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Title

Prognostic Role of Ammonia in Cirrhotic Patients

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Abbreviations

AUROC; area under receiver operator characteristic curve

ACLF; acute on chronic liver failure

AD; acute decompensationc

CLIF-C; Chronic Liver Failure Consortium

CLIF – OF; Chronic Liver Failure - Organ Failure

HE; hepatic encephalopathy

MELD; Model for end stage Liver disease

TLC; Total Leucocyte Count

UCL; University College London

WH; West Haven

Author Contributions

Conception and design of the study: RJ, SA; collection, analysis and interpretation of data: S, MFS, RM, BA; drafting of the manuscript: S, MFS, RJ, BA; All authors approved the authorship list.

Potential Conflict of Interest.

Rajiv Jalan: Rajiv Jalan has research collaborations with Yaqrit and Takeda. Rajiv Jalan is the inventor of OPA, which has been patented by UCL and licensed to Mallinckrodt Pharma. He is also the founder of Yaqrit limited, a spin out company from University College London.

STROBE

We confirm this study follows the STROBE guidelines for reporting observational studies

Abstract

Ammonia is thought to be central to the pathogenesis of hepatic encephalopathy (HE), but its prognostic role in cirrhotic patients with acute decompensation (AD) is unknown. The aims of this study were to determine the relationship between ammonia levels and severity of HE, association with organ dysfunction and short-term mortality. We identified 498 patients from two institutions as part of prospective observational studies in cirrhotic patients. Plasma ammonia levels were measured on admission and Chronic Liver Failure-Sequential Organ Failure Assessment criteria was used to determine the presence of organ failures. 28-day patient survival was determined. Receiver operator characteristic analysis was used to identify the cut-off points for ammonia value and multivariable analysis was performed using Cox proportional hazard regression model. 28-day mortality was 43.4%. Plasma ammonia correlated with severity of HE ($p<0.001$), was significantly higher in non-survivors (93 [73-121] vs. 67 [55-89] µmol/L, $p<0.001$) and was an independent predictor of 28-day mortality (HR 1.009, $p<0.001$). An ammonia level of 79.5µmol/L had sensitivity of 68.1% and specificity of 67.4% for prediciting 28-day mortality. An ammonia level of ≥79.5µmol/L was associated with a higher frequency of organ failures (liver [$p=0.004$], coagulation [$p<0.001$], kidney [$p=0.004$] and respiratory [$p<0.001$]). Lack of improvement in baseline ammonia at day 5 was associated with high mortality (70.6%). Ammonia levels correlate not only with the severity of HE but also the failure of other organs and is an independent risk factor for mortality. Lack of improvement in ammonia levels is associated with high risk of death making it an important biomarker and a therapeutic target.

Introduction

Ammonia homeostasis is a multi-organ process involving not the liver but also the kidneys, brain, gastro-intestinal tract and muscle. Hyperammonaemia is thought to play a central role in the pathogenesis of hepatic encephalopathy (HE). In acute liver failure, elevated plasma ammonia levels are predictive of cerebral oedema and herniation. (1) In cirrhotic patients with acute decompensation (AD), this relationship is less well established. (2) Infection and inflammation which are important contributory factors in the pathogenesis of HE in acute liver failure, are also thought to be the key mediators of HE in cirrhosis. (3, 4) In cirrhotics, clinically relevant cerebral oedema is an infrequent occurrence observed in only up to 5% of patients with acute on chronic liver failure (ACLF). (5) However, in cirrhosis ammonia levels can predict risk and frequency of HE episodes (6) and HE related admissions, and in ACLF patients higher levels are observed in those with HE compared with those without. A failure in the reduction of ammonia levels in ACLF over time increases the probability for death. (7) Even in ACLF survivors without HE, a serial decrease in ammonia levels over 7 days has been observed possibly suggesting an additional role of ammonia in outcomes of ACLF other than HE. (7) Admission ammonia levels have also been shown to be predictive of in-hospital survival in patients with alcoholic hepatitis, (8) transplant free survival of acutely decompensated cirrhotics, (9) and are lower in ACLF patients who survive compared to those that die. (10)

In addition to the well-known neurotoxic effects, hyperammonaemia has a deleterious effect on several organ systems, which may augment inflammation and/or organ injury. (11) Hyperammonaemia may directly induce hepatic injury, (12) potentiate immune dysfunction, (13) (14) and hepatic stellate cell activation (15) and

contribute to the pathogenesis of sarcopenia, (16) (17) which is an independent predictor of mortality in cirrhosis. (18)

Thus, while a direct pathophysiological link between hyperammonaemia and non-neurological organ injury is not well established, it is possible that ammonia may play an indirect role in mediating multiorgan dysfunction through stellate cell activation and inflammation, increased susceptibility to new infections and reduced capacity for recovery through sarcopaenia in cirrhosis and AD other than HE thereby contributing independently to mortality. The aims of this study were to firstly, determine whether ammonia is increased in HE patients and whether this defines outcome. Second, we aimed to determine whether ammonia is associated with organ dysfunction and therefore mortality and thirdly whether a change in ammonia levels defines outcomes.

Methods

Patients

The study included a total of 498 cirrhotic patients from a combination of three different cohorts from 2 cities; London, UK and New Delhi, India.

Cohort 1

Consecutive AD patients (n=291) admitted to Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi between January 2012 to December 2015 were prospectively recruited. All patients had baseline arterial ammonia measured at admission.

