

## Original Article



## Association between Serum Chloride Levels and Prognosis in Patients with Hepatic Coma in the Intensive Care Unit

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### Abstract

**Objective** To explore the relationship between serum chloride levels and prognosis in patients with hepatic coma in the intensive care unit (ICU).

**Methods** We analyzed 545 patients with hepatic coma in the ICU from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database. Associations between serum chloride levels and 28-day and 1-year mortality rates were assessed using restricted cubic splines (RCSs), Kaplan-Meier (KM) curves, and Cox regression. Subgroup analyses, external validation, and mechanistic studies were also performed.

**Results** A total of 545 patients were included in the study. RCS analysis revealed a U-shaped association between serum chloride levels and mortality in patients with hepatic coma. The KM curves indicated lower survival rates among patients with low chloride levels (< 103 mmol/L). Low chloride levels were independently linked to increased 28-day and 1-year all-cause mortality rates. In the multivariate models, the hazard ratio (HR) for 28-day mortality in the low-chloride group was 1.424 (95% confidence interval [CI]: 1.041–1.949), while the adjusted hazard ratio for 1-year mortality was 1.313 (95% CI: 1.026–1.679). Subgroup analyses and external validation supported these findings. Cytological experiments suggested that low chloride levels may activate the phosphorylation of the NF- $\kappa$ B signaling pathway, promote the expression of pro-inflammatory cytokines, and reduce neuronal cell viability.

**Conclusion** Low serum chloride levels are independently associated with increased mortality in patients with hepatic coma.

**Key words:** Hepatic coma; Chloride; Mortality; Intensive care unit

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## INTRODUCTION

**H**epatic encephalopathy (HE) is a common complication of both acute and chronic liver diseases, and is characterized by neurological and psychiatric symptoms<sup>[1]</sup>. Approximately 30%–45% of patients with liver cirrhosis develop significant HE<sup>[2]</sup>. The condition manifests with varying degrees of cognitive impairment, including deficits in psychomotor speed, working memory, and more severe neuropsychiatric symptoms. Hepatic coma, the most severe form of HE, is defined as complete unconsciousness or unresponsiveness to the external environment<sup>[3]</sup>. HE is associated with reduced survival in patients with liver cirrhosis<sup>[4]</sup>, and hepatic coma further elevates mortality risk<sup>[5]</sup>. Timely prognostic assessment is crucial for improving the outcomes of these patients.

Critically ill patients often experience both hypochloremia and hyperchloremia because of underlying conditions or treatments<sup>[6]</sup>. Chloride levels have recently gained attention as a prognostic marker in critically ill patients, with studies demonstrating that hyperchloremia is associated with acute kidney injury (AKI) and in-hospital mortality in severe sepsis<sup>[7,8]</sup>. Javier et al. found that in critically ill patients with sepsis with hyperchloremia (chloride levels of  $\geq 110$  mmol/L) at intensive care unit (ICU) admission, worsening hyperchloremia within 72 h correlated with increased hospital mortality. Similarly, Bandarn et al. reported that hyperchloremia frequently occurs in severe sepsis and septic shock, and is independently associated with AKI. Even patients without initial hyperchloremia may develop AKI with a moderate increase in serum chloride levels (a change in serum chloride  $\geq 5$  mmol/L). Hypochloremia has also been identified as an independent prognostic factor in conditions such as hypertension<sup>[9]</sup>, pulmonary arterial hypertension<sup>[10]</sup>, AKI<sup>[11]</sup>, and chronic heart failure<sup>[12,13]</sup>. Therefore, it can serve as a predictive factor for mortality in these diseases.

However, the effect of serum chloride levels on the prognosis of patients with severe hepatic coma remains unclear. This study aimed to investigate the association between serum chloride levels and 28-day and 1-year all-cause mortality in patients with hepatic coma. In addition, we explored potential interventions that could improve outcomes and reduce the healthcare burden associated with severe hepatic coma.

## MATERIALS AND METHODS

### *Data Source*

This retrospective observational study used data from the Medical Information Mart for Intensive Care IV (MIMIC-IV, v2.2). The MIMIC-IV is a large, publicly available, single-center database that includes over 40,000 patients admitted to the Beth Israel Deaconess Medical Center's (BIDMC) ICUs from 2008 to 2019<sup>[14]</sup>. Although patients in the MIMIC-IV database are no longer identifiable, it contains comprehensive records including demographic information, physiological readings from bedside monitors, laboratory results, diagnoses, treatment information, and other clinical data collected during routine medical care. The use of this database was approved by the Institutional Review Board (IRB) of the Massachusetts Institute of Technology (Cambridge, Massachusetts, USA). One author of this study has completed the "Protecting Human Research Participants" course and obtained database access certification (Certificate Number: 47937607). Additionally, patients with hepatic coma who presented to the emergency department of Beijing Chaoyang Hospital between March 2022 and September 2024 were included for external validation.

### *Inclusion Criteria for the Study Population*

After a comprehensive screening, all patients with hepatic coma were included in the analysis. For patients with multiple admissions, only data from the first ICU admission were analyzed. The inclusion criteria were as follows: (1) diagnosis of hepatic coma based on diagnostic codes, including ICD-9 codes (700, 7020, 7021, 7022, 7023, 7041, 7042, 7043, 7044, 7049, 706, 7071, 709, 5722) and ICD-10 codes (B150, B160, B162, B1711, B190, B1911, B1921, K7041, K7111, K7201, K7211, K7291); and (2) age over 16 years. The exclusion criteria were (1) patients with ICU stays of  $< 24$  h and (2) missing serum chloride measurements.

### *Data Extraction*

We collected comprehensive patient data, including baseline characteristics such as age and sex, and vital signs, including body temperature, heart rate, respiratory rate (RR), diastolic blood pressure (DBP), systolic blood pressure (SBP), mean arterial pressure (MAP), and peripheral oxygen saturation (SpO<sub>2</sub>). Comorbidities, such as atrial fibrillation (AF), heart failure (HF), respiratory failure

(RF), chronic kidney disease (CKD), AKI, and sepsis, were also noted. Laboratory tests included albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, hematocrit, hemoglobin, international normalized ratio (INR), lactate, platelet count, potassium, prothrombin time (PT), partial thromboplastin time (PTT), lactate dehydrogenase (LDH), sodium, total bilirubin (Tbil), white blood cell count (WBC), and magnesium levels. Life support therapies, including continuous renal replacement therapy (CRRT) and invasive mechanical ventilation (IMV), were also recorded along with the sequential organ failure assessment (SOFA) score. For variables with repeated measurements, only the initial values were included in the analysis.

### Outcome

The primary outcome analyzed in this study was 28-day all-cause mortality, whereas the secondary outcome was 1-year all-cause mortality.

### Statistical Analyses

Continuous variables are expressed as means  $\pm$  standard deviations for normally distributed data or medians (interquartile ranges) for non-normally distributed data. Categorical variables are presented as counts and percentages. Between-group comparisons were made using Student's *t*-tests for normally continuous variables, Mann-Whitney U tests for non-normally continuous variables, and the chi-squared test for categorical variables.

