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# The value of ammonia as a biomarker in patients with cirrhosis

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**Conflict of Interest:** Rajiv Jalan has research collaborations with Yaqrit. 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 Ltd, a spin out company from University College London, Cyberliver Ltd and Hepyx Ltd. The other authors declare no conflicts of interest.

**Abstract:**
Ammonia is a product of amino acid metabolism that accumulates in the blood of patients with cirrhosis and plays a pivotal role in the pathogenesis of hepatic encephalopathy (HE). Despite being one of the main drivers of brain dysfunction, for many years international societies stated that increased blood ammonia does not add any diagnostic, staging, or prognostic value for HE in patients with cirrhosis. Nonetheless, in the last decades, evidence is emerging that supports the utility of ammonia for risk stratification, but its role in guiding HE diagnosis, staging and treatment is unclear and there is equipoise in its use in clinical practice. This review provides the latest evidence on the value of ammonia as a biomarker in patients with cirrhosis. Although correct measurement of ammonia requires disciplined sample collection, it provides extremely useful clinical guidance for the diagnosis of HE, offers prognostic information and it defines a therapeutic target.

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The value of ammonia as a biomarker in patients with cirrhosis

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## ABSTRACT

Ammonia is a product of amino acid metabolism that accumulates in the blood of patients with cirrhosis and plays a pivotal role in the pathogenesis of hepatic encephalopathy (HE). Despite being one of the main drivers of brain dysfunction, for many years international societies stated that increased blood ammonia does not add any diagnostic, staging, or prognostic value for HE in patients with cirrhosis. Nonetheless, in the last decades, evidence is emerging that supports the utility of ammonia for risk stratification, but its role in guiding HE diagnosis, staging and treatment is unclear and there is equipoise in its use in clinical practice. This review provides the latest evidence on the value of ammonia as a biomarker in patients with cirrhosis. Although correct measurement of ammonia requires disciplined sample collection, it provides extremely useful clinical guidance for the diagnosis of HE, offers prognostic information and it defines a therapeutic target.

## KEYWORDS

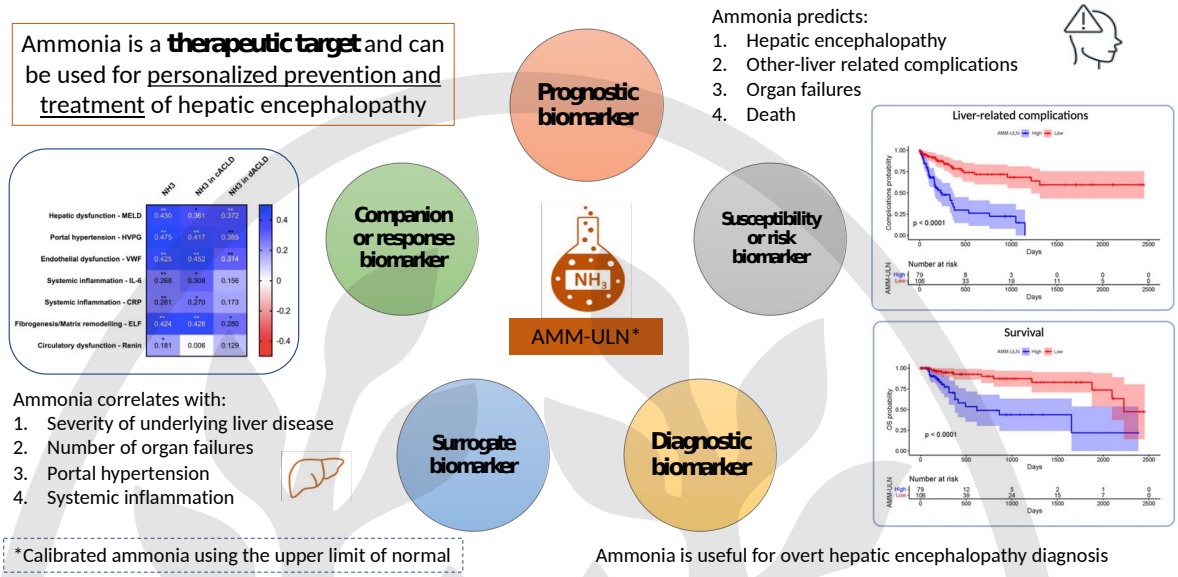
Ammonia, hepatic encephalopathy, cirrhosis, biomarker.

## LAY SUMMARY

Ammonia is a product of amino acid metabolism that accumulates in blood of patients with cirrhosis. It is one of the main drivers of hepatic encephalopathy (HE) but it is not routinely used in clinical practice for diagnostic, staging, or prognostic purposes. This review aims to provide the latest evidence on the value of ammonia as a biomarker in patients with cirrhosis to illustrate its clinical utility for the diagnosis of HE, for identification of patients at risk of developing overt HE and for personalised prevention and treatment of HE.

## GRAPHICAL ABSTRACT

## The value of ammonia as a biomarker in patients with cirrhosis




### Introduction

Hepatic encephalopathy (HE) is a complex behavioural, neuropsychiatric and neurodevelopmental disorder that occurs in about 30-80% of patients with cirrhosis (1-2). Worldwide, there are about 10 million people with decompensated cirrhosis and about 30% