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Non-linear relationship between serum iron levels and 28-day mortality in sepsis patients: a retrospective study

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Recent studies have shown a significant association between iron and the development and prognosis of sepsis, but the relationship between iron levels and mortality in sepsis patients remains unclear, with previous studies examining this relationship under a linear assumption. This retrospective observational study aimed to assess the possible non-linear relationship between serum iron (SI) levels and 28-day all-cause mortality (28-DACM) in individuals with sepsis. We used multiple imputation for data with less than 30% missing values and developed Cox models to calculate the hazard ratio (HR) with a 95% confidence interval (CI) for the main outcome (28-DACM). To accurately assess the relationship between SI levels and the HR for 28-DACM, we utilized a restricted cubic spline (RCS) regression model with five knots. We also conducted subgroup analyses to evaluate the robustness of the primary results. The study found that SI levels upon ICU admission are an independent predictor of 28-DACM in sepsis patients, showing a J-shaped correlation with mortality. Patients with extremely high and low SI levels had higher mortality compared to those within the normal range. However, this study has limitations inherent to its retrospective observational design, such as potential bias and unmeasured confounding factors. Future studies should address these limitations through prospective designs.

Keywords Serum iron, Sepsis, Mortality, MIMIC-IV, Risk factor

Sepsis is a serious condition characterized by organ dysfunction due to an uncontrolled immune response to infection[1]. The global incidence of sepsis is estimated to be between 30 and 48.9 million cases in 2017, resulting in the death of 11 million people[2]. In China, the standardized mortality rate associated with sepsis is recorded at 66.7 per 100,000 population in 2018[3,4]. Recent statistics from 2022 indicate that the pooled mortality rate associated with sepsis in China was recorded at 29% in 2022, which is higher than the rates observed in European and American countries[5].

Iron metabolism disorders play a crucial role in the occurrence and development of sepsis[6]. Iron is a vital micronutrient that plays a critical role in important biological processes as it facilitates electron transfer, oxygen transport, and various biochemical reactions through its ability to switch between ferrous and ferric oxidation states[7–9]. In sepsis, pathogens and their products, including endotoxins, induce macrophages to produce IL-6, stimulating hepcidin production. Hepcidin downregulates iron transport proteins, inhibiting intestinal iron absorption and promoting macrophage iron storage[10]. This reduces plasma iron levels, representing a protective mechanism as pathogens require iron for growth[11]. However, an imbalance in the hepcidin-ferroprotein axis leads to cellular iron overload, accumulating significant iron ions that trigger oxygen free radical and hydrogen peroxide production via the Fenton reaction, ultimately causing oxidative stress cell damage[12]. On the other hand, changes in iron-binding capacities, such as increased total iron-binding capacity (TIBC) or decreased unsaturated iron-binding capacity (UIBC), can also affect iron distribution and utilization. This can disrupt the delicate balance between providing sufficient iron for the host's essential functions while restricting it from pathogens.

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Previous studies investigating the relationship between serum iron (SI) levels and mortality in sepsis patients have reported conflicting results. Some studies have indicated that elevated SI is associated with higher mortality rates in individuals with sepsis[13,14]. However, a large multicenter study uncovered that low SI levels were associated with bloodstream infections and higher mortality rates[15]. This study aims to examine the potential non-linear relationship between SI levels and mortality in sepsis patients. Specifically, we utilize the Medical Information Mart for Intensive Care IV (MIMIC-IV) database of the United States to investigate the relationship between SI levels and 28-day all-cause mortality (28-DACM) in sepsis patients.

Material and methods

Data source

This retrospective observational study relied on data from the Medical Information Mart for Intensive Care-IV (MIMIC-IV) dataset[16,17]. MIMIC-IV is an openly accessible critical care medicine dataset. The most recent version (2.2) was released on January 6, 2023. This database contains over 50,000 de-identified patient records from individuals admitted to the Intensive Care Unit (ICU) at Beth Israel Deaconess Medical Center in Boston, Massachusetts between 2008 and 2019. The Institutional Review Board of Beth Israel Deaconess Medical Center approved the waiver of informed consent and the sharing of research resources. The authors obtained permission to access the database, as indicated by Certificate Number 56161429. This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[18].

Study population

We obtained a cohort of 9,645 adult patients (aged 18 years or older) from the MIMIC IV database who were diagnosed with sepsis and admitted to the ICU. Patients who were missing SI data on the first day of hospitalization were excluded from the analysis. The diagnosis of sepsis was based on the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), which required either suspected or documented infection and a minimum two-point increase in the Sequential Organ Failure Assessment (SOFA) score. Because pre-hospital organ-specific data are unavailable in MIMIC-IV, we assumed a baseline SOFA of 0 for all patients and defined organ dysfunction as a first-day ICU SOFA ≥ 2. For patients with multiple admissions, only their first ICU admission was considered. The final cohort was divided into high and low SI groups based on the optimal SI cut-off value (100 μg/dL)[19,20]. The flow chart depicting the patient screening process is presented in Fig. 1.

Data extraction

To extract the data, structured query language (SQL) script codes were obtained from the GitHub website (https://github.com/MIT-LCP/mimic-iv) [21] Various characteristics of the patients, including their age, sex, body mass index, and Charlson comorbidity index, were collected for analysis. Using the International Classification of Diseases coding systems 9 and 10, we extracted information on comorbidities such as hypertension,