We employed restricted cubic spline (RCS) Cox regression models to explore potential linear or nonlinear associations between serum chloride levels and 28-day/1-year all-cause mortality. The cohort was then stratified into subgroups based on RCS-derived optimal cutoff points. Kaplan–Meier (KM) curves with log-rank tests were generated to compare the survival probabilities across the chloride-level subgroups. Univariate and multivariate Cox proportional hazard models were used to assess associations, with results reported as hazard ratios (HRs) and 95% confidence intervals (CIs). Model 1 represents the unadjusted univariate Cox regression analysis. Model 2 accounts for adjustments based on age and sex, whereas Model 3 includes additional adjustments for age, sex, comorbidities, and the SOFA score.

Additional analyses were conducted to verify the robustness of the findings. First, we examined the potential interactions between serum chloride levels and key stratification variables. Second, subgroup

analyses were conducted to determine whether the association between serum chloride levels and 28-day mortality persisted after accounting for potential confounders. Finally, we validated the primary outcome (28-day all-cause mortality) using data from Beijing Chaoyang Hospital, reproducing both the KM survival analyses and Cox regression models.

Statistical significance was set at  $P < 0.05$ . All statistical analyses were performed using the R software version 4.3.1.

### Cellular Experiments

To investigate the mechanism by which chloride ions influence hepatic encephalopathy, we extracted primary neurons from 6–8-week-old C57BL/6J mice and cultured them in media containing different concentrations of chloride ions. Neuronal viability was assessed, and the expression of inflammatory cytokines, as well as the phosphorylation level of the NF- $\kappa$ B signaling pathway, was measured using polymerase chain reaction (PCR) and western blot (WB) analysis. The detailed procedures were as follows:

C57BL/6J mice aged 6–8 weeks, weighing 18–20 g (purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.), were selected, and brain tissue was aseptically collected. After dissecting the cerebral cortex, the tissues were incubated with 0.25% trypsin (25200072; Gibco) at 37 °C for 15 min. The digestion was terminated and neutralized with DMEM (PM150220A, Procell System) containing 10% fetal bovine serum (FBS) (10099141C, Gibco), followed by filtration through a cell strainer. After counting, the cells were seeded into six-well plates pre-coated with poly-D-lysine (PDL) (E607014-0002, Sangon Biotech) and cultured in Neurobasal medium (PM151223, Procell System) supplemented with B27 and L-glutamine. Once the cells adhered and reached stable growth (day 3), the medium was replaced with one containing different chloride ion concentrations.

Control group: standard neurobasal medium (PM151223; Procell system); low-chloride group 1: chloride concentration reduced by 10% compared to standard neurobasal medium; low-chloride group 2: chloride concentration reduced by 20%. Both the low-chloride media were custom-formulated by Procell Systems (PM151223).

After 48 h of incubation, neuronal viability was assessed using a CCK-8 assay (ab228554, Abcam). Ten microliters of CCK-8 reagent was added to each well and incubated for 2 h, after which the absorbance was measured at 450 nm.

After 12 h of incubation, total RNA was extracted from the cells, and complementary DNA (cDNA) was synthesized using a reverse transcription kit (ANG0818A, TAKARA). Real-time quantitative PCR using SYBR Green (11199ESO8, YEASEN) was performed to measure the mRNA expression levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Fwd: 5'- AGTGGT GCCAGCCGATGGGTTGT -3'; Rev: 5'- GCTGAGTTGGT CCCCTTCTCCAG -3'), interleukin-1 $\beta$ (IL-1 $\beta$ ) (Fwd: 5'- GCCACCTTTTGACAGTGATG -3'; Rev: 5'- GCTCTTGTG ATGTGCTGCT -3'), and interleukin-6 (IL-6) (Fwd: 5'- CCCCAATTTCCAATGCTCTCC -3'; Rev: 5'- GGATGGT CTTGGTCCTTAGCC -3'). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fwd: 5'- CCCAG CTTAGGTTTCATCAGG -3'; Rev: 5'- CCAAATCCGTTTCCACA CCGAC -3') served as the internal control, and relative expression levels were calculated using the  $2^{-\Delta\Delta C_t}$  method. For protein analysis, total cellular proteins were extracted, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto membranes. The membranes were then incubated with antibodies against NF- $\kappa$ B p65 (1:1000, ab32536, Abcam), phosphorylated p65 (p-p65) (1:1000, ab76302, Abcam), and GAPDH (1:1000, 60004-1-Ig, Proteintech). Protein expression was detected using an enhanced chemiluminescence (ECL) chemiluminescence system, and grayscale intensity was analyzed using the ImageJ software.

Statistical analyses were performed using GraphPad Prism version 9. Comparisons between the groups were performed using *t*-tests, one-way analysis of variance (ANOVA), and Tukey's multiple comparison tests. Statistical significance was set at  $P < 0.05$ .

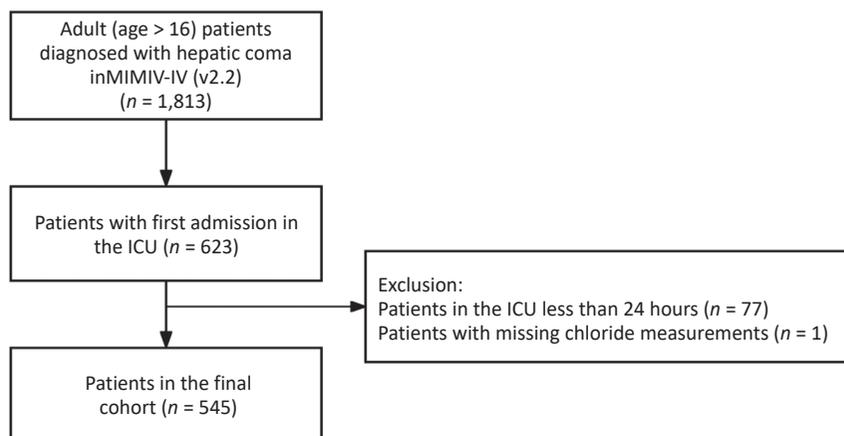
## RESULTS

### Baseline Characteristics

Figure 1 illustrates the participant selection process; 545 patients diagnosed with hepatic coma were included. Of these, 333 were male and 212 were female. Upon a 28-day follow-up, 356 patients (65.32%) survived and 189 patients (34.68%) died. Upon 1-year follow-up, 245 patients (44.95%) survived and 300 (55.05%) died. Tables 1 and 2 display the clinical characteristics of survivors and non-survivors during the 28-day and 1-year follow-ups, respectively. During both follow-up periods, the non-survivor group exhibited lower serum chloride concentrations and higher BUN, creatinine, lactate, Tbil, and INR levels than the survivor group. A higher proportion of patients in the non-survivor group received CRRT and IMV, although the difference in IMV use was not statistically significant ( $P > 0.05$ ). Comorbidity analysis indicated a significantly higher prevalence of AKI, respiratory failure, and sepsis among non-survivors at both follow-up intervals (all  $P > 0.05$ ).

