Prognosis of Patients with Sepsis and Non-Hepatic Hyperammonemia: A Cohort Study

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Background:
Hyperammonemia has been reported in some critically ill patients with sepsis who do not have hepatic failure. A significant proportion of patients with non-hepatic hyperammonemia have underlying sepsis, but the association between non-hepatic hyperammonemia and prognosis is unclear.

Material/Methods:
Information about patients with sepsis and non-hepatic hyperammonemia was retrieved from the Medical Information Mart for Intensive Care-III database. Survival rates were analyzed using the Kaplan-Meier method. Multivariate logistic regression models were employed to identify prognostic factors. Receiver operating characteristic (ROC) curve analysis was used to measure the predictive ability of ammonia in terms of patient mortality.

Results:
A total of 265 patients with sepsis were enrolled in this study. Compared with the non-hyperammonemia group, the patients with hyperammonemia had significantly higher rates of hospital (59.8% vs. 43.0%, P=0.007), 30-day (47.7% vs. 34.8%, P=0.036), 90-day (61.7% vs. 43.7%, P=0.004), and 1-year mortality (67.3% vs. 49.4%, P=0.004). In the survival analysis, hyperammonemia was associated with these outcomes. Serum ammonia level was an independent predictor of hospital mortality. The area under the ROC curve for the ammonia levels had poor discriminative capacity. The hyperammonemia group also had significantly lower Glasgow Coma Scale scores (P=0.020) and higher incidences of delirium (15.9% vs. 8.2%, P=0.034) and encephalopathy (37.4% vs. 19.6%, P=0.001). Intestinal infection and urinary tract infection with organisms such as Escherichia coli may be risk factors for hyperammonemia in patients who have sepsis.

Conclusions:
Higher ammonia levels are associated with poorer prognosis in patients with sepsis. Ammonia also may be associated with sepsis-associated encephalopathy. Therefore, we recommend that serum ammonia levels be measured in patients who are suspected of having sepsis.

MeSH Keywords: Escherichia coli Infections • Hyperammonemia • Prognosis Sepsis

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Background

Sepsis is a serious medical condition responsible for approximately 19.77% of all deaths worldwide [1,2]. The mortality is a result of the systemic inflammation and end-organ dysfunction associated with these infections [3]. The rate of mortality in patients diagnosed with sepsis is 30%, and 50% in individuals with severe sepsis. In patients in whom the disease progresses to septic shock, the mortality rate can rise to as high as 80%. As an individual’s infection worsens, the risk of mortality gradually increases [4]. Sepsis-associated encephalopathy (SAE) can be found in up to 70% of patients with severe sepsis and it is a common neurological complication [5], with a mortality rate of up to 70% [6].

Ammonia is a major factor in the pathogenesis of hepatic encephalopathy and it crosses the blood-brain barrier readily, resulting in significant neurotoxicity [7]. Disorders of ammonia metabolism can lead to hyperammonemia, which usually is a consequence of hepatic failure. Hyperammonemia also can occur in critically ill patients who do not have hepatic disease [8], including individuals with sepsis, gastrointestinal bleeding, kidney failure, elevations in sodium, and exposure to valproate [8,9]. In recent reports, serum ammonia has been suggested as a possible predictor of 28-day mortality and hospital stay in patients with sepsis. While elevation in ammonia level has been reported as a novel biomarker for sepsis, severe sepsis, or septic shock according to International Classification of Diseases, Ninth Revision (ICD-9) codes; (2) age ≥18 and ≤89 years; (3) admission for >24 hours in the intensive care unit (ICU); and documentation of blood ammonia levels. A blood ammonia level >35 μmol/L was defined as hyperammonemia in the MIMIC-III database.

Exclusion criteria for the study were as follows: (1) a history of acute or chronic liver disease, including hepatitis, hepatic cirrhosis, hepatic encephalopathy, hepatorenal syndrome, hepatic injury, and other chronic liver disease, according to ICD-9 diagnosis codes on patient discharge (Supplementary Table 1); and (2) no documentation of vital signs or ICD-9 diagnostic codes.

Data extraction

R statistical software (R Foundation for Statistical Computing, Vienna, Austria) was used to collect data on baseline characteristics information such as age, sex, and vital signs and laboratory parameters during the first 24 hours of ICU admission. The maximum value for ammonia during each patient’s ICU stay also was retrieved. Infection type (Supplementary Table 2), microbiology type (Supplementary Table 3), and patient comorbidities (Supplementary Table 4) were determined according to the primary ICD-9 codes, as documented in each patient’s discharge summary. We retrieved the SQL scripts from the GitHub website (https://github.com/MIT-LCP/mimic-code/tree/master/concepts/severity-scores) and used them to calculate the severity scores. Simplified Acute Physiology Score (SAPSIII), Sequential Organ Failure Assessment (SOFA) score, and Glasgow Coma Scale (GCS) ratings also were recorded during the first 24 hours of each patient’s ICU stay. Outcomes of patient conditions such as delirium, encephalopathy, mechanical ventilation, renal replacement therapy (Supplementary Table 5), and survival status were recorded. Relevant information was obtained about patients who were diagnosed with “sepsis,” “severe sepsis,” and “septic shock” on discharge, according to ICD-9 codes (Supplementary Table 6).

Patients were assigned to the hyperammonemia and non-hyperammonemia groups based on serum ammonia levels. They were also divided into conscious (GCS=15), sub-coma (GCS 9–14), and deep coma groups (GCS 3–8) based on GCS scores.
Statistical analysis

The statistical analysis compared the hyperammonemia and non-hyperammonemia groups. Data distribution was tested using the Shapiro-Wilk test. Continuous variables were expressed as means with standard deviation for normally distributed data, and for non-normally distributed data, medians (interquartile range [IQR]) were expressed. Categorical variables were represented as frequencies with percentage and compared using a chi-square test. Variables with missing data are relatively common in the MIMIC-III database and we replaced them with median values (Supplementary Material 1).

