.6	65.0 ± 16.6	66.1 ± 15.5	< 0.001
Gender (male) [n (%)]	805 (45.2)	590 (42.7)	1096 (46.1)	0.133
BMI (Kg/m^2)	30.8 ± 8.9	34.1 ±13.5	35.2 ± 39.5	0.001
Ethnicity				
White [n (%)]	1202 (67.5)	987 (71.5)	1761 (74.0)	0.754
Black [n (%)]	180 (10.1)	101 (7.3)	146 (6.1)	< 0.001
Asian [n (%)]	39 (2.2)	30 (2.1)	71 (2.9)	0.169
Emergency [n (%)]	1641 (92.1)	1284 (93.0)	2229 (93.7)	0.138
ICU type				
MICU [n (%)]	953 (53.5)	727 (52.6)	1362 (57.2)	0.008
CCU/CSRU [n (%)]	413 (23.2)	323 (23.4)	453 (19.0)	0.001
TSICU/SICU [n (%)]	414 (23.2)	330 (23.9)	562 (23.6)	0.908
Vasopressors				
Norepinephrine [n (%)]	566 (31.7)	374 (27.1)	711 (29.9)	0.016
Dopamine [n (%)]	198 (11.1)	151 (10.9)	256 (10.7)	0.013
Epinephrine [n (%)]	67 (3.7)	28 (2.0)	37 (1.5)	< 0.001
Vasopressin [n (%)]	156 (8.7)	88 (6.3)	172 (7.2)	0.033
Overall vasopressor use	701 (39.3)	482 (34.9)	858 (36.1)	0.022
Fluid input/output				
Fluid intake (ml/kg/48hr)	99.9 ± 60.9	90.7 ± 57.6	97.2 ± 61.2	< 0.001
Urine output (ml/kg/48hr)	42.0 ± 32.0	42.9 ± 30.3	41.9 ± 29.5	0.5659
Fluid balance (ml/kg/48hr)	46.7 ± 59.4	38.3 ± 55.1	46.0 ± 60.4	< 0.001
Infection site				
Respiratory infection	1048 (58.8)	929 (67.3)	1580 (66.4)	< 0.001
Blood infection	768 (43.1)	509 (36.8)	998 (41.9)	0.001
Urinary infection	549 (30.8)	409 (29.6)	682 (28.6)	0.323
Abdominal infection	245 (13.7)	159 (11.5)	334 (14.0)	0.072
Cerebral infection	153 (8.5)	106 (7.6)	169 (7.1)	0.206
Disease severity scores				
SOFA on ICU admission median (IQR)	6 (4–9)	5 (4–8)	5 (3–7)	< 0.001
Maximum SOFA during ICU stay median (IQR)	10 (7–14)	9 (7 – 12)	9 (7 – 12)	< 0.001
Laboratory outcomes				
Maximum serum creatinine (mg/L)	2.5 ± 2.7	2.2 ± 2.1	2.1 ± 1.9	< 0.001
Minimum hemoglobin level (g/dl)	8.3 ± 1.7	8.69 ± 1.7	8.4 ± 1.6	< 0.001
Maximum serum sodium (mmol/L)	145.1 ± 5.4	145.0 ± 5.2	144.6 ± 5.1	0.009
Maximum serum lactate (mmol/L)	4.1 ± 3.8 (n=1536)	3.4 ± 3.1 (n=1174)	3.1 ± 3.0 (n=2112)	< 0.001
Platelet count (10^9/L)	146.7 ± 88.0	225.1 ± 107.2	197.5 ± 163.4	< 0.001