Cohort 2

This population was a nested cohort of 101 patients within a previously reported larger prospective cohort study of cirrhotics with AD admitted to University College London (UCL) Hospital, London between 2000 and 2006. (7) Those requiring admission to the intensive care unit were included. All patients had arterial ammonia measured on admission.

Patients were included in both these protocols if they had a clinical, radiological, or histological diagnosis of cirrhosis. The Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) score was used to determine the presence of organ failures and grade of ACLF. (19) West Haven (WH) classification (20) was used to determine the severity of HE and accordingly patients were divided into three groups; no or mild HE (WH grade 0-1), moderate HE (WH grade 2), and severe HE (WH grade 3-4). Exclusion criteria included admissions for reasons other than AD, severe co-morbid diseases, especially established cardiovascular or renal disease, malignancy (extra-hepatic or hepatocellular carcinoma), pregnancy and those who had undergone any major surgery.

Cohort 3

This population of 106 patients were a part of a prospective study at UCL Hospitals between 2008-2010 to determine the characteristics and outcomes of cirrhotic patients with Grade 1 and minimal hepatic encephalopathy. (21) This group of patients were clinically stable cirrhotics and all had baseline venous ammonia measured at enrolment. This cohort comprised cirrhotics without overt HE (less than

WH Grade 2). Exclusion criteria included active infection, presence of any organ failure, and hepatic or extra hepatic malignancy.

For the patients included in the study in New Delhi, India, the study was approved by the Institute's ethics committee and patients included prospectively. The UCL Hospital ethics committee approved the studies in the UK, all patients provided informed consent and were recruited prospectively. If a patient did not have capacity to consent, assent from next of kin was obtained with retrospective consent from the patient, in accordance with the 1975 Declaration of Helsinki.

Management

Local protocols were used to manage hospitalised patients. All patients had protocol screening tests to identify possible sepsis, including blood, urine, and ascitic fluid cultures, and imaging as clinically indicated. Broad-spectrum antibiotics were used to treat infection as per hospital guidelines. Intravenous crystalloids and/or human albumin were used for fluid resuscitation and terlipressin if treating hepatorenal syndrome. In those with HE, bowel-cleansing agents (lactulose with or without phosphate enemas) were used to achieve 2-3 bowel motions per day. Organ support with continuous veno-venous haemofiltration for renal failure, intubation and ventilation for respiratory failure and vasopressors for circulatory failure were used as and when indicated.

Data collection

Baseline clinical, demographic, and biochemical data were recorded prospectively at time of enrolment. Prognostic scores (including Child Pugh, Model for end-stage liver disease [MELD], CLIF-SOFA and CLIF-C ACLF) were subsequently calculated using parameters obtained at baseline. Follow up was for 28-days from inclusion or until death or liver transplantation, if before. Ammonia samples were taken prior to institution of any organ support and transported on ice and plasma ammonia measured either by an enzymatic method (Randox Lab Ltd, UK) or analysed spectrophotmetrically (CobasMiraS, Hoffman-LaRoche, Switzerland). Where possible repeat ammonia measurements were made at 5 days after baseline.

Statistical analyses

Continuous variables were expressed as median (interquartile range). Categorical data was presented as proportions. Comparison of demographics and clinical features was done using Chi-square or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables. Multivariable analysis was performed using Cox proportional hazard regression model.

Receiver Operator Characteristic curves were used to identify the cut-off points for baseline ammonia values. Kaplan-Meier method was used to generate survival curves. The data was analyzed using SPSS statistics software (version 20.0, Chicago, IL, USA), and Medcalc software (version 15.11.4, MedCalc Software, Ostend, Belgium).

Results

A total of 498 patients with cirrhosis were included. The majority of patients (72%) were male, median age 48 (39-56) years. The commonest aetiology of cirrhosis was alcohol (53.4%) followed by hepatitis B (14.7%). 190 (38.2%) had no ACLF, 79 (15.9%) had ACLF Grade 1, 135 (27.1%) had ACLF Grade 2 and 94 (18.9%) had ACLF Grade 3. Overall, 28-day mortality was 43.4%. Overt encephalopathy (Grade 2 or above) was present in 39.6% (197) patients. Baseline demographic and clinical characteristics with respect to varying grades of HE are described in Table 1.

Factors associated with HE

301 patients (60.4%) had no or mild HE (Grade 0/1). Of the 197 patients with overt HE, 116 (58.9%) patients had Grade 2 HE whereas 81 (41.1%) had severe HE (Grade 3 or 4). ACLF was commoner in those with overt encephalopathy; 55.1% of patients with Grade 0/1 HE had ACLF compared with 58.6% in those with Grade 2 HE and 91.4% in those with Grade 3/4. Bacterial infection was the most common precipitating event in those with Grade 3/4 HE (28.4%).

Ammonia levels were significantly higher in patients with Grade 3/4 HE (97µmol/L [79-121]) compared to those with Grade 2 (77 µmol/L [60-99]) and Grade 0/1 HE (73µmol/L [57-97]) (P<0.001). On univariate analysis, total leucocyte count (TLC), creatinine, ammonia, respiratory and kidney failures were predictive of overt HE (Supplementary Table 1). Different multivariate models were made to analyse which factors remained associated with overt HE. Model one included TLC, creatinine, bilirubin and ammonia. Ammonia (OR 1.006, P=0.022) and creatinine (OR 1.189, p=0.004) remained independently predictive of overt HE but in model two including

TLC, ammonia, and liver, kidney, coagulation and respiratory failures, only TLC (OR 1.028, p= 0.032), coagulation (OR 1.599, p=0.044) and respiratory failure (OR 2.059, p=0.001) remained significanty associated. Factors associated with Grade 3/4 HE were age, TLC, INR, creatinine, ammonia, kidney, coagulation and respiratory failures (Table 2). In a multivariate model excluding organ failures, ammonia (OR 1.010, p=0.005), INR (OR 1.353, p=0.0044) and creatinine (OR 1.463, p<0.001) remained independently associated with Grade 3/4 HE. In multivariate analysis including organ failures, kidney (OR 2.384, p=0.013) and respiratory (OR 8.149, p<0.001) failures remained associated with Grade 3/4 HE.