A non-parametric test (Mann-Whitney U or Kruskal-Wallis) was used for comparisons between the baseline characteristics and outcomes in the hyperammonemia and non-hyperammonemia groups. The relationship between serum ammonia and consciousness. Kaplan-Meier curves were analyzed using log-rank tests for comparison of hospital mortality between the hyperammonemia and non-hepatic hyperammonemia groups. A Cox regression model was used to screen for variables associated with hospital mortality in survivors versus non-survivors. A 2-tailed \( P<0.05 \) was considered statistically significant. All statistical analyses were performed with R software (version 3.4.3).

Results

Baseline patient characteristics

The patient inclusion flowchart is shown in Figure 1. A total of 2159 patients were tested for blood ammonia according to information in the MIMIC-III database. Using the inclusion and exclusion criteria, 1051 patients were identified for further screening. Of those patients, 265 were diagnosed with “sepsis,” “severe sepsis,” or “septic shock” on discharge, according to ICD-9 codes, and were enrolled in the study. The incidence of non-hepatic hyperammonemia was 40.4% with a 67.3% rate of 1-year mortality.

Information on the patients’ baseline characteristics, vital signs, laboratory parameters, infection type, microbiology type, and comorbid diseases is summarized in Table 1. There were 107 patients in the hyperammonemia group and 158 patients in the non-hyperammonemia group.

Patients in the hyperammonemia group had significantly more intestinal infections (23.4% vs. 13.3%, \( P=0.034 \)) and urinary tract infections (UTIs) (45.8% vs. 24.7%, \( P<0.001 \)) than patients in the non-hyperammonemia group. Patients with hyperammonemia were more likely to be infected with \textit{Escherichia coli} (42.1% vs. 22.8%, \( P=0.001 \)). Patients in the hyperammonemia group had lower GCS scores than patients in the non-hyperammonemia group (\( P=0.020 \)). No correlation was found between ammonia levels and respiratory infection, gastrointestinal bleeding, heart failure, kidney failure, or infection in other tissues by \textit{E. coli}. In addition, there were no significant differences in SAPSII or SOFA scores between the 2 groups.

Patient outcomes

Table 2 shows the outcomes in the hyperammonemia and non-hyperammonemia groups. As illustrated, a greater proportion of patients in the hyperammonemia group were diagnosed with delirium (15.9% vs. 8.2%, \( P=0.034 \)) and encephalopathy (37.4% vs. 19.6%, \( P=0.001 \)). Patients with hyperammonemia also had higher rates of short- and long-term mortality.
| Baseline variable                          | Hyperammonemia group n=107 | Non-hyperammonemia group n=158 | P value |
|-------------------------------------------|-----------------------------|---------------------------------|---------|
| Age, median (IQR)                         | 69.0 (56.1–76.6)            | 66.8 (55.6–75.6)                | 0.451   |
| Sex, n (%)                                |                             |                                 |         |
| Female                                    | 43 (40.2)                   | 67 (42.4)                       | 0.719   |
| Male                                      | 64 (59.8)                   | 91 (57.6)                       |         |
| Vital signs, median (IQR)/(x±s)           |                             |                                 |         |
| Heart rate (bpm)                          | 89.7±15.3                   | 91.6±16.6                       | 0.435   |
| Systolic blood pressure (mmHg)            | 111.7 (104.3–123.9)         | 110.1 (102.6–126.5)             | 0.654   |
| Diastolic blood pressure (mmHg)           | 56.1 (49.4–61.5)            | 57.7 (52.1–65.4)                | 0.058   |
| Respiratory rate (bpm)                    | 19.5 (16.8–22.8)            | 19.8 (16.7–23.9)                | 0.729   |
| Temperature (°C)                          | 36.9±0.76                   | 36.9±0.76                       | 0.537   |
| Laboratory parameters, median (IQR)       |                             |                                 |         |
| Alanine aminotransferase (IU/L)           | 23 (15–35)                  | 24 (12–39)                      | 0.922   |
| Aspartate aminotransferase (IU/L)         | 32 (21–52.0)                | 36 (23–52.3)                    | 0.468   |
| Creatinine (mg/dL)                        | 1.7 (1.0–2.8)               | 1.5 (1.0–2.6)                   | 0.355   |
| Hemoglobin(g/dL)                          | 9.3 (8.2–10.7)              | 9.2 (8.4–10.5)                  | 0.917   |
| Platelet (×10^9/L)                        | 197 (121–275)               | 167.0 (104.8–244.5)             | 0.091   |
| Partial thromboplastin time(s)            | 36.3 (29.8–46.9)            | 38.4 (30.5–49.9)                | 0.341   |
| International normalized ratio             | 1.4 (1.2–1.8)               | 1.4 (1.2–1.8)                   | 0.996   |
| Prothrombin time(s)                       | 15.6 (13.8–19.0)            | 15.7 (13.9–19.0)                | 0.822   |
| Blood urea nitrogen (mg/dL)               | 36 (22–55)                  | 31 (18–57)                      | 0.255   |
| White blood cell count (×10^9/L)          | 12.6 (8.6–18.2)             | 13.1 (8.9–19.1)                 | 0.373   |
| Ammonia (µmol/L)                          | 63 (46–131)                 | 24 (18–30)                      | <0.001  |
| Infection type, n (%)                     |                             |                                 |         |
| Intestinal infection                      | 25 (23.4)                   | 21 (13.3)                       | 0.034   |
| Urinary tract infection                   | 49 (45.8)                   | 39 (24.7)                       | <0.001  |
| Lung infection                            | 63 (58.9)                   | 86 (54.4)                       | 0.474   |
| Microbiology type, n (%)                  |                             |                                 |         |
| Pseudomonas aeruginosa                    | 21 (19.6)                   | 32 (20.3)                       | 0.900   |
| Klebsiella pneumonia                      | 22 (20.6)                   | 44 (27.8)                       | 0.178   |
| Viridans streptococci                     | 0 (1)                       | 8 (5.1)                         | 0.018   |
| Stenotrophomonas maltophilia              | 10 (9.3)                    | 9 (5.7)                         | 0.259   |
| Pneumocysts carinii positive              | 1 (0.9)                     | 0 (0)                           | 0.223   |
| Staphylococcus, coagulate negative        | 45 (42.1)                   | 82 (51.9)                       | 0.116   |
| Staphylococcus aureus coagulate positive  | 37 (34.6)                   | 62 (39.2)                       | 0.442   |
| Positive for methicillin-resistant Staphylococcus aureus | 10 (9.3) | 18 (11.4) | 0.595 |
| Enterococcus sp.                          | 30 (28.0)                   | 43 (27.2)                       | 0.883   |
| Enterococcus faecium                      | 9 (8.4)                     | 21 (13.3)                       | 0.219   |

