SHOCK, Vol. 60, No. 4, pp. 525–533, 2023

OPEN

U-SHAPED ASSOCIATION BETWEEN SERUM CALCIUM LEVELS AND 28-DAY MORTALITY IN PATIENTS WITH SEPSIS: A RETROSPECTIVE ANALYSIS OF THE MIMIC-III DATABASE

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Received 3 Jun 2023; first review completed 24 Jun 2023; accepted in final form 28 Jul 2023

ABSTRACT—**Background:** Serum calcium levels disorder have been reported to be associated with poor prognosis in different diseases. Studies on the association between serum calcium and outcomes of septic patients remained limited. The aim of this study is to investigate the association between serum calcium and 28-day mortality in septic patients. **Method:** Patients diagnosed with sepsis in the Medical Information Mart for Intensive Care III database were included. Patients were divided into five groups according to the quintiles of serum calcium levels, and their baseline characteristics were compared. Multivariate Cox regression models were used to assess the association between serum calcium and 28-day mortality. Smooth curve fitting and segmented regression models were used to visualize the association between serum calcium levels and 28-day mortality risk. The 28-day survival probability between five groups was analyzed using Kaplan-Meier curves. **Results:** A total of 3,016 patients with sepsis were enrolled, and the 28-day mortality rate was 35.64%. After adjusting for confounders, compared with the reference quintile (Q4: 9.00–9.50), the lowest serum calcium level quintile (Q1: 5.70–8.20) was independently associated with an increased risk of 28-day mortality (hazard ratio [HR], 2.12; 95% CI, 1.76–2.56). Smooth spline fitting revealed a U-shaped association between serum calcium and 28-day mortality. When serum calcium was <9.0 mg/dL, 28-day mortality risk increased by 58% per unit decrease in serum calcium (HR, 0.42; 95% CI, 0.37–0.48). When serum calcium was >9.0 mg/dL, the 28-day mortality risk increased by 12% per unit increase in serum calcium (HR, 1.12; 95% CI, 1.04–1.20). **Conclusion:** A U-shaped association was observed between serum calcium levels and 28-day mortality in septic patients. Lower or higher serum calcium levels were associated with increased risk of 28-day mortality in septic patients.

KEYWORDS—Serum calcium; sepsis; intensive care unit; mortality; MIMIC-III

INTRODUCTION

Sepsis, a complex disorder caused by a dysregulated host response to infection, is associated with acute organ dysfunction and a high risk of mortality (1). In critically ill patients, the in-hospital mortality rate attributable to sepsis has been documented to range between 30% and 45% (2–4). An overactive proinflammatory response is considered as a primary driver of sepsis-related mortality (5). Despite advancements in antibiotic treatment that have demonstrated partial efficacy in reducing 28-day mortality, the overall mortality rate of sepsis still remains relatively high (6). Thus, the identification of high-risk patients with poor prognosis could prompt clinicians to intervene timely and adequately.

Calcium is involved in various physiological regulatory mechanisms such as myocardial contraction and relaxation, renal function, platelet adhesion, and coagulation (7–10). Calcium dyshomeostasis could lead to serious cardiovascular complications. Wray *et al.* revealed that hypocalcemia could prolong the QT interval, thereby increasing likelihood of dysrhythmias. In addition, hypocalcemia could suppress cardiac contractility, thereby contributing to acute cardiovascular decompensation (11). In addition, Can *et al.* proposed that hypocalcemia may lead to hemorrhage event and prolong bleeding time by impairing platelet function and affecting the coagulation cascade (12). Certainly, calcium derangements could result from the diseases per se. Zaloga *et al.* suggested that hypocalcemia could be caused by impaired secretion or action of parathyroid hormone, impaired synthesis or action of vitamin D, and calcium chelation/precipitation in critically ill patients (13). Hendy *et al.* demonstrated that the proinflammatory cytokines IL-1β and IL-6 could upregulate the expression of calcium-sensing receptor in the parathyroid and kidney, resulting in decreased levels of parathyroid hormone and 1,25-dihydroxyvitamin D, thus leading to hypocalcemia (14). Calcium metabolic disorder has been reported to be associated with poor prognosis in different diseases such as acute myocardial infarction (15), acute pulmonary thromboembolism (16), chronic kidney disease (17), and COVID-19 (18).

Studies have indicated that the calcium derangements could activate calcium-sensing receptors located on T cell surfaces,

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This work was funded by the Youth Fund of Xiangya Hospital (grant/award number: 2021Q13), Natural Science Foundation of Hunan Province (grant/award number: 2022JJ40840, 2022JJ70076). The funding bodies did not have role in the design of the study, in data collection and analysis, nor in the interpretation and dissemination of the results.

The authors report no conflict of interests.

Consent for publication: Not applicable.

Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.shockjournal.com).

DOI: 10.1097/SHK.0000000000002203

thereby promoting the release of reactive oxygen species (ROS) and cytokine, ultimately leading to endothelial barrier damage, fluid leakage, myocardial cell apoptosis, and aggravating sepsis (19). Furthermore, inflammatory mediator-induced calcium influx could also lead to the disruption of adhesion connections and rearrangement of the cytoskeleton, thereby disrupting endothelial integrity, increasing permeability, and exacerbating the inflammatory response in sepsis (20,21). Researches on the association between serum calcium levels and mortality of sepsis were limited. Li *et al.* reported that serum calcium had a certain value for judging disease severity of elderly patients with sepsis (22). Liu *et al.* (23) demonstrated a significant association between lower calcium levels and organ dysfunction, as well as sepsis-related mortality in neonates. However, it remains insufficiently studied that whether higher or lower serum calcium is associated with short-term mortality in sepsis. Furthermore, the dose-response association has not been elucidated. In this study, we aimed to explore the association between serum calcium levels and risk of 28-day mortality in critically ill patients with sepsis.