Those with Grade 3/4 HE had higher MELD and CLIF-C ACLF scores compared to Grade 0/1 HE (32 vs. 25 and 54.2 vs 49.0, respectively) p<0.001. Patients with more advanced stages of HE had increased mortality at 28-days (75.3%) compared to 35.5% mortality in those with Grade 0/1 HE (p<0.001), survival curves stratified by severity of HE are presented in figure 1.

Ammonia as a predictor of overt HE had an area under the receiver operator characteristic curve (AUROC) of 0.600 (0.550-0.650) (supplementary Figure 1). The optimal cut-off (Youden's index) of baseline ammonia as a predictor of overt HE was 79.5 µmol/L, with a sensitivity of 56% and specificity of 57%. AUROC of ammonia as a predictor of Grade 3/4 was 0.690 (0.632-0.749). Overall, 239 patients (48.0%) had baseline ammonia ≥79.5µmol/L (table 3). In the group with ammonia ≥79.5 µmol/L, the incidence of overt HE was significantly higher at 46.1% compared to 33.6% in those with ammonia ≤79.5µmol/L (p <0.001).

Ammonia levels and its association with other organ failures

An ammonia ≥79.5μmol/L was associated with a higher frequency of organ failures (liver 52.7% vs 39.8% [p=0.004], coagulation 32.2% vs 16.3% [p<0.001], kidney 29.7% vs 18.5% [p = 0.004] and respiratory 38.1% vs 22.4% [p<0.001]). Accordingly, in the higher ammonia group a larger proportion of patients had ACLF; 75.3% vs. 49.4% in the lower ammonia group, p<0.001.

Ammonia as a predictor of survival

Overall mortality at 28-days was 43.4% (n=216) (Table 4). Non-survivors were younger (45 vs 50, p<0.001), more likely to be male (p=0.009) and were more likely to have alcohol related cirrhosis and alcohol abuse as a precipitating illness. Ammonia levels were higher in non-survivors compared with survivors (93 vs. 67μmol/L, P < 0.001). Non-survivors had a higher frequency of all organ failures (liver, kidney, brain, coagulation, respiratory, p<0.001) except circulatory failure (p=0.052). 84.7% of those who died had ACLF at baseline compared to only 44.3% of survivors, p<0.001). Baseline MELD, Child Pugh and CLIF-C ACLF scores were all higher in non-survivors compared to survivors (30.5 vs 22.4,12 vs 10, and 53.1 vs 47.5, respectively [p<0.001]).

In univariate analysis, increased ammonia was significantly associated with reduced 28-day survival (HR 1.011, p<0.001) (Table 5). On multivariate analysis ammonia remained independently predictive of death (HR 1.009, p<0.001). Ammonia as a predictor of 28-day mortality had an AUROC of 0.726 (0.682-0.770) (supplementary figure 2). Those with an ammonia level of ≥79.5 μmol/L had a greater probability of death compared with those with an ammonia of ≤79.5 μmol/L (61.5% vs. 26.6%, p<

0.001) (figure 2a) (sensitivity of 68.1% and specificity of 67.4%). To determine whether ammonia levels affected mortality independent of the degree of HE, we calculated Kaplan Meier curves within the three severity categories of HE using an ammonia of 79.5µmol/L as a cut off (figures 2b, 2c, 2d). In patients with Grade 0/1 or Grade 2 HE, those that have an ammonia ≥79.5µmol/L have significantly higher mortality compared to those with an ammonia concentration <79.5µmol/L (P<0.001).

Change in Ammonia levels and, severity of hepatic encephalopathy and mortality

In 86 patients with AD repeat ammonia was measurement was available at day 5. Of these, 74% were male (n=64), alcohol was the leading cause of chronic liver disease 37(43%) and 71% (n=61) had ACLF (Table 6). None of these patients were receiving renal replacement therapy. A decrease in ammonia at day 5 from baseline (n=28) was associated with no change or improvement in HE for the majority of patients (85.7%). In those patients where there was no change or an increase in ammonia levels between admission and day 5 (n=58), 82.7% had worsening of or no improvement in HE grade. Progression of HE from grade 0/1 on day 1 to grades 2-4 on day 5 was associated with an average increase in ammonia of 17µmol/l (p=0.025). However, no significant change in ammonia levels were observed if the grades of HE remained the same or improved (supplementary table 2).

No change or worsening of ammonia at day 5 (n=58) was associated with a 28-day mortality of 70.6% (n=41) compared to 35.7% (n=10) mortality in those with an improvement in baseline ammonia (n=28) (P=0.007). In those that survived the change in ammonia (day 1 – day 5) was 10umol/L (-11 – 40) compared to a ammonia change (day 1 – day 5) of -13umol/L (-26 – 2) in non-survivors (p= 0.004).