Table 1. Baseline characteristics of the hyperammonemia and non-hyperammonemia groups.
### Table 1 continued. Baseline characteristics of the hyperammonemia and non-hyperammonemia groups.

|                        | Hyperammonemia group n=107 | Non-hyperammonemia group n=158 | P value |
|------------------------|-----------------------------|---------------------------------|---------|
| **Gram negative rod(s)** |                             |                                 |         |
|                        | 22 (20.6)                   | 47 (29.7)                       | 0.095   |
| **Escherichia coli**    | 45 (42.1)                   | 36 (22.8)                       | 0.001   |
| **Clostridium difficile** |                            | 10 (9.3)                         | 0.262   |
| **Bacteroides fragilis group** |                        | 6 (5.6)                            | 0.487   |
| **Aspergillus fumigatus** |                             | 4 (3.7)                             | 0.014   |
| **Yeast**               | 61 (57.0)                   | 90 (57.0)                        | 0.994   |
| **Candida albicans**    | 20 (18.7)                   | 24 (15.2)                        | 0.452   |
| **Comorbid disease, n (%)** |                           |                                  |         |
| Gastrointestinal bleeding | 12 (11.2)                   | 33 (20.9)                        | 0.004   |
| Heart failure           | 77 (72.0)                   | 120 (75.9)                       | 0.466   |
| Kidney failure          | 32 (29.9)                   | 50 (31.6)                        | 0.764   |
| **Score system, median (IQR)** |                        |                                   |         |
| SAPSII                  | 42 (34–52)                  | 40 (30–53)                       | 0.400   |
| SOFA                    | 6.0 (4.0–8.0)               | 6.0 (4.0–9.0)                    | 0.422   |
| GCS                     | 14.0 (9.0–15.0)             | 15 (13–15)                       | 0.020   |

IQR – interquartile range; SAPSII – Simplified Acute Physiology Score; SOFA – Sequential Organ Failure Assessment score; GCS – Glasgow Coma Scale. P<0.05 indicates statistical significance.

### Table 2. Outcomes in the hyperammonemia and non-hyperammonemia groups.

| Outcome                  | Hyperammonemia group n=107 | Non-hyperammonemia group n=158 | P value |
|--------------------------|-----------------------------|---------------------------------|---------|
| Mechanical ventilation, n (%) |                            |                                 |         |
| Renal replacement therapy, n (%) |                        |                                  |         |
| Delirium, n (%)           |                            |                                 |         |
| Encephalopathy, n (%)     |                            |                                 |         |
| Length of stay, median (IQR) |                        |                                  |         |
| In ICU                    | 4.0 (2.1–13.3)             | 5.0 (2.1–13.7)                  | 0.748   |
| In hospital               | 14 (6–28)                  | 14 (7–28)                       | 0.726   |
| Mortality, n (%)          |                            |                                 |         |
| Hospital mortality        | 64 (59.8)                  | 68 (43.0)                       | 0.007   |
| 30-day                    | 51 (47.7)                  | 55 (34.8)                       | 0.036   |
| 90-day                    | 66 (61.7)                  | 69 (43.7)                       | 0.004   |
| 1-year                    | 72 (67.3)                  | 78 (49.4)                       | 0.004   |

ICU – intensive care unit; IQR – interquartile range. P<0.05 indicates statistical significance.
Ammonia was an independent prognostic predictor in patients with sepsis.

Patients in the hyperammonemia group had worse survival rates (in-hospital, 90-day, and 1-year mortality) (Figure 2). Furthermore, univariate and multivariate Cox analysis was performed of baseline variables (age and sex) and results of laboratory tests (alanine aminotransferase, aspartate aminotransferase, creatinine, blood urea nitrogen, hemoglobin, platelet count, partial thromboplastin time, international normalized ratio, prothrombin time, white blood cell count, and ammonia). The factors significantly correlated with survival were adjusted for in the multivariate analysis. The analysis revealed that ammonia remained an independent prognostic factor in patients with sepsis. ($P<0.01$ or $P<0.05$) (Table 3).

### Table 3. Univariate and multivariate analysis of risk factors for hospital mortality.

|                      | Univariate analysis |                      | Multivariate analysis |                      |
|----------------------|---------------------|----------------------|-----------------------|---------------------|
|                      | RR                  | 95.0% CI             | RR                    | 95.0% CI            |
|                      | Lower               | Upper                | $P$ value             | Lower               | Upper | $P$ value |
| Age, median (IQR)   | 1.015               | 1.003                | 1.028                 | 0.015               | 1.014 | 1.002 | 1.027 | 0.027 |
| Sex n (%)            | 0.955               | 0.671                | 1.360                 | 0.799               |       |       |       |       |
| Laboratory parameters, median (IQR) |
| Alanine aminotransferase (IU/L) | 1.002 | 0.997 | 1.008 | 0.404 |
| Aspartate aminotransferase (IU/L) | 1.001 | 0.999 | 1.003 | 0.425 |
| Creatinine (mg/dL)   | 1.089               | 1.011                | 1.172                 | 0.024               | 1.072 | 0.994 | 1.155 | 0.071 |
| Blood urea nitrogen (mg/dL) | 1.001 | 0.995 | 1.006 | 0.839 |
| Platelet ($\times 10^9$/L) | 0.999 | 0.998 | 1.001 | 0.390 |
| Partial thromboplastin time(s) | 0.998 | 0.991 | 1.004 | 0.512 |
| International normalized ratio | 1.072 | 0.921 | 1.247 | 0.370 |
| Prothrombin time     | 1.002               | 0.988                | 1.017                 | 0.745               |       |       |       |       |
| White blood cell count ($\times 10^9$/L) | 0.997 | 0.981 | 1.013 | 0.689 |
| Ammonia (umol/L)     | 1.010               | 1.006                | 1.014                 | <0.001              | 1.009 | 1.006 | 1.013 | <0.001 |

CI – confidence interval; IQR, interquartile range; RR – relative risk. $P<0.05$=statistically significant.