MATERIALS AND METHODS

Database

Data in this study were extracted from Medical Information Mart for Intensive Care (MIMIC) III, which is a large, single-center, publicly available critical care database (24). It includes unidentified health-related data of 52,963 intensive care unit (ICU) stays at the Beth Israel Deaconess Medical Center (BIDMC) between June 2001 and October 2012. The variables recorded in this database included demographics, vital signs, laboratory tests, medications, nursing progress records, and other related clinical variables. The MIMIC-III was built by researchers at the Massachusetts Institute of Technology (MIT) Laboratory for Computational Physiology and collaborating research groups. To obtain access to the database, we completed the course "Protecting Human Research Participants" on the website of the National Institutes of Health and obtained certification (record ID: 40060500). The project was approved by the institutional review boards of MIT and BIDMC and was granted a waiver of informed consent.

Patient selection

Adult patients diagnosed as sepsis were eligible for inclusion. We analyzed the first ICU stay of patients who had multiple ICU admissions. The exclusion criteria were (1) younger than 18 years of age, (2) ICU stays <24 h, (3) Sequential Organ Failure Assessment (SOFA) <2 (25), and (4) no serum total calcium levels data.

Data extraction and management

Structured query language (SQL) and PostgreSQL tools (https://www.postgresql.org/, PostgreSQL Global Development Group, version 9.6) were used for data extraction. We extracted basic data for each patient from MIMIC-III, and baseline variables included age, sex, ethnicity, and body mass index (BMI). Vital signs included heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), respiratory rate, temperature, and oxygen saturation (Spo_2). Blood gas analysis indices included partial pressure of carbon dioxide (Pco_2), partial pressure of oxygen (PO_2), pH, potassium, sodium, and lactate. Laboratory parameters included creatinine (Cr), serum urea nitrogen (BUN), red blood cell (RBC) count, hemoglobin, white blood cell (WBC) count, platelet (PLT) count, activated partial thromboplastin time (APTT), international normalized ratio (INR), prothrombin time (PT), and albumin (Alb). Furthermore, the SOFA score (26), Logistic Organ Dysfunction System (LODS) score (27), Simplified Acute Physiology Score II (SAPSII) (28), and systemic inflammatory response syndrome (SIRS) score (29) were calculated using the SQL code provided by Johnson *et al.* (30). The intervention measures included ventilation, continuous renal replacement therapy (CRRT), and vasopressors. Vasopressor use was defined as the use of norepinephrine, epinephrine, dopamine, dobutamine, or vasopressin during ICU hospitalization. Comorbidities included congestive heart failure, neurological disease, chronic obstructive pulmonary disease, diabetes, renal failure, and metastatic cancer. Serum total calcium levels were measured within 24 h of ICU admission. The worst values were adopted when baseline data were tested multiple times within 24 h of ICU admission. The main endpoint was 28-day mortality.

Definition

Sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. The diagnostic criteria for sepsis included suspected or documented infection and an acute increase in the total SOFA score $\geq$2 points as a proxy for organ dysfunction (31). The calculation method of SOFA score was shown in the Supplemental Table 1, http://links.lww.com/SHK/B747.

Statistical analysis

Continuous variables, presented as mean $\pm$ SD or median with interquartile range, were tested using one-way ANOVA (normal distribution) and Kruskal-Wallis H (skewed distribution). Shapiro-Wilk tests were used to assess variable distributions. Categorical data, summarized as numbers (percentage), were compared using the χ^2 test and Fisher exact test. The missing values for Alb (33.5%), BMI (31.9%), Pco_2 (12.9%), pH (10.5%), lactate (8.3%), APTT (4.6%), INR (4.5%), PT (4.5%), heart rate (0.8%), SBP (0.9%), DBP (0.9%), MAP (0.9%), respiratory rate (0.9%), temperature (0.8%), Spo_2 (0.9%), potassium (0.03%), Cr (0.03%), BUN (0.03%), RBC count (0.1%), hemoglobin (0.1%), WBC count (0.2%), and PLT (0.1%) were imputed by means (normal distribution) or medians (abnormal distribution).

All patients were stratified by quintiles according to their serum total calcium levels, and their baseline characteristics were compared. Univariate analysis was used to evaluate the associations between the variables and 28-day mortality. We

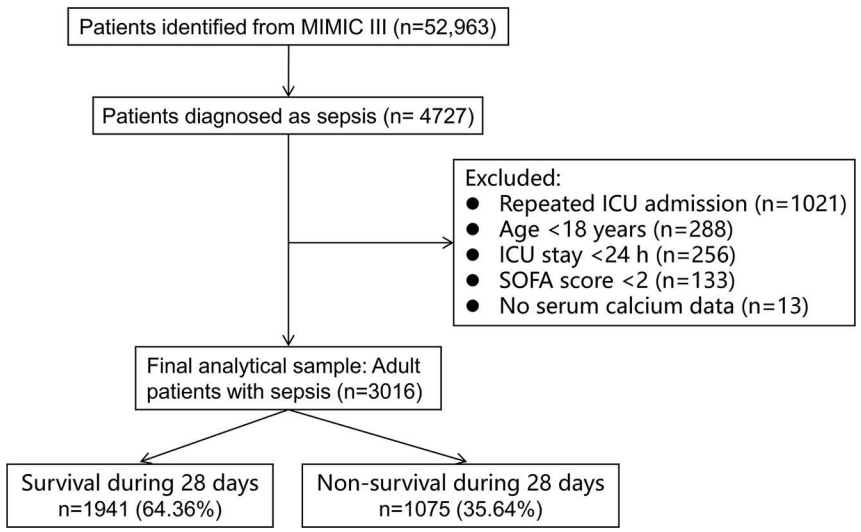

Fig. 1. **Flow chart of the study.**

Table 1. **Baseline and clinical characteristics of the study population according to serum total calcium**