### Association between Chloride Levels and All-cause Mortality

The RCS analysis revealed a significant U-shaped association between serum chloride levels and mortality, with inflection points at 103 and 113 mmol/L (Figure 2). Based on these thresholds, the patients were stratified into three groups: low chloride ( $< 103$  mmol/L,  $n = 269$ ), moderate chloride (103–113 mmol/L,  $n = 232$ ), and high chloride ( $> 113$  mmol/L,  $n = 44$ ) levels. Both the low- and high-chloride groups showed elevated mortality risks



**Figure 1.** Patient selection process. MIMIC, Medical Information Mart for Intensive Care; ICU, intensive care unit.

**Table 1.** Comparisons of the baseline characteristics at the 28-day follow-up

Variables	Survivors	Non-survivors	P-value
N	356	189	
Age, years	57.11 (12.80)	59.33 (14.52)	0.067
Albumin, g/dL	3.08 (0.73)	2.95 (0.72)	0.063
ALT <sup>*</sup> , U/L	34.00 [22.00, 101.00]	51.00 [31.00, 240.00]	0.001
AST <sup>*</sup> , U/L	72.00 [43.00, 191.00]	128.00 [64.50, 392.50]	< 0.001
BUN <sup>*</sup> , mg/dL	27.00 [15.00, 47.75]	37.00 [20.75, 57.00]	< 0.001
Calcium, mmol/L	8.29 (1.20)	8.22 (1.05)	0.520
Chloride, mmol/L	104.16 (7.20)	101.05 (9.33)	< 0.001
Creatinine <sup>*</sup> , mg/dL	1.20 [0.70, 2.10]	1.70 [1.00, 2.60]	0.001
DBP, mmHg	66.93 (18.17)	65.95 (18.93)	0.556
Glucose <sup>*</sup> , mg/dL	122.00 [100.00, 158.00]	116.00 [91.50, 151.00]	0.065
Hematocrit (%)	29.09 (5.96)	29.80 (6.93)	0.213
Hemoglobin, g/dL	9.71 (2.04)	9.87 (2.24)	0.403
HR, number/min	92.45 (19.54)	95.64 (22.36)	0.085
INR <sup>*</sup>	1.70 [1.40, 2.20]	2.10 [1.70, 2.80]	< 0.001
Lactate <sup>*</sup> , mmol/L	2.30 [1.50, 3.60]	2.90 [2.00, 4.85]	< 0.001
Platelet <sup>*</sup> , K/ $\mu$ L	112.00 [64.50, 165.00]	104.00 [66.00, 183.00]	0.909
Potassium, mmol/L	4.15 (0.82)	4.32 (0.90)	0.027
PT <sup>*</sup> , seconds	18.70 [15.80, 23.42]	22.20 [18.40, 29.50]	< 0.001
PTT <sup>*</sup> , seconds	38.90 [32.82, 48.18]	45.50 [36.35, 57.55]	< 0.001
RR <sup>*</sup> , number/min	19.00 [16.00, 23.00]	20.00 [16.00, 23.00]	0.130
SBP, mmHg	120.19 (23.02)	118.29 (23.28)	0.362
LDH <sup>*</sup> , U/L	280.50 [208.50, 458.75]	388.50 [246.75, 733.75]	< 0.001
Sodium, mmol/L	137.10 (6.09)	135.93 (8.22)	0.061
SpO <sub>2</sub> , %	97.32 (3.52)	96.59 (3.35)	0.019
Tbil <sup>*</sup> , $\mu$ mol/L	3.90 [1.70, 9.05]	5.90 [2.30, 16.90]	0.001
WBC <sup>*</sup> , k/ $\mu$ L	8.80 [5.70, 13.00]	11.40 [7.30, 17.50]	< 0.001
MAP <sup>*</sup> , mmHg	78.00 [69.00, 90.50]	75.50 [65.00, 88.00]	0.116
Temperature, °C	36.72 (0.78)	36.53 (0.95)	0.016
SOFA <sup>*</sup> , scores	8.00 [6.00, 10.25]	10.00 [8.00, 13.00]	< 0.001
Magnesium, mmol/L	2.01 (0.47)	2.14 (0.48)	0.002
Sex			0.042
Female	150 (42.1)	62 (32.8)	
Male	206 (57.9)	127 (67.2)	
CRRT (%)	44 (12.4)	36 (19.0)	0.049
IMV (%)	216 (60.7)	126 (66.7)	0.199
AF (%)	62 (17.4)	35 (18.5)	0.839
AKI (%)	199 (55.9)	149 (78.8)	< 0.001
CKD (%)	55 (15.4)	23 (12.2)	0.362
HF (%)	50 (14.0)	27 (14.3)	> 0.999

Continued

Variables	Survivors	Non-survivors	P-value
RF	128 (36.0)	104 (55.0)	< 0.001
Sepsis	78 (21.9)	85 (45.0)	< 0.001

**Note.** \* Mann-Whitney U test. Categorical variables are presented as *n* (%); Continuous variables are expressed as means  $\pm$  standard deviations for normally distributed data or medians [interquartile ranges] for non-normally distributed data; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; DBP, diastolic blood pressure; HR, heart rate; INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RR, respiratory rate; SBP, systolic blood pressure; LDH, lactate dehydrogenase; SpO<sub>2</sub>, peripheral oxygen saturation; Tbil, total bilirubin; WBC, white blood cells; MAP, mean arterial pressure; SOFA, sequential organ failure assessment; CRRT, continuous renal replacement therapy; IMV, invasive mechanical ventilation; AF, atrial fibrillation; AKI, acute kidney injury; CKD, chronic kidney disease; HF, heart failure; RF, respiratory failure.

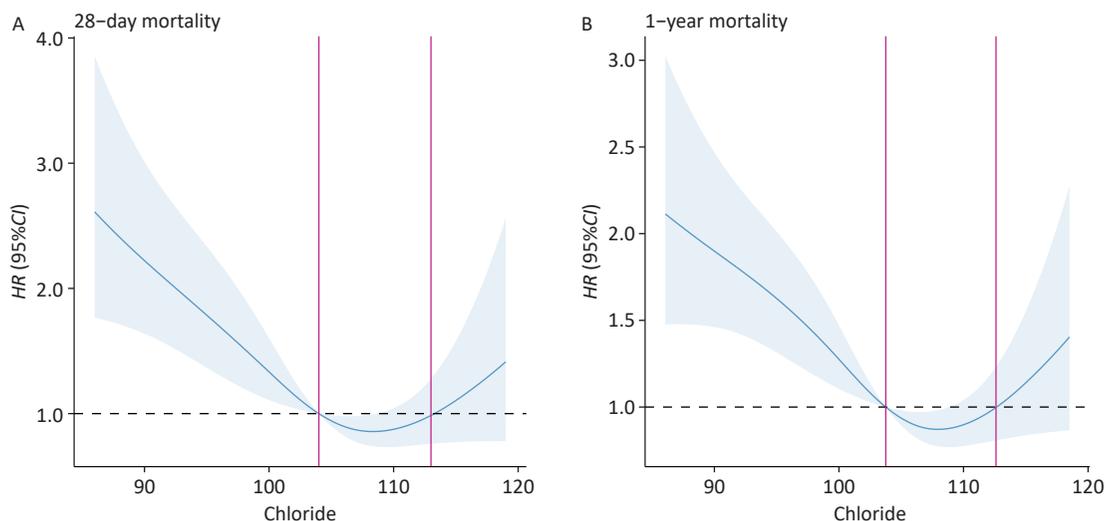
**Table 2.** Comparisons of the baseline characteristics at the 1-year follow-up

