ORIGINAL ARTICLE

Persistent or incident hyperammonemia is associated with poor outcomes in acute decompensation and acute-on-chronic liver failure

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Declaration of conflict of interest: The authors report no conflict of interest relevant to the present study.

Author contribution: Shalimar contributed to acquisition of data, draft writing, study concept, design, study supervision, statistical analysis, and critical revision of the manuscript. Gyanranjan Rout contributed to acquisition of data and drafting of the manuscript. Ramesh Kumar contributed to study concept and critical revision of the manuscript. Achintya D Singh contributed to draft writing and critical revision of the manuscript. Sanchit Sharma contributed to data acquisition and draft writing. Deepak Gunjan contributed to data acquisition. Anoop Saraya contributed to data acquisition. Baibaswata Nayak contributed to draft writing. Subrat K Acharya contributed to draft writing.
Guarantor of the article: Shalimar.

Abstract

Background and Aim: The effect of elevated ammonia on organ failures (OF), apart from hepatic encephalopathy, in patients with acute decompensation (AD) of cirrhosis and acute-on-chronic liver failure (ACLF) is unclear. We aimed to assess the effect of persistent or incident hyperammonemia on OF and outcomes in patients with AD and ACLF.

Methods: A total of 229 patients with ACLF and 83 with AD were included. Arterial ammonia was measured on day 1 and day 3 of admission. Persistent or incident hyperammonemia was defined as a level of ≥ 79.5 μmol/L on day 3. The changes in ammonia levels during the first 3 days were analyzed with respect to the complications and outcomes.

Results: At admission, the median level of arterial ammonia was higher in ACLF compared to AD patients (103 *vs* 86 μmol/L, $P < 0.001$). Persistent or incident hyperammonemia was noted in 206 (66.0%) patients and was more frequent in ACLF compared to AD patients (70.7 *vs* 53.0%, $P = 0.013$). Patients with persistent or incident hyperammonemia, compared to those without it, developed a higher proportion of new-onset OF during hospitalization involving liver ($P = 0.018$), kidney ($P = 0.001$), brain ($P = 0.005$), coagulation ($P = 0.036$), circulation ($P = 0.002$), and respiratory ($P = 0.003$) issues and had higher 28-day mortality (log-rank test, $P < 0.001$). After adjustment for chronic liver failure consortium ACLF score, persistent or incident hyperammonemia (hazard ratio, 3.174) was independently associated with 28-day mortality. The presence of infection was an independent predictor of persistent or incident hyperammonemia.

Conclusion: Persistent or incident hyperammonemia during first 3 days of hospitalization in patients with AD or ACLF is associated with increased risk of OF and death.

Introduction

Elevated arterial ammonia levels at the time of hospital admission,[1,2] as well as persistent arterial hyperammonemia (defined as ≥ 122 μmol/L for three consecutive days)[3] in patients with acute liver failure (ALF), is a harbinger of increased hepatic encephalopathy (HE), cerebral edema, and mortality risks. Hyperammonemia is associated with immune dysfunction and impaired neutrophil function, predisposing these patients to infection, and contributes to worsening of HE and poor outcomes.[4,5]

Similarly, higher levels of arterial ammonia have been reported in acute-on-chronic liver failure (ACLF) patients with HE.[6,7] HE is an independent predictor of poor outcomes in patients of cirrhosis with acute decompensation (AD) and ACLF.[8] An elevated ammonia level at admission is associated with poor outcomes in cirrhotic patients with AD.[7,9,10] In addition, an increase in ammonia levels within the first 24 h after admission has been reported to be independently associated with poor outcomes in ACLF.[6] Furthermore, hyperammonemia is associated with stellate cell activation[11] and sarcopenia, which independently

predict mortality in patients with liver cirrhosis.[12] The ammonia levels are dynamic, and the association between the early dynamicity of arterial ammonia and the complications and outcomes in patients with ACLF and AD is unclear. The factors associated with hyperammonemia in patients with ACLF or AD are even more obscure. We hypothesized that persistent or new-onset (incident) hyperammonemia in such patients during the initial 3 days of hospitalization would be associated with higher complications compared to those with lower or declining ammonia levels. Therefore, the present study was conducted to evaluate the association between the dynamic changes of ammonia levels over the first 3 days after admission, in patients of ACLF and AD, and the development of new organ failure (OF), the severity of ACLF, and 28-day outcome and also to identify predictors of hyperammonemia.

Methods

Consecutive patients with cirrhosis and AD and ACLF admitted to the Department of Gastroenterology at All India Institute of Medical Sciences, New Delhi, India from January 2012 to May 2018 were screened for inclusion in the study. Only patients with paired ammonia measurements on day 1 and day 3 of admission were included. Patients with incomplete data, suspicion of ALF, prominent portosystemic shunts, and severe comorbidities and those who refused to grant consent were excluded. Informed consent was obtained from patients or their nearest relatives in cases of altered sensorium. The institute's ethics committee approved the study.

Definitions. ACLF and AD were defined per the CANONIC study.[13] OF was determined on day 1 and days 3–7 using Chronic Liver Failure (CLIF)-OF score, and the grade of ACLF was defined based on the types and number of OF.[13] Hepatic encephalopathy (HE) was defined and graded as per West Heaven criteria.[14] Early HE was defined as grades 1 and 2, and advanced HE included grades 3 and 4. The chronic liver failure consortium (CLIF-C) ACLF score, Child-Turcotte-Pugh (CTP), and model for end-stage liver disease-sodium (MELD-Na) score were calculated on day 1 and between days 3 and 7 after admission.