Discussion

This study of cirrhotic patients, many with ACLF, adds significantly to the evidence that ammonia levels correlate not only with the severity of HE but also the failure of other organs in cirrhosis and is an independent risk factor for 28-day mortality. The data provide evidence that ammonia levels have a clinically relevant utility not only in determining the severity of HE but can provide important prognostic information, signifying its potential role as a biomarker in identifying patients at high risk of mortality. A reduction in ammonia levels was associated with improved survival confirming it as a potential therapeutic target.

Studies assessing the role of ammonia in HE have had variable results, in part due to differing patient cohorts and study designs. (2, 3, 7, 22) This study demonstrates a significant correlation between increasing ammonia levels and severity of HE, which supports a role for ammonia in the development of HE. The data presented here confirm that the severity of HE is associated with increased mortality. (4, 23) However, in patients with the same grade of HE, an ammonia level ≥79.5µmol/L is associated with increased mortality indicating an additional role of ammonia in dictating clinical outcomes other than HE severity. At present, it is not clear whether reducing ammonia levels would result in a reduction in mortality. Sidhu et al. (24) compared L-ornithine L-aspartate with placebo in patients with HE and acute decompensation. Although there was a significant reduction in ammonia in the drug arm, no differences in survival were observed possibly because of the relatively low sample size. In a metanalysis of L-ornithine L-aspartate for prevention and treatment of HE that included 1489 patients, L-ornithine L-aspartate administration

was associated with a relative reduction in mortality compared to placebo (RR 0.42 (0.24 to 0.72) suggesting that ammonia lowering may improve survival of cirrhotic patients with AD. (25) The data in our paper showing that an increase in ammonia levels between days 5 and admission was associated with increased risk of death and with worsening of HE provides support for the argument that ammonia should be a therapeutic target. This hypothesis will need to be tested in prospective studies.

Our results are in agreement with two recent U.S. single-centre retrospective studies showing that ammonia levels on admission are predictive of in hospital mortality in decompensated cirrhosis. (8, 9) Our study adds to these previous studies through inclusion of a larger number of patients from two separate institutions in two countries and the large cohort of patients with ACLF of various grades allowing analysis of the relationship between organ failure and ammonia. Although the data were obtained from retrospective studies, all patients were prospectively recruited. An ammonia of ≥79.5μmol/L correlated with liver, kidney, respiratory and coagulation failure and trended towards an association with circulatory failure although this did not reach statistical significance.

The pathogenic effects of ammonia in modulating the dysfunction of organs other than the brain may explain the association of its concentration with mortality. The kidneys can adapt in early hyperammonaemia but with worsening renal function exacerbated by clinical scenarios such as acidosis, hypokalemia (11) and upper GI bleeding (26), renal ammoniagenesis exceeds its clearence capacity thus the kidneys are responsible for a net release in circulating ammonia. (16) Ammonia levels are indicative of portosystemic shunting, (27) and thus higher ammonia levels

maybe be due to worse portal hypertension, which is known to be associated with worse outcomes. (28) Hyperammonaemia may not only reflect the degree of underlying sarcopenia but be a driver for it, (18) as ammonia is critically involved in regulating myoststain. (17) Sarcopenia is associated with worse outcomes in cirrhosis. (29) Ammonia also impairs neutrophil function thus pre-disposing to infection. (14) It is therefore, a potentially important therapeutic target. It was interesting to note that the patients in whom ammonia levels were reduced at day-5 had significantly better survival compared with the patients in whom this was unchanged or increased. These data are in keeping with previous studies both in patients with ACLF and acute liver failure (7, 8, 30). From this study, it is not possible to ascertain whether the relationship between the change in ammonia and survival is a cause or effect.

The limitations of this study include the merging of three distinct patient cohorts into one group over a large study time period. We compared the baseline demographic and clinical profile of patients at both institutions (Supplementary Table 3). The cohort at from New Delhi compared with London was younger (43 vs. 55, p<0.001), comprised more males (76.6% vs 65.7%, p=0.008), had a greater incidence of hepatitis B induced cirrhosis (19.6% vs 8%, P<0.001), had higher MELD and Child-Pugh scores (29 vs 20 and 12 vs 9, respectively P<0.001 and comprised a greater incidence of ACLF patients (69.1% vs 51.7 %, p<0.001). Therefore, it was not surprising that there were significant differences in baseline ammonia values (91 vs 65µmol/L, respectively [p<0.001]). Despite these potential limitations, the data were collected prospectively and contain a large number of patients with ACLF, which allows evaluation of the relationship between ammonia levels and organ failures.

Although we combined results of venous and arterial ammonia, previous studies have shown no significant differences between venous and arterial ammonia values with respect to HE. (2) Venous blood sampling was only performed in the mHE population cohort due to ethical considerations. Renal replacement therapy such as continuous venous-veno haemofltration was used where required for patients with renal failure and can lead to a reduction in plasma ammonia. This is a potential confounding factor when assessing survival. However, renal failure (thus use of renal support) was higher in non-survivors (38%) compared to survivors (13%). Therefore, the use of renal replacement therapy would only potentially confound to decrease the strength of any relationship between hyperammonema and mortality. The fact that statistical significance exists between hyperammonaemia and survival in this study despite a potential negative confounding bias only serves to strengthen the conclusions of the study.

In conclusion, this study supports a role for ammonia as an important toxin that independently defines the risk of organ failures and mortality of cirrhotic patients with AD and ACLF both with and without HE. Improvement in HE and survival are strongly linked with a lowering of ammonia levels suggesting that ammonia is a potential therapeutic target to improve not only HE but also other organ failures and survival. Further studies are required to assess whether targeted ammonia lowering can improve the mortality of this patient group.