### Figure 2. Probability of mortality curve for patients with sepsis by ammonia levels.

(A) Hospital mortality. (B) Ninety-day survival. (C) One-year mortality. $P$ values were calculated using log-rank and Mantel tests. $P<0.05$ was considered statistically significant.

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Receiver operating characteristic curves of ammonia indices for predicting mortality

To further confirm the reliability of ammonia, we plotted the area under the receiver operating characteristic (ROC) curve for 90-day and 1-year survival, and in-hospital mortality. The discriminative ability of ammonia levels based on the ROC curve analysis was 0.625 for in-hospital mortality, 0.620 for 90-day survival, and 0.624 for 1-year survival (Figure 3).

Relationship between serum ammonia and consciousness

Patients were divided into conscious (n=109), sub-coma (n=112), and deep coma groups (n=44) based on GCS score. As shown in Figure 4, patients with lower GCS scores had higher serum ammonia levels. The serum ammonia levels were highest in the deep coma group, compared with the other 2 groups (P<0.001), and they were significantly higher in the sub-coma group than in the conscious group (P<0.001) (Figure 4).

Discussion

Our study demonstrated that the incidence of non-hepatic hyperammonemia is 40.4% in patients with sepsis and the incidence of sepsis with encephalopathy in patients with non-hepatic hyperammonemia is 37.4%. Serum ammonia level may be a predictor of mortality in patients with sepsis who do not have hepatic disease.

In addition, we found that intestinal infection, UTI, and infections in other tissues caused by E. coli were risk factors for non-hepatic hyperammonemia in patients with sepsis.

We also found that the rate of hospital mortality in patients with sepsis who had non-hepatic hyperammonemia was 59.8%, which was significantly higher than in patients with sepsis who had normal serum ammonia levels (46.4%) [1]. A higher serum ammonia level may be a risk factor for mortality. Our results are consistent with the findings of Zhao et al., which showed that in patients with sepsis, an increased serum ammonia level on admission to the emergency department was correlated...
with an increased rate of mortality at 28 days. Our study explored mortality levels for up to 1 year, and we found that serum ammonia is an independent risk factor for long-term prognosis in patients with sepsis. In a case series, McCaw et al. suggested that higher serum ammonia levels are related to adverse clinical outcomes, which correlates with our findings. However, Zhao et al. showed that serum ammonia levels had a robust ability to predict the 28-day mortality rate in patients with sepsis, with an area under the ROC curve of 0.813, which is in contrast to our findings. That discrepancy may be attributable to differences in basic patient characteristics between the 2 studies. It suggests that serum ammonia level may be a new prognostic marker for patients with sepsis.

An interesting finding in our study is that non-hepatic hyperammonemia may be associated with an increased risk of SAE [12]. SAE is mainly characterized by symptoms of delirium with changes in a patient’s consciousness, and it also can lead to coma [13]. Our study demonstrated that patients with hyperammonemia had lower GCS scores. In the absence of previous cerebrovascular and encephalopathic brain disease, SAE is more likely to occur as the serum ammonia level increases. SAE is a diffuse brain dysfunction that occurs secondary to sepsis in the body without overt infection of the central nervous system. Its pathogenesis is multifaceted and is attributed to a combination of astrocyte swelling, an increase in glutamine synthesis, and a disproportionate ratio of aromatic amino acids to branched chain amino acids [14–16]. Based on our study results, we hypothesize that non-hepatic hyperammonemia may be associated with SAE. Unfortunately, in our study, some primary brain diseases (such as cerebral hemorrhage and cerebral infarction) and some secondary brain diseases (such as metabolic encephalopathy and pulmonary encephalopathy) were not excluded. The association between non-hepatic hyperammonemia and SAE needs to be validated in future well-designed experimental trials.

Intestinal infection, UTI, and infection of other tissues by E. coli may be risk factors for non-hepatic hyperammonemia in patients with sepsis. Our results showed that the incidence of intestinal infections in the hyperammonemia group was 23.4% higher than in the non-hyperammonemia group. This is consistent with research by Wang et al., which found that in patients with infection-induced hepatic encephalopathy, levels of plasma ammonia were significantly higher in association with intestinal tract infection compared with other sites of infection. Their results, along with our findings, support the notion that intestinal infection is related to hyperammonemia [17]. A possible explanation for the link between intestinal infection and non-hepatic hyperammonemia is intestinal flora. Colonic bacteria have been known to produce ammonia from amino acid deamination or via urease, the hydrolysis of urea into carbon dioxide and ammonia [18]. When the body develops sepsis, the composition of intestinal microbes changes, due to factors such as antibiotic usage, systemic inflammation, and intestinal leakage [19]. In the patient’s feces, the composition of the microbial components changes rapidly, the microbial diversity is largely lost, and the proportion of anaerobic bacteria significantly reduced and of Enterobacteriaceae increased [20]. Ammonia production is increased by converting nitrate to nitrite, and subsequently to ammonia [21].

Our results are consistent with previous studies, in which an increase in ammonia was associated with higher rates of infection by Enterobacteriaceae [3, 13, 22]. Therefore, serum ammonia should be measured when risk factors are present, such as intestinal infection or infection by E. coli. Our study showed that UTI is significantly associated with non-hepatic hyperammonemia in patients with sepsis, which is in line with the literature [23–25]. The possible explanation for the link between non-hepatic hyperammonemia and UTI is urease-producing bacteria and distal renal tubular acidosis [26]. With the entry of urea into the urinary tract, urease-producing bacteria form “ammonia,” which results in alkalization of the urine. The pH of the urine, when relatively high compared with that of the blood, enhances the diffusion of “ammonia” into the bloodstream [27, 28].

Another plausible explanation for the linkage between hyperammonemia and UTI is distal renal tubular acidosis. Severe UTIs occasionally are accompanied by altered distal renal tubular function, which results in reduced bicarbonates, and in turn, leads to increased renal “ammonia” production [29]. The last explanation could be urinary retention associated with a neurogenic bladder. As the pressure in the bladder increases, the area of the bladder expands and promotes drainage of more ammonia directly into the inferior vena cava via the internal iliac veins [30]. Therefore, in patients with UTIs, serum ammonia levels should be closely monitored and timely measures taken to reduce them.

Several limitations of the present study must be acknowledged. First, the result suggests a link between higher serum ammonia levels and lower GCS scores. Because of the nature of the retrospective analysis, the onset times of coma were not always available or documented, and some patients with primary and secondary encephalopathy in this study were not excluded. Therefore, whether there is a causal relationship between ammonia and SAE cannot be determined based on our results. Second, due to the limitations of the database, information was missing on some clinical variables, such as bilirubin, albumin, and intravenous nutrition. Inclusion of those data may have led to a more comprehensive understanding of the role of other biomarkers in sepsis with non-hepatic hyperammonemia. Third, our cohort study used ICD-9 diagnostic codes for sepsis, severe sepsis, and septic shock, but the
concept of severe sepsis was eliminated in Sepsis 3.0, which may have led to bias in our research results.

**Conclusions**

Non-hepatic hyperammonemia is associated with mortality in patients with sepsis. The present study was essentially a pilot that requires validation. We recommend that serum ammonia levels be measured in patients who have risk factors, such as intestinal infection, UTI, and *E. coli* infection. Infection caused by *E. coli* is a potential biomarker for sepsis in patients who have non-hepatic hyperammonemia. Our study also demonstrated a correlation between non-hepatic hyperammonemia and an increased risk of SAE.

**Conflict of interests**

None.

**Availability of data and materials**

The MIMIC-III database (version 1.4) is publically available from https://mimic.physionet.org/. Any researcher who adheres to the data use requirements is permitted access to the database.

**Ethics approval and consent to participate**

The use of the database was approved by the Massachusetts Institute of Technology (Cambridge, Massachusetts, U.S.A.) and the Institutional Review Boards of Beth Israel Deaconess Medical Center (Boston, Massachusetts, U.S.A.).

**Conflict of interests**

None.

**Supplementary Data**

**Supplementary Table 1.** Exclusion of patients with acute and chronic liver disease from the MIMIC-III database according to International Classification of Diseases, Ninth Revision codes.

| ICD9-code | Description |
|-----------|-------------|
| 700       | Hepatitis A with coma |
| 0701      | Viral hepatitis A without mention of hepatic coma |
| 07020     | Viral hepatitis B with hepatic coma, acute or unspecified, without mention of hepatitis delta |
| 07021     | Viral hepatitis B with hepatic coma, acute or unspecified, with hepatitis delta |
| 07022     | Chronic viral hepatitis B with hepatic coma without hepatitis delta |
| 07023     | Chronic viral hepatitis B with hepatic coma with hepatitis delta |
| 07030     | Viral hepatitis B without mention of hepatic coma, acute or unspecified, without mention of hepatitis |
| 07031     | Viral hepatitis B without mention of hepatic coma, acute or unspecified, with hepatitis delta |
| 07032     | Chronic viral hepatitis B without mention of hepatic coma without mention of hepatitis delta |
| 07033     | Chronic viral hepatitis B without mention of hepatic coma with hepatitis delta |
| 07041     | Acute hepatitis C with hepatic coma |
| 07042     | Hepatitis delta without mention of active hepatitis B disease with hepatic coma |
| 07043     | Hepatitis E with hepatic coma |
| 07044     | Chronic hepatitis C with hepatic coma |
| 07049     | Other specified viral hepatitis with hepatic coma |
| 07051     | Acute hepatitis C without mention of hepatic coma |
| 07052     | Hepatitis delta without mention of active hepatitis B disease or hepatic coma |
| 07053     | Hepatitis E without mention of hepatic coma |
| 07054     | Chronic hepatitis C without mention of hepatic coma |
| 07059     | Other specified viral hepatitis without mention of hepatic coma |
| 0706      | Unspecified viral hepatitis with hepatic coma |
Supplementary Table 1 continued. Exclusion of patients with acute and chronic liver disease from the MIMIC-III database according to International Classification of Diseases, Ninth Revision codes.

| ICD9-code | Description                                                                 |
|-----------|------------------------------------------------------------------------------|
| 07070     | Unspecified viral hepatitis C without hepatic coma                           |
| 07071     | Unspecified viral hepatitis C with hepatic coma                              |
| 0709      | Unspecified viral hepatitis without mention of hepatic coma                  |
| 5712      | Alcoholic cirrhosis of liver                                                 |
| 5713      | Alcoholic liver damage, unspecified                                          |
| 57140     | Chronic hepatitis, unspecified                                               |
| 57141     | Chronic persistent hepatitis                                                  |
| 57142     | Autoimmune hepatitis                                                         |
| 57149     | Other chronic hepatitis                                                       |
| 5715      | Cirrhosis of liver without mention of alcohol                                |
| 5716      | Biliary cirrhosis                                                            |
| 5718      | Other chronic nonalcoholic liver disease                                     |
| 5719      | Unspecified chronic liver disease without mention of alcohol                 |
| 5722      | Hepatic encephalopathy                                                       |
| 5724      | Hepatorenal syndrome                                                         |
| 5730      | Chronic passive congestion of liver                                          |
| 5734      | Hepatic infarction                                                           |
| 5733      | Hepatitis, unspecified                                                       |
| 5731      | Hepatitis in viral diseases classified elsewhere                             |
| 5735      | Hepatopulmonary syndrome                                                     |
| 5738      | Other specified disorders of liver                                           |
| 5740      | Other sequelae of chronic liver disease                                      |
| 5749      | Other chronic liver disease                                                  |
| V0260     | Viral hepatitis carrier, unspecified                                          |
| V0261     | Hepatitis B carrier                                                          |
| V0269     | Other viral hepatitis carrier                                                |
| 86400     | Injury to liver without mention of open wound into cavity, unspecified injury|
| 86401     | Injury to liver without mention of open wound into cavity, hematoma and contusion|
| 86402     | Injury to liver without mention of open wound into cavity, laceration, minor  |
| 86403     | Injury to liver without mention of open wound into cavity, laceration, major  |
| 86405     | Injury to liver without mention of open wound into cavity laceration, unspecified|
| 86409     | Other injury to liver without mention of open wound into cavity               |
| 86410     | Injury to liver with open wound into cavity, unspecified injury               |
| 4560      | Esophageal varices with bleeding                                              |
| 4561      | Esophageal varices without mention of bleeding                                |
| 45620     | Esophageal varices in diseases classified elsewhere, with bleeding            |
| 45621     | Esophageal varices in diseases classified elsewhere, without mention of bleeding|
### Supplementary Table 2. Type of Microbiology type and org_itemid.

| org_itemid | Description                                      |
|------------|--------------------------------------------------|
| 80026      | Pseudomonas aeruginosa                           |
| 80155      | Staphylococcus, coagulase negative               |
| 80223      | Probable enterococcus                            |
| 8023       | Staph aureus coag +                              |
| 80155      | Staphylococcus, coagulase negative               |
| 80280      | Viridans streptococci                            |
| 80081      | Gram positive bacteria                            |
| 80075      | Yeast                                            |
| 80004      | Klebsiella pneumoniae                            |
| 80060      | Albicans                                         |
| 80254      | Candida albicans, presumptive identification      |
| 80058      | Gram negative rod(s)                             |
| 80260      | Positive for pneumocystis carinii                |
| 80002      | Escherichia coli                                 |
| 80053      | Enterococcus sp.                                 |
| 80293      | Positive for methicillin resistant staph aureus  |
| 80168      | Enterococcus faecium                             |
| 80139      | Clostridium difficile                            |
| 80112      | Bacteroides fragilis group                       |
| 80087      | Stenotrophomonas (xanthomonas) maltophilia       |
| 80066      | Aspergillus fumigatus                            |

### Supplementary Table 3. Type of infection and International Classification of Diseases, Ninth Revision codes.

| ICD9 code | Description                                                   |
|-----------|---------------------------------------------------------------|
| 845       | Intestinal infection due to *Clostridium difficile*           |
| 847       | Intestinal infection due to other gram-negative bacteria       |
| 88        | Intestinal infection due to other organism, not elsewhere classified |
| 90        | Infectious colitis, enteritis, and gastroenteritis            |
| 93        | Diarrhea of presumed infectious origin                       |
| 56081     | Intestinal or peritoneal adhesions with obstruction (postoperative) (postinfection) |
| 56982     | Ulceration of intestine                                       |
| 56983     | Perforation of intestine                                      |
| 5990      | Urinary tract infection                                       |

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**Supplementary Table 3 continued.** Type of infection and International Classification of Diseases, Ninth Revision codes.

| ICD9 code | Description |
|-----------|-------------|
| lung infection | |
| 322 | *Salmonella pneumonia* |
| 1160 | *Tuberculous pneumonia [any form], unspecified* |
| 1161 | *Tuberculous pneumonia [any form], bacteriological or histological examination not done* |
| 1162 | *Tuberculous pneumonia [any form], bacteriological or histological examination unknown (at present)* |
| 1163 | *Tuberculous pneumonia [any form], tubercle bacilli found (in sputum) by microscopy* |
| 1164 | *Tuberculous pneumonia [any form], tubercle bacilli not found (in sputum) by microscopy, but found by bacterial culture* |
| 1165 | *Tuberculous pneumonia [any form], tubercle bacilli not found by bacteriological examination, but tuberculosis confirmed histologically* |
| 413 | *Klebsiella pneumoniae* |
| 551 | *Postmeasles pneumonia* |
| 382 | *Pneumococcal septicemia* (*Streptococcus pneumoniae* septicemia) |
| 11505 | *Histoplasma caps pneumon* |
| 11515 | *Infection by Histoplasma duboisii, pneumonia* |
| 11595 | *Histoplasmosis, unspecified, pneumonia* |
| 730 | *Ornithosis with pneumonia* |
| 48249 | *Other Staphylococcus pneumonia* |
| 48281 | *Pneumonia due to anaerobes* |
| 48282 | *Pneumonia due to Escherichia coli* |
| 48283 | *Pneumonia due to other gram-negative bacteria* |
| 4800 | *Pneumonia due to adenovirus* |
| 4801 | *Pneumonia due to respiratory syncytial virus* |
| 4802 | *Pneumonia due to parainfluenza virus* |
| 4803 | *Pneumonia due to SARS-associated coronavirus* |
| 4808 | *Pneumonia due to other virus not elsewhere classified* |
| 4809 | *Viral pneumonia, unspecified* |
| 481 | *Pneumococcal pneumonia* (*Streptococcus pneumoniae* pneumonia) |
| 4820 | *Pneumonia due to Klebsiella pneumoniae* |
| 4821 | *Pneumonia due to Pseudomonas* |
| 4822 | *Pneumonia due to Hemophilus influenzae* (*H. influenzae*) |
| 48230 | *Pneumonia due to Streptococcus, unspecified* |
| 48231 | *Pneumonia due to Streptococcus, group A* |
| 48232 | *Pneumonia due to Streptococcus, group B* |
Supplementary Table 3 continued. Type of infection and International Classification of Diseases, Ninth Revision codes.

| ICD9 code | Description                                                |
|-----------|------------------------------------------------------------|
| 48239     | Pneumonia due to other Streptococcus                       |
| 48240     | Pneumonia due to Staphylococcus, unspecified               |
| 48241     | Methicillin susceptible pneumonia due to *Staphylococcus aureus* |
| 48242     | Methicillin resistant pneumonia due to *Staphylococcus aureus* |
| 48284     | Pneumonia due to Legionnaires’ disease                     |
| 48289     | Pneumonia due to other specified bacteria                   |
| 4829      | Bacterial pneumonia NOS Bacterial pneumonia, unspecified   |
| 4830      | Pneumonia due to mycoplasma pneumoniae                     |
| 4831      | Pneumonia due to chlamydia                                 |
| 4838      | Pneumonia due to other specified organism                   |
| 4841      | Pneumonia in cytomegalic inclusion disease                  |
| 4843      | Pneumonia in whoop cough                                   |
| 4845      | Pneumonia in anthrax                                       |
| 4846      | Pneum in aspergillosis                                     |
| 4847      | Pneumonia in other systemic mycoses                        |
| 4848      | Pneumonia in other infectious diseases classified elsewhere |
| 485       | Bronchopneumonia, organism unspecified                     |
| 486       | Pneumonia, organism unspecified                            |
| 4870      | Influenza with pneumonia                                   |
| 4871      | Influenza with other respiratory manifestations             |
| 4878      | Influenza with other manifestations                        |
| 48801     | Influenza due to identified avian influenza virus with pneumonia |
| 48802     | Influenza due to identified avian influenza virus with other respiratory manifestations |
| 48809     | Influenza due to identified avian influenza virus with other manifestations |
| 48811     | Influenza due to identified 2009 H1N1 influenza virus with pneumonia |
| 48812     | Influenza due to identified 2009 H1N1 influenza virus with other respiratory manifestations |
| 48819     | Influenza due to identified 2009 H1N1 influenza virus with other manifestations |
| 48881     | Influenza due to identified novel influenza A virus with pneumonia |
| 48882     | Influenza due to identified novel influenza A virus with other respiratory manifestations |
| 48899     | Influenza due to identified novel influenza A virus with other manifestations |
| 51630     | Idiopathic interstitial pneumonia, not otherwise specified |
| 51635     | Idiopathic lymphoid interstitial pneumonia                  |
| 51636     | Cryptogenic organizing pneumonia                           |
### Supplementary Table 3 continued. Type of infection and International Classification of Diseases, Ninth Revision codes.

| ICD9 code | Description |
|-----------|-------------|
| 51637     | Desquamative interstitial pneumonia |
| 5171      | Rheumatic pneumonia |
| 7700      | Congenital pneumonia |
| V066      | Need for prophylactic vaccination and inoculation against *Streptococcus pneumoniae* [pneumococcus] and influenza |
| 99731     | Ventilator associated pneumonia |
| 99732     | Postprocedural aspiration pneumonia |
| V0382     | Other specified vaccinations against *Streptococcus pneumoniae* [pneumococcus] |
| 1166      | Tuberculous pneumonia [any form], tubercle bacilli not found by bacteriological or histological examination, but tuberculosis confirmed by other methods [inoculation of animals] |
| 3453      | Grand mal status |

### Supplementary Table 4. Type of disease and International Classification of Diseases, Ninth Revision codes.

| Disease                       | ICD9-Code            | Description |
|-------------------------------|----------------------|-------------|
| Gastrointestinal bleeding     |                      |             |
| 5789                          | Hemorrhage of gastrointestinal tract, unspecified |
| 5780                          | Hematemesis          |
| 5781                          | Blood in stool       |
| 5693                          | Hemorrhage of rectum and anus |
| 4560                          | Esophageal varices with bleeding |
| 45620                         | Esophageal varices in diseases classified elsewhere, with bleeding |
| 53100                         | Acute gastric ulcer with hemorrhage, without mention of obstruction |
| 53101                         | Acute gastric ulcer with hemorrhage, with obstruction |
| 53120                         | Acute gastric ulcer with hemorrhage and perforation, without mention of obstruction |
| 53121                         | Acute gastric ulcer with hemorrhage and perforation, with obstruction |
| 53300                         | Acute peptic ulcer of unspecified site with hemorrhage, without mention of obstruction |
| 53320                         | Acute peptic ulcer of unspecified site with hemorrhage and perforation, without mention of obstruction |
| 53321                         | Acute peptic ulcer of unspecified site with hemorrhage and perforation, with obstruction |
| 53200                         | Acute duodenal ulcer with hemorrhage, without mention of obstruction |
| 53201                         | Acute duodenal ulcer with hemorrhage, with obstruction |
| 53220                         | Acute duodenal ulcer with hemorrhage and perforation, without mention of obstruction |
| 53221                         | Acute duodenal ulcer with hemorrhage and perforation, with obstruction |
| 53400                         | Acute gastrojejunal ulcer with hemorrhage, without mention of obstruction |
| 53401                         | Acute gastrojejunal ulcer, with hemorrhage, with obstruction |
| 53420                         | Acute gastrojejunal ulcer with hemorrhage and perforation, without mention of obstruction |
| 53421                         | Acute gastrojejunal ulcer with hemorrhage and perforation, with obstruction |
| 53501                         | Acute gastritis, with hemorrhage |
Supplementary Table 4 continued. Type of disease and International Classification of Diseases, Ninth Revision codes.

| Disease | ICD9-Code | Description                                                                 |
|---------|-----------|-----------------------------------------------------------------------------|
| Heart failure | 4280 | Congestive heart failure, unspecified |
|         | 4281 | Left heart failure |
|         | 42830 | Diastolic heart failure, unspecified |
|         | 42831 | Acute diastolic heart failure |
|         | 42832 | Chronic diastolic heart failure |
|         | 42833 | Acute on chronic diastolic heart failure |
|         | 42840 | Combined systolic and diastolic heart failure, unspecified |
|         | 42841 | Acute combined systolic and diastolic heart failure |
|         | 42842 | Chronic combined systolic and diastolic heart failure |
|         | 42843 | Acute on chronic combined systolic and diastolic heart failure |
| Kidney failure | 39891 | Rheumatic heart failure (congestive) |
|         | 5845 | Acute kidney failure with lesion of tubular necrosis |
|         | 5846 | Acute kidney failure with lesion of renal cortical necrosis |
|         | 5848 | Acute kidney failure with other specified pathological lesion in kidney |
|         | 5849 | Acute kidney failure, unspecified |
|         | 5852 | Chronic kidney disease, Stage II (mild) |
|         | 5853 | Chronic kidney disease, Stage III (moderate) |
|         | 5854 | Chronic kidney disease, Stage IV (severe) |
|         | 5855 | Chronic kidney disease, Stage V |
|         | 5856 | End stage renal disease |

Supplementary Table 5. Outcome of patients in the hyperammonemia and non-hyperammonemia groups and International Classification of Diseases, Ninth Revision codes.

| ICD9-code | Description                                                                 |
|-----------|-----------------------------------------------------------------------------|
| Delirium  | 29041 | Vascular dementia, with delirium |
|           | 29281 | Drug-induced delirium |
|           | 2930 | Delirium due to conditions classified elsewhere |
|           | 29043 | Vascular dementia, with depressed mood |
| Encephalopathy | 4372 | Hypertensive encephalopathy |
|           | 34982 | Toxic encephalopathy |
|           | 34831 | Metabolic encephalopathy |
|           | 34830 | Encephalopathy, unspecified |
|           | 34839 | Other encephalopathy |
**Supplementary Table 6.** Definition of sepsis based on International Classification of Diseases, Ninth Revision codes.

| ICD9-code | Description |
|-----------|-------------|
| 99591     | Sepsis      |
| 99592     | Severe sepsis |
| 78552     | Septic shock |

ICD-9 – International Classification of Diseases, Ninth Revision.