Lymphocyte count (10^9/L)	2.1 ± 5.7	1.1 ± 0.5	0.68 ± 0.4	< 0.001
PLR	91.8 ± 37.1	195.8 ± 28.6	557.5 ± 484.8	< 0.001
Clinical outcomes				
ICU LOS	9.9 ± 10.1	9.3 ± 8.7	10.1 ± 9.9	0.071
Hospital LOS	17.7 ± 15.1	16.6 ± 13.5	17.2 ± 13.7	0.082
AKI [n (%)]	861 (48.3)	601 (43.5)	1080 (45.4)	0.022
Hospital mortality [n (%)]	475 (26.6)	291 (21.0)	621 (26.1)	< 0.001

Abbreviations:

PLR: platelet to lymphocyte ratio; BMI body mass index; MICU, multiple intensive care unit; CCU, coronary care unit; CSRU, cardiac surgery care unit; TSICU, traumatic surgical intensive care unit; SICU, surgical intensive care unit; SOFA, sequential organ failure assessment; SAPS II, Simplified Acute Physiology Score II; IQR, interquartile range; LOS length of stay; AKI, acute kidney injury.

Table 2 multivariable logistic regressions of PLR using linear spline function

Variables	Crude Odds ratio	95% CI	p	Adjusted Odds ratio	95% CI	p
PLR (≤ 200)	0.997	0.996 – 0.998	< 0.001	0.9993	0.9980 – 1.0006	0.319
PLR (> 200)	1.0002	1.0001 – 1.0004	0.001	1.0002	1.0000 – 1.0003	0.025
Age (> 65)	1.77	1.56 – 2.11	< 0.001	2.32	1.99 – 2.64	< 0.001
Maximum SOFA	1.20	1.18 – 1.22	< 0.001	1.18	1.16 – 1.20	< 0.001
Urinary infection	0.66	0.57 – 0.76	< 0.001	0.65	0.56 – 0.76	< 0.001
Respiratory infection	1.29	1.13 – 1.47	< 0.001	1.25	1.09 – 1.45	0.002
Blood infection	2.14	1.89 – 2.42	< 0.001	1.49	1.29 – 1.71	< 0.001
Fluid balance (ml/kg/48hrs)	1.006	1.005 – 1.007	< 0.001	1.002	1.0008 – 1.0031	0.001
MICU	1.34	1.15 – 1.56	< 0.001	1.15	0.97 – 1.37	0.089
CCU/CSRU	1.22	1.01 – 1.47	0.032	1.03	0.84 – 1.26	0.752

Note: The mean variance inflation factor was 2.89 and p value of goodness of fit was 0.632.

Abbreviation: PLR platelet to lymphocyte ratio; CI confidence interval; SOFA sequential organ failure assessment; MICU multiple intensive care unit; CCU coronary care unit; CSRU cardiac surgery care unit.

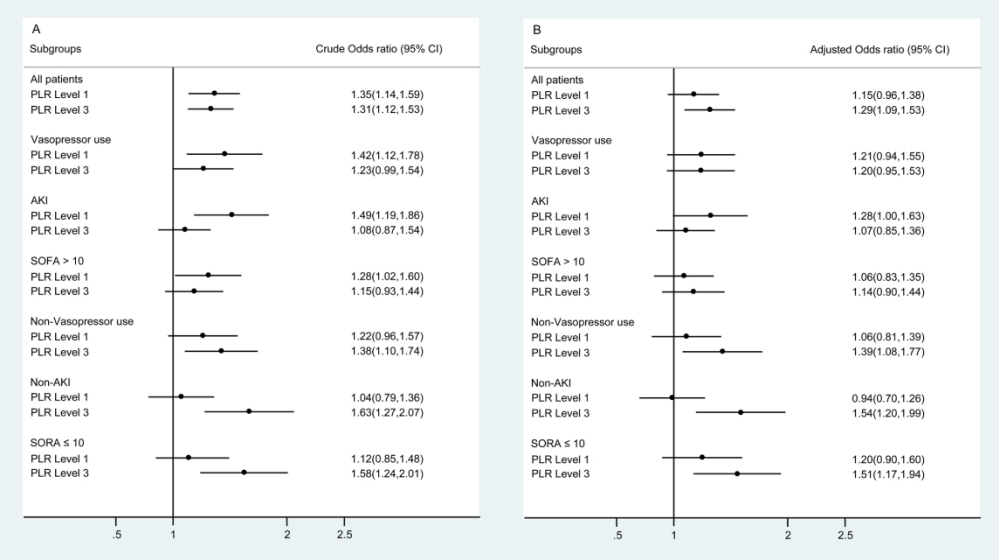
Table 3 Association between three PLR levels and hospital mortality