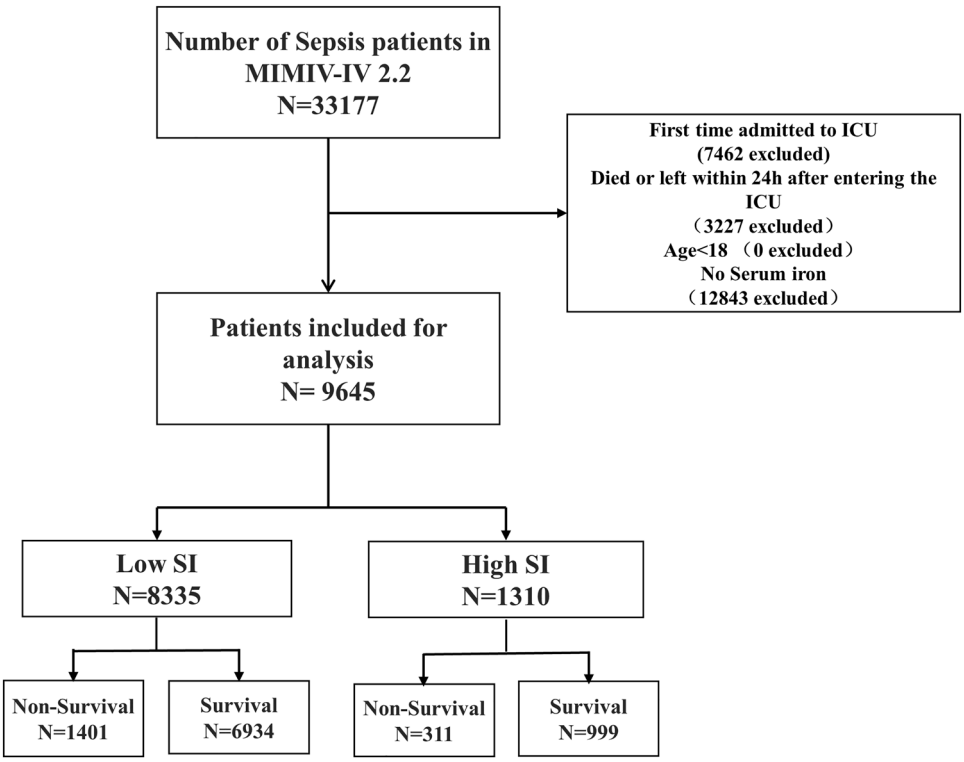

Fig. 1. The flowchart of extracting patients in this study.

cerebrovascular disease, myocardial infarction, congestive heart failure, chronic lung disease, liver disease, diabetes mellitus, renal disease, cancer, and AIDS.

To operationalize the Sepsis-3 criteria, we defined the onset of sepsis as the first co-occurrence of suspected infection (antibiotic administration and culture sampling) and acute organ dysfunction (SOFA score ≥ 2) within a 24-h window of ICU admission.,Baseline data were collected, including the severity of illness (Sequential Organ Failure Assessment [SOFA] score, Oxford Acute Severity of Illness Score [OASIS], Acute Physiological Score III [APSIII]), vital signs (systolic blood pressure (mmHg), diastolic blood pressure (mmHg), mean arterial pressure (mmHg), heart rate (beats min^{-1}), respiratory rate (min^{-1}), pulse oximetry (%), and body temperature (℃)), and laboratory results (SI level(ug/dl), hemoglobin level(g/dl), platelet count(K/ul), white blood cell count(K/ul), prothrombin time(S), partial thromboplastin time(S), international normalized ratio, blood urea nitrogen level(mg/dl), creatinine level(mg/dl), electrolytes, and blood gas analysis.

Data on different types of microorganisms and therapies, including mechanical ventilation and vasopressors, were also collected. If a variable was recorded more than once during the first 24 h, the mean value was used.

Primary outcome and secondary outcomes

The primary outcome of this study was 28-DACM. Secondary outcomes included in-hospital mortality, 90-day all-cause mortality, length of stay in days in the intensive care unit (ICU), and total hospital length of stay. For the assessment of outcomes, including in-hospital and 28-day mortality, we utilized the MIMIC-IV database, which captures both in-hospital death records and death dates from state records for up to one-year post-discharge. This comprehensive data collection allowed us to accurately determine the mortality status of patients for a continuous 28-day period following their hospitalization, regardless of whether the death occurred within the hospital or after discharge.

Statistical analysis

The optimal cut-off value for the SI in a receiver operating characteristic (ROC) curve was determined as the value with the highest sum of sensitivity and specificity. This approach was selected to pinpoint the SI level that best balances sensitivity and specificity in predicting 28-DACM, offering a potentially more insightful threshold compared to quartiles or the median. Given that deleting data with missing values would result in the loss of a significant amount of useful information, we adopted a multiple imputation approach for data with less than 30% missing values (Supplementary Figure S1). This allows us to make full use of the existing data information to enhance the accuracy of our predictions. For variables with missing values less than 30%, the MICE package for multiple imputations was used[22]. Categorical variables were expressed as frequencies or percentages and analyzed using the chi-squared test or Fisher's exact test. The Shapiro–Wilk test was performed on continuous variables to determine the normality assumption. Continuous variables were described by their mean ± standard deviation or median and interquartile range, depending on the normality or skewness of the variables. For the comparison of continuous variables, normally distributed data were analyzed using the independent samples t-test, while non-normally distributed data were assessed with the Mann–Whitney U test. Cox models were developed to calculate the hazard ratio (HR) with a 95% confidence interval (CI) for the main outcome (i.e. (28-DACM)). Model I comprised age + race + sex + BMI, + Charlson Comorbidity Index. Model II: Model I + mean heart rate + MAP + mean respiratory rate + + mean temperature + + mean SpO2 + mean platelets + white blood cells + lactate + pH + creatinine + sodium + potassium, and INR. Model III Model II + SOFA score + OASIS score + APSIII. Model IV, based on Model II, was further adjusted for the use of vasoactive medications, mechanical ventilation, and isolated microorganisms. The cumulative incidence of 28-DACM was determined using Kaplan–Meier estimates, and differences were assessed using the log-rank test. To accurately assess the relationship between SI levels and the hazard ratio (HR) for 28-DACM, we utilized a restricted cubic spline (RCS) regression model, employing five knots as per Stone's recommendation[23] suitable for our sample size exceeding 500. The application of five knots is strategic for attaining a nuanced model fit that ensures the curve's smoothness, thereby preventing overfitting and maintaining the precision of our estimates. This approach is particularly beneficial for capturing the subtleties of potential non-linear trends within our extensive dataset[24]. We conducted Pearson correlation analysis to assess the linear relationships between SI levels and other continuous variables included in Model IV. This step is a common practice in regression analysis to ensure that the assumptions of the linear model are not violated, particularly multicollinearity, which can inflate the variance of the regression coefficients and affect the stability of the model. This preliminary analysis ensures that our model is robust and the results are reliable. Statistical analyses were performed using R software version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria), and a 2-tailed P-value < 0.05 was considered statistically significant.