Variables	Survivors	Non-survivors	P-value
<i>N</i>	245	300	
Age, years	55.34 (12.48)	59.95 (13.87)	< 0.001
Albumin, g/dL	3.04 (0.65)	3.02 (0.79)	0.735
ALT*, U/L	39.00 [23.00, 146.00]	42.00 [24.00, 105.00]	0.842
AST*, U/L	79.00 [45.00, 299.00]	94.00 [50.00, 235.00]	0.470
BUN*, mg/dL	24.50 [14.00, 47.00]	35.00 [19.25, 53.00]	< 0.001
Calcium, mmol/L	8.18 (1.25)	8.34 (1.06)	0.112
Chloride, mmol/L	104.30 (7.20)	102.09 (8.70)	0.002
Creatinine*, mg/dL	1.10 [0.70, 2.00]	1.60 [1.00, 2.50]	< 0.001
DBP, mmol/L	67.56 (18.57)	65.79 (18.30)	0.266
Glucose*, mg/dL	123.50 [100.00, 162.00]	118.00 [94.00, 150.00]	0.125
Hematocrit (%)	29.55 (6.01)	29.17 (6.56)	0.489
Hemoglobin, g/dL	9.89 (2.07)	9.67 (2.14)	0.224
HR, number/min	92.66 (19.19)	94.29 (21.68)	0.358
INR*	1.70 [1.40, 2.30]	1.90 [1.60, 2.50]	0.009
Lactate*, mmol/L	2.30 [1.50, 3.70]	2.70 [1.90, 4.50]	0.003
Platelet*, k/ $\mu$ L	118.50 [65.00, 173.00]	102.00 [64.75, 166.50]	0.239
Potassium, mmol/L	4.15 (0.86)	4.25 (0.85)	0.152
PT*, seconds	18.70 [16.15, 24.45]	20.65 [17.28, 26.60]	0.012
PTT*, seconds	38.40 [32.60, 48.80]	43.80 [35.32, 54.45]	0.001
RR*, number/min	19.00 [16.00, 23.00]	19.00 [16.00, 23.00]	0.459
SBP, mmol/L	119.91 (21.93)	119.23 (24.07)	0.735
LDH*, U/L	285.50 [213.25, 529.00]	347.00 [230.25, 540.75]	0.276
Sodium, mmol/L	137.16 (6.04)	136.31 (7.55)	0.152
SpO <sub>2</sub> , %	97.40 (3.29)	96.79 (3.60)	0.041
Tbil*, umol/L	3.50 [1.70, 8.00]	5.20 [2.20, 13.20]	< 0.001
WBC*, k/ $\mu$ L	9.25 [6.10, 13.20]	9.90 [6.40, 15.53]	0.089
MAP*, mmHg	78.00 [69.00, 92.00]	76.00 [66.00, 88.00]	0.083
Temperature, °C	36.77 (0.81)	36.56 (0.86)	0.006

Continued

Variables	Survivors	Non-survivors	P-value
SOFA*, scores	8.00 [6.00, 11.00]	9.00 [7.00, 12.00]	< 0.001
Magnesium, mmol/L	2.02 (0.49)	2.09 (0.46)	0.083
Sex			0.204
Female	103 (42.0)	109 (36.3)	
Male	142 (58.0)	191 (63.7)	
CRRT (%)	27 (11.0)	53 (17.7)	0.039
IMV (%)	158 (64.5)	184 (61.3)	0.503
AF (%)	38 (15.5)	59 (19.7)	0.250
AKI (%)	126 (51.4)	222 (74.0)	< 0.001
CKD (%)	28 (11.4)	50 (16.7)	0.107
HF (%)	32 (13.1)	45 (15.0)	0.601
RF (%)	91 (37.1)	141 (47.0)	0.026
Sepsis (%)	43 (17.6)	120 (40.0)	< 0.001

**Note.** \* Mann-Whitney *U* test. Categorical variables are presented as n(%); Continuous variables are expressed as means  $\pm$  standard deviations for normally distributed data or medians [interquartile ranges] for non-normally distributed data; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; DBP, diastolic blood pressure; HR, heart rate; INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RR, respiratory rate; SBP, systolic blood pressure; LDH, lactate dehydrogenase; SpO<sub>2</sub>, peripheral oxygen saturation; Tbil, total bilirubin; WBC, white blood cells; MAP, mean arterial pressure; SOFA, sequential organ failure assessment; CRRT, continuous renal replacement therapy; IMV, invasive mechanical ventilation; AF, atrial fibrillation; AKI, acute kidney injury; CKD, chronic kidney disease; HF, heart failure; RF, respiratory failure.



**Figure 2.** Association between chloride levels and hazard ratio of 28-day (A) and 1-year (B) all-cause mortality. *HR*, hazard ratio; *CI*, confidence interval.

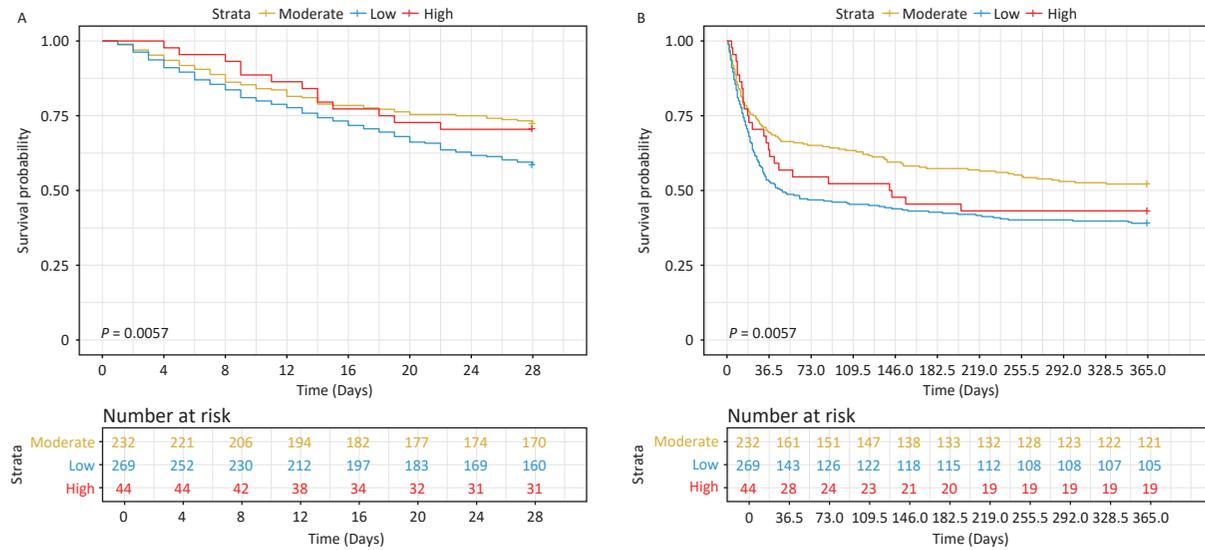
( $HR > 1$ ), with KM curves confirming significantly worse 28-day and 1-year survival compared to the moderate group ( $P < 0.05$ ; Figure 3A–B).