Categorization of patients on the basis of serial ammonia levels. Hyperammonemia was defined using ammonia levels (≥79.5 μmol/L).[7] Patients were categorized into high (≥79.5 μmol/L) and low (<79.5 μmol/L) ammonia groups based on the day 1 ammonia values.[7] These groups were further classified into three subgroups based on ammonia levels on day 3:

Persistent or incident hyperammonemia: patients with baseline hyperammonemia (day 1) who still had elevated (≥79.5 μmol/L) ammonia levels on day 3 of admission or those with nonelevated (<79.5 μmol/L) ammonia levels at baseline but hyperammonemia on day 3.

Nonpersistent hyperammonemia: patients with baseline hyperammonemia who had a decrease in ammonia levels below 79.5 μmol/L on day 3.

No hyperammonemia: patients with nonelevated ammonia levels at baseline and on day 3.

Management protocol. All patients were managed according to standard management protocol, which included close monitoring, optimization of metabolic derangement, and intensive hemodynamic/organ support. Broad-spectrum antibiotics were given to those who developed an infection after inclusion, subsequent to the collection of blood, urine, and ascitic fluid for culture, and were modified as per the sensitivity reports. In cases with Hepatitis B virus (HBV) infection, oral antiviral drugs (entecavir or tenofovir) were used. Strict abstinence was maintained from alcohol consumption. Patients who developed spontaneous bacterial peritonitis and hepatorenal syndrome were managed as per the recommendations.[15] Lactulose and rifaximin were used in patients with HE. Renal replacement therapy and ventilator support were provided whenever required. Appropriate treatment for HE was instituted. Inotropes were administered to maintain mean arterial pressure above 60 mmHg.

Determination of ammonia levels. Arterial ammonia samples in heparinized plasma at admission were obtained in fasting state on day 1 and day 3 postadmission. Samples were collected in a heparinized syringe and transported in ice packs to the laboratory for measurement. Arterial ammonia was estimated using an enzymatic method (Randox Lab Ltd., Crumlin, UK).[2]

Statistical analysis. The normally distributed variables were expressed as mean ± SD and continuous variables with skewed distribution as median (interquartile range [IQR]). Categorical data were presented as proportions. Normally distributed continuous variables were compared among two groups using an independent *t*-test or Mann–Whitney U test as appropriate. Chi-square or Fisher's exact test was used for categorical variables. Predictors of survival were assessed using the Cox proportional hazard model. Predictors of hyperammonemia were assessed using the logistic regression model. All variables significantly associated with mortality on univariate analysis (with $P < 0.10$) were entered in the multivariable model for adjustment. Survival curves were calculated using the Kaplan–Meier method and compared with the log-rank test. A *P*-value of <0.05 was considered
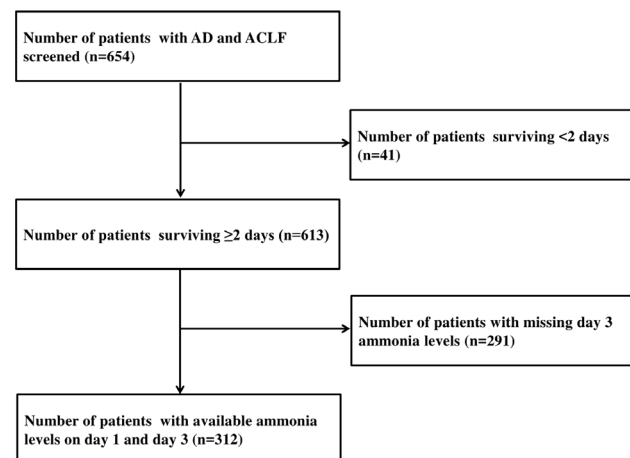
Figure 1 Flow chart of patients included in the study. ACLF, acute-on-chronic liver failure; AD, acute decompensation.

Table 1 Comparison of baseline characteristics of cirrhosis in patients with acute decompensation who survived and those who died (*n* = 312)