Sensitivity analysis

Subgroup analyses were conducted to evaluate the robustness of the primary results according to prespecified patient characteristics that could impact outcomes. To explore potential interaction effects, we examined the interactions between SI and key subgroup-defining variables by introducing product terms into our models. The subgroups included: age (< 60 vs ≥ 60 years), sex (female vs male), Charlson Comorbidity Index (< 6 vs 6 or higher)[25], use of mechanical ventilation (yes vs no), and use of vasopressors (yes vs no). Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated from Cox proportional hazard models for each subgroup. Tests for interaction were performed to determine whether the association between SI levels and 28-DACM differed significantly across the subgroups.

Results

Baseline characteristic

As shown in Table 1, baseline characteristics are grouped by SI level. The SI cut-off value of 100 μg/dL was determined based on the ROC curve. Among the 1310 patients with SI ≥ 100 μg/dL, the mean age is 61 years (IQR 51–70), 60.8% are male, and 23.7% died within 28 days. Patients in the high SI group were younger, had higher severity of illness based on SOFA and APSIII scores on admission, a higher prevalence of liver disease and cancer, greater vasoactive drug use, higher lactate, INR, PT and PTT levels, lower white blood cell and platelet counts, and fewer positive microbial culture results compared to the low group.

Primary outcome

The 28-DACM rate was 23.7% (311/1310) in the high SI group and 16.8% (1401/8335) in the low SI group. The Kaplan–Meier curve in Fig. 2 illustrates 28-DACM according to SI in the study cohort. The primary Kaplan–Meier curve illustrates the 28-DACM rates, where the red line indicates the high serum iron group and the blue line indicates the low serum iron group. To enhance the visualization of survival probabilities, particularly in the early critical phase post-admission, an inset graph is presented. This inset graph zooms in on the Y-axis to provide a detailed view of the survival rates during the initial period. The early separation of the curves is noteworthy, as it may reflect the rapid changes in survival probabilities associated with serum iron levels. The log-rank test was used to statistically compare the survival distributions between the two groups, with a significant difference indicated (HR 1.484, 95% CI 1.290–1.709, log-rank $P < 0.001$).

The estimates were derived from Cox proportional hazards regression models. In the analysis of using SI levels as a continuous variable, higher SI levels were significantly associated with increased risk of all-cause mortality in all models:(Model I: HR 1.002, 95% CI 1.001–1.003, $p < 0.001$, Model II: HR 1.003,95% CI 1.002–1.004, $p < 0.001$, Model III: HR 1.003, 95% CI 1.002–1.003, $p < 0.001$, Model IV: HR 1.002, 95% CI 1.001–1.003, $p < 0.001$). Patients were stratified into a low iron group (SI < 100 μg/dl) and a high iron group (SI ≥ 100 μg/dl). The high iron group had a significantly higher risk of mortality compared to the low iron group in all models: (Model I: HR 1.487, 95% CI 1.315–1.681, $p < 0.001$, Model II: HR 1.736, 95% CI 1.533–1.967, $p < 0.001$, Model III: HR 1.572, 95% CI 1.382–1.788, $p < 0.001$, Model IV: HR 1.513, 95% CI 1.332–1.717, $p < 0.001$) (Table 2). Based on the heatmap, the results suggest that there are no strong correlations between SI levels and the other clinical variables. Most of the correlations between SI levels and the other variables appear light in color, suggesting relatively weak correlations. The correlation between SI and PLT has the largest magnitude at -0.236, while the correlation with SOFA is 0.139. However, even these represent relatively low levels of linear association based on the color scale presented (Fig. 3). There was a non-linear relationship between SI and 28-DACM based on the RCS regression model (P for non-linearity = 0.0285), with significantly decreased HR (< 0.001) observed when SI levels ranged from 31.6ug/dl to 45.3ug/dl. Both SI level deficiency and excess were associated with an increased risk of 28-DACM (Fig. 4).

Sensitivity analysis

We conducted sensitivity analyses by subgroups to examine the robustness of the association between SI levels and mortality. The results of the subgroup analyses for 28-DACM in the study cohort are shown in Fig. 4. SI values > 100ug/dl were associated with higher mortality in subgroups of male [HR (95% CI) 1.44(1.22–1.69)], age < 60 years [HR (95% CI) 1.86(1.47–2.36)], age ≥ 60 years [HR (95% CI) 1.23(1.04–1.45)], Charlson comorbidity index < 6 [HR (95% CI) 1.53(1.22–1.92)], Charlson comorbidity index ≥ 6 [HR (95% CI) 1.29(1.1–1.51)], vasopressor use [HR (95% CI) 1.57(1.1.25–1.98)], no vasopressor use [HR (95% CI) 1.22(1.04–1.43)] and mechanical ventilation [HR (95% CI) 1.55(1.27–1.89)] (all $P < 0.05$). Interestingly, the predictive value of SI seems to be more prominent in patients who are younger than 60 years old (P for interaction < 0.001), male patients (P for interaction = 0.038), and patients using vasodilator drugs (P for interaction = 0.047) (Fig. 5).

Secondary outcomes

In the high SI group, the in-hospital and 90-day all-cause mortality rates were 22.5% (295/1310) and 33.5% (439/1310), respectively. In the low SI group, the mortality rates were 14.8% (1233/8335) and 26.4% (2200/8335). Both univariate and multivariate analyses showed an association between high SI and higher in-hospital mortality rates ($P < 0.001$). The median length of hospital stay was 10 days (IQR 6–19) in the high SI group and 11 days (IQR 6–19) in the low SI group. However, the median ICU stay duration was 3 days (IQR 1.84–5.54) in the high SI group and 3.11 days (IQR 1.86–6.47) in the low SI group. SI levels were found to be associated with ICU stay duration ($P < 0.001$), but not with overall hospital stay duration (Table 3).