Variable	Total (5.7–19.9) (*N* = 3,016)	Serum total calcium, mg/dL					*P*
		Q1 (≤8.20) (*n* = 587)	Q2 (8.30–8.60) (*n* = 590)	Q3 (8.70–8.90) (*n* = 493)	Q4 (9.00–9.50) (*n* = 697)	Q5 (≥9.60) (*n* = 649)	
Baseline variables							
Age, y	65.29 ± 15.19	68.34 ± 14.53	66.93 ± 14.98	67.07 ± 14.61	64.22 ± 15.56	60.85 ± 14.88	<0.001
Female	1,289 (42.74%)	243 (41.40%)	245 (41.53%)	215 (43.61%)	287 (41.18%)	299 (46.07%)	0.333
Ethnicity							<0.001
White	2,204 (73.08%)	446 (75.98%)	466 (78.98%)	374 (75.86%)	492 (70.59%)	426 (65.64%)	
Asian	89 (2.95%)	30 (5.11%)	18 (3.05%)	9 (1.83%)	19 (2.73%)	13 (2.00%)	
Black	297 (9.85%)	36 (6.13%)	41 (6.95%)	55 (11.16%)	70 (10.04%)	95 (14.64%)	
Hispanic or Latino	92 (3.05%)	17 (2.90%)	14 (2.37%)	7 (1.42%)	29 (4.16%)	25 (3.85%)	
Other	334 (11.07%)	58 (9.88%)	51 (8.64%)	48 (9.74%)	87 (12.48%)	90 (13.87%)	
BMI, kg/m^2	29.70 ± 9.74	28.13 ± 7.95	28.72 ± 7.70	30.31 ± 10.41	30.76 ± 10.40	29.83 ± 8.47	<0.001
Vital signs							
Heart rate, b/min	92.82 ± 17.45	95.33 ± 18.45	92.82 ± 17.17	91.19 ± 17.11	92.05 ± 16.81	92.60 ± 17.51	0.001
SBP, mm Hg	109.21 ± 13.56	106.27 ± 12.39	108.91 ± 13.50	109.36 ± 12.05	110.62 ± 14.15	110.48 ± 14.59	<0.001
DBP, mm Hg	57.38 ± 9.44	56.21 ± 9.42	56.83 ± 8.90	57.72 ± 8.67	58.54 ± 9.98	57.42 ± 9.77	<0.001
MAP, mm Hg	72.71 ± 9.41	70.85 ± 8.66	72.38 ± 9.16	72.88 ± 8.65	73.79 ± 9.87	73.37 ± 10.05	<0.001
Respiratory rate, b/min	21.43 ± 4.64	21.83 ± 4.86	21.56 ± 4.37	21.18 ± 4.40	21.44 ± 4.64	21.13 ± 4.83	0.066
Temperature, °C	36.88 ± 0.79	36.81 ± 0.77	36.92 ± 0.74	36.90 ± 0.82	36.91 ± 0.82	36.87 ± 0.80	0.024
SpO_2, %	96.76 ± 2.94	96.36 ± 3.93	96.98 ± 2.49	96.74 ± 2.45	96.87 ± 2.59	96.80 ± 2.93	0.005
Blood gas analysis							
PCO_2, mm Hg	53.90 ± 18.43	47.88 ± 15.72	50.64 ± 17.24	53.99 ± 16.81	55.79 ± 19.89	59.58 ± 19.02	<0.001
PO_2, mm Hg	50.00 (38.00–69.00)	52.00 (40.00–75.00)	53.00 (39.00–72.00)	53.50 (38.00–70.00)	50.00 (38.00–68.00)	45.00 (35.00–61.00)	<0.001
pH	7.24 ± 0.13	7.25 ± 0.13	7.26 ± 0.12	7.25 ± 0.12	7.25 ± 0.13	7.20 ± 0.13	<0.001
Potassium, mEq/L	4.72 ± 0.96	4.63 ± 0.94	4.61 ± 0.87	4.76 ± 0.93	4.74 ± 1.02	4.87 ± 1.01	<0.001
Sodium, mEq/L	135.94 ± 6.00	136.11 ± 6.21	136.20 ± 6.34	135.83 ± 5.54	135.88 ± 5.77	135.68 ± 6.06	0.548
Lactate, mmol/L	2.80 (1.70–4.62)	3.00 (1.80–5.00)	2.80 (1.80–4.70)	2.70 (1.70–4.40)	2.50 (1.65–4.30)	2.90 (1.70–5.00)	<0.001
Laboratory parameters							
Cr, mg/dL	1.70 (1.10–2.80)	1.60 (1.10–2.70)	1.60 (1.02–2.60)	1.60 (1.10–2.60)	1.70 (1.10–2.80)	2.00 (1.20–3.60)	<0.001
BUN, mg/dL	35.00 (22.00–55.00)	35.00 (22.00–54.00)	35.00 (21.00–55.75)	32.00 (21.00–50.00)	34.00 (22.00–54.00)	36.00 (24.00–61.00)	0.015
RBC, M/uL	2.79 ± 0.56	2.90 ± 0.55	2.88 ± 0.54	2.85 ± 0.53	2.78 ± 0.53	2.57 ± 0.55	<0.001
Hemoglobin, g/L	9.54 ± 1.89	9.36 ± 1.82	9.70 ± 1.86	9.63 ± 1.84	9.67 ± 1.90	9.33 ± 2.00	<0.001
WBC, K/uL	15.30 (9.83–22.10)	15.65 (9.20–23.30)	15.20 (10.45–22.25)	15.70 (10.90–22.30)	15.30 (10.10–21.55)	14.40 (8.60–21.40)	0.018
PLT, K/uL	163.00 (97.00–245.00)	155.50 (92.00–235.75)	173.50 (113.75–258.75)	168.00 (104.00–245.00)	175.00 (107.00–257.25)	142.00 (70.00–224.00)	<0.001
APTT, s	37.30 (30.90–52.30)	37.60 (31.10–51.00)	35.45 (29.70–46.40)	37.10 (31.20–51.00)	36.30 (30.70–52.40)	40.10 (31.70–60.20)	<0.001
INR	1.50 (1.30–2.10)	1.60 (1.30–2.10)	1.40 (1.30–1.80)	1.50 (1.20–2.00)	1.50 (1.30–2.00)	1.60 (1.30–2.30)	<0.001
PT, s	16.30 (14.20–20.45)	16.60 (14.50–20.70)	15.80 (14.00–19.10)	16.05 (13.97–19.70)	16.10 (14.20–20.20)	17.00 (14.30–22.90)	0.023
Alb, g/dL	2.83 ± 0.56	2.64 ± 0.56	2.80 ± 0.53	2.90 ± 0.520	2.92 ± 0.51	2.87 ± 0.60	<0.001
Score system							
SOFA	7.00 (5.00–10.00)	8.00 (5.00–10.00)	7.00 (5.00–9.00)	7.00 (5.00–9.00)	7.00 (5.00–10.00)	8.00 (5.00–11.00)	<0.001
LODS	6.00 (4.00–8.00)	7.00 (5.00–9.00)	6.00 (4.00–8.00)	6.00 (4.00–8.00)	6.00 (4.00–8.00)	7.00 (5.00–9.00)	<0.001
SAPSII	47.33 ± 15.34	50.38 ± 16.37	46.41 ± 15.36	46.22 ± 14.90	45.57 ± 14.69	48.13 ± 14.97	<0.001
SIRS	3.28 ± 0.81	3.29 ± 0.79	3.35 ± 0.79	3.29 ± 0.82	3.26 ± 0.80	3.22 ± 0.86	0.063
Interventions							
Ventilation	701 (56.40%)	289 (49.23%)	308 (52.20%)	284 (57.61%)	405 (58.11%)	415 (63.94%)	<0.001
CRRT	353 (11.70%)	23 (3.92%)	20 (3.39%)	27 (5.48%)	71 (10.19%)	212 (32.67%)	<0.001
Vasopressor	2,233 (74.04%)	439 (74.79%)	450 (76.27%)	352 (71.40%)	499 (71.59%)	493 (75.96%)	0.141
Comorbidity							

