Predictive value of Arterial Ammonia for Complications and Outcome in Acute Liver Failure

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COMPETING INTEREST STATEMENT

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ABSTRACT

Background and Aim: In acute liver failure (ALF), brain is exposed to high levels of ammonia. Human studies defining the clinical significance of ammonia in ALF are lacking. This prospective study evaluated the relation of arterial ammonia levels at admission to complications and survival among patients with ALF.

Methods: 80 consecutive ALF patients admitted from March 2001 to December 2003 were followed up till death or complete recovery. All had arterial ammonia estimation at admission (Enzymatic method). Logistic regression analysis was performed to identify the independent predictors of mortality.

Results: 42 (52.5%) patients died. Non-survivors had significantly higher median ammonia levels than the survivors (174.7 µmol/L vs. 105.0 µmol/L; p<0.001). An arterial ammonia level of ≥ 124 µmol/L was found to predict mortality with 78.6% sensitivity and 76.3% specificity, and had 77.5% diagnostic accuracy. Patients with higher ammonia levels also developed more complications including deeper encephalopathy (p=0.055) cerebral edema (p=0.020), need for ventilation (p=<0.001), and seizures (p=0.006). Logistic regression analysis showed pH, presence of cerebral edema and arterial ammonia at admission, were independent predictors of mortality (OR: 6.6, 12.6, and 10.9 respectively). Incorporating these variables, a score predicting mortality risk at admission was derived: 2.53 + 2.91 ammonia + 2.41 edema + 1.40 pH, where ammonia is scored as 0 (if <124 µmol/L) or 1 (if ≥ 124 µmol/L); edema is scored as 0 (absent) or 1 (present); and pH is scored as 1 (if ≤ 7.40) or 0 (if > 7.40). Levels of partial pressure of ammonia were equally correlated with the outcome.

Conclusion: Arterial ammonia at presentation is predictive of outcome and can be used for risk stratification. Ammonia lowering therapies in patients with ALF should be evaluated.
INTRODUCTION
Ammonia neurotoxicity is an important component of cerebral dysfunction in patients with liver failure. [1][2][3][4] Higher ammonia levels are found in patients with acute liver failure (ALF) compared to those with compensated and decompensated cirrhosis. [5] The brain is exposed to high ammonia concentration in ALF, and unidirectional brain ammonia uptake has been well documented. [1] Ammonia exposure induces multiple cellular abnormalities in brain such as impaired bioenergetics and neurotransmission, astrocyte swelling, excitotoxicity, oxidative and nitrosative stress and increased neuro-steroid biosynthesis. [2][3][4]
ALF has a high mortality with most non-survivors succumbing soon after admission. The natural course of the disease is often interrupted by liver transplantation and liver replacement therapies. Therefore dissecting out the role of any single factor such as ammonia is difficult. Despite an impressive progress at the molecular level, it is still not known if ammonia levels are related to cerebral dysfunction or patient outcome. To date only one human study has reported that high ammonia levels among ALF patients are associated with subsequent cerebral herniation. [5] If these observations are validated, use of ammonia lowering therapies in such patients would have a rational basis.
Ammonia predominantly exists in an ionized form (NH₄⁺) in the blood. Only a small pH dependent fraction, reflected by the partial pressure of ammonia (pNH₃) exists in a diffusible form. [6] No data comparing the total ammonia levels and pNH₃ exists for ALF patients. We therefore studied 80 conservatively managed ALF patients till recovery or demise, to determine the relation of ammonia levels with patient outcome. We also created a mortality risk score by incorporating ammonia levels at admission, and compared the prognostic value of simultaneously estimated total ammonia levels and pNH₃ levels.