Discussion

To the best of our knowledge, this study is the largest examination to date of the relationship between SI levels and 28-DACM in sepsis patients. Our findings suggest that SI levels are an independent strong predictor of increased 28-DACM in sepsis patients, after adjusting for underlying diseases, vital signs, and laboratory tests. Using the restricted cubic spline regression model, we observed a non-linear association between SI levels and 28-DACM. The Kaplan–Meier curve showed a significant association between high SI levels (SI ≥ 100 μg/dL) and an increased risk of 28-DACM. Subgroup analysis revealed lower mortality rates in female patients and in patients who did not require mechanical ventilation. Additionally, we identified interactions between high SI levels and gender, age, and the use of vasopressors on 28-DACM in sepsis patients. These findings may help guide the development of more personalized treatment approaches for sepsis patients.

Previous research has extensively explored the relationship between SI levels and mortality in sepsis patients, showing a mixed finding in the shape of the association between SI levels and mortality. For example, a study of

Variables	Total(N = 9645)	SI < 100ug/dl (N = 8335)	SI ≥ 100ug/dl(N = 1310)	P
Mean age (IQR)	67.00(56.00,78.00)	68.00(57.00, 79.00)	61.00(51.00, 70.00)	< 0.001
Gender, n (%)				< 0.001
Male	5321 (55.2%)	4525 (54.3%)	796 (60.8%)	
Female	4324 (44.8%)	3810 (45.7%)	514 (39.2%)	
Race, n (%)				0.002
White	6538 (67.8%)	5662 (67.9%)	876 (66.9%)	
Black	1186 (12.3%)	1050 (12.6%)	136 (10.4%)	
Asian	291 (3.0%)	234 (2.8%)	57 (4.4%)	
Hispanic/Latino	411 (4.3%)	344 (4.1%)	67 (5.1%)	
Others	1219 (12.6%)	1045 (12.5%)	174 (13.3%)	
BMI, Median (IQR)	27.11(23.18,31.89)	27.06(23.17, 31.88)	27.45(23.39, 32.06)	0.078
Comorbidities, n (%)				
Myocardial infarct				< 0.001
No	8287 (85.9%)	7102 (85.2%)	1185 (90.5%)	
Yes	1358 (14.1%)	1233 (14.8%)	125 (9.5%)	
Congestive heart failure				< 0.001
No	6680 (69.3%)	5621 (67.4%)	1059 (80.8%)	
Yes	2965 (30.7%)	2714 (32.6%)	251 (19.2%)	
Cerebrovascular disease				0.008
No	8595 (89.1%)	7400 (88.8%)	1195 (91.2%)	
Yes	1050 (10.9%)	935 (11.2%)	115 (8.8%)	
Chronic pulmonary disease				< 0.001
No	7121 (73.8%)	6077 (72.9%)	1044 (79.7%)	
Yes	2524 (26.2%)	2258 (27.1%)	266 (20.3%)	
Liver disease				< 0.001
No	7685 (79.7%)	6936 (83.2%)	749 (57.2%)	
Yes	1960 (20.3%)	1399 (16.8%)	561 (42.8%)	
Diabetes mellitus				< 0.001
No	6378 (66.1%)	5410 (64.9%)	968 (73.9%)	
Yes	3267 (33.9%)	2925 (35.1%)	342 (26.1%)	
Renal disease				< 0.001
No	6944 (72.0%)	5898 (70.8%)	1046 (79.8%)	
Yes	2701 (28.0%)	2437 (29.2%)	264 (20.2%)	
Cancer				< 0.001
No	8049 (83.5%)	7027 (84.3%)	1022 (78.0%)	
Yes	1596 (16.5%)	1308 (15.7%)	288 (22.0%)	
AIDS				0.770
No	9542 (98.9%)	8247 (98.9%)	1295 (98.9%)	
Yes	103 (1.1%)	88 (1.1%)	15 (1.1%)	
Hypertension				0.017
No	4168 (43.2%)	3562 (42.7%)	606 (46.3%)	
Yes	5477 (56.8%)	4773 (57.3%)	704 (53.7%)	
Scoring systems (IQR)				
Charlson comorbidity index	6.00(4.00,8.00)	6.00(4.00, 8.00)	5.00(4.00, 7.00)	< 0.001
OASIS	35.00(28.00,41.00)	35.00(28.00, 41.00)	34.00(28.00, 41.00)	0.153
APSIII	55.00(42.00,74.00)	55.00(42.00, 73.00)	58.50(43.00, 81.00)	< 0.001
SOFA	3.00(2.00,5.00)	3.00(2.00, 5.00)	4.00(2.00, 6.00)	< 0.001
First-day vital signs (IQR)				
Heart rate mean (beats min^{-1})	87.26(75.96,99.71)	87.16(75.88, 99.66)	87.86(76.32, 99.93)	0.223
SBP (mmHg)	112.27(103.58,124.28)	112.21(103.69, 124.38)	112.75(103.00, 123.75)	0.401
DBP (mmHg)	60.26(54.04,67.46)	60.08(53.89, 67.23)	61.52(54.97, 68.99)	< 0.001
MAP (mmHg)	74.67(68.88,81.70)	74.56(68.80, 81.52)	75.55(69.36, 82.90)	0.007
Respiratory rate (min^{-1})	19.43(16.97,22.50)	19.53(17.04, 22.52)	18.83(16.61, 22.30)	< 0.001
Temperature (℃)	36.84(36.58,37.19)	36.85(36.59, 37.20)	36.81(36.54, 37.10)	< 0.001
Spo2 (%)	97.09(95.79,98.56)	97.12(95.80, 98.57)	97.00(95.72, 98.52)	0.301
First-day laboratory tests (IQR)				
Continued				