Continued next page

TABLE 1. *(Continued)*

Variable	Total (5.7–19.9) (N = 3,016)	Serum total calcium, mg/dL					P
		Q1 (≤8.20) (n = 587)	Q2 (8.30–8.60) (n = 590)	Q3 (8.70–8.90) (n = 493)	Q4 (9.00–9.50) (n = 697)	Q5 (≥9.60) (n = 649)	
Congestive heart failure	1,156 (38.33%)	188 (32.03%)	216 (36.61%)	192 (38.95%)	297 (42.61%)	263 (40.52%)	0.002
Neurological disease	462 (15.32%)	82 (13.97%)	94 (15.93%)	61 (12.37%)	111 (15.93%)	114 (17.57%)	0.135
COPD	718 (23.81%)	111 (18.91%)	132 (22.37%)	113 (22.92%)	210 (30.13%)	152 (23.42%)	<0.001
Diabetes	761 (25.23%)	158 (26.92%)	129 (21.86%)	126 (25.56%)	197 (28.26%)	151 (23.27%)	0.057
Renal failure	723 (23.97%)	106 (18.06%)	126 (21.36%)	115 (23.33%)	170 (24.39%)	206 (31.74%)	<0.001
Metastatic cancer	229 (7.59%)	86 (14.65%)	48 (8.14%)	30 (6.09%)	29 (4.16%)	36 (5.55%)	<0.001
28-Day mortality	1,075 (35.64%)	304 (51.79%)	177 (30.00%)	150 (30.43%)	207 (29.70%)	237 (36.52%)	<0.001

Results are expressed as mean ± SD, median (Q1–Q3) or n (%).
Alb, albumin; APTT, partial thromboplastin time; BMI, body mass index; BUN, serum urea nitrogen; COPD, chronic obstructive pulmonary disease; Cr, creatinine; CRRT, continuous renal replacement therapy; DBP, diastolic blood pressure; INR, international normalized ratio; LODS, Logistic Organ Dysfunction System; MAP, mean arterial pressure; P_{CO_2}, carbon dioxide; PLT, platelet; PO_2, partial pressure of oxygen; PT, prothrombin time; RBC, red blood cell count; SAPSII, Simplified Acute Physiology Score II; SBP, systolic blood pressure; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; Sp_{O_2}, oxygen saturation; WBC, white blood cell count.