PATIENTS AND METHODS
Patients
One hundred three consecutive patients with acute liver failure (ALF) admitted to the Gastroenterology ward of the All India Institute of Medical Sciences (AIIMS), New Delhi during March 2001 to December 2003, were evaluated for inclusion. ALF was diagnosed according to the criteria laid down by the International Association for the Study of Liver (IASL). [7] Twenty three patients were excluded because of diagnostic uncertainty at presentation, or because of prior administration of ammonia lowering therapies, sedative drugs, or enteral alimentation. Hence a total of 80 patients with ALF were included in the study. In nine patients, arterial pH and ammonia levels could not be estimated in the same sample, and hence pNH₃ levels were available for 71 ALF patients.

Study variables
Acute liver failure (ALF) was defined by the occurrence of encephalopathy within 4 weeks of onset of symptoms in the absence of preexisting liver disease. [7][8][9] The diagnosis was confirmed by the presence of sub-massive or massive necrosis in the postmortem liver biopsy specimen of patients who died.
Pre-encephalopathy (PE) and Icterus-encephalopathy (IE) periods were defined as the interval from the onset of prodrome and jaundice respectively, to the onset of hepatic encephalopathy. [8][9]

**Admission to death interval** was defined as the time interval from admission to death among the non-survivors.

**Awake time** was defined as the period between admission and improvement to grade 0 encephalopathy.

**Grading of encephalopathy** [8] [10] was done as follows:

**Grade 1:** Loss of sleep rhythm, drowsiness, confusion and flapping tremors.

**Grade 2:** Features of grade 1 encephalopathy with loss of sphincter control in addition.

**Grade 3:** Unconsciousness with no response to oral commands, but responding to painful stimuli.

**Grade 4:** Deep unconscious state, with no response to pain.

**Cerebral edema** was defined clinically by the presence of spontaneous or inducible decerebrate posturing, or by the presence of any two of: hypertension (BP>150/90mmHg), bradycardia, pupillary changes or neurogenic hyperventilation. [8][9]

**Infection** was diagnosed by the presence of pyrexia (temperature > 101°F) or hypothermia (temperature < 98°F) and neutrophil leucocytosis (total leucocyte count >15,000/mm³, with > 80% polymorphs), and one or more of: positive blood culture, positive urine culture, or radiological evidence of pneumonitis. [8][9]

**Renal failure** was diagnosed if patients developed decreased urine output (< 400mL in 24 hours), with serum creatinine >1.5 mg/dL and blood urea > 40mg/dL, despite hydration, objectively assessed by central venous pressure of 10 cm of saline or more.

**Methods**

All patients were admitted to the gastroenterology intensive care unit (ICU) and a uniform management protocol was followed. History was obtained from the attendants of the patient and a detailed clinical examination was performed at admission. All patients were subsequently evaluated every two hours. Each patient had continuous, non-invasive cardiac, oxygen saturation and blood pressure monitoring. Blood glucose was monitored two hourly. Neurological assessment was done once daily and on any sign of deterioration. The parameters noted were: grade of encephalopathy, presence of spontaneous or induced decerebration, pupillary size and reaction, and presence of any focal neurological deficit. Complete blood counts, biochemistry and body fluid surveillance cultures were obtained daily.

Each patient was treated with prophylactic parenteral antibiotics (cefotaxime and cloxacillin), stress ulcer prophylaxis, and, inotropic support with an aim to maintain mean arterial pressure (MAP) at more than 60 mm Hg. Elective ventilation was used for all patients in grade 4 encephalopathy and those in grade 3 encephalopathy with evidence of cerebral edema. Sedation or long acting muscle-paralyzing agents were not used in any patient. Cerebral edema was managed conservatively by mannitol given in standard doses. Seizures were initially treated with diazepam and an additional mannitol bolus, followed by phenytoin.