Variables	Total(N = 9645)	SI < 100ug/dl (N = 8335)	SI ≥ 100ug/dl(N = 1310)	P
SI (ug/dL)	44.00(26.00,73.00)	38.00(24.00, 58.00)	134.00(115.00, 171.00)	< 0.001
Glucose (mg/dL)	133.33(112.00,167.17)	133.67(112.00, 167.00)	131.78(110.50, 168.83)	0.325
Hemoglobin (g/dL)	9.70(8.50,11.15)	9.70(8.55, 11.15)	9.62(8.30, 11.20)	0.097
PLT (K/uL)	182.50(121.50,262.00)	192.00(132.00, 271.25)	125.00(75.00, 191.50)	< 0.001
WBC (K/uL)	11.45(7.90,15.75)	11.65(8.10, 15.85)	10.32(6.90, 14.80)	< 0.001
Creatinine (mg/dL)	1.20(0.80,2.05)	1.20(0.80, 2.05)	1.15(0.80, 2.04)	0.150
BUN (mg/dL)	25.50(16.00,42.00)	25.50(16.00, 42.00)	25.00(15.00, 43.00)	0.426
Chlorine (mmol/L)	103.50(99.00,107.50)	103.50(99.50, 107.50)	103.00(98.50, 107.50)	0.007
Sodium (mmol/L)	138.00(135.00,141.00)	138.00(135.00, 141.00)	137.50(134.00, 140.50)	< 0.001
Potassium (mmol/L)	4.20(3.80,4.65)	4.20(3.80, 4.65)	4.20(3.85, 4.70)	0.316
Ionic calcium mean (mmol/L)	1.10(1.05,1.16)	1.11(1.05, 1.16)	1.09(1.04, 1.16)	< 0.001
INR	1.35(1.20,1.70)	1.35(1.20, 1.65)	1.45(1.20, 2.00)	< 0.001
PT (S)	14.85(13.10,18.45)	14.70(13.00, 18.00)	16.00(13.60, 21.45)	< 0.001
PTT (S)	32.80(28.10,42.75)	32.50(28.00, 42.10)	34.88(29.20, 45.89)	< 0.001
Magnesium (mg/dL)	1.90(1.70,2.20)	1.90(1.70, 2.10)	1.90(1.70, 2.20)	0.021
Phosphate (mg/dL)	3.50(3.00,4.20)	3.60(3.00, 4.20)	3.50(2.90, 4.20)	0.111
Lactate mean (mmol/L)	1.80(1.25,2.70)	1.80(1.20, 2.65)	2.10(1.45, 3.30)	< 0.001
pH	7.38(7.33,7.43)	7.38(7.33, 7.43)	7.38(7.33, 7.43)	0.343
PO2 (mmHg)	121.00(82.00,195.50)	121.00(82.00, 196.00)	124.00(82.50, 193.50)	0.727
PCO2 (mmHg)	39.50(35.00,45.00)	40.00(35.00, 45.50)	38.50(33.50, 43.50)	< 0.001
Mechanical ventilation, n (%)				0.743
No	5790 (60.0%)	5009 (60.1%)	781 (59.6%)	
Yes	3855 (40.0%)	3326 (39.9%)	529 (40.4%)	
Vasopressor, n (%)				< 0.001
No	8395 (87.0%)	7309 (87.7%)	1086 (82.9%)	
Yes	1250 (13.0%)	1026 (12.3%)	224 (17.1%)	
Microorganism, n (%)				0.001
Negative	8142 (84.4%)	6995 (83.9%)	1147 (87.6%)	
Gram negative	663 (6.9%)	602 (7.2%)	61 (4.7%)	
Gram positive	678 (7.0%)	603 (7.2%)	75 (5.7%)	
Fungus	153 (1.6%)	128 (1.5%)	25 (1.9%)	
Others	9 (0.1%)	7 (0.1%)	2 (0.2%)	

Table 1. Baseline Characteristics of study patients grouped according to SI level. Continuous variables are presented as mean ± standard deviation for normally distributed data and median (interquartile range) for non-normally distributed data. Statistical comparisons between groups for continuous variables were performed using the independent samples t-test for normally distributed data and the Mann–Whitney U test for non-normally distributed data, as determined by the Shapiro–Wilk test. *SI* serum iron, *BMI* body mass index, *AIDS* acquired immunodeficiency syndrome, *OASIS* Oxford acute severity of illness, *APSIII* acute physiology score III, *SOFA* sequential organ failure assessment, *SBP* systolic blood pressure, *MAP* mean arterial pressure, *DBP* diastolic blood pressure, *PLT* platelet, *WBC* White blood cell, *PT* prothrombin time, *PTT* partial thromboplastin time, *INR* international normalized ratio, *SpO2* pulse blood oxygen saturation, *BUN* blood urea nitrogen, Los length of stay.

1,891 sepsis patients in the MIMIC-III database found a correlation between high SI levels upon ICU admission and 90-day mortality, with rising mortality as SI levels increased[13]. However, this study does not find a threshold for SI levels. Similarly, a prospective study of 61 sepsis patients found that higher SI and ferritin levels upon admission were associated with higher SOFA scores and mortality, suggesting a positive correlation with patient prognosis[14]. Conversely, a study conducted in Norway with 61,852 participants revealed that individuals with SI levels ≤ 6 μmol/L had a 72% higher risk of bloodstream infection and a 52% higher mortality rate[15]. Iron deficiency can negatively impact T cell function, and reduce the bactericidal capacity of macrophages, ultimately weakening the body's immune defenses. In an iron-deficient state, the suppression of immune function may be more significant relative to bacteria's ability to acquire iron, resulting in an elevated susceptibility to infections. While these three findings may initially appear contradictory, they align with our conclusions, indicating a J-shaped correlation between SI levels and 28-DACM among sepsis patients. Notably, both excessively high and low SI levels contribute to increased mortality rates. Iron plays a dual role in sepsis, acting as both a beneficial and harmful factor. On the one hand, reduced SI levels can inhibit microbial growth and improve sepsis prognosis[26–28]. On the other hand, excessive iron accumulation can lead to oxidative stress and tissue damage[29–31]. Therefore, maintaining a balanced SI level is crucial for sepsis patients to avoid both high and low