Variable	Total (*n* = 312)	Survived (*n* = 128)	Died (*n* = 184)	*P* value
Age (years)	41 (33–49)	40 (32–48)	41 (34–50)	0.442
Males: females	255 (81.7%): 57 (18.3%)	99 (77.3%): 29 (22.7%)	156 (84.8%): 28 (15.2%)	0.103
Hemoglobin (g/dL)	9.0 (7.2–10.2)	9.1 (7.3–11.0)	8.9 (7.2–10.0)	0.253
Platelet count ($\times10^3$/mm^3)	98 (62–141)	100 (67–141)	93 (60–142)	0.316
Total leucocyte count ($\times10^3$/mm^3)	10.5 (7.0–17.1)	9.4 (6.3–15.0)	12.2 (7.5–18.5)	0.003
Bilirubin (mg/dL)	13.3 (6.2–24.5)	10.0 (5.4–21.0)	15.2 (7.0–25.0)	0.013
Alanine aminotransferase (IU/L)	50 (31–86)	50 (30–83)	51 (31–91)	0.628
Albumin (g/dL)	2.5 (2.1–2.9)	2.6 (2.2–3.0)	2.5 (2.0–2.9)	0.019
Creatinine (mg/dL)	1.5 (0.8–2.8)	1.3 (0.7–2.2)	1.8 (0.9–3.2)	<0.001
Ammonia (μmol/L)	98 (75–126)	87 (68–115)	104 (84–130)	<0.001
INR	2.4 (1.9–3.1)	2.2 (1.8–2.7)	2.6 (1.9–3.4)	<0.001
Organ failure				
Liver	169 (54.2%)	56 (43.8%)	113 (61.4%)	0.003
Kidney	126 (40.4%)	36 (28.1%)	90 (48.9%)	<0.001
Brain	87 (27.9%)	21 (16.4%)	66 (35.9%)	<0.001
Coagulation	140 (44.9%)	44 (34.4%)	96 (52.2%)	0.003
Circulation	47 (15.1%)	18 (14.1%)	29 (15.8%)	0.749
Respiratory	68 (21.8%)	13 (10.2%)	55 (29.9%)	<0.001
Prognostic scores				
CTP	12 (11–13)	12 (10–13)	12 (11–14)	<0.001
MELD-Na	31.8 (26.1–37.9)	29.3 (23.7–33.4)	34.2 (27.7–39.7)	<0.001
CLIF-C ACLF	45.5 (38.3–52.9)	43.5 (38.8–49.3)	50.9 (44.4–56.7)	<0.001
Etiology acute				0.677
Alcohol	136 (43.6%)	56 (43.8%)	80 (43.5%)	
Bacterial infection	53 (17.0%)	18 (14.1%)	35 (19.0%)	
Other/unidentified	22 (7.1%)	8 (6.3%)	14 (7.6%)	
Hepatitis virus A/B/E	33 (10.6%)	16 (12.5%)	17 (9.2%)	
Cryptogenic	68 (21.8%)	30 (23.4%)	38 (20.7%)	
Etiology chronic				0.408
Viral	38 (12.2%)	18 (14.1%)	20 (10.9%)	
Alcohol	191 (61.2%)	77 (60.2%)	114 (62.0%)	
Cryptogenic	42 (13.5%)	20 (15.6%)	22 (12.0%)	
Others	41 (13.1%)	13 (10.2%)	28 (15.2%)	
No ACLF: ACLF Grade 1/2/3	83 (26.6%): 32 (10.3%): 91 (29.2%): 106 (34.0%)	56 (43.8%): 16 (12.5%): 33 (25.8%): 23 (18.0%)	27 (14.7%): 16 (8.7%): 58 (31.5%): 83 (45.1%)	<0.001

All data are expressed as *n* (%) or median (interquartile range) unless otherwise specified.
ACLF, acute-on-chronic liver failure; CLIF-C, chronic liver failure consortium; CTP, Child-Turcotte-Pugh; INR, International normalized ratio; MELD-Na, model for end-stage liver disease-sodium.

statistically significant. Data were analyzed using IBM SPSS Statistics software (version 20.0, Chicago, IL, USA) and MedCalc software (version 15.11.4, MedCalc Software, Ostend, Belgium).

Results

A total of 654 patients with a diagnosis of ACLF or AD were screened between January 2012 and May 2018 for inclusion in the study. Of these, 41 patients survived for less than 2 days, and in 291 patients, day 3 ammonia levels were not available. Finally, 312 subjects, which included 229 ACLF and 83 AD patients, formed the study cohort (Fig. 1). The patients included in the study had lower platelet count, total leucocyte count, alanine aminotransferase levels, and frequency of respiratory failure compared to patients who were not included. In addition, the CLIF-C ACLF score was lower, whereas alcohol as an acute precipitant was more common in included patients. The remaining

characteristics were similar between patients included and not included in the study (Table S1).

The baseline demographic, clinical, and laboratory characteristics of 312 patients with ACLF or AD are shown in Table 1. On day 1, the median ammonia levels were higher in ACLF compared with AD patients (103 *vs* 86 μmol/L, *P* < 0.001). Overall, 128 (41.0%) patients survived, and 184 (59.0%) died at 28 days. Patients who died had higher total leucocyte count (*P* = 0.003), serum bilirubin (*P* = 0.013), serum creatinine (*P* < 0.001), international normalized ration (INR) (*P* < 0.001), and lower albumin levels (*P* = 0.019) than those who survived (Table 1). The median arterial ammonia level in patients who died was significantly higher than those who survived (104 [84–130] μmol/L *vs* 87 [68–115] μmol/L), *P* < 0.001. Except for circulatory failure, all OFs—liver, kidney, brain, coagulation, and respiratory failures; ACLF grades; and prognostic scores (CTP, MELD, CLIF-C ACLF) were significantly higher at

Table 2 Comparison of complications and outcomes on admission day in patients with day 1 ammonia ≥79.5 μmol/L and those with ammonia <79.5 μmol/L

	Ammonia <79.5 μmol/L (*n* = 91)	Ammonia ≥79.5 μmol/L (*n* = 221)	*P* value
Organ failure at day 1			
Liver	46 (50.5%)	123 (55.7%)	0.454
Kidney	30 (33.0%)	96 (43.4%)	0.099
Brain	10 (11.0%)	77 (34.8%)	<0.001
Coagulation	36 (39.6%)	104 (47.1%)	0.260
Circulatory	11 (12.1%)	36 (16.3%)	0.388
Respiratory	6 (6.6%)	62 (28.1%)	<0.001
Infection (no infection: infection at admission or within 48 h: infection after 48 h)	32 (35.2%): 40 (43.9%): 19 (20.9%)	58 (26.2%): 124 (56.1%): 39 (17.6%)	0.139
No ACLF: ACLF Grade 1/2/3 on day 1	36 (39.6%): 14 (15.4%): 23 (25.3%): 18 (19.8%)	47 (21.2%): 18 (8.1%): 68 (30.8%): 88 (39.8%)	<0.001
28-day mortality	36 (39.6%)	148 (67.0%)	<0.001

All data are expressed as *n* (%) or median (interquartile range) unless otherwise specified.
ACLF, acute-on-chronic liver failure.

baseline in patients who died compared to those who survived. For predicting 28-day mortality, CLIF-C ACLF had the highest Area Under the Receiver Operating Characteristics (AUROC), 0.703 (0.638–763), compared to MELD-Na, 0.615 (0.547–0.680), *P* = 0.033 and CTP 0.590 (0.522–0.656), *P* = 0.030.