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Figure Legends

Figure 1 Kaplan-Meier graph of 28 day survival stratified by severity of HE (no/mild, moderate and advanced) ($p<0.001$)

Figure 2a Kaplan-Meier graph of 28 day survival stratified by ammonia ≥ 79.5 µmol/L or < 79.5 µmol/L. ($p< 0.001$)

Figure 2b Kaplan-Meier graph of 28 day survival in patients with grade 0/1 HE stratified by ammonia ≥ 79.5 µmol/L or < 79.5 µmol/L. ($P<0.001$)

Figure 2c Kaplan-Meier graph of 28 day survival in patients with grade 2 HE stratified by ammonia ≥ 79.5 µmol/L or < 79.5 µmol/L. ($P<0.001$)

Figure 2d Kaplan-Meier graph of 28 day survival in patients with grade 3/4 HE stratified by ammonia ≥ 79.5 µmol/L or < 79.5 µmol/L

Table 1 Comparison of baseline patient characteristics stratified by differing grades of Hepatic Encephalopathy (n=498)

Baseline characteristic	Grade 0/1 HE (n=301)	Grade 2 HE (n=116)	Grade 3/4 HE (n=81)	P value
PREDISPOSITION				
Age (years)	47 (39-56)	52 (43-62)	45 (35-55)	0.001*†
Males : Females	215 (71.4%): 86 (28.6%)	79 (68.1%): 37 (31.9%)	65 (80.2%): 16 (19.8%)	0.160
Etiology (CLD)				0.089
Alcohol	151 (50.2%)	66 (56.9%)	49 (60.5%)	
Autoimmune	13 (4.3%)	6 (5.2%)	4 (4.9%)	
HBV	47 (15.6%)	13 (11.2%)	13 (16.0%)	
HCV	18 (6.0%)	12 (10.3%)	0	
HBV + Alcohol	4 (1.3%)	0	0	
HCV + Alcohol	4 (1.3%)	3 (2.6%)	0	
Cryptogenic	34 (11.3%)	7 (6.0%)	11 (13.6%)	
Others	30 (10.0%)	9 (7.8%)	4 (4.9%)	
PRECIPITATING ILLNESS				
Alcohol	77 (25.6%)	10 (8.6%)	21 (25.9%)	
GI bleeding	15 (5.0%)	16 (13.8%)	11 (13.6%)	
Bacterial Infection	34 (11.3%)	10 (8.6%)	23 (28.4%)	
Acute Hepatitis A/B/E	49 (16.3%)	13 (11.2%)	11 (13.6%)	
Other/unidentified	126 (41.9%)	67 (57.8%)	15 (18.5%)	
ORGAN FAILURES				
Liver	155 (51.5%)	33 (28.4%)	41 (50.6%)	<0.001*†
Kidney	62 (20.6%)	21 (18.1%)	36 (44.4%)	<0.001†‡
Coagulation	64 (21.3%)	22 (19.1%)	33 (40.7%)	<0.001†‡
Circulation	17 (5.6%)	7 (6.0%)	5 (6.2%)	0.978
Respiratory	70 (23.3%)	33 (28.4%)	46 (56.8%)	<0.001†‡
LABORATORY VALUES				
Ammonia (µmol/L)	73 (57-97)	77 (60-99)	97 (79-121)	<0.001†‡
TLC (x10^9)	9.5 (6.4-14.6)	9.5 (5.7-15.2)	13.3 (8.8-19.1)	0.001†‡
Platelets (x10^9)	110 (70.0-184.0)	106 (65-182)	110 (69-162)	0.621
Bilirubin (mg/dL)	13.0 (4.7-22.8)	7.0 (3.3-16.0)	10.5 (5.1-23.1)	0.002*†
INR	1.8 (1.5-2.3)	1.7 (1.4-2.1)	2.3 (1.7-2.9)	<0.001†‡
Albumin (g/dL)	2.6 (2.2-3.0)	2.8 (2.4-3.1)	2.6 (2.3-3.0)	0.568
Creatinine (mg/dL)	1.1 (0.7-1.7)	1.0 (0.7-1.7)	1.9 (1.1-3.6)	<0.001†‡
SCORES				
ACLF grades				<0.001
ACLF 0	135(44.9%)	48(41.4%)	7(8.6%)	
ACLF 1	47(15.6%)	29(25.0%)	3(3.7%)	
ACLF 2	91(30.2%)	29(25.0%)	15(18.5%)	

ACLF 3	28(9.3%)	10(8.6%)	56(69.1%)	
MELD	25 (20-31)	22 (16-30)	32 (23- 40)	<0.001*†‡
Child Pugh	11 (9-12)	9 (8-12)	12 (11-13)	<0.001†‡
CLIF- C ACLF (those with ACLF)	49.0 (43.6-54.7)	51.3 (44.7-56.8)	54.2 (48.3-61.7)	<0.001†‡
28 day mortality (%)	107 (35.5%)	48 (41.4%)	61 (75.3%)	<0.001†‡

NOTE: All data are expressed as n (%) or median (IQR), unless otherwise specified.
*significant between grade 0/1 HE and grade 2 HE, †significant between grade 2 HE and grade 3/4 HE, ‡significant between grade 0/1 and grade 3/4 HE.