evaluated the effect of serum total calcium levels on 28-day mortality in septic patients using multiple Cox regression models. In model I, no variables were adjusted. In model II, the covariates age, sex, and BMI were adjusted. We adjusted for age, sex, BMI, and potential confounders (ethnicity, SOFA, LODS, SAPSII, SIRS, heart rate, SBP, DBP, MAP, respiratory rate, temperature, Sp_{O_2}, ventilation, CRRT, vasopressor use, neurological disease, metastatic cancer, P_{CO_2}, PO_2, pH, potassium, sodium, lactate, Cr, BUN, RBC count, hemoglobin, PLT, APTT, INR, PT and Alb) on the basis of their associations with 28-day mortality ($P < 0.10$) or a change in effect estimate of more than 10% in model III (32). Smooth curve fitting was performed to determine the association between serum calcium levels and the risk of 28-day mortality in patients with sepsis. Furthermore, a segmented regression model and logarithmic likelihood ratio test (LRT) were used to analyze the threshold effect between serum calcium levels and 28-day mortality. The results of the multivariate analysis were presented as hazard ratio (HR) with 95% CI. The Kaplan-Meier method was used to plot survival curves, and log-rank testing was used to compare survival rates among five groups.

All statistical analyses were performed using R (http://www.R-project.org, The R Foundation) and EmpowerStats software version 3.0 (http://www.empowerstats.com/cn/, X&Y solutions, Inc, Boston, MA). $P < 0.05$ was considered statistically significant.

RESULTS

Selection of participants

Among the 52,963 patients identified from MIMIC-III, 4727 patients were diagnosed with sepsis. We excluded 1,021 patients with duplicate ICU admission, 288 patients younger than 18 years, 256 patients with ICU stays <24 h, 133 patients with SOFA scores <2, and 13 patients with missing serum calcium data. The remaining 3,016 patients with sepsis were enrolled in the final analysis. The overall incidence of 28-day death was 35.64% (1,075/3,016) (Fig. 1).

Demographics and baseline characteristics

The baseline characteristics of the patients according to serum calcium quintiles (Q1, ≤8.20 mg/dL; Q2, 8.30–8.60 mg/dL; Q3, 8.70–8.90 mg/dL; Q4, 9.00–9.50 mg/dL; Q5, ≥9.6 mg/dL) were shown in Table 1. The 28-day mortality significantly differed among the serum calcium quintiles. The 28-day mortality was highest in the Q1 (51.79%) group and lowest in the Q4 (29.70%) group.

Participants in the lowest calcium level group (Q1) were older and had a lower BMI than the other groups. In group Q1, SBP, DBP, MAP, temperature, Sp_{O_2}, P_{CO_2}, and Alb were the lowest, whereas heart rate, lactate, RBC count, and INR were the highest. Patients in the highest calcium level group (Q5) had higher values of potassium, Cr, BUN, APTT, INR, and PT and lower values of PO_2, pH, RBC, hemoglobin, WBC, and PLT than the other groups. Moreover, the SOFA and LODS scores in the Q5 and Q1 groups were significantly higher than those in the other groups. SAPSII scores were highest in the Q1 group. In addition, the proportions of patients with renal failure, ventilation, and CRRT were higher in the Q5 group. There were significant differences in the incidence of congestive heart failure, metastatic cancer, and chronic obstructive pulmonary disease among the groups. No statistically significant differences were found in sex, respiratory rate, sodium, SIRS, vasopressor use, neurological disease, and diabetes between the groups.

Univariate analysis of risk factors associated with 28-day mortality in patients with sepsis

Univariate Cox regression analysis showed that serum calcium (HR, 0.88; 95% CI, 0.82–0.94), BMI (HR, 0.99; 95% CI, 0.98–1.00), SBP (HR, 0.99; 95% CI, 0.98–0.99), DBP (HR,

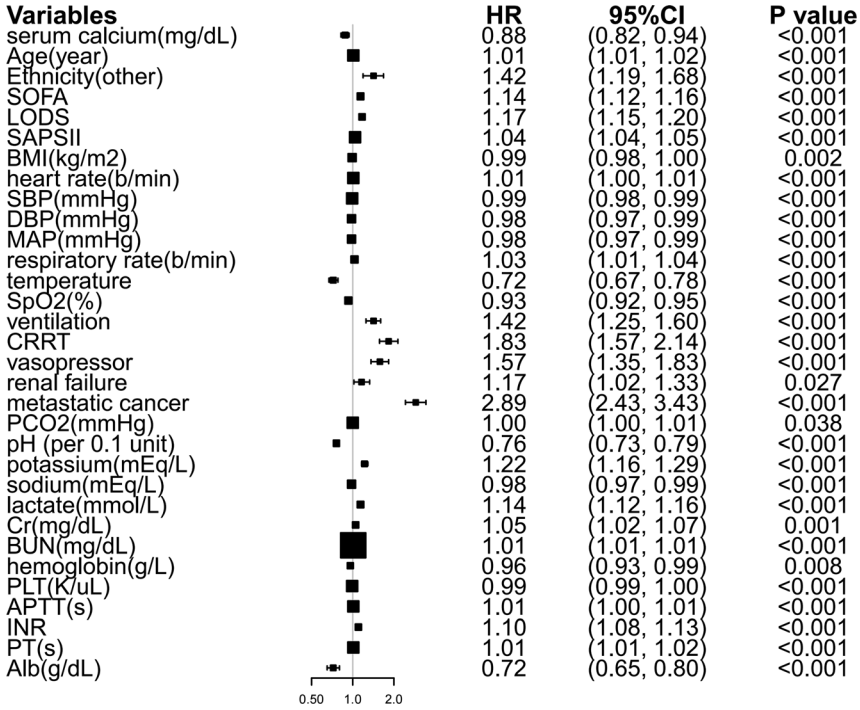

Fig. 2. **Univariate analysis.** HR, hazard ratio.