Comparison of complications and outcomes between patients with and without hyperammonemia on day 1. Patients with hyperammonemia had a higher proportion of brain failure (34.8 *vs* 11.0%, *P* < 0.001) and respiratory failure (28.1 *vs* 6.6%, *P* < 0.001) compared to patients without hyperammonemia. In addition, these patients had higher grades of ACLF on day 1 (*P* < 0.001) and higher 28-day mortality (67.0 *vs* 39.6%, *P* < 0.001) compared to patients with ammonia <79.5 μmol/L (Table 2). There was no difference in the proportion of patients with infection (*P* = 0.139) between the two groups.

Comparison of complications and outcomes between patients with and without hyperammonemia on day 3. Compared to patients with without hyperammonemia on day 3, patients with hyperammonemia on day 3 had a higher proportion of new-onset OF during days 3–7 of hospital stay: liver failure (22.1 *vs* 4.2%, *P* = 0.007), kidney failure (22.6 *vs* 2.8%, *P* < 0.001), brain failure (29.1 *vs* 11.0%, *P* = 0.002), coagulation failure (54.1 *vs* 36.1%, *P* = 0.026), circulatory failure (28.8 *vs* 10.5%, *P* = 0.001), and respiratory failure (29.8 *vs* 10.8%, *P* < 0.001) (Table 3). In addition, these patients had higher ACLF grades on day 3 (*P* < 0.001) and higher 28-day mortality (70.4 *vs* 36.8%, *P* < 0.001). Progression from AD to ACLF occurred in a higher proportion (59.1 *vs* 23.1%) of patients with hyperammonemia on day 3 compared to those without hyperammonemia on day 3. New-onset infections were more common among patients with hyperammonemia compared to patients without hyperammonemia.

Table 3 Comparison of complications and outcomes at days 3–7 in patients with day 3 ammonia ≥79.5 μmol/L and those with ammonia <79.5 μmol/L

	Ammonia <79.5 μmol/L on day 3 (*n* = 106)	Ammonia ≥79.5 μmol/L on day 3 (*n* = 206)	*P* value
New OF at days 3–7 among those without OF on day 1			
Liver (23/143)	2/48 (4.2%)	21/95 (22.1%)	0.007
Kidney (28/186)	2/71 (2.8%)	26/115 (22.6%)	<0.001
Brain (49/225)	10/91 (11.0%)	39/134 (29.1%)	0.002
Coagulation (82/172)	22/61 (36.1%)	60/111 (54.1%)	0.026
Circulatory (59/265)	10/95 (10.5%)	49/170 (28.8%)	0.001
Respiratory (55/244)	10/93 (10.8%)	45/151 (29.8%)	<0.001
New infection (58/148)	17/60 (28.3%)	41/88 (46.6%)	0.027
AD progression to ACLF (35/83)	9/39 (23.1%)	26/44 (59.1%)	0.002
No ACLF: ACLF Grade 1/2/3 (days 3–7)	43 (40.6%): 9 (8.5%): 26 (24.5%): 28 (26.4%)	22 (10.7%): 17 (8.3%): 45 (21.8%): 122 (59.2%)	<0.001
28-day mortality	39 (36.8%)	145 (70.4%)	<0.001

All data are expressed as *n* (%) unless otherwise specified.
ACLF, acute-on-chronic liver failure; AD, acute decompensation; OF, organ failure.

Table 4 Comparison of complications and outcomes on days 3–7 among patients with nonpersistent, persistent/incident, and no hyperammonemia

	Persistent or incident hyperammonemia (*n* = 206)	Nonpersistent hyperammonemia (*n* = 46)	No hyperammonemia (*n* = 60)	*P* value
New organ failure at days 3–7				
Liver (23/143)	21/95 (22.1%)	0	2/28(7.1%)	0.018
Kidney (28/186)	26/115 (22.6%)	1/32 (3.1%)	1/39 (2.6%)	0.001
Brain (49/225)	39/134 (29.1%)	4/36 (11.1%)	6/55 (10.9%)	0.005
Coagulation (82/172)	60/ 111 (54.1%)	7/26 (26.9%)	15/35 (42.9%)	0.036
Circulatory (55/265)	49/170 (28.8%)	3/40 (7.5%)	7/55 (12.7%)	0.002
Respiratory (55/244)	45/151 (29.8%)	4/37 (10.8%)	6/56 (10.7%)	0.003
New-onset infections (58/148)	41/88 (46.6%)	7/26 (26.9%)	10/34 (29.4%)	0.081
ACLF on days 3–7/No ACLF on day 1	26/44 (59.1%)	3/16 (18.8%)	6/23 (26.1%)	0.004
No ACLF: ACLF grade 1/2/3 on days 3–7, among patients with no ACLF on day 1	18 (40.9%): 6 (13.6%): 12 (27.3%): 8 (18.2%) Total *n* = 44	13 (81.3%): 2 (12.5%): 1 (6.3%): 0 Total *n* = 16	17 (73.9%): 1 (4.3%): 5 (21.7%): 0 Total *n* = 23	0.017
Overall AD: ACLF 1/2/3 on days 3–7 (65:247)	22 (10.7%): 17 (8.3%): 45 (21.8%): 122 (59.2%)	18 (39.1%): 5 (10.9%): 9 (19.6%): 14 (30.4%)	25 (41.7%): 4 (6.7%): 17 (28.3%): 14 (23.3%)	<0.001
28-day mortality	145/206 (70.4%)	21/46 (45.7%)	18/60 (30.0%)	<0.001

All data are expressed as *n* (%) unless otherwise specified.
ACLF, acute-on-chronic liver failure; AD, acute decompensation.