Table 2 Univariate and multivariate analysis for predictors of advanced HE

	Univariate Model		Multivariate model (all significant continuous variables)		Multivariate Model (includes all OFs)		Multivariate model (**3)- without respiratory failure**	
	OR	**P value**	**OR**	**P value**	**OR**	**P value**	**OR**	**P value**
Age	0.975 (0.958-0.993)	0.005	0.976 (0.952-1.001)	0.061	0.970 (0.946-0.995)	0.018	0.976 (0.953-0.999)	0.045
Sex (Female)	0.588 (0.327-1.057)	0.076	0.897 (0.415-1.937)	0.782	0.892 (0.405-1.967)	0.777	0.829 (0.391-1.757)	0.625
TLC	1.049 (1.022-1.076)	<0.001	1.016 (0.982-1.051)	0.360	1.011 (0.977-1.046)	0.522	1.018 (0.986-1.052)	0.273
INR	1.564 (1.245-1.964)	<0.001	1.353 (1.009-1.815)	0.044	NI		NI	
Creatinine	1.428 (1.252-1.628)	<0.001	1.463 (1.231-1.738)	<0.001*	NI		NI	
Total Bilirubin	1.017 (0.997-1.037)	0.090	0.978 (0.947-1.010)	0.167				
AST	1.002 (1.000-1.003)	0.100	1.000 (0.998-1.003)	0.650	0.999 (0.997-1.001)	0.467	1.000 (0.998-1.002)	0.906
ALT	1.001 (0.999-1.003)	0.230						
Albumin	0.812 (0.530-1.244)	0.340						
Ammonia	1.011 (1.006-1.017)	<0.001	1.010 (1.003-1.017)	0.005	1.002 (0.995-1.009)	0.544	1.009 (1.002-1.015)	0.008
MAP	0.992 (0.974-1.011)	0.409						
Organ Failures								
Liver	1.249 (0.775-2.010)	0.361						
Kidney	3.219 (1.953-5.307)	<0.001			2.384 (1.200-4.737)	0.013	3.239 (1.719-6.105)	<0.001
Coagulation	2.630 (1.591-4.348)	<0.001			1.882 (0.936-3.781)	0.076	1.534 (0.803-2.931)	0.195

Circulation	1.077 (0.399-2.912)	0.883			
Respiratory	4.007 (2.448-6.558)	< 0.001	8.149 (3.933-16.887)	<0.001	NI

NI, not included
Variables selected for multivariate model if P value ≤ 0.10

Table 3 Comparison of complications and outcome in patients with and without an elevated ammonia at baseline

	Ammonia <79.5 (n=259)	Ammonia ≥79.5 (n=239)	P Value
HE			<0.001
Grade 0/1	172 (66.4%)	129 (54.0%)	
Grade 2	64 (24.7%)	52 (21.8%)	
Grade 3/4	23 (8.9%)	58 (24.3%)	
Organ Failures			
Liver	103 (39.8%)	126 (52.7%)	0.004
Kidney	48 (18.5%)	71 (29.7%)	0.004
Brain	23 (8.9%)	58 (24.3%)	< 0.001
Coagulation	42 (16.3%)	77 (32.2%)	<0.001
Circulation	10 (3.9%)	19 (7.9%)	0.057
Respiratory	58 (22.4%)	91 (38.1%)	<0.001 *
ACLF Grade			
Grade 0	131 (50.6%)	59 (24.7%)	<0.001
Grade 1	38 (14.7%)	41 (17.2%)	
Grade 2	64 (24.7%)	71 (29.7%)	
Grade 3	26 (10.0%)	68 (28.5%)	
28 day Mortality	69 (26.6%)	147 (61.5%)	< 0.001

Table 4 Baseline characteristics of patients stratified by survivors versus those who died

	Survived (n=282)	Died (n=216)	P value
PREDISPOSITION			
Age (years)	50 (41-59)	45 (36-55)	<0.001
Males : Females	190 (67.4%): 92 (32.6%)	169 (78.2%): 47 (21.8%)	0.009
Etiology (CLD)			<0.001
Alcohol	140 (49.6%)	129 (57.1%)	
Autoimmune	12 (4.3%)	12 (5.3%)	
HBV	43 (15.2%)	31 (13.7%)	
HCV	30 (10.6%)	0	
Cryptogenic	27 (9.6%)	25 (11.6%)	
Others	30 (10.6%)	19 (8.8%)	
PRECIPITATING ILLNESS			
Alcohol	37(13.1%)	71 (32.8%)	
GI bleeding	17 (6.0%)	20 (9.3%)	
Bacterial Infection	30 (10.6%)	37 (17.1%)	
Acute Hepatitis A/B/E	43 (15.2%)	30 (13.9%)	
Other/unidentified	155 (55.0%)	58 (26.9%)	
ORGAN FAILURES			
Liver	98 (34.8%)	131 (60.6%)	<0.001
Kidney	37 (13.1%)	82 (38.0%)	<0.001
Cerebral	20 (7.1%)	61 (28.2%)	< 0.001
Coagulation	37 (13.2%)	82 (38.0%)	<0.001
Circulation	11 (3.9%)	18 (8.3%)	0.052
Respiratory	61 (21.6%)	88 (40.7%)	<0.001
LABORATORY VALUES			
Ammonia(µmol/L)	67 (55-89)	93 (73-121)	< 0.001
TLC (x10^9)	8.2 (5.4-11.7)	13.5 (9.0-18.5)	< 0.001
Platelets (x10^9)	114 (81-184)	106 (65-176)	0.148
Bilirubin (mg/dL)	7.1 (3.4-17.8)	16.9 (7.1-24.8)	< 0.001
INR	1.6 (1.4-2.1)	2.2 (1.7-2.9)	< 0.001
Albumin (g/dL)	2.7 (2.4-3.1)	2.5 (2.1-3.0)	<0.001
Creatinine (mg/dL)	1.0 (0.7-1.4)	1.6 (0.9-2.7)	< 0.001