**References:**

1. Rudd KE, Johnson SC, Agea KM et al: Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. Lancet, 2020; 395(10219): 200–11
2. Lu X, Wang X, Gao Y et al: Efficacy and safety of corticosteroids for septic shock in immunocompromised patients: A cohort study from MIMIC. Am J Emerg Med, 2020 [Online ahead of print]
3. Kurtz CB, Millet YA, Puurunen MK et al: An engineered E. coli Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. Sci Transl Med, 2019; 11(475): eaau7975
4. Jawad I, Lukisch I, Rafnsson SB: Assessing available information on the burden of sepsis: Global estimates of incidence, prevalence and mortality. J Glob Health, 2012; 2(1): 010404
5. Chung HY, Wickel J, Brunkhorst FM, Geis C: Sepsis-associated encephalopathy: from delirium to dementia? J Clin Med, 2020; 9(3): 703
6. Ren C, Yao RQ, Zhang H et al: Sepsis-associated encephalopathy: A vicious cycle of immunosuppression. J Neuroinflammation, 2020; 17(1): 14
7. Hadjihami A, Arias N, Sheik M, Jalan R: Hepatic encephalopathy: A critical current review. Hepatol Int, 2018; 12(Suppl. 1): 135–47
8. Jacoby KI, Singh P, Prekker ME, Leatherman JW: Characteristics and outcomes of critically ill patients with severe hyperammonemia. J Crit Care, 2020; 56: 177–81
9. Sakusic A, Sabov M, Mccambridge AJ et al: Features of adult hyperammonemia not due to liver failure in the ICU. Crit Care Med, 2018; 46(9): e897–903
10. Zhao J, He Y, Xu P et al: Serum ammonia levels on admission for predicting sepsis patient mortality at D28 in the emergency department: A 2-center retrospective study. Medicine (Baltimore), 2020; 99(11): e19477
11. Numan Y, Jawad Y, Hizallah H et al: Ammonia vs. lactic acid in predicting positivity of microbial culture in sepsis: The ALPS pilot study. J Clin Med, 2018; 7(8): 182
12. Mazeraud A, Bozza FA, Sharshar T: Sepsis-associated encephalopathy is septic. Am J Respir Crit Care Med, 2018; 197(6): 698–99
13. Hassan AA, Ibrahim W, Subahi A, Mohamed A: ‘All that glitters is not gold’: Wen hyperammonemia is not from hepatic aetiology. BMJ Case Rep, 2017; 2017: bcr2017129441
14. Flati Kenston SS, Song X, Li Z, Zhao J: Mechanistic insight, diagnosis, and treatment of ammonia-induced hepatic encephalopathy. J Gastroenterol Hepatol, 2019; 34(1): 31–39
15. Ninan J, Feldman L: Ammonia levels and hepatic encephalopathy in patients with known chronic liver disease. J Hosp Med, 2017; 12(8): 659–61
16. Sivolap YP: Prevention and treatment of hepatic encephalopathy. Zh Nevrol Psihiatr Im S S Korsakova, 2017; 117(10): 144–47
17. Wang QM, Ji Q, Duan ZJ et al: A study on the position and etiology of infection in cirrhotic patients: A potential precipitating factor contributing to hepatic encephalopathy. Exp Ther Med, 2013; 6(2): 584–90
18. Vince A, Dawson AM, Park N, O’Grady F: Ammonia production by intestinal bacteria. Gut, 1973; 14(3): 171–77
19. Fay KT, Ford ML, Coopersmith CM: The intestinal microenvironment in sepsis. Biochim Biophys Acta Mol Basis Dis, 2017; 1863(10 Pt B): 2574–83
20. Haak BW, Wiersinga WJ: The role of the gut microbiota in sepsis. Lancet Gastroenterol Hepatol, 2017; 2(2): 135–43
21. Tiso M, Schechter AN: Nitrate reduction to nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions. PloS One, 2015; 10(3): e0119712
22. Marco-Marín C, Gil-Ortiz F, Pérez-Arellano I et al: A novel Two-domain architecture within the amino acid kinase enzyme family revealed by the crystal structure of Escherichia coli glutamate 5-kinase. J Mol Biol, 2007; 367(5): 1431–46
23. Nakamori H, Fujimura M, Shiraishi T et al: [A case of consciousness disturbance due to hyperammonemia associated with urinary tract infections]. Hinyokika Kyō, 2019; 65(5): 163–66 [in Japanese]
24. Hanai S, Iwata M, Terasawa T: Relapsing hypoglycaemia associated with hypocarnitinemia following treatment with cefcapene pivoxil in an elderly man. Intern Med, 2019; 58(19): 2891–94
25. Li GZ, Tio MC, Pak LM et al: Nonscirrhotic hyperammonemia after deceased donor kidney transplantation: A case report. Am J Transplant, 2019; 19(11): 3197–201
26. ClericiCt MM, Milani GP, Lava SAG et al: Hyperammonemia associated with distal renal tubular acidosis or urinary tract infection: A systematic review. Pediatr Nephrol, 2018; 33(3): 485–91
27. Goda T, Watanabe K, Kobayashi J et al: [A case of hyperammonemia with obstructive urinary tract infection by urease-producing bacteria.] Rinsho Shinkeigaku, 2017; 57(3): 130–33 [in Japanese]
28. Emura M, Tsuchihashi K, Shimizu Y et al: [A case of hyperammonemia caused by urinary tract infection due to urease-producing bacteria.] Hinyokika Kyō, 2016; 62(6): 421–25 [in Japanese]
29. Hsu KH, Cheng CH, Tseng MH et al: Hyperammonemia in distal renal tubular acidosis: A new case and review of the literature. Pediatr Neonatol, 2015; 56(6): 432–34
30. Oliver RM, Talbot S, Raman GV: Hyperammonaemic coma in ureterosigmoid urinary diversion. Postgrad Med J, 1989; 65(765): 502–4