	PLR ≤ 150		150 < PLR ≤ 250		PLR > 250	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Model 1	1.41 (1.19 – 1.67)	< 0.001	Ref.	–	1.28 (1.09 – 1.51)	0.002
Model 2	1.34 (1.13 – 1.59)	0.001	Ref.	–	1.23 (1.05 – 1.45)	0.009
Model 3	1.35 (1.14 – 1.61)	0.001	Ref.	–	1.21 (1.03 – 1.43)	0.018
Model 4	1.15 (0.96 – 1.38)	0.123	Ref.	–	1.29 (1.09 – 1.53)	0.003

Adjusted covariates: Model 1 = age, admitted ICU type. Model 2 = Model 1+ (fluid balance at 48 hours after ICU admission). Model 3= Model 2 + (infection sites). Model 4 = Model 3+ (Maximum SOFA score during ICU stay).

The mean variance inflation factor was 2.53 and p value of goodness of fit was 0.665 for Model 4.

Abbreviations: PLR platelet to lymphocyte ratio; OR = odds ratio; CI= confidence interval; Ref reference category.



The crude and adjusted odds ratios in the subgroup analysis. PLR level 2 was used as the reference level in all the logistic models.
Abbreviations: PLR, platelet-to-lymphocyte ratio; AKI, acute kidney injury; SOFA, Sequential Organ Failure Assessment; CI, confidence interval

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Table S1 Adjusted odds ratio of hospital mortality using PLR as design variable in multivariable logistic regression

Variables	Odds ratio	95% CI	p
PLR Level 1 (≤ 150)	1.15	0.96 – 1.38	0.123
PLR Level 2 (151 ~ 250)	Ref.	–	–
PLR Level 3 (> 250)	1.29	1.09 – 1.53	0.003
Age (> 65)	2.27	1.97 – 2.62	< 0.001
Maximum SOFA	1.19	1.16 – 1.21	< 0.001
Urinary infection	0.65	0.56 – 0.76	< 0.001
Respiratory infection	1.25	1.08 – 1.44	0.002
Blood infection	1.49	1.29 – 1.71	< 0.001
Fluid balance (ml/kg/48hrs)	1.002	1.0008 – 1.0031	0.001
MICU	1.16	0.98 – 1.37	0.082
CCU/CSRU	1.04	0.84 – 1.27	0.700

Note: The mean variance inflation factor (VIF) was 2.53 and p value of goodness of fit was 0.665.

Abbreviation: PLR platelet to lymphocyte ratio; CI confidence interval; SOFA sequential organ failure assessment; MICU multiple intensive care unit; CCU coronary care unit; CSRU cardiac surgery care unit.

Table S2 Comparisons of subgroups according to the existence of vasopressor use, AKI and SOFA score