0.98; 95% CI, 0.97–0.99), MAP (HR, 0.98; 95% CI, 0.97–0.99), temperature (HR, 0.72; 95% CI, 0.67–0.78), Sp_{O_2} (HR, 0.93; 95% CI, 0.92–0.95), pH (per 0.1 unit) (HR, 0.76; 95% CI, 0.73–0.79), sodium (HR, 0.98; 95% CI, 0.97–0.99), hemoglobin (HR, 0.96; 95% CI, 0.93–0.99), PLT (HR, 0.99; 95% CI, 0.99–1.00), and Alb (HR, 0.72; 95% CI, 0.65–0.80) were negatively associated with the risk of 28-day death in patients with sepsis. In contrast, age (HR, 1.01; 95% CI, 1.01–1.02), ethnicity (HR, 1.42; 95% CI, 1.19–1.68), SOFA score (HR, 1.14; 95% CI, 1.12–1.16), LODS score (HR, 1.17; 95% CI, 1.15–1.20), SAPSII score (HR, 1.04; 95% CI, 1.04–1.05), heart rate (HR, 1.01; 95% CI, 1.00–1.01), respiratory rate (HR, 1.03; 95% CI, 1.01–1.04), ventilation (HR, 1.42; 95% CI, 1.25–1.60), CRRT (HR, 1.83; 95% CI, 1.57–2.14), vasopressor use (HR, 1.57; 95% CI, 1.35–1.83), renal failure (HR, 1.17; 95% CI, 1.02–1.33), metastatic cancer (HR, 2.89; 95% CI, 2.43–3.43), P_{CO_2} (HR, 1.00; 95% CI, 1.00–1.01), potassium (HR, 1.22; 95% CI, 1.16–1.29), lactate (HR, 1.14; 95% CI, 1.12–1.16), Cr (HR, 1.05; 95% CI, 1.02–1.07), BUN (HR, 1.01; 95% CI, 1.01–1.01), APTT (HR, 1.01; 95% CI, 1.00–1.01), INR (HR, 1.10; 95% CI, 1.08–1.13), and PT (HR, 1.01; 95% CI, 1.01–1.02) were positively correlated with the risk of 28-day death (Fig. 2).

Association between serum calcium and 28-day mortality in sepsis

The independent association between serum calcium levels and 28-day mortality in sepsis was shown in Table 2. When serum calcium was handled as a continuous variable, it was negatively correlated with 28-day mortality. In nonadjusted model I, the risk of 28-day mortality decreased by 12% per unit increase in serum calcium level (HR, 0.88; 95% CI, 0.82–0.94). In model

Table 2. **Association between serum total calcium and 28-day mortality in patients with sepsis**

Variables	Model I, HR (95% CI)	Model II, HR (95% CI)	Model III, HR (95% CI)
Serum total calcium	0.88 (0.82–0.94) *	0.91 (0.85–0.98) *	0.82 (0.76–0.88) *
Serum total calcium quintiles			
Q1 (≤8.20)	2.36 (1.97–2.81) *	2.22 (1.86–2.65) *	2.12 (1.76–2.56) *
Q2 (8.30–8.60)	1.04 (0.85–1.27)	0.99 (0.81–1.22)	1.04 (0.84–1.27)
Q3 (8.70–8.90)	1.04 (0.84–1.28)	1.01 (0.82–1.24)	0.98 (0.79–1.21)
Q4 (9.00–9.50)	Ref	Ref	Ref
Q5 (≥9.60)	1.28 (1.06–1.54) *	1.34 (1.11–1.61) *	1.02 (0.83–1.24)
P for trend	<0.001	<0.001	<0.001

* *P* value <0.05.

Model I: adjusted for none.

Model II: adjusted for age, sex, and BMI.

Model III: adjusted for age, sex, BMI, ethnicity, SOFA, LODS, SAPSII, SIRS, heart rate, SBP, DBP, MAP, respiratory rate, temperature, Sp_{O_2}, ventilation, CRRT, vasopressor, neurological disease, metastatic cancer, P_{CO_2}, P_{O_2}, pH, potassium, sodium, lactate, Cr, BUN, RBC, hemoglobin, PLT, APTT, INR, PT, and Alb.

CI, confidence interval; HR, hazard ratio; Ref, reference.