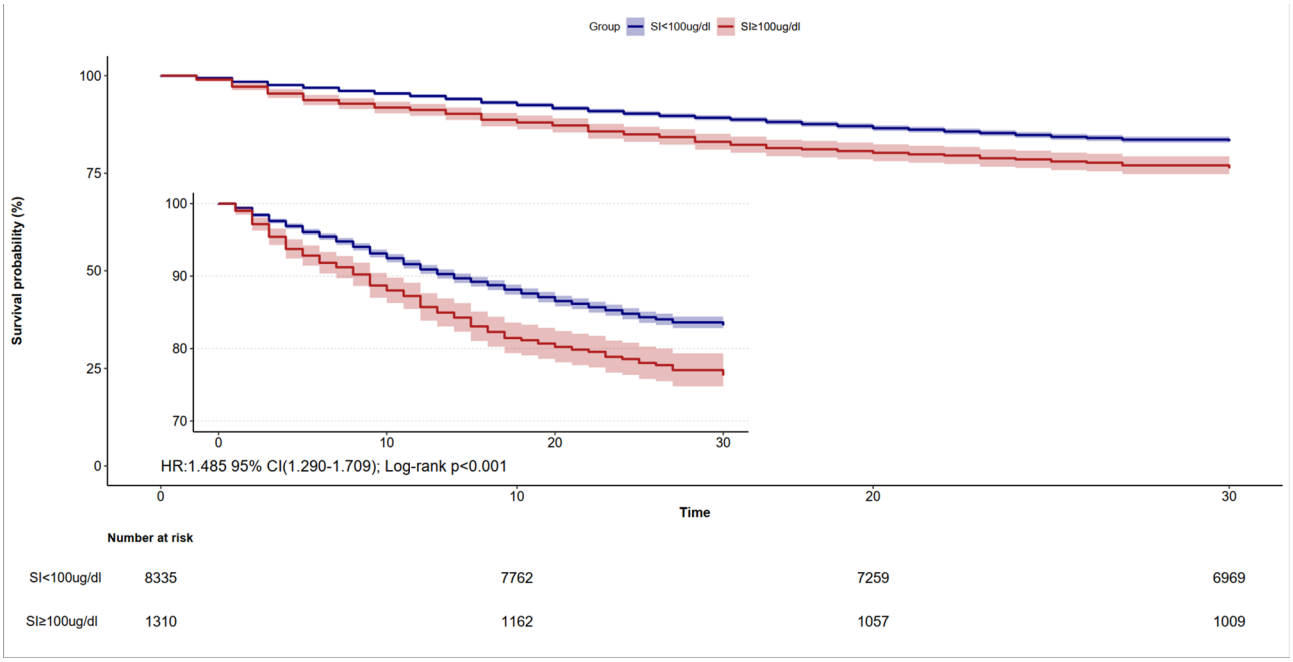

Fig. 2. Kaplan–Meier curve for 28-day all-cause mortality according to serum iron group (The red line indicates the high serum iron group and the blue line indicates the low serum iron group).

	Non-adjusted		Model I		Model II		Model III		Model IV	
Serum Iron	**HR(95% CI)**	**P-value**	**HR(95% CI)**	**P-value**	**HR(95% CI)**	**P-value**	**HR(95% CI)**	**P-value**	**HR(95% CI)**	**P-value**
Continuous	1.002(1.001,1.003)	< 0.001	1.003(1.002,1.004)	< 0.001	1.003(1.002,1.003)	< 0.001	1.002(1.001,1.003)	< 0.001	1.002(1.001,1.003)	< 0.001
Stratification										
Low Iron (SI < 100ug/dl)	1.0 (Ref)		1.0 (Ref)		1.0 (Ref)		1.0 (Ref)		1.0 (Ref)	
High Iron (SI ≥ 100ug/dl)	1.487(1.315,1.681)	< 0.001	1.736(1.533,1.967)	< 0.001	1.572(1.382,1.788)	< 0.001	1.513(1.332,1.717)	< 0.001	1.513(1.332,1.718)	< 0.001

Table 2. The association between 28-day mortality and SI. *HR* hazard ratio, *CI* confidence interval, *Ref* reference group. Model I: age + race + gender + BMI + Charlson comorbidity index. Model II: Model I + heart rate mean + MAP mean + respiratory rate mean + temperature mean + SpO2 mean + platelet mean + white blood cell + lactate + pH, creatinine + sodium + potassium + INR. Model III: Model II + SOFA + OASIS + APSIII. Model IV: Model III + vasopressor + mechanical ventilation + microorganism.

levels, as they can contribute to increased mortality rates. Our restricted cubic spline regression findings further indicate that SI levels above 45.3 μg/dL and below 31.6 μg/dL may both be associated with increased mortality rates.

Consistent with our findings, previous research suggests that women may have a survival advantage under extreme conditions[32]. This is primarily attributed to women typically having lower iron stores than men[33]. This reduces their susceptibility to infections caused by iron-dependent microorganisms, such as sepsis pathogens. During adolescence, iron plays a vital role in growth and development, leading to relatively high SI levels[34]. In older individuals, the presence of low-grade chronic inflammation tends to increase with age[35]. This weakens the regulatory effects of hepcidin and reduces the efficiency of iron absorption, potentially resulting in lower SI levels in sepsis[36]. Additionally, as individuals age, the accumulation of iron in the brain can lead to a decrease in SI levels[37]. Consistent with our subgroup analysis findings, it has been observed that patients requiring vasoactive drugs generally have more severe conditions. This heightened severity may contribute to higher mortality rates[38].