Univariate and multivariate Cox regression analyses were also performed (Table 3). Univariate

analysis revealed that the low chloride group was significantly associated with increased 28-day (unadjusted *HR*, 1.615; 95% *CI*, 1.188–2.195) and 1-year all-cause mortality (unadjusted *HR*, 1.482; 95% *CI*, 1.164–1.887) in patients with hepatic coma. After

adjusting for age and sex, the association remained significant for both 28-day (adjusted *HR*, 1.624; 95% *CI*, 1.194–2.209) and 1-year all-cause mortality (adjusted *HR*, 1.509; 95% *CI*, 1.185–1.921). Further adjustment for age, sex, SOFA score, CRRT, IMV, AKI,

RF, and sepsis did not alter this trend, and the low chloride group remained significantly associated with higher 28-day (adjusted *HR*, 1.424; 95% *CI*, 1.041–1.949) and 1-year all-cause mortality (adjusted *HR*, 1.313; 95% *CI*, 1.026–1.679). In



**Figure 3.** Kaplan-Meier survival curves of patients with hepatic coma with moderate (yellow, chloride 103–103 mmol/L ), high (red, chloride > 113 mmol/L ), and low (blue, chloride < 103 mmol/L) chloride levels at 28-day (A) and 1-Year (B) follow-up.

**Table 3.** Findings of the univariate and multivariable analyses

Chloride	Model 1		Model 2		Model 3	
	<i>HR</i> , 95% <i>CI</i>	<i>P</i> -value	<i>HR</i> , 95% <i>CI</i>	<i>P</i> -value	<i>HR</i> , 95% <i>CI</i>	<i>P</i> -value
<b>28-day death</b>						
Continuous	0.964 (0.948, 0.981)	< 0.001	0.962 (0.946, 0.979)	< 0.001	0.964 (0.948, 0.982)	< 0.001
<b>Categorical</b>						
Moderate	Reference	Reference	Reference	Reference	Reference	Reference
High	1.052 (0.580, 1.911)	0.866	1.126 (0.619, 2.049)	0.697	0.867 (0.471, 1.594)	0.646
Low	1.615 (1.188, 2.195)	0.002	1.624 (1.194, 2.209)	0.002	1.424 (1.041, 1.949)	0.027
<b>1-year death</b>						
Continuous	0.973 (0.959, 0.987)	< 0.001	0.970 (0.956, 0.984)	< 0.001	0.974 (0.960, 0.989)	0.001
<b>Categorical</b>						
Moderate	Reference	Reference	Reference	Reference	Reference	Reference
High	1.246 (0.807, 1.923)	0.321	1.335 (0.863, 2.065)	0.195	1.071 (0.686, 1.672)	0.764
Low	1.482 (1.164, 1.887)	0.001	1.509 (1.185, 1.921)	0.001	1.313 (1.026, 1.679)	0.031
<b>External 28-day death</b>						
Non-Low	Reference	Reference	Reference	Reference	Reference	Reference
Low	2.673 (1.085, 6.560)	0.032	2.626 (1.044, 6.603)	0.040	4.311 (1.495, 12.432)	0.007

**Note.** *HR*, hazard ratio; *CI*, confidence interval. Model 1: Unadjusted. Model 2: Adjusted for sex and age. Model 3: Adjusted for sex, age, SOFA score, CRRT, IMV, AKI, RF, and sepsis.

contrast, the high chloride group showed no significant association with mortality in either the univariate or multivariate analyses ( $P > 0.05$ ).

**Interaction and Subgroup Analysis**

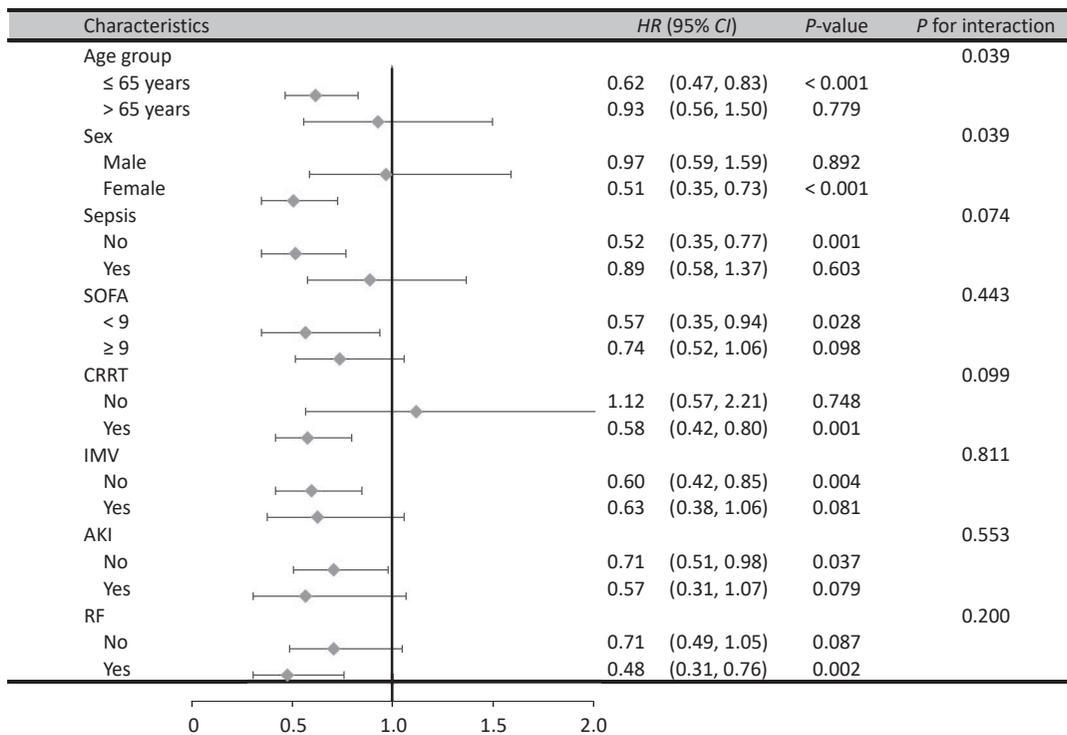
Based on these findings, we stratified the patients into two distinct groups: a low-chloride group and a combined non-low-chloride group (incorporating both moderate- and high-chloride categories). To further investigate these associations, we performed comprehensive interaction tests and subgroup analyses to examine the potential effect modifications and differential associations across clinically relevant patient subgroups.