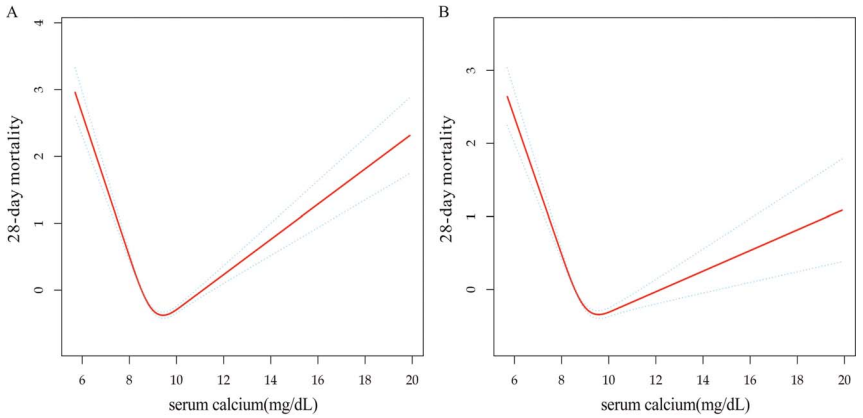

FIG. 3. **Smoothing spline fitting curve.** A, Unadjusted model. B, Adjusted model: after adjusting for sex, age, BMI, ethnicity, SOFA, LODS, SAPSII, SIRS, heart rate, SBP, DBP, MAP, respiratory rate, temperature, SpO_2, ventilation, CRRT, vasopressor, neurological disease, metastatic cancer, PCO_2, PO_2, pH, potassium, sodium, lactate, Cr, BUN, RBC, hemoglobin, PLT, APTT, INR, PT, Alb. Nonlinear plots are displayed with red dotted lines, and the blue dotted lines represent 95% CIs. Alb, albumin APTT, partial thromboplastin time; BMI, body mass index; BUN, serum urea nitrogen; Cr, creatinine; CRRT, continuous renal replacement therapy; DBP, diastolic blood pressure; INR, international normalized ratio; LODS, Logistic Organ Dysfunction System; MAP, mean arterial pressure; PCO_2, carbon dioxide; PLT, platelet; PO_2, partial pressure of oxygen; PT, prothrombin time; SAPSII, Simplified Acute Physiology Score II; SBP, systolic blood pressure; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; SpO_2, oxygen saturation.

II (adjusted for age, sex, and BMI), the result remained significant (HR, 0.91; 95% CI, 0.85–0.98). In model III (adjusted for age, sex, BMI, and potential confounders), the result did not show obvious changes (HR, 0.82; 95% CI, 0.76–0.88), and a rise of 1 mg/dL in the serum calcium level was associated with an 18% decrease in the risk of 28-day mortality. Furthermore, group Q4 (9.00–9.50 mg/dL) was used as the reference group, compared with the group Q4, the risk of 28-day mortality was increased by 136% in the Q1 group (HR, 2.36; 95% CI, 1.97–2.81) and by 28% in the Q5 group (HR, 1.28; 95% CI, 1.06–1.54) in model I. The overall trend in models II and III remained consistent with model I. For the sensitivity analysis, we handled serum calcium as five equal categorical variables and continuous variables separately, and the trend of the association between serum calcium level and 28-day mortality was consistent ($P < 0.001$).

Smoothing spline fitting curve

A U-shaped association was observed between serum calcium levels and 28-day mortality in patients with sepsis (Fig. 3A). After adjusting for potential confounders, a turning point value of serum calcium (9.0 mg/dL) was found by the segmentation regression model between serum calcium and the risk of 28-day mortality (Fig. 3B, Table 3). When serum calcium levels were less than 9.0 mg/dL, 28-day mortality risk decreased by 58% with each 1.0 mg/dL increase in serum calcium (HR, 0.42; 95% CI, 0.37–0.48). When serum calcium exceeded 9.0 mg/dL, 28-day mortality risk increased by 12% with each 1.0 mg/dL increase in serum calcium (HR, 1.12; 95% CI, 1.04–1.20). The LRT ($P < 0.001$) demonstrated a nonlinear association between serum calcium levels and risk of 28-day mortality in patients with sepsis (Table 3).

Survival analysis

Among the 3,016 patients included, 1,075 (35.64%) died during the first 28 days. The 28-day mortality rates of the Q1 (≤8.20 mg/dL), Q2 (8.30–8.60 mg/dL), Q3 (8.70–8.90 mg/dL), Q4 (9.00–9.50 mg/dL), and Q5 (≥9.6 mg/dL) groups were 51.79%, 30.00%, 30.43%, 29.70%, and 36.52%, respectively. Kaplan-Meier curves were constructed to visualize the association between serum calcium quintiles and 28-day mortality. The survival probability of five groups differed significantly without (Fig. 4A) or with (Fig. 4B) adjusting for potential confounders (log-rank test: $P < 0.001$), and patients in the lowest serum calcium quintile had the lowest survival probability.

DISCUSSION

In this retrospective cohort study, we found a U-shaped association between serum calcium levels and 28-day mortality in patients with sepsis. Serum calcium was negatively correlated with 28-day mortality risk for serum calcium <9.0 mg/dL and positively correlated for serum calcium >9.0 mg/dL. Serum calcium close to 9.0 mg/dL might be associated with the lowest risk of 28-day mortality in patients with sepsis. The serum calcium threshold (9.0 mg/dL) found in this study was just within the range of normal range of serum calcium (8.8–10.8 mg/dL)

TABLE 3. **Threshold effect analysis**

	Unadjusted model		Adjusted model	
Models	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Model I				
One line slope	0.88 (0.82–0.94)	<0.001	0.82 (0.76–0.88)	<0.001
Model II				
Turning point (K1)				
<9.0 slope 1	0.38 (0.34–0.43)	<0.001	0.42 (0.37–0.48)	<0.001
>9.0 slope 2	1.26 (1.19–1.34)	<0.001	1.12 (1.04–1.20)	0.002
LRT	<0.001*		<0.001*	

Data are presented as HR (95% CI) and *P* value.

*Indicates that model II is significant different from model I. Model I, linear analysis; model II, nonlinear analysis. Logarithmic likelihood ratio test ($P < 0.05$ means model II is significantly different from model I, which indicates a nonlinear association). The adjusted variables are the same as those in model III of multiple Cox regression.

CI, confidence interval; HR, hazard ratio; LRT, logarithmic likelihood ratio test.