Development of complications (days 3–7) among patients with different ammonia levels on day 1 and day 3. Persistent or incident hyperammonemia was noted in 206 (66.0%) patients. It was seen more frequently in patients with ACLF than those with AD (70.7 *vs* 53.0%, *P* = 0.013). New-onset OFs—liver, kidney, brain, coagulation, circulatory, and respiratory—were seen more frequently among patients with persistent or incident hyperammonemia (Table 4). Patients with persistent or incident hyperammonemia also had higher ACLF grades on days 3–7 (*P* < 0.001) and higher 28-day mortality (*P* < 0.001). Progression from AD to ACLF occurred in a higher proportion of patients with persistent or incident hyperammonemia (*P* = 0.004). New-onset infections were more common among patients with persistent or incident hyperammonemia but were not significantly different (*P* = 0.081) (Table 4).

The change in ammonia levels (median, IQR) between day 3 and day 1 (delta ammonia) among survivors and nonsurvivors was −10.0 (−25.5–6.0) μmol/L *versus* 5.5 (−18.0–22.7), respectively, *P* < 0.001. The percentage change in ammonia between day 3 and day 1 (ammonia day 3 − day 1/day 1 × 100) among survivors and nonsurvivors was −10.4% (−25.2 to 9.2%) *versus* 5.4% (−15.8 to 24.2%), respectively, *P* < 0.001 (Fig. S1).

Table 5 Univariate and Multivariate predictors of 28-day survival

Variables	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)- Model 1	*P* value	Model 2	*P* Value
Age	1.005 (0.993–1.017)	0.390	–		–	
Sex (female)	0.693 (0.463–1.037)	0.074	0. 827 (0.538–1.272)	0.388	0.899 (0.583–1.385)	0.629
Total leucocyte count x 10^3/mm^3 (log)	1.029 (1.015–1.043)	<0.001	–		–	
Creatinine, mg/dl	1.187 (1.097–1.285)	<0.001	–		–	
Platelet count x 10^3, per mm^3 (log)	0.999 (0.997–1.001)	0.999	–		–	
Total bilirubin, mg/dl	1.020 (1.007–1.034)	0.003	–		–	
Albumin, g/dl	0.774 (0.602–0.995)	0.046	0.780 (0.585–1.041)	0.091	0.817 (0.617–1.082)	0.159
International normalized ratio	1.263 (1.140–1.400)	<0.001	–		–	
No–hyperammonemia	1		1		–	
Non–persistent hyperammonemia	1.669 (0.889–3.133)	0.111	1.470 (0.734–2.946)	0.277		
Persistent or incident hyperammonemia	3.590 (2.196–5.867)	<0.001	3.174 (1.851–5.442)	<0.001		
Delta ammonia (day 3–day 1)	1.005 (1.002–1.008)	0.003	–		1.005 (1.002–1.009)	0.003
CLIF–C ACLF (only patients with ACLF)	1.042 (1.026–1.059)	<0.001	1.040 (1.024–1.057)	<0.001	–	
Delta CLIF–C ACLF (day 3–day 1)	–		–		1.046 (1.032–1.060)	<0.001

Abbreviations: MELD, model for end stage liver disease; OF, organ failure; CLIF-C, chronic liver failure consortium.
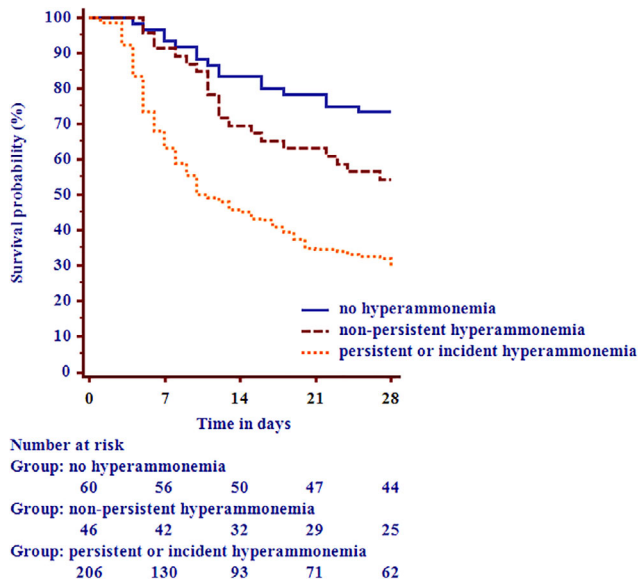
Figure 2 Survival probability among patients with no hyperammonemia, nonpersistent, and persistent or incident hyperammonemia. The 28-day mortality among patients with persistent or incident hyperammonemia was higher than among those with nonpersistent and no hyperammonemia (log-rank test, *P* < 0.001).

Survival analysis. We performed univariate and multivariate analyses to assess the independent predictors of 28-day mortality. The variables included in the univariate analysis are shown in Table 5. In the final multivariate analysis, we included gender, albumin level, ammonia, and CLIF-C ACLF. We excluded total leucocyte count, serum creatinine, bilirubin, INR, and different OFs that were significant in the univariate analysis as these are components of the CLIF-C ACLF score. After adjustment, persistent or incident hyperammonemia {adjusted hazard ratio [HR] [95% confidence interval (CI)]: 3.174 [1.851–5.442],

P < 0.001} and CLIF-C ACLF (adjusted HR [95% CI]: 1.04 [1.024–1.057], *P* < 0.001) were the only variables significantly associated with 28-day mortality. We assessed the effect of dynamic change (delta ammonia) in ammonia level and CLIF-C ACLF (delta) between day 3 and day 1 in a separate multivariable model. After adjustment, delta ammonia (day 3-day 1) (adjusted HR [95% CI]: 1.005 [1.002–1.009], *P* = 0.003) and delta CLIF-C ACLF (day 3-day 1) (adjusted HR [95% CI]: 1.046 [1.032–1.060], *P* < 0.001) were the only variables significantly associated with 28-day mortality.