Lactulose or other ammonia lowering therapies were not used in any case. Enteral feeding was not initiated in the initial three days in any patient. Liver transplantation and liver replacement therapies were unavailable and all patients were followed up until recovery or demise.
Viral markers: Serum sample from each patient was tested for hepatitis B surface antigen (HBsAg), IgM antibody against hepatitis B core antigen (IgM anti-HBc), and IgM antibody against hepatitis A virus (IgM anti-HAV) using commercial immunosorbent assay (ELISA) test kits (Organon, Teknika, Netherlands). IgM antibody to ORF-1, ORF-2 and ORF-3 of hepatitis E virus (HEV) and HEV RNA were tested by methods developed at our institute. [11][12] Anti-hepatitis C (HCV) antibody was tested by using a 3rd generation, commercial ELISA (Xcyton, Bangalore, India) method. HCV-RNA was tested by reverse transcription nested polymerase chain reaction, using primers from the 5’ non–translated region, if appropriate by methods already described. [11][12]

Estimation of ammonia: Arterial ammonia was estimated within 24 hours of admission by enzymatic method (Randox Lab Ltd., UK) in heparinized plasma. The assay was based on the reaction of ammonia with α-oxoglutarate in the presence of glutamate dehydrogenase to yield glutamate and NAD+. The normal ammonia value for the assay used was 10 – 47 µmol/L. Samples were obtained before the next dose of mannitol if possible, and with the ventilator settings unaltered for a minimum of one hour. The site of sampling was the radial artery in all cases. PH and bicarbonate levels were also obtained from the same sample. All samples were transported in ice and processed within 15 minutes.

Estimation of partial pressure (pNH₃) of ammonia [13]

Combining the mass balance equation and Henry’s law, the formula for p[NH₃] can be derived as:

$$p[NH₃] = \frac{K_H \times [NH₃]_T}{1 + [H+] / K_a}$$

where, $[NH₃]_T$ = total blood ammonia,

$K_H$ for ammonia =1.75 x 10⁻⁵,

$K_a$ =9.8 x 10⁻¹⁰, and

$[H+]$ is derived from the pH values, as antilog (-pH)

Data analysis and statistics

Skewness of data distribution was checked before analysis. Median values and range is provided for skewed data. Survivors and non-survivors were compared using the Mann-Whitney U test for continuous variables, and Chi-square or Fishers test for discrete variables, wherever applicable. The level of significance was set at p=0.05. A ROC curve was made using the outcome variable as death and admission arterial ammonia levels as independent variable to find out an appropriate cut off ammonia value for predicting mortality. [14] The sensitivity, specificity, and odds ratio (OR) for the cut off value were also derived.

Univariate analysis of all relevant clinical and biochemical variables that may influence mortality was carried out. The significant variables were dichotomized using discriminant values derived by constructing ROC curves for each variable, and tested using forward stepwise logistic regression analysis taking a probability 0.15 to enter the covariates into the model and 0.10 to exclude the covariate from the model. Odds ratio (OR) with 95% confidence intervals (95%CI), and diagnostic accuracy for each predictor was calculated.

The predicted probability of outcome (mortality) was derived from the variables found significant on logistic regression analysis using the equation:[15]
\[ P_X = \frac{1}{1+e^{-Z}} \]

where, \( P_X \) = probability of outcome (mortality),
\[ Z = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 - \cdots - \beta_n X_n \]
and \( \alpha \) is the constant, \( \beta \) is an unknown parameter, and \( X \) (1-n) is the covariate.

A ROC curve for the predicted probability of death was made to derive area under the curve. The \(-2\) log likelihood ratios function for total ammonia and \( pNH_3 \) were estimated from multivariate analysis of patients with available simultaneous estimations. The difference between the ratios was compared as Chi square value at two degrees of freedom for the significance level. Statistical software SPSS (version 10, SPSS Inc. Chicago, IL), and STATA (version 8.0) were used for statistical analysis.

**RESULTS**

The baseline clinical and biochemical characteristics of the included patients, and the etiology of ALF are summarized in table 1. Over 80% of the cases had a viral etiology. Acute hepatitis E infection, either alone or as dual infection was the most common cause (53.5% cases). Forty two (52.5%) patients died. The median time from admission to death was 4.0 days (range: 1 to 17 days). The awake time among the survivors was 4.0 days (range: 1 to 12 days). Infection occurred in 47 (58.8%) patients. Mechanical ventilation was required in 40 (50.0%) cases.