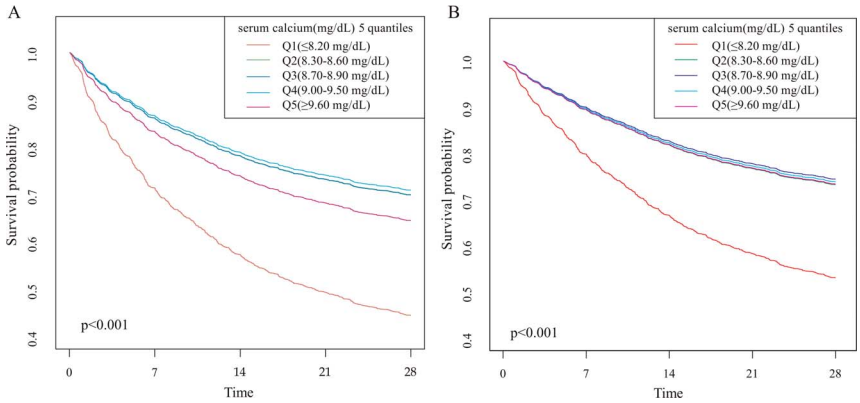

Fig. 4. **Kaplan-Meier survival curves for sepsis patients with different groups of serum calcium levels.** A, Unadjusted model. B, Adjusted model: the adjusted variables are the same as those in model III of multiple Cox regression.

(33). Wang *et al.* (34) also observed a U-shaped association between serum calcium and in-hospital mortality in critically ill patients. They indicated that patients with a decreased or increased serum calcium had significantly increased in-hospital mortality compared to those with the reference serum calcium level (8.6–9.0 mg/dL). Nguyen *et al.* (35) reported an independent association between serum calcium and 30-day mortality in septic patients based on a study involving 91 participants. Fei *et al.* (36) found that 28-day mortality in a hypocalcemia group was significantly higher than that in a normal serum calcium group in a sample of 119 sepsis patients. Likewise, Zaloga *et al.* (37) reported a higher mortality rate (50%) in sepsis patients with hypocalcemia compared with those with normocalcemia (29%). Our study found that lower or higher serum calcium levels were associated with increased 28-day mortality risk. The identification of the threshold holds potential value for adjusting nutritional support, treatment, and monitoring regimens for septic patients.

Multiple risk factors, such as advanced age (38), hyperlactatemia (39), cancer (40), hypotension (41), hypothermia (42), thrombocytopenia (43), hypocoagulability (44), and high procalcitonin (45), were associated with poor outcomes in patients with sepsis. Consistent with the previous study, univariate analysis in this study also revealed that advanced age, decreased SBP, DBP and MAP, low body temperature, presence of metastatic cancer, high lactate levels, low platelet count, and elevated APTT, PT, and INR were positively associated with 28-day mortality in sepsis. Therefore, early intervention and management of septic patients with these risk factors were needed to improve prognosis.

The specific physiological mechanism underlying the association between serum calcium levels and the prognosis of patients with sepsis remains unclear. The regulation of calcium levels by parathyroid hormone, vitamin D (25-hydroxyvitamin D or 1,25-dihydroxyvitamin D), albumin, and other factors had been extensively studied (46). Under sepsis condition, the proinflammatory cytokines IL-1β and IL-6 could upregulate the expression of calcium-sensing receptor in the parathyroid and kidney, ultimately reducing serum calcium levels (14). In turn, calcium derangements could also activate calcium-sensing receptors on T cell surfaces, promote the release of ROS and cytokine, damage endothelial cells and barrier function, and ultimately lead to fluid leakage, tissue inflammation and poor prognosis in sepsis (19). In addition, calcium influx could destroy the adhesion connections and cytoskeleton of endothelial cells, impairing endothelial cell integrity and exacerbating tissue damage and organ failure in septic patients (20,47). Calcium serves as a crucial cofactor in the coagulation cascade. Dysregulated serum calcium can lead to bleeding events and poor prognosis in septic patients by impairing platelet function and affecting the clotting cascade (11,12,48). It was reported that calcium dysregulation was significantly associated with increased incidence of acute kidney injury, myocardial damage, hypotension, disseminated intravascular coagulation, and organ failure. These heightened adverse events might contribute to an elevated risk of mortality in septic patients (49–52). In brief, calcium derangements and sepsis processes might interact and influence each other, leading to a vicious cycle of disease progression.

Our study had some limitations. First, retrospective cohort studies inevitably have some bias. However, we adjusted for potential confounding factors as much as possible in the data analysis to reduce potential bias. Second, intracellular calcium is an indispensable second messenger in endothelial cells and plays a key role in sepsis prognosis. Because of the limitations of retrospective clinical analysis, information on intracellular calcium cannot be obtained from database, and it is difficult to explore the effect of intracellular calcium on the prognosis of septic patients. In addition, it is difficult to determine the causal relationship and the mechanism between calcium derangements and outcomes in septic patients. Further basic experiments and prospective studies will be conducted to explore the mechanism and key roles of intracellular calcium on the prognosis of sepsis and to verify the causal relationship between calcium metabolism and sepsis mortality. Third, we did not explore the association between serum calcium levels and the long-term prognosis of patients with sepsis, which will be further explored.

CONCLUSION

The association between serum calcium and 28-day mortality in septic patients followed a U-shaped curve. Higher or lower serum calcium levels were associated with increased risk of 28-day mortality in patients with sepsis. Thus, serum calcium might serve as a factor to predict the risk of short-term mortality in septic patients and to help risk stratification and early management of septic patients.

Ethics Approval and Informed Consent

The studies involving human participants were reviewed and approved by the institutional review boards of MIT and BIDMC. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. No animal studies are presented in this manuscript. No potentially identifiable human images or data are presented in this study.

Data Availability Statement

Data analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

All authors contributed to the study concept and design; DY and RY collected and assembled data; DY operated the software and statistical analysis; RY, DY, and XX performed the data analysis and interpretation; DY contributed to writing the original draft; and NL and RY obtained the funding. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Editage (http://www.editage.cn) for the English language editing.

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