A seemingly contradictory finding in our study is that, although the low-iron group has lower mortality, it shows longer median ICU and hospital stays. This paradox likely reflects survivorship (immortal-time) bias: the higher proportion of early deaths in the high-iron group truncates observation time and thus lowers the calculated average length of stay. Analyzing length of stay only among survivors or using hospital-free days as an endpoint would avoid favoring early deaths and could be considered in future work to address this bias. This study attempts to clarify the significant role of iron levels in sepsis and provide possible implications for clinical practice. The results indicate that a uniform approach to iron management in sepsis may not be suitable,

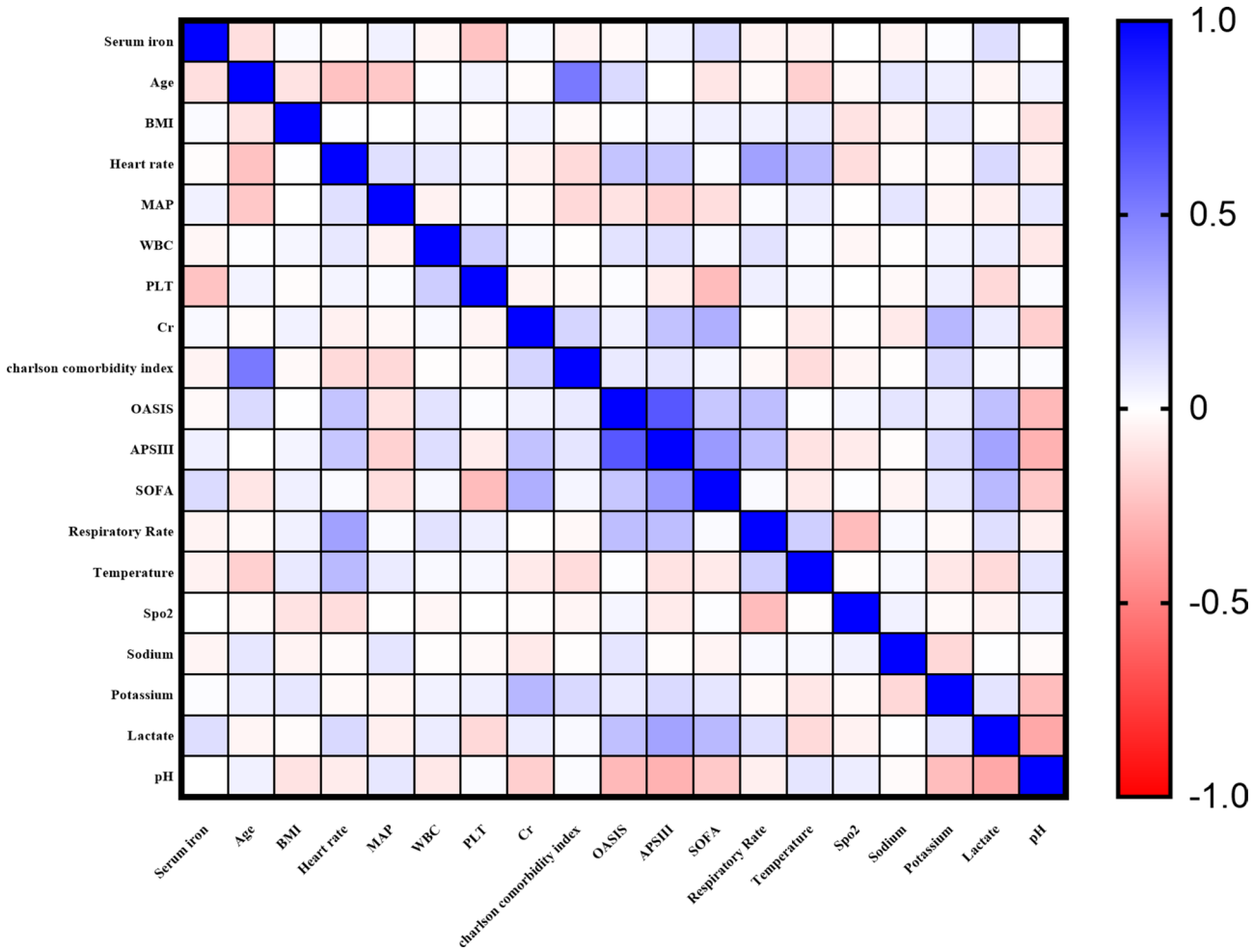

Fig. 3. The heatmap shows the correlation coefficients between SI and the continuous variables included in the final model. The correlations are represented by color, with blue indicating a positive correlation and red negative correlation. Paler hues denote weaker magnitude.

emphasizing the need for personalized strategies based on individual patient factors including serum iron levels, illness severity, and comorbidities.

This study has limitations inherent to its retrospective observational design. Firstly, we used the ROC curve to determine the serum iron cutoff, while aiming for clinical relevance, which may introduce bias and limit generalizability. Secondly, the multiple comparisons in Table 1, were performed without adjusting for multiple testing, which may increase the false positive rates. Thirdly, ferritin and transferrin were excluded from analyses because more than half of the cohort lacked these measurements, precluding reliable modeling of iron-profile–specific outcome trajectories; Lastly, as a retrospective observational study, there are inherent limitations such as the potential for residual bias and unmeasured confounding factors. The inclusion of only sepsis patients with available iron measurement results may have introduced selection bias. Future prospective studies should track post-baseline interventions—particularly RBC transfusion practices and subsequent infectious complications—to determine whether they mediate or modify the relationship between baseline iron status and patient outcomes.

Conclusion

This retrospective study found that SI levels upon ICU admission are an independent predictor of 28-DACM in sepsis patients, showing a J-shaped correlation with mortality. Patients with extremely high and low SI levels had higher mortality compared to those within the normal range.