Raised blood urea levels (>40 mg/dl) were present in 17 patients at admission. Of them, 12 patients normalized their renal parameters with hydration alone, and 5 of them had renal failure. Overall, renal failure occurred in 11 (14.1%) patients during the course of their illness, all of whom died. Ongoing uncontrolled infection was present in 10 of these patients.
Table 1: Clinical and biochemical parameters among patients with ALF (n=80)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Sex (females)</td>
</tr>
<tr>
<td>Encephalopathy grade 3,4</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Cerebral edema</td>
<td>Ammonia (µmol/L)</td>
</tr>
<tr>
<td>Pre-encephalopathy interval (days)</td>
<td>pNH₃ # (X 10⁻⁵ mmHg)</td>
</tr>
<tr>
<td>Icterus-encephalopathy interval (days)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>Bicarbonate (meq/L)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>Sodium (meq/L)</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>Blood pH</td>
</tr>
<tr>
<td>PT-increase (sec)</td>
<td>Admission to death (days)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>Awake time (days)</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
</tr>
<tr>
<td>Post viral**</td>
<td></td>
</tr>
<tr>
<td>ATT induced</td>
<td></td>
</tr>
</tbody>
</table>

Quantitative data are median (range).

# pNH₃ = partial pressure of ammonia; data for 71 patients
** Markers of acute hepatitis A, B and / or E viral infection 71.8% patients; isolated HBsAg or anti-HCV antibody in 9.9% patients; acute hepatitis E infection, either alone or as dual infection detected in 53.5% cases.

Ammonia levels at admission: The median arterial ammonia level at admission was 128.6µmol/L (range: 9.3 to 735.0µmol/L). Fifteen (18.8%) patients had ammonia levels within normal range (<47µmol/L) at admission.

Prognostic value of ammonia levels: The median ammonia levels were significantly higher among non-survivors than survivors (174.7µmol/L vs. 105.0µmol/L; p<0.001) as shown in figure 1. A receiver operator curve (ROC) with ammonia levels of each patient as independent variable and mortality as the outcome variable was made (Figure 2). The area under the ROC curve was 0.76 (95% CI: 0.65-0.87). An arterial ammonia level of ≥ 124µmol/L was found to be 78.6% sensitive and 76.3% specific for predicting mortality, with a diagnostic accuracy of 77.5%. Thirty-three (78.6%) of the 42 non-survivors and only nine (23.7%) of the 38 survivors had ammonia levels of ≥ 124µmol/L at initial presentation (p< 0.001).

Ammonia levels and complications: Patients with ammonia levels above the derived cut off value of 124µmol/L had a higher frequency of cerebral edema (p=0.020) and deeper encephalopathy grades at admission (p=0.055). These patients also developed other complications like need for ventilation (p=0.001), seizures (p=0.006) and renal failure (p=0.009) more often as compared to patients with lower ammonia levels (Table 2).
Table 2: Mortality, advanced encephalopathy, and complications in ALF patients as a function of plasma ammonia levels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ammonia&lt;124µmol/L (n=38)</th>
<th>Ammonia ≥ 124µmol/L (n=42)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>9(23.7)</td>
<td>33(78.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal failure</td>
<td>1(2.7)</td>
<td>10(23.3)</td>
<td>0.009</td>
</tr>
<tr>
<td>Ventilation</td>
<td>10 (27.0)</td>
<td>30(69.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infection</td>
<td>19(51.4)</td>
<td>28(65.1)</td>
<td>0.212</td>
</tr>
<tr>
<td>Cerebral edema</td>
<td>8(21.6)</td>
<td>20(46.5)</td>
<td>0.020</td>
</tr>
<tr>
<td>Encephalopathy grade (3/4)</td>
<td>23(62.2)</td>
<td>35(81.4)</td>
<td>0.055</td>
</tr>
<tr>
<td>Seizures</td>
<td>3 (8)</td>
<td>15(35)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values are number (percentage)

Ammonia levels and cerebral dysfunction: At presentation, patients in grade 3 or 4 encephalopathy had higher median ammonia levels, than patients in grade 1 or 2 encephalopathy (148.3µmol/L vs. 105.0µmol/L respectively, p=0.021). Similarly patients with clinical cerebral edema at presentation had higher median ammonia levels, than patients without overt cerebral edema, although the difference did not reach statistical significance (156.0µmol/L vs. 119.0µmol/L, p=0.064).