Variables	Vasopressor-use (n = 2554)	Non-Vasopressor-use (n = 2983)	p	AKI (n = 2542)	Non-AKI (n = 2995)	p	SOFA > 10 (n = 2390)	SOFA <= 10 (n = 2147)	p
age	66.3 ± 15.2	63.6 ± 16.8	< 0.001	65.1 ± 15.4	64.6 ± 16.8	0.211	64.0 ± 15.7	65.5 ± 16.4	< 0.001
Vasopressor-use [n (%)]	-	-	-	1321	1233	< 0.001	1554	1000	< 0.001
Fluid intake (ml/kg/48hr)	114.4 ± 661.	81.1 ± 50.0	< 0.001	94.3 ± 62.0	98.3 ± 58.8	0.013	110.7 ± 65.5	85.6 ±53.6	< 0.001
Fluid balance (ml/kg/48hr)	63.2 ± 64.1	28.1 ± 48.5	< 0.001	48.4 ± 60.9	40.8 ± 56.9	< 0.001	62.0 ± 64.1	30.8 ± 50.6	< 0.001
Maximum SOFA median (IQR)	12 (9 – 14)	8 (6 – 11)	< 0.001	11 (8 – 14)	9 (7 – 11)	< 0.001	13 (12 – 15)	7 (6 – 9)	< 0.001
Platelet count (10^9/L)	225.0 ± 240.1	236.0 ± 148.7	0.005	219.2 ± 143.4	240.9 ± 145.5	< 0.001	208.4 ± 147.1	248.1 ± 140.8	< 0.001
Lymphocyte count (10^9/L)	1.26 ± 3.32	1.28 ± 3.32	0.890	1.21 ± 2.21	1.32 ± 4.03	0.246	1.24 ± 3.42	1.29 ± 3.24	0.529
Hospital LOS	17.8 ± 14.3	16.7 ± 14.1	0.002	19.7 ± 15.6	15.1 ± 12.3	< 0.001	19.6 ± 14.6	15.5 ± 13.4	< 0.001
AKI [n (%)]	1321	1221	< 0.001	-	-	-	1360	1182	< 0.001
Hospital mortality [n (%)]	777	612	< 0.001	875	514	< 0.001	884	505	< 0.001

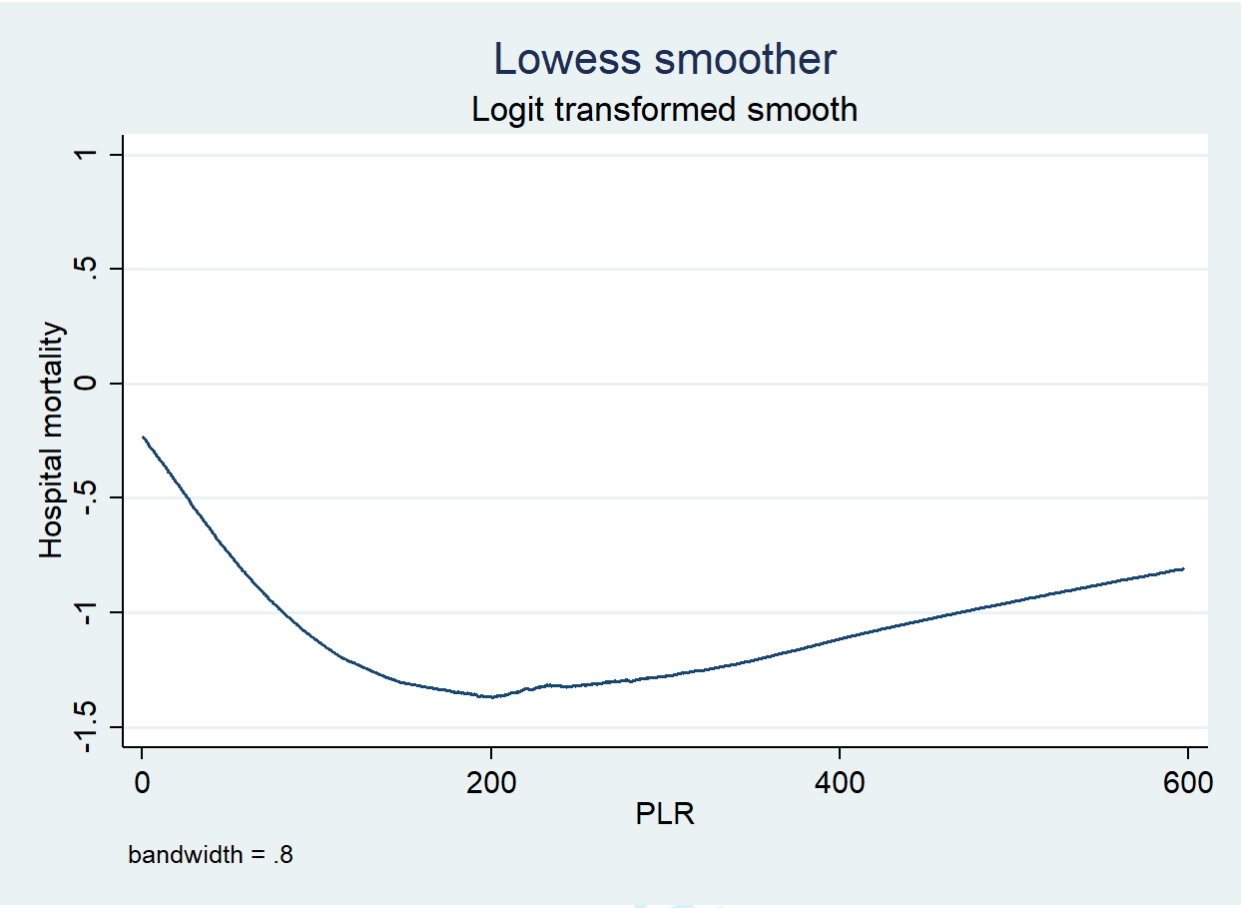
Abbreviation: SOFA sequential organ failure assessment; IQR interquartile range; LOS length of stay; AKI acute kidney injury.