SCORES			
ACLF 0	157 (55.7%)	33 (15.3%)	<0.001
ACLF 1	49 (16.3%)	33 (15.3%)	
ACLF 2	60 (21.3%)	75 (34.7%)	
ACLF 3	19 (6.7%)	75 (34.7%)	
MELD	22.4 (16.5-27.5)	30.5 (25.0-37.5)	< 0.001
Child Pugh	10 (9-12)	12 (10-13)	< 0.001
CLIF- C ACLF (those with ACLF)	47.5 (42.5-54.0)	53.1 (47.0-59.7)	< 0.001

Table 5 Univariate analysis of predictors of 28 days mortality

		Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)	P value
Hepatic Encephalopathy	None, minimal HE Moderate HE Advanced HE	1 1.208 (0.859-1.697) 3.213 (2.341-4.410)	 0.278 < 0.001	NI	
Age		0.983 (0.974-0.993)	< 0.001	1.005 (0.994-1.016)	0.390
Sex	Male Female	1 0.650(0.470-0.898)	 0.009	0.632 (0.449-0.888)	0.008
MAP		0.983 (0.973-0.994)	0.002	NI	
TLC		1.065 (1.051-1.078)	< 0.001	1.039 (1.024-1.055)	<0.001
Creatinine		1.187 (1.128-1.248)	<0.001	NI	
Total bilirubin		1.026 (1.016-1.037)	<0.001	NI	
Ammonia		1.011 (1.009-1.013)	< 0.001	1.009 (1.006-1.012)	<0.001
INR		1.632 (1.462-1.822)	<0.001		
Organ Failures					
Liver		2.152 (1.637-2.829)	< 0.001	1.686 (1.259-2.257)	<0.001
Kidney		2.791 (2.116 – 3.680)	< 0.001	1.728 (1.284-2.326)	<0.001
Cerebral		3.041 (2.256-4.099)	< 0.001	1.604 (1.151-2.235)	0.005
Coagulation		2.672 (2.027-3.524)	< 0.001	1.973 (1.452-2.680)	<0.001
Circulation		1.790 (1.105-2.900)	0.018	1.459 (0.882-2.413)	0.141
Respiratory		2.116 (1.612-2.777)	<0.001	1.433 (1.059-1.938)	0.020

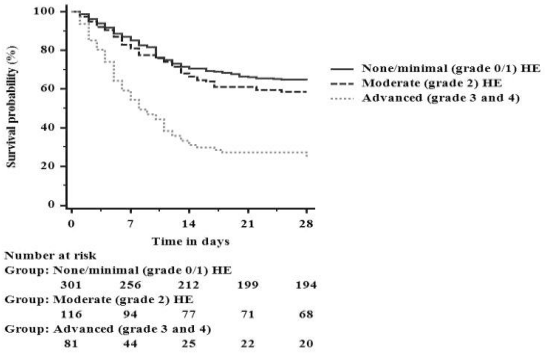
NI, not included

Table 6 Baseline patient characteristics and outcomes stratified by ammonia change in those with repeat ammonia measurements at day 1 and day 5 (n=86)