Ammonia levels and biochemical parameters: No correlation was found between the ammonia levels and aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time (PT) prolongation, pH, bicarbonate, urea levels, and albumin. Only the bilirubin levels were found to correlate with the ammonia levels (Pearson correlation coefficient=0.29, p=0.01).

Seizures: Seizures occurred in 18 patients (22.5% overall, 43% among non-survivors). Seizures in these patients were recalcitrant to standard anti-seizure medications and were uniformly fatal. All patients had features of cerebral edema when seizures occurred, and no contributing metabolic factor was found. Patients admitted with higher ammonia levels had a significantly higher seizure incidence (Table 2). Fifteen of 43(35%) patients with ammonia levels ≥
124μmol/L developed seizures compared to 3 of 37 (8%) patients with ammonia levels < 124μmol/L (p=0.006).

**Ammonia levels and time to death:** The ammonia levels were not correlated with the time to death among the non-survivors (Pearson correlation coefficient = 0.013, p=0.936). The 23 patients who died early (within 4 days) and the 19 patients who died later had similar median ammonia levels at admission (172.9μmol/L vs. 186.0μmol/L; p=0.723).

**Comparison of survivors and non-survivors:** Thirteen clinical and biochemical variables were found to significantly influence outcome on univariate analysis (Table 3). Of these 13 variables - infection, need for ventilation, and renal failure developed in most patients subsequent to admission. Hence these three factors were excluded, and only those variables were included in the multivariate analysis that would be available at the initial evaluation. Additionally, since development of seizures was universally predictive of mortality, it could not be used as a covariate. A total of 9 remaining variables were analyzed using forward stepwise logistic regression analysis, taking death as the dependent variable (survival=0, death=1). These variables were dichotomized for best discrimination between survivors and non-survivors using cut of values derived by the construction of individual ROC curves (Table 3). Three variables: arterial pH≤7.40, presence of clinical cerebral edema and arterial ammonia levels>124μmol/L were found to be significantly predictive of death on multivariate analysis. The adjusted OR, and diagnostic accuracy of these three variables are given in table 3.
Table 3: Dichotomous clinical and biochemical variables significantly influencing mortality in patients with ALF