Table S3 Risk factors associated with in-hospital mortality

Variables	Odds ratio	95% CI	p
Age (> 65)	1.77	1.56 – 2.01	< 0.001
Gender (male)	0.91	0.81 – 1.03	0.173
BMI (Kg/m^2)	1.00	0.99 – 1.00	0.485
TSICU/SICU	Ref.	–	–
MICU	1.34	1.15 – 1.56	< 0.001
CCU/CSRU	1.22	1.01 – 1.47	0.032
Fluid balance (ml/kg/48hrs)	1.006	1.005 – 1.007	< 0.001
Urine output (ml/kg/48hrs)	0.98	0.98 – 0.98	< 0.001
Respiratory infection	1.29	1.13 – 1.47	< 0.001
Blood infection	2.14	1.89 – 2.42	< 0.001
Urinary infection	0.66	0.57 – 0.76	< 0.001
Abdominal infection	0.93	0.77 – 1.11	0.458
Cerebral infection	0.66	0.51 – 0.85	0.002
SOFA on ICU admission	1.15	1.13 – 1.17	< 0.001
Maximum SOFA	1.20	1.18 – 1.22	< 0.001
Maximum serum creatinine (mg/L)	1.15	1.12 – 1.19	< 0.001
Minimum hemoglobin level (g/dl)	0.84	0.81 – 0.88	< 0.001
Maximum serum sodium (mmol/L)	1.02	1.01 – 1.02	< 0.001
Platelet count (10^9/L)	0.998	0.997 – 0.998	< 0.001
Lymphocyte count (10^9/L)	0.972	0.934 – 1.01	0.163

Abbreviations: BMI body mass index; MICU, multiple intensive care unit; CCU, coronary care unit; CSRU, cardiac surgery care unit; TSICU, traumatic surgical intensive care unit; SICU, surgical intensive care unit; SOFA, sequential organ failure assessment;

Figure S1 Crude relationship between hospital mortality and PLR



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
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*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
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	*4*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
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	7-8
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A.
		(*e*) Describe any sensitivity analyses	N/A.
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	8
		(b) Give reasons for non-participation at each stage	N/A.
		(c) Consider use of a flow diagram	N/A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) Summarise follow-up time (eg, average and total amount)	N/A.
Outcome data	15*	Report numbers of outcome events or summary measures over time	9
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	7
		(*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	N/A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A.
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	16

*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.