Our analysis revealed significant interaction effects between chloride levels and demographic factors (Figure 4). During the 28-day follow-up, we observed a notable age-dependent interaction ( $P$  interaction = 0.039), where patients aged  $\leq 65$  years with non-low chloride levels exhibited a significantly reduced mortality risk ( $HR$ , 0.62; 95%  $CI$ , 0.47–0.83;  $P < 0.001$ ). A similar sex-based interaction was observed ( $P$  interaction = 0.039), with female patients showing a strong inverse association between chloride levels and 28-day mortality ( $HR$ ,

0.51; 95%  $CI$ , 0.35–0.73;  $P < 0.001$ ). These patterns persisted at 1-year follow-up (Figure 5), with an even more pronounced sex-based interaction ( $P$  interaction = 0.009). While female patients continued to show a protective association of non-low chloride levels ( $HR$ , 0.56; 95%  $CI$ , 0.42–0.75;  $P < 0.001$ ), no significant association was found among male patients ( $HR$ , 1.04; 95%  $CI$ , 0.72–1.52;  $P = 0.823$ ), highlighting a striking sex-based difference in the prognostic value of serum chloride.

**External Validation**

The validation cohort comprised 68 patients. In addition to chloride levels, data were collected on sex, age, SOFA score, CRRT, IMV, and comorbidities, including AKI, RF, and sepsis. The baseline characteristics are presented in Table 4. The cohort included 46 survivors (67.6%) and 22 non-survivors (32.4%). Notably, non-survivors had significantly lower serum chloride levels ( $100.18 \pm 5.76$  mmol/L vs.  $105.93 \pm 6.35$  mmol/L in survivors,  $P = 0.001$ ) and higher disease severity as reflected by SOFA scores ( $10.00 [7.25–14.00]$  vs.  $7.50 [5.00–10.00]$ , respectively;  $P = 0.004$ ). However, no significant differences were found between the groups



**Figure 4.** Subgroup analysis of patients with hepatic coma for 28-day mortality rates. *HR*, hazard ratio; *CI*, confidence interval; SOFA, Sequential Organ Failure Assessment; CRRT, continuous renal replacement therapy; IMV, invasive mechanical ventilation; AKI, acute kidney injury; RF, respiratory failure.

regarding age, sex, IMV, CRRT, or distribution of comorbidities (AKI, RF, or sepsis) ( $P > 0.05$ ). The KM curve (Figure 6) demonstrated significantly worse survival rates in the hypochloremia group (log-rank  $P = 0.026$ ). Cox regression models consistently showed an increased mortality risk in the low chloride group across all three models (Model 1–3), with HRs consistently exceeding 1 (Table 3).

**Cellular Experiments**

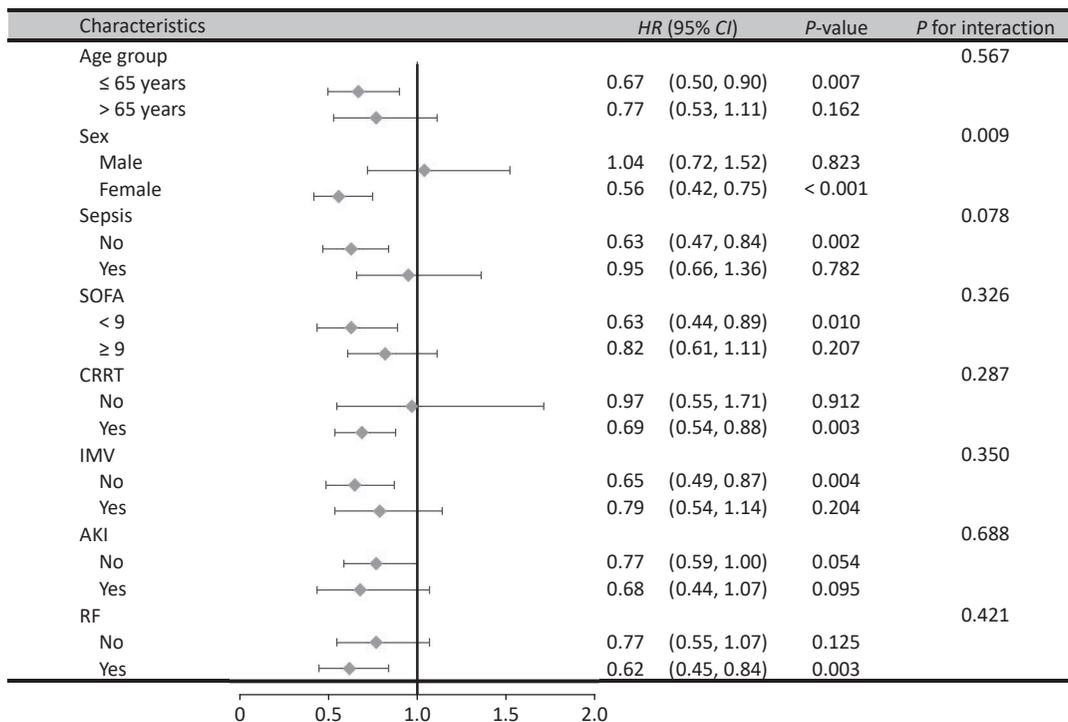
The results of the cellular experiments (Figure 7) showed that neuronal cell viability was significantly reduced in the group with a 20% reduction in chloride concentration compared to that in the standard medium ( $P < 0.001$ ). This group also exhibited markedly increased phosphorylation of NF- $\kappa$ B ( $P < 0.001$ ) and elevated mRNA levels of pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 ( $P < 0.001$ ). These findings suggest that low chloride levels may activate NF- $\kappa$ B pathway phosphorylation, promote the expression of pro-inflammatory cytokines, and reduce neuronal cell viability.

**DISCUSSION**

This study represents the first investigation of

the correlation between serum chloride levels and both short- and long-term all-cause mortality in patients in the ICU diagnosed with hepatic coma. The primary finding of this study was that diminished serum chloride levels served as a significant and independent predictor of increased 28-day and 1-year all-cause mortality in patients with hepatic coma. Notably, this association remained consistent even after controlling for other variables. The findings of this study provide a simple and effective biomarker for accurately assessing short- and long-term prognoses of hepatic coma. In addition, the cell experiments further validated the potential mechanism by which a low chloride concentration affects neuronal cells, providing evidence for the impact of hypochloremia on the prognosis of patients with HE.

In the RCS curve, there is a "U"-shaped correlation between serum chloride levels and 28-day and 1-year all-cause mortality in patients with hepatic coma. Specifically, the lowest mortality was observed when the chloride ion concentration was within the range of 103–113 mmol/L. The KM survival curve demonstrated that patients in the low-chloride group had the highest risk of mortality, while those in the high-chloride group had a higher



**Figure 5.** Subgroup analysis of patients with hepatic coma for 1-year mortality rates. HR, hazard ratio; CI, confidence interval; SOFA, Sequential Organ Failure Assessment; CRRT, continuous renal replacement therapy; IMV, invasive mechanical ventilation; AKI, acute kidney injury; RF, respiratory failure.