<table>
<thead>
<tr>
<th>Variables</th>
<th>Survivors *</th>
<th>Non-survivors*</th>
<th>p-value</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>Diagnostic accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
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<tr>
<td>Age</td>
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<tr>
<td>&lt; 30 years</td>
<td>31 (81.6)</td>
<td>25 (59.5)</td>
<td>0.032</td>
<td>3.0 (1.1-8.4)</td>
<td></td>
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<tr>
<td>≥ 30 years</td>
<td>7 (18.42)</td>
<td>17 (40.5)</td>
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<tr>
<td><strong>Cerebral edema</strong></td>
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<tr>
<td>Absent</td>
<td>33 (86.8)</td>
<td>19 (45.2)</td>
<td>&lt;0.00</td>
<td>8.0 (2.6-24.5)</td>
<td>12.6 (1.5-108.5)</td>
<td>70.0</td>
</tr>
<tr>
<td>Present</td>
<td>5 (13.2)</td>
<td>23 (54.8)</td>
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<tr>
<td><strong>Grade 3,4 encephalopathy</strong></td>
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<tr>
<td>Absent</td>
<td>16 (42.1)</td>
<td>6 (14.3)</td>
<td>0.005</td>
<td>4.4 (1.5-12.8)</td>
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<tr>
<td>Present</td>
<td>22 (57.9)</td>
<td>36 (85.7)</td>
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<tr>
<td><strong>Biochemical parameters</strong></td>
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<td><strong>Bilirubin</strong></td>
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<tr>
<td>&lt; 15 mg/dL</td>
<td>29 (78.4)</td>
<td>19 (45.2)</td>
<td>0.003</td>
<td>4.4 (1.6-11.8)</td>
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<tr>
<td>≥ 15 mg/dL</td>
<td>8 (21.6)</td>
<td>23 (54.8)</td>
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<tr>
<td><strong>Albumin</strong></td>
<td></td>
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<tr>
<td>≤ 3mg/dL</td>
<td>17 (54.8)</td>
<td>30 (81.1)</td>
<td>0.020</td>
<td>3.5 (1.2-10.4)</td>
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<tr>
<td>&gt; 3 mg/dL</td>
<td>14 (45.2)</td>
<td>7 (18.9)</td>
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<tr>
<td><strong>PT increase</strong></td>
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<tr>
<td>&lt; 25 seconds</td>
<td>23 (67.7)</td>
<td>11 (26.2)</td>
<td>&lt;0.00</td>
<td>5.9 (2.2-15.9)</td>
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<tr>
<td>≥ 25 seconds</td>
<td>11 (32.3)</td>
<td>31 (73.8)</td>
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<tr>
<td><strong>Bicarbonate</strong></td>
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<tr>
<td>≤ 20 mmol/L</td>
<td>13 (39.4)</td>
<td>22 (61.1)</td>
<td>0.071</td>
<td>2.4 (0.9-6.4)</td>
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<tr>
<td></td>
<td>&gt; 20 mmol/L</td>
<td>≤ 7.40</td>
<td>&gt; 7.40</td>
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<tr>
<td></td>
<td>20 (60.6)</td>
<td>4 (12.50)</td>
<td>28 (87.5)</td>
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</tr>
<tr>
<td></td>
<td>14 (38.9)</td>
<td>14 (38.9)</td>
<td>22 (61.1)</td>
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<td></td>
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</tr>
<tr>
<td>pH</td>
<td></td>
<td>0.014 (1.3-15.5)</td>
<td>4.5 (0.8-57.5)</td>
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<tr>
<td></td>
<td></td>
<td>6.6 (12.50)</td>
<td>61.8 (12.50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
<td>&lt;0.00 (4.1-33.8)</td>
<td>11.8 (4.1-33.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 124µmol/L</td>
<td>29 (76.3)</td>
<td>9 (21.4)</td>
<td>1 (4.1-33.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.00 (1.3-15.5)</td>
<td>11.8 (4.1-33.8)</td>
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</tr>
<tr>
<td>≥ 124µmol/L</td>
<td>9 (23.7)</td>
<td>33 (78.6)</td>
<td>10.9 (5.9-284.0)</td>
<td></td>
<td></td>
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**Predicted probability of death:** Using the formula for Z value given earlier and the results of logistic regression, the derived value for Z is:

\[-2.53 + 2.91 \text{ammonia} + 2.41 \text{edema} + 1.40 \text{pH}\]

where, ammonia is scored as 1 if it is \( \geq 124 \mu\text{mol/L} \), and 0 if it is \(< 124 \mu\text{mol/L} \), edema is scored as 1 if present and 0 if absent, and pH is scored as 1 if \(< 7.40 \) and 0 if \( > 7.40 \).

The Z value was calculated for each case and the predicted probability of death was derived using the equation: 

\[ P_X = 1/(1+e^{-Z}) \]

as described earlier. A ROC curve was made from the individual derived probability scores as the independent variable, and mortality as the dependent variable. The area under the ROC curve was found to be 0.93 (Figure 3).