The Kaplan–Meier survival curves of patients with levels of ammonia ≥79.5 μmol/L and ammonia <79.5 μmol/L on day 1 and day 3 are shown in Figure S2a and b. Patients with ammonia <79.5 μmol/L on both day 1 and day 3 had higher survival probability than patients with ammonia ≥79.5 μmol/L (log-rank test, *P* < 0.001).

The Kaplan–Meier survival curves of the patients with persistent or incident, nonpersistent, and no hyperammonemia are shown in Figure 2. They indicate that patients having either persistent or incident hyperammonemia had significantly higher mortality compared to patients having either nonpersistent or no hyperammonemia (log-rank test, *P* < 0.001).

Risk-stratified analysis for 28-day mortality based on CLIF-C ACLF score and persistent or incident hyperammonemia (≥79.5 μmol/L) at day 3. On the basis of a cutoff value of CLIF-C ACLF score of 47 obtained on a receiver operating characteristic (ROC) curve, patients were categorized into low-risk (CLIF-C ACLF < 47) and high-risk groups (CLIF-C ACLF ≥ 47) for 28-day mortality. Patients in the low-risk group had significantly lower mortality compared to the high-risk group (54.0 *vs* 79.8%, *P* < 0.001). Among patients initially classified as having a low risk of mortality as per CLIF-C ACLF score <47, those with persistent or incident hyperammonemia had high mortality rates (65.8%) compared to patients with no hyperammonemia (17.6%) and nonpersistent hyperammonemia (30.0%) (*P* < 0.001). Mortality in those with CLIF-C ACLF <47
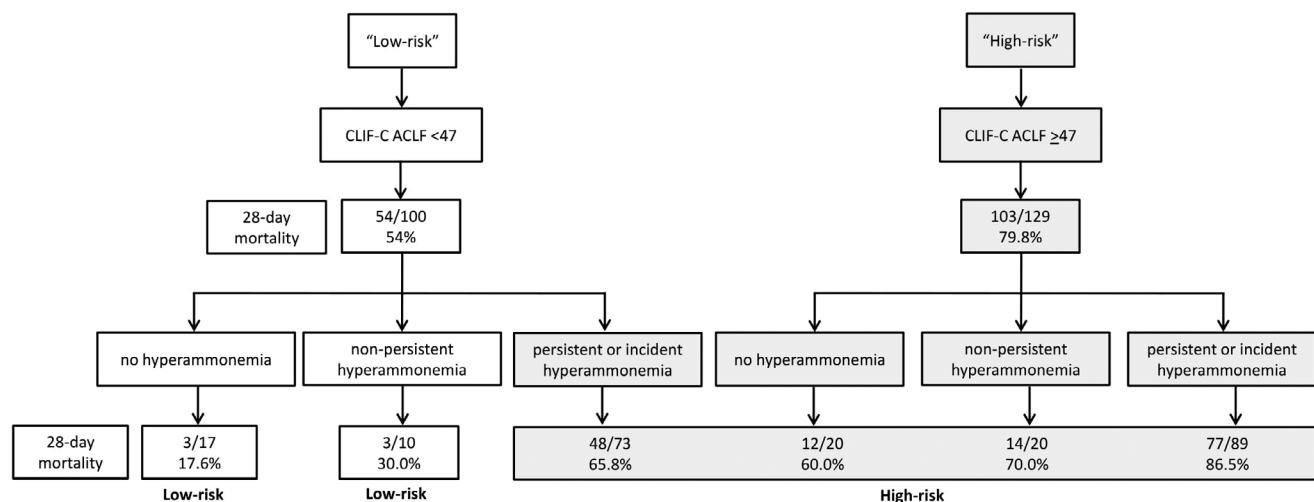
Figure 3 Risk-stratified 28-day mortality among patients with acute-on-chronic liver failure (ACLF) based on chronic liver failure consortium (CLIF-C) ACLF score and no hyperammonemia, nonpersistent, and persistent or incident hyperammonemia.

and persistent or incident hyperammonemia (65.8%) was comparable to patients classified as high risk as per CLIF-C ACLF ≥47 criteria with no hyperammonemia (60%) and nonpersistent hyperammonemia (70%) (*P* = 0.799) (Fig. 3).

Predictors of persistent or incident hyperammonemia (day 3) in AD and ACLF patients. On multivariate logistic regression analysis, presence of infection (odds ratio [OR] [95% CI]: 2.232 [1.340–3.717], *P* = 0.002) was the only independent predictor of persistent or incident hyperammonemia (Table S2).

Discussion

In the present retrospective study, we explored the prognostic utility of serial measurements of ammonia. Patients presenting with hyperammonemia at baseline and those with persistent or worsening ammonia levels at day 3 had worse outcomes compared to patients with improving ammonia levels. In addition, the progression of AD to ACLF, OF, and the 28-day mortality rates were higher among patients with persistent or incident hyperammonemia on day 3. Furthermore, persistent or incident hyperammonemia could categorize ACLF patients with lower CLIF-C ACLF (<47) scores into high-risk and low-risk groups. The delta ammonia levels were significantly associated with 28-day mortality rates, emphasizing their utility in predicting the outcome.