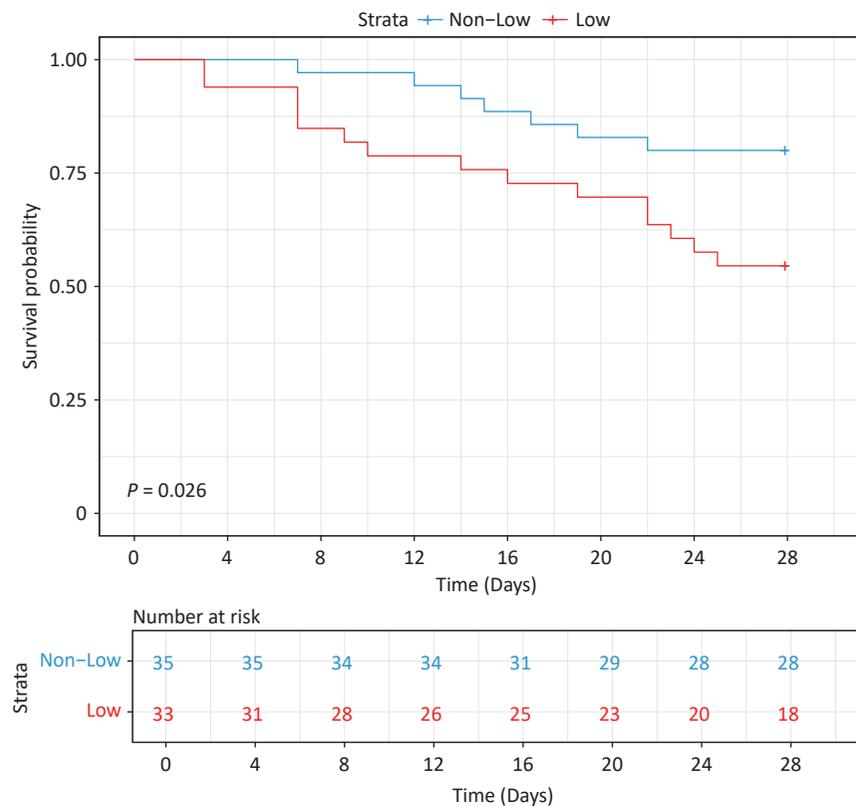
mortality risk than those in the moderate-chloride group. This finding was further supported by Cox regression analysis, which indicated that patients in

the low-chloride group had significantly higher overall mortality than those in the moderate- and high-chloride groups. Subgroup analysis confirmed

**Table 4.** Comparisons of the baseline characteristics at the 28-day follow-up of the external cohort

Variables	Survivors	Non-survivors	P-value
N	46	22	
Age, years	59.13 ± 13.78	62.09 ± 10.19	0.374
Chloride, mmol/L	105.93 (6.35)	100.18 (5.76)	0.001
SOFA, scorces	7.50 [5.00, 10.00]	10.00 [7.25, 14.00]	0.004
Sex			> 0.999
Female	20 (43.5)	9 (40.9)	
Male	26 (56.5)	13 (59.1)	
CRRT	6 (13.0)	1 ( 4.5)	0.514
IMV	13 (28.3)	12 (54.5)	0.067
AKI	17 (37.0)	14 (63.6)	0.071
RF	24 (52.2)	10 (45.5)	0.795
Sepsis	13 (28.3)	6 (27.3)	> 0.999

**Note.** SOFA, sequential organ failure assessment; CRRT, continuous renal replacement therapy; IMV, invasive mechanical ventilation; AKI, acute kidney injury; RF, respiratory failure.



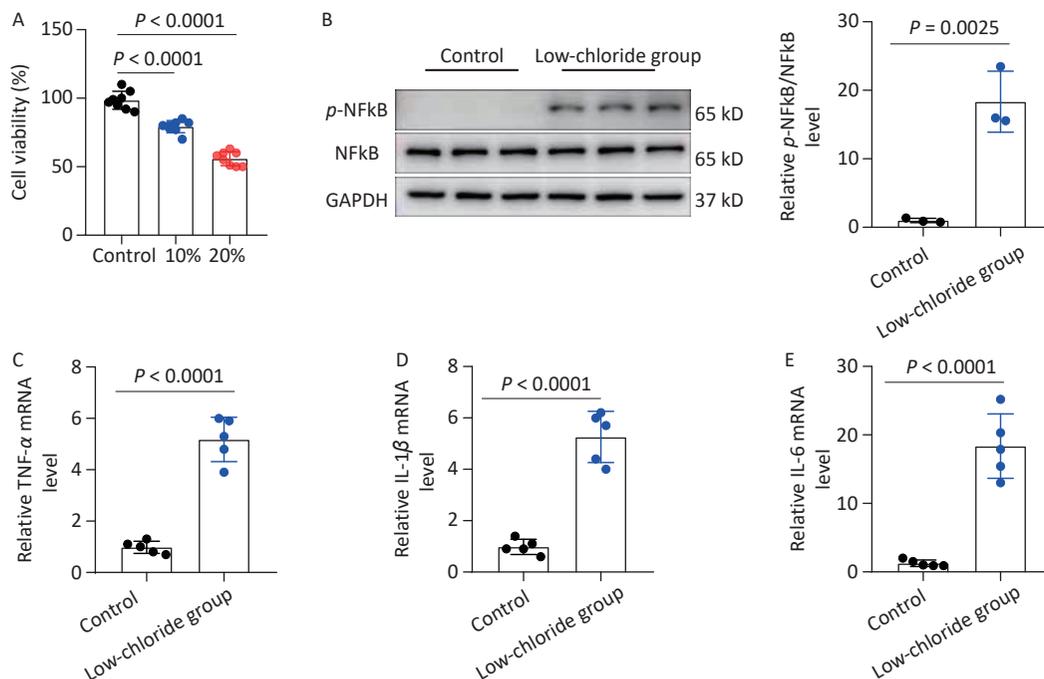
**Figure 6.** Kaplan-Meier survival curves of patients with hepatic coma with low (red, chloride < 103 mmol/L) and non-low chloride (blue, chloride ≥ 103 mmol/L) levels at the 28-Day follow-Up.

this finding. In summary, this study underscores the utility of serum chloride levels in risk stratification and identification of high-risk patients with hepatic coma, thereby providing valuable insights for the clinical management of these patients.

Most studies have focused on the prognosis of patients with cirrhosis or HE. Only few research articles have specifically addressed the prognostic prediction in patients with hepatic coma<sup>[15-17]</sup>. It is important to note that hepatic coma can arise from causes other than liver cirrhosis and represent the most severe manifestation of HE. Consequently, the prognosis of patients with hepatic coma is particularly concerning compared with that of those with mild HE. Therefore, it is important to conduct prognostic assessments that are specifically tailored for patients with hepatic coma. We carefully collected comprehensive and detailed baseline data, including demographic information, vital signs, laboratory tests, disease severity scores, and treatment approaches. To ensure the reliability of our findings, we used multivariate Cox regression and subgroup analyses to account for these variables. We have comprehensively and robustly

confirmed the prognostic value of low serum chloride levels in patients with hepatic coma.