**Comparative prognostic value of total ammonia and pNH\(_3\):** The prognostic value of pNH\(_3\) levels and total ammonia levels at admission were compared in 71 patients, in whom simultaneous estimations were available. Like total ammonia, median pNH\(_3\) levels were significantly higher among non-survivors as compared to the survivors (9.00 X 10\(^{-5}\) mmHg vs. 4.73 X 10\(^{-5}\) mmHg; \( p < 0.001 \)). From the ROC curves constructed with mortality as outcome variable, it was found that the area under curve for both total ammonia and pNH\(_3\) levels were similar (Figure 4). Additionally, by using multivariate logistic regression analysis the \(-2 \log likelihood ratios for total ammonia and pNH\(_3\) were found to be 46.7 and 49.8 respectively, which were not significantly different at two degrees of freedom.

**DISCUSSION**

The present study defined the clinical utility of ammonia levels among patients with ALF. The advantage of our study cohort was its homogenous etiology, with viral hepatitis as the cause in over 80% cases (Table 1), similar to previously reported studies from our center. [8] [16] In western series the etiological profile of the patients have been heterogeneous. [17][18][19] Drug induced and non A-E hepatitis induced ALF have been repeatedly associated with a poorer prognosis. [20][21] In addition, a uniform treatment protocol was followed in all cases. No patient was given enteral feedings for initial 72 hours or ammonia lowering therapies, which would have confounded interpretation of ammonia levels. In the only published human study relating ammonia to outcome, all patients were already on lactulose when ammonia levels were
estimated, and a subgroup was being treated with N-acetyl cysteine. Liver transplantation was not available at our center at the time of study, and all patients could be followed up to survival or demise. Such studies would not be ethically possible at western centers.

The present study documented that ammonia levels at admission were significantly higher among the non-survivors than the survivors (Figure 1 and Table 3). Majority of the patients could be stratified for mortality risk by a cut off ammonia level of 124µmol/L (Figure 2). This observation was further supported by the fact that those patients whose ammonia levels were above 124µmol/L within 24 hours of hospitalization had advanced encephalopathy, higher frequency of overt cerebral edema, renal failure, infection and seizures. Each of these complications was associated with higher mortality among ALF patients (Table2). Clemmesen et al had reported higher ammonia levels in the 14 patients with ALF related to acetaminophen toxicity, who later died from cerebral herniation than in the 30 patients who survived. They found ammonia levels of > 146µmol/L to be predictive of subsequent cerebral herniation. [5] This was an arbitrary level, which was chosen because all patients who developed cerebral herniation had ammonia higher than this level. No estimates of predictive accuracy were provided. Furthermore, their time of ammonia estimation was within 24 hours of developing grade 3 encephalopathy. [5] While the time trends in ammonia levels among ALF patients remain undefined, we found that ammonia levels at admission itself can be used to predict subsequent outcome. From a clinical viewpoint it is encouraging that single arterial ammonia estimations can be so informative. However multiple factors can influence ammonia values, and a wide range of ammonia levels were found. In the present study 18.8% patients had ammonia levels within normal limits at presentation. Thus a single normal ammonia value may not rule out the diagnosis of ALF, or portend a favorable prognosis. More frequent ammonia estimations may further refine the conclusions determined in the present study.

Using logistic regression analysis, arterial pH, presence of cerebral edema and arterial ammonia levels at admission were predictive of mortality (Table3). We excluded infection, need for ventilation and renal failure because these factors were absent at the time of hospitalization, and any mortality estimate incorporating these three variables would require following the entire clinical course of the patient. Such risk estimates would lack any practical utility. In our patients, initial presentation with renal failure or sepsis was infrequent, and these complications usually developed later.

The adjusted OR for mortality attributable to ammonia was 10.9, which was similar to the OR of overt cerebral edema in the present study (Table3). The latter complication in ALF has been documented as the major cause of death. [5] [8][18][19] [20] [21] [22] Therefore, arterial ammonia levels may be used to identify high risk patients for mortality during the early course of the illness. From the multivariable analysis, a quantitative estimate of the mortality risk was derived, and was found to adequately demarcate the non-survivors from the survivors. This is depicted by figure 3, which illustrates the fit of the model. However, the predictive ability of any score is expected to be high when validated on the dataset used to generate the score. Thus, our prognostic model must be validated prospectively in an external dataset. Another limitation of our study is the small sample size available for analysis. This means that our study will have reduced power to detect effects of a moderate size.