ACLF is associated with high short-term mortality, and higher grades of ACLF are associated with poor outcomes. High ammonia levels at admission have been associated with the development of complications and poor outcomes in patients with AD and ACLF.[9,10] To date, there are no large studies evaluating the effect of persistently elevated ammonia in these patients. With this in mind, we explored the prognostic utility of serial measurements of ammonia. Indeed, in our study, the development of complications (specifically OFs) was greater in patients who showed persistent or incident hyperammonemia on day 3.

The development of OFs is associated with higher mortality in patients with ACLF or AD. Therefore, it is essential to identify the subgroup of patients at risk of developing OF. Early prediction of OF can help triage patients for better management and even consideration for early liver transplantation. Liver transplantation has been shown to improve survival even among the sickest group of advanced ACLF-3.[16] We have previously demonstrated that ammonia levels correlate with the grades of HE.[7] Our results provide new information regarding the importance of serial ammonia estimation for predicting the development of new OFs other than HE. In our study, OFs were seen in a greater proportion of patients who had ammonia levels ≥79.5 μmol/L on day 1. In addition, patients with persistent or incident hyperammonemia developed new OFs—liver, kidney, brain, coagulation, circulatory, and respiratory failures—more frequently compared to patients with nonpersistent and no hyperammonemia. A higher proportion of AD with persistent or incident hyperammonemia progressed to ACLF. These facts highlight the possible role of ammonia in the progression from AD to ACLF. The mechanisms by which ammonia increases the likelihood of OF are not clear. Inflammatory cytokines—VCAM-1, ICAM-1, and GM-CSF— have been associated with poor outcomes in patients with ACLF.[17] The effect of ammonia on inflammatory cytokines and vice versa needs further exploration. Moreover, patients with AD and ACLF are at risk of infections, which in turn are associated with the development

of OF.[8,18] Ammonia has direct hepatotoxic effects and induces liver cell apoptosis.[19] A recent study in a bile duct model of cirrhosis reported ammonia to be associated with deleterious effects on the structure and function of stellate cells[11]; reduction of ammonia was associated with the deactivation of stellate cells and reduction in portal hypertension.[11] Hyperammonemia may be a marker of severity of portal hypertension and resultant portosystemic shunting of blood.[20] Furthermore, portal hypertension is a poor prognostic marker in ACLF. In addition, hyperammonemia may exaggerate sarcopenia by NF-κB–dependent upregulation of myostatin, leading to a poor outcome.[21] This pleiotropic action of ammonia could result in an increased risk of complications and death, highlighting the additional prognostic value of ammonia.

The role of ammonia as a prognostic indicator for neurological complications and death is well defined in patients with ALF. There is a scant amount of data on the influence of ammonia levels on outcomes in patients with ACLF. Patwardhan *et al.* have reported elevated ammonia levels to be associated with poor outcomes in patients with AD and ACLF.[10] In our study, persistent or incident hyperammonemia was independently associated with 28-day mortality even after adjusting for CLIF-C ACLF scores. Importantly, the delta ammonia (day 3–day 1) was significantly different between those who survived compared to those who died. The delta ammonia was also independently associated with 28-day mortality after adjusting for delta CLIF-C ACLF, suggesting that even absolute dynamic changes in ammonia levels predict outcome in ACLF patients. Our results suggest that the estimation of ammonia may be useful in stratifying patients with low CLIF-C ACLF scores into low-risk and high-risk groups for predicting mortality. Patients with persistent or incident hyperammonemia are at high risk of death, even with low CLIF-C ACLF scores. These results need to be validated in other cohorts of patients, especially among those undergoing liver transplantation.

Potential ammonia-lowering therapies include L-Ornithine L-Aspartate,[22,23] continuous renal replacement therapy,[24] and high-volume plasma exchange. The role of these ammonia-lowering therapies is unclear in patients with AD and ACLF. Our results suggest that a higher fraction of AD patients with persistent or incident hyperammonemia progressed to ACLF compared to those with declining and with ammonia levels <79.5 μmol/L. Whether the reduction of ammonia levels in patients with AD leads to a reduction in the progression to ACLF needs further exploration. Our results provide evidence that ammonia can be an important prognostic marker and a potential therapeutic target to improve outcomes in patients presenting with AD and ACLF. Future prospective studies are needed to evaluate the effect of ammonia lowering and its effect on OF and outcomes in patients with AD and ACLF.

The strength of our study is an analysis based on commonly used parameters of a large number of patients from a single center, which ensures a homogeneous cohort managed with a similar treatment protocol. However, the present study has some limitations. The retrospective design is the main limitation of our study. We did not have day 3 ammonia values in almost half of the patients. With a better understanding of the role of ammonia,[7] we started exploring the role of serial ammonia estimation. There were a few differences between patients included and those excluded from the study, which limit the generalization of the results, and future prospective studies are needed to validate the findings. This study was conducted in a tertiary care center, which could have

led to a referral bias for more sick patients. Third, the arterial ammonia level was measured rather than the partial pressure of ammonia. The partial pressure of ammonia may be more important as ammonia crosses the blood–brain barrier readily in a gaseous state.[25] Use of renal replacement therapy and ammonia-lowering agents in patients could have led to a reduction in ammonia levels in our study cohort on day 3 and possibly led to a survival advantage in some patients in the nonpersistent hyperammonemia group. We did not assess for sarcopenia, portosystemic shunts, and gastrointestinal bleeding, which may have affected the ammonia levels.

In conclusion, patients with AD and ACLF with persistent or incident arterial hyperammonemia for 3 days are more likely to develop OF and have greater mortality.

Acknowledgment

REFERENCES

1 Clemmesen JO, Larsen FS, Kondrup J, Hansen BA, Ott P. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. *Hepatology*. 1999; **29**: 648–53.