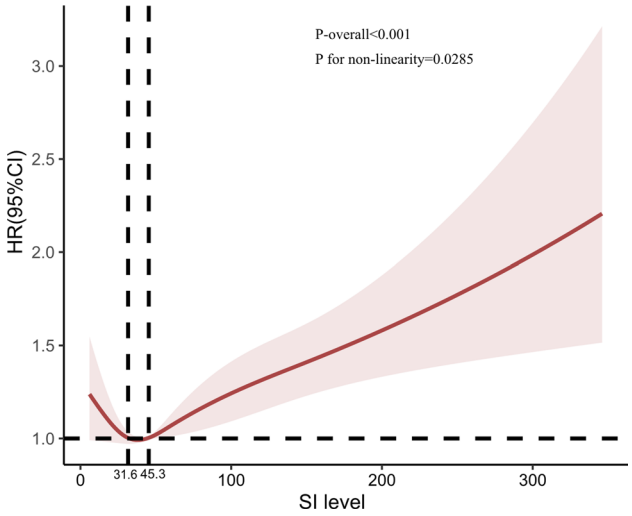

Fig. 4. Relationship between SI level and 28-day all-cause mortality. Adjusted for age; race; gender; BMI; Charlson comorbidity index; heart rate mean; MAP mean; respiratory rate mean; temperature mean; SpO2 mean; platelet mean; white blood cell; lactate; pH, creatinine; sodium; potassium; INR; SOFA; OASIS; APSIII; vasopressor; mechanical ventilation; microorganism. Restricted cubic spline regression analysis of SI level with 28-day all-cause mortality. Heavy central lines represent the estimated adjusted hazard ratios, with shaded ribbons denoting 95% confidence intervals. SI 31.6 and 45.3 were selected as the reference level represented by the vertical dotted lines. The horizontal dotted lines represent the hazard ratio of 1.0.

Subgroup	SI<100ug/dl	SI≥100ug/dl	HR (95% CI)	P-value	P for interaction
	Death/Total	Death/Total			
Age					<0.001
<60yr	233/2472(9.4%)	139/598(23.2%)	1.86 (1.47~2.36)	<0.001	
≥60yr	1168/5863(19.9%)	172/712(24.2%)	1.23 (1.04~1.45)	0.013	
Gender					0.038
Male	750/4525(16.6%)	197/796(24.7%)	1.44 (1.22~1.69)	<0.001	
Female	651/3810(17.1%)	114/514(22.2%)	1.21 (0.98~1.49)	0.08	
Charlson comorbidity index					0.053
<6	383/3658(10.5%)	114/659(17.3%)	1.53 (1.22~1.92)	<0.001	
≥6	1018/4677(21.8%)	197/651(30.3%)	1.29 (1.1~1.51)	0.002	
Vasopressor					0.047
No	1055/7309(14.4%)	193/1086(17.8%)	1.22 (1.04~1.43)	0.016	
Yes	346/1026(33.7%)	118/224(52.7%)	1.57 (1.25~1.98)	<0.001	
Mechanical ventilation					0.129
No	842/5009(16.8%)	171/781(21.9%)	1.16 (0.98~1.38)	0.085	
Yes	559/3326(16.8%)	140/529(26.5%)	1.55 (1.27~1.89)	<0.001	

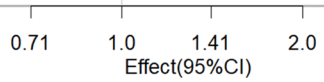

Fig. 5. Forest plots of hazard ratios for the 28-day all-cause in different subgroups. The multivariable Cox models were developed to calculate the Hazard ratio (HR) proportional hazards model was adjusted for race; BMI; heart rate mean; MAP mean; respiratory rate mean; temperature mean; SpO2 mean; platelet mean; white blood cell; lactate; pH, creatinine; sodium; potassium; INR; SOFA; OASIS; APSIII; microorganism.*HR* hazard ratio, *CI* confidence interval, *SI* Serum iron.

Outcomes	SI < 100ug/dl N = 8335	SI ≥ 100ug/dl N = 1310	Univariable analysis		Multivariate analysis	
			OR /Coefficient (95% CI)	p-value	OR /Coefficient (95% CI)	p-value
Primary outcome						
28-day all-cause mortality, n(%)	1401 (16.8%)	311 (23.7%)	1.54(1.34,1.772)	< 0.001	1.59 (1.353,1.884)	< 0.001
Secondary outcome						
In-hospital mortality, n(%)	1233 (14.8%)	295 (22.5%)	1.67(1.451,1.932)	< 0.001	1.53(1.287,1.808)	< 0.001
90-day all-cause mortality, n(%)	2200 (26.4%)	439 (33.5%)	1.36(1.23,1.5)	< 0.001	1.40(1.26,1.56)	< 0.001
Length of ICU stay (days), median (IQR)	3.11(1.86, 6.47)	3.00(1.84, 5.54)	-0.561(-0.959, -0.163)	0.006	-0.850(-1.228, -0.472)	< 0.001
Length of hospital stay (days), median (IQR)	11.00(6.00, 19.00)	10.00(6.00, 19.00)	0.295(-0.602,1.193)	0.519		

Table 3. The association between outcomes and serum iron. *CI* confidence interval, *OR* odds ratio, *IQR* interquartile range; *Adjusted for age; race; gender; BMI; Charlson comorbidity index; heart rate mean; MAP mean; respiratory rate mean; temperature mean; SpO2 mean; platelet mean; white blood cell; lactate; pH, creatinine; sodium; potassium; INR; SOFA; OASIS; APSIII; vasopressor; mechanical ventilation; microorganism. OR with 95% CI was calculated using a logistic regression model.

Data availability

The datasets presented in this study are available online. Here are the names and accession numbers of the repository/repositories: MIMIC-IV(https://mimic.physionet.org/).

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Author contributions

JJ: collecting data, writing, analysis, funding acquisition LY: revision, analysis. QZ: revision, funding acquisition. KC： analysis, editing. KC and CTTW: revision. ZD: organized, validated and cleaned the source data. YL: writing – review & editing. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

This study used publicly available datasets with institutional review board (IRB) approval from the original studies and was conducted after obtaining a CITI certificate. The review for ethics approval was exempted from the ethics approval statement by the Ethics Committee of Shenzhen Hospital of Southern Medical University.

Additional information

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