HE is a neuropsychiatric syndrome resulting from hepatic insufficiency or portosystemic shunting, and is one of the most severe complications of decompensated cirrhosis. Clinically, HE presents with a spectrum of neurological disturbances ranging from subtle cognitive impairments to profound coma. The underlying pathophysiology is multifactorial, with hyperammonemia serving as the principal pathogenic mechanism. Additional contributing factors include oxidative stress<sup>[18,19]</sup> and systemic inflammation<sup>[20]</sup>, which collectively exacerbate neuronal dysfunction. Most toxic metabolic products are typically produced in the intestines, and before being detoxified and cleared by the liver, enter the systemic circulation through collateral pathways, subsequently crossing the blood-brain barrier and leading to brain dysfunction<sup>[20]</sup>. Serum chloride levels are closely associated with various neurological disorders<sup>[21]</sup>. Chloride plays a vital role in the physiological functions of the central nervous system (CNS)<sup>[22]</sup>. Changes in serum chloride levels have been



**Figure 7.** Effects of low-chloride environment on cell viability and the NF-κB inflammatory pathway. (A) Effects of culture media containing different chloride ion concentrations on neuronal cell viability. (B) Expression of p-NF-κB protein in mouse neurons cultured in a medium containing 20% reduced chloride ions. (C) Comparison of TNF-α mRNA expression between the low-chloride group and the normal group. (D) Comparison of IL-1β mRNA expression between the low-chloride and normal groups. (E) Comparison of IL-6 mRNA expression between the low-chloride and normal groups.

considered as potential targets for the treatment of various neurological diseases<sup>[23]</sup>. Previous studies have shown that hyperchloremia can predict early mortality in severe traumatic brain injury<sup>[24]</sup>. Hyperchloremia often occurs in more severe cases and is independently associated with death or disability within 90 days. Avoiding hyperchloremia may reduce mortality or disability observed in patients with cerebral hemorrhage<sup>[25]</sup>. However, some studies have reported that hyponatremia during hospitalization is associated with in-hospital mortality in patients with acute stroke<sup>[26]</sup>. Hyponatremia may be an important prognostic factor for determining the risk of death in patients with severe traumatic brain injury<sup>[27]</sup>. In our study, we found that lower serum chloride ion levels were associated with a poor prognosis in patients with hepatic coma. Although there are inconsistencies in the research results regarding chloride levels and the prognosis of neurological diseases, targeted interventions to normalize serum chloride levels may be a potential approach to improve the prognosis of severe neurological disorders.

Multiple studies have indicated a close association between the changes in serum chloride levels and adverse outcomes in various diseases, including liver cirrhosis. Several studies have assessed the prognostic significance of serum chloride levels in patients with liver cirrhosis. Sumarsono et al. found that serum chloride levels were independently and negatively correlated with the 180-day mortality in patients with decompensated cirrhosis in the ICU<sup>[28]</sup>. Yun et al. found that ICU mortality was higher in patients with hypochloremia than in those without. They also observed that serum chloride levels were independently correlated with ICU mortality in patients with severe liver cirrhosis<sup>[29]</sup>. In addition, one study found an association between low serum chloride levels and long-term mortality in patients with advanced chronic liver disease. Low serum chloride levels were also associated with ICU mortality<sup>[30]</sup>. However, to date, no study has evaluated the significance of serum chloride level as a prognostic indicator in patients with hepatic coma.

Serum chloride levels are often overlooked; however, chloride is the second most abundant electrolyte in the serum after sodium. It plays a crucial role in regulating the fluid and electrolyte balance, maintaining electrical neutrality, and managing the acid-base status. Abnormal chloride levels on serum electrolyte evaluations often indicate severe underlying metabolic disturbances

such as metabolic acidosis or alkalosis<sup>[6]</sup>. Chloride levels have several advantages as a prognostic biomarker. First, chloride can be conveniently and rapidly measured in most hospitals, offering the benefits of timeliness and low cost. Second, as mentioned earlier, serum chloride levels can serve as an independent prognostic factor in patients with liver cirrhosis<sup>[31-33]</sup>. Additionally, patients with HE often experience electrolyte imbalances. Hence, there is a theoretical basis for using serum chloride levels as a predictor of prognosis in patients with hepatic coma. This study also demonstrated the role of low chloride levels in predicting 28-day and 1-year all-cause mortality in patients with hepatic coma.

In addition, we explored the regulatory effects of chloride ion concentrations on neuronal activity and inflammatory responses using *in vitro* experiments. The results demonstrated that reducing the chloride concentration in the culture medium significantly suppressed neuronal viability, accompanied by activation of the NF- $\kappa$ B signaling pathway and upregulation of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6). These findings provide new experimental evidence for understanding the potential role of chloride ions in HE. They further confirm that chloride deficiency may aggravate neuronal injury by activating inflammatory pathways that are closely related to the pathogenesis of neurological dysfunction in HE. Moreover, activation of the NF- $\kappa$ B signaling pathway may be a key mechanism through which chloride ions regulate inflammation. NF- $\kappa$ B is a central transcription factor in the inflammatory response, and its phosphorylation promotes the transcription of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$ <sup>[34]</sup>. However, the specific underlying mechanisms require further investigation.

Although our study confirmed that low chloride levels can serve as an effective prognostic factor in clinical practice, we must acknowledge certain limitations. First, this was a retrospective analysis of an observational study; thus, causal relationships could not be definitively established. However, careful, multifaceted, and rigorous statistical methods were employed to obtain valid and reliable results. Further research is required to determine whether interventions targeting chloride levels have a positive impact on clinical outcomes. Second, the data for validation was sourced from a single-center database, and further validation is required to determine its applicability to other settings. Finally, the more mechanism linking elevated serum chloride levels to increased mortality in hepatic coma patients requires further exploration.

## CONCLUSION

Chloride levels are independently associated with mortality in patients with hepatic coma in the ICU. Patients with low chloride levels (< 103 mmol/L) had higher 28-day and 1-year all-cause mortality rates than those with high chloride levels. Our findings may provide a rationale for future studies, including targeted interventions to avoid low serum chloride levels and improve outcomes in patients with hepatic coma.

**Competing Interests** The authors declare that they have no conflicts of interest.

**Ethics** All methods in this study were performed in accordance with the relevant guidelines and regulations (the Declaration of Helsinki). The MIMIC-IV is an anonymous public database. The project was approved by the Institutional Review Boards of both the Massachusetts Institute of Technology (MIT) and Beth Israel Deaconess Medical Center (BIDMC), with an informed consent waiver.

**Authors' Contributions** Methodology design, data collection, and manuscript drafting: Shuxing Wei and Xiya Wang; Literature review and data analysis: Yuan Du and Ying Chen; Manuscript revision and data collection assistance: Jinlong Wang, Yue Hu, Wenqing Ji, and Xingyan Zhu; Conceptualization and communication management: Xue Mei and Da Zhang.

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**Data Sharing** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

**Consent for Publication** Not applicable.

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