Prognostic criteria for ALF have been reported from both the East and West. [22] Both King’s college and Clichy criteria had positive predictive value of 0.89, and negative predictive value of 0.47 and 0.36, respectively, when assessed in non-transplanted patients with non-paracetamol...
induced ALF. No prognostic model till date has incorporated ammonia levels. Any refinement of mortality estimate of ALF patients would be of immense utility to allocate expensive treatment options, like liver transplantation. This model also illustrates the fundamental conclusion drawn from this study, that higher ammonia levels confer higher mortality risk (Table 2 and Figure 1).

Prothrombin time prolongation has been identified as one of the important prognostic variables among patients with ALF in many studies. However in the present study, multivariate analysis did not find it to be an independent predictor of mortality. It is possible that due to the smaller sample size of the present study as compared to some previous reports [8] [19][20][21] the association of prothrombin time prolongation with outcome was not evident. Further, none of the previous studies has included ammonia as a covariate of mortality. Arterial ammonia may have a stronger association with the outcome than prothrombin time, and may have masked the influence of the latter factor, particularly when the sample size is small in which only robust influencing factors of prognosis are likely to be identified.

It has been stated that a relation between the biological toxicity of ammonia and pH of the milieu is “universal throughout nature from onion to man.” [6] However, pNH₃ was not superior to total arterial ammonia levels for prognostication, among our ALF patients. Since estimation of the former is more cumbersome, total ammonia levels may suffice for purpose of risk stratification. Despite the extensive supporting experimental literature, the uncertain role of ammonia is reflected by the fact that hepatology textbooks still quote that therapies to lower ammonia are of no use in ALF. [24][25][26][27] While targeting the gut to lower ammonia production by lactulose or antibiotics may be too slow to influence the outcome in ALF, increasing peripheral utilization of ammonia by ornithine aspartate or sodium benzoate may be useful. Rose et al reported that ornithine aspartate infusion resulted in normalization of plasma ammonia and a significant decrease in brain water content in rats with ALF. [28] No comparable human data exists.

There is a significant unidirectional blood to brain transfer of ammonia in ALF, with brain-blood ammonia ratios increased four fold or more. [29] Measures like hypothermia which reduces this cerebral uptake of ammonia, have been documented to decrease cerebral edema and improve encephalopathy. [1] [30] Therefore higher ammonia levels might lead to a greater degree of cerebral dysfunction in ALF. We found a significantly higher ammonia levels among patients with deeper encephalopathy as expected. While seizures have been commonly reported in the congenital hyperammonemic syndromes, they are rarely reported in ALF. [31][32][33] We do not routinely paralyze or sedate ALF patients, and have observed a high frequency of seizures among our ALF patients. [9] In this study, patients with ammonia levels ≥ 124µmol/L at admission had increased seizure frequency (Table 2). It is likely that this cut off value of ammonia identified patients with advanced cerebral dysfunction. More direct evidence is required to link ammonia levels and seizures in patients with ALF.

In summary, we have demonstrated that high arterial ammonia levels as measured within 24 hours of patient presentation, are associated with a worse outcome among patients with ALF. Our findings encourage viewing ALF as a ‘hyperammonemic state’, and provide a rational background for the use of ammonia lowering therapies.
REFERENCES


**Figure Legend**

Figure 1: Comparison of arterial ammonia levels at admission between survivors and non-survivors among ALF patients.

Figure 2: Receiver Operated Curve (ROC) of ammonia levels and mortality. Arterial ammonia levels > 124µmol/L: 78.6% sensitive and 76.3% specific for predicting mortality.

Figure 3: ROC curve of the predicted probability of death derived from the independent variables associated with mortality.

Figure 4: ROC curves of total ammonia and pH3 levels, and mortality in 71 ALF patients. AUC for total ammonia is 0.77 (95% CI: 0.65-0.89), and for pH3 is 0.76 (95% CI: 0.65-0.88).
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