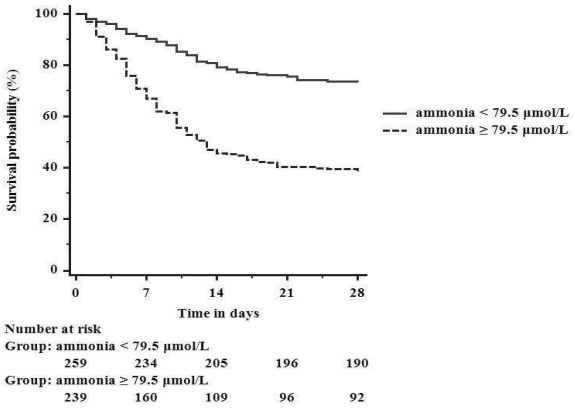
Baseline characteristic	Ammonia decreased (n=28)	Ammonia no change (n=17)	Ammonia increased (n=41)	P value
PREDISPOSITION				
Age (years)	35 (26-46)	43 (39-55)	41 (33-52)	0.064
Males : Females	16 (57.1%) : 12 (42.9%)	16 (94.1%) : 1 (5.9%)	32 (78.0%) : 9 (22.0%)	0.017*†
Etiology (CLD)				0.430
Alcohol	10 (35.7%)	10 (58.8%)	17 (41.5%)	
Autoimmune	3 (10.7%)	0	4 (9.8%)	
HBV	7 (25.0%)	3 (17.6%)	6 (14.6%)	
Cryptogenic	4 (14.3%)	4 (23.5%)	6 (14.6%)	
Others	4 (14.3%)	0	8 (19.5%)	
PRECIPITATING ILLNESS				0.662
Alcohol	9 (32.1%)	8 (47.1%)	13 (31.7%)	
GI bleeding	2 (7.1%)	2 (11.8%)	1 (2.4%)	
Bacterial Infection	5 (17.9%)	1 (5.9%)	5 (12.2%)	
Acute Hepatitis A/B/E	6 (21.5%)	3 (17.6%)	10 (24.4%)	
Other/unidentified	6 (21.3%)	3 (17.6%)	12 (29.3%)	
ORGAN FAILURES				
Liver	12 (42.9%)	11 (64.7%)	29 (70.7%)	0.062
Kidney	6 (21.4%)	7 (41.2%)	15 (36.6%)	0.293
Brain	6 (21.4%)	3 (17.6%)	11 (26.8%)	0.724
Coagulation	9 (32.1%)	5 (29.4%)	18 (43.9%)	0.464
Circulation	3 (10.7%)	0	3 (7.3%)	0.390
Respiratory	8 (28.6%)	3 (17.6%)	10 (24.4%)	0.710
LABORATORY VALUES				
Ammonia (µmol/L)	104 (88-152)	78 (68-105)	91 (73-111)	0.036*
TLC ($x10^9$)	9.2 (6.9-14.6)	12.8 (8.9-18-4)	10 (7.9-17.5)	0.148
Platelets ($x10^9$)	138 (73- 208)	144 (76-225)	114 (79-193)	0.782
Bilirubin (mg/dL)	8.8 (4.7-19.9)	18.4 (9.1-22.5)	17.9 (8.7-24.9)	0.116
INR	2.1 (1.7-3.3)	2.1 (1.6-2.5)	2.2 (1.8-3.3)	0.621
Albumin (g/dL)	2.6 (2.0-3.0)	2.5 (1.9-2.9)	2.5 (2.2-3.0)	0.832
Creatinine (mg/dL)	0.9 (0.8-1.9)	1.2 (0.8-3.5)	1.4 (0.9-2.5)	0.118
SCORES				
ACLF grades				0.311
ACLF 0	9(32.1%)	4(23.5%)	12(29.3%)	
ACLF 1	4(14.3%)	2(11.8%)	1(2.4%)	
ACLF 2	10(35.7%)	8(47.1%)	13(31.7%)	
ACLF 3	5(17.9%)	3(17.6%)	15(36.6%)	
MELD	27 (23-33)	29 (24-37)	31 (24-37)	0.144
Child Pugh	11 (10-13)	11 (10-13)	11 (10-13)	0.907
CLIF- C ACLF (those with ACLF)	42.7 (40.0-46.2)	47.3 (40.0-60.0)	51.3 (45.6-59.7)	0.005*‡
Delta ammonia (day 5-day 1)	37 (17-55)	-1 (-3 – 2)	-23 (-50 - -14)	<0.001*†‡
HE change (improved:no change:worsened)	4 (14.3%): 22 (78.6%): 2 (7.1%)	2 (11.8%): 11 (64.7%): 4 (23.6%)	8 (19.5%): 23 (56.1%): 10 (24.4%)	0.312

28 day mortality (%)	10 (35.7%)	11 (64.7%)	30 (73.2%)	0.007

NOTE: All data are expressed as n (%) or median (IQR), unless otherwise specified.
*significant between ammonia decrease and no change, †significant between ammonia no change and increase, ‡significant between ammonia decrease and increase.

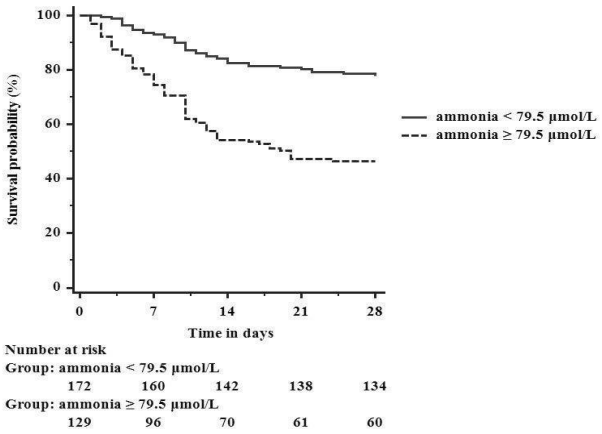


Number at risk					
Group: None/minimal (grade 0/1) HE					
	301	256	212	199	194
Group: Moderate (grade 2) HE					
	116	94	77	71	68
Group: Advanced (grade 3 and 4)					
	81	44	25	22	20



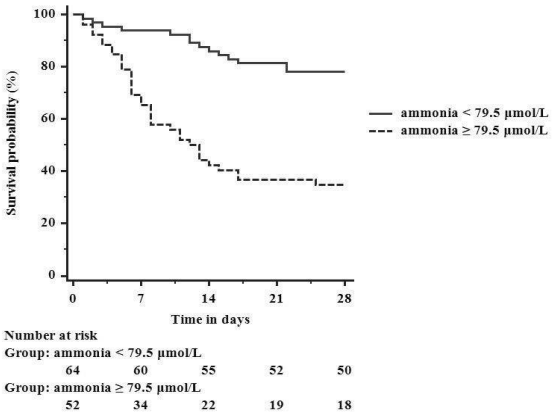
Number at risk

Group: ammonia < 79.5 µmol/L				
259	234	205	196	190
Group: ammonia ≥ 79.5 µmol/L				
239	160	109	96	92



Number at risk

Group: ammonia < 79.5 µmol/L	172	160	142	138	134
Group: ammonia ≥ 79.5 µmol/L	129	96	70	61	60



Number at risk

Group: ammonia < 79.5 µmol/L	64	60	55	52	50
Group: ammonia ≥ 79.5 µmol/L	52	34	22	19	18