2 Bhatia V, Singh R, Acharya SK. Predictive value of arterial ammonia for complications and outcome in acute liver failure. *Gut*. 2006; **55**: 98–104.

3 Kumar R, Shalimar, Sharma H *et al.* Persistent hyperammonemia is associated with complications and poor outcomes in patients with acute liver failure. *Clin. Gastroenterol. Hepatol.* 2012; **10**: 925–31.

4 Shawcross DL, Wright GAK, Stadlbauer V *et al.* Ammonia impairs neutrophil phagocytic function in liver disease. *Hepatology*. 2008; **48**: 1202–12.

5 Shalimar, Kedia S, Sharma H *et al.* Predictors of infection in viral-hepatitis related acute liver failure. *Scand. J. Gastroenterol.* 2017; **12**: 1413–19.

6 Sawhney R, Holland-Fischer P, Rosselli M, Mookerjee RP, Agarwal B, Jalan R. Role of ammonia, inflammation, and cerebral oxygenation in brain dysfunction of acute-on-chronic liver failure patients. *Liver Transpl.* 2016; **22**: 732–42.

7 Shalimar, Sheikh MF, Mookerjee RP, Agarwal B, Acharya SK, Jalan R. Prognostic role of ammonia in patients with cirrhosis. *Hepatology*. 2019; **70**: 982–94.

8 Shalimar, Rout G, Jadaun SS *et al.* Prevalence, predictors and impact of bacterial infection in acute on chronic liver failure patients. *Dig. Liver Dis.* 2018; **50**: 1225–31.

9 Ravi S, Bade KS, Hasanin M, Singal AK. Ammonia level at admission predicts in-hospital mortality for patients with alcoholic hepatitis. *Gastroenterol. Rep.* 2017; **5**: 232–6.

10 Patwardhan VR, Jiang ZG, Risech-Neiman Y *et al.* Serum ammonia in associated with transplant-free survival in hospitalized patients with acutely decompensated cirrhosis. *J. Clin. Gastroenterol.* 2016; **50**: 345–50.

11 Jalan R, De Chiara F, Balasubramaniyan V *et al.* Ammonia produces pathological changes in human hepatic stellate cells and is a target for therapy of portal hypertension. *J. Hepatol.* 2016; **64**: 823–33.

12 Dasarathy S. Consilience in sarcopenia of cirrhosis. *J. Cachexia. Sarcopenia Muscle*. 2012; **3**: 225–37.

13 Moreau R, Jalan R, Gines P *et al.* Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology*. 2013; **144**: 1426–37.

14 Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy–definition, nomenclature, diagnosis,

and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology*. 2002; **35**: 716–21.

15 EASL. Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J. Hepatol.* 2018; **69**: 406–60.

16 Artru F, Louvet A, Ruiz I *et al.* Liver transplantation in the most severely ill cirrhotic patients: a multicenter study in acute-on-chronic liver failure grade 3. *J. Hepatol.* 2017; **67**: 708–15.

17 Solé C, Solà E, Morales-Ruiz M *et al.* Characterization of inflammatory response in acute-on-chronic liver failure and relationship with prognosis. *Sci. Rep.* 2016; **31**: 32341.

18 Fernández J, Acevedo J, Wiest R *et al.* Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut*. 2018; **67**: 1870–80.

19 Jia B, Yu Z-J, Duan Z-F *et al.* Hyperammonaemia induces hepatic injury with alteration of gene expression profiles. *Liver Int.* 2014; **34**: 748–58.

20 Sanyal AJ, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. *Gastroenterology*. 2008; **134**: 1715–28.

21 Qiu J, Thapaliya S, Runkana A *et al.* Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-κB-mediated mechanism. *Proc. Natl. Acad. Sci.* 2013; **110**: 18162–7.

22 Bai M, Yang Z, Qi X *et al.* l-ornithine-l-aspartate for hepatic encephalopathy in patients with cirrhosis: a meta-analysis of randomized controlled trials. *J. Gastroenterol. Hepatol.* 2013; **28**: 783–92.

23 Sidhu SS, Sharma BC, Goyal O, Kishore H, Kaur N. L-ornithine L-aspartate in bouts of overt hepatic encephalopathy. *Hepatology*. 2018; **67**: 700–10.

24 Cardoso FS, Gottfried M, Tujios S, Olson JC, Karvellas CJ; For the US Acute Liver Failure Study Group. Continuous renal replacement therapy is associated with reduced serum ammonia levels and mortality in acute liver failure. *Hepatology*. 2018; **67**: 711–20.

25 Kramer L, Tribl B, Gendo A *et al.* Partial pressure of ammonia versus ammonia in hepatic encephalopathy. *Hepatology*. 2000; **31**: 30–4.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1 The change in ammonia levels (median, interquartile range) between day 3 and day 1 (delta ammonia) among survivors and nonsurvivors. The delta ammonia levels were −10.0 (−25.5–6.0) μmol/L *versus* 5.5 (−18.0–22.7), $P < 0.001$.

Figure S2 (a) Survival probability among patients with day 1 ammonia <79.5 μmol/L was higher as compared to those with ammonia ≥79.5 μmol/L (log-rank test, $P < 0.001$). (b) Survival probability among patients with day 3 ammonia <79.5 μmol/L was higher as compared to those with ammonia ≥79.5 μmol/L (log-rank test, $P < 0.001$).

Table S1 Comparison of baseline characteristics of cirrhosis with acute decompensation who were included ($n = 312$) and those not included in the study ($n = 291$).

Table S2 Predictors of hyperammonemia (day 3) in acute decompensation and acute-on-chronic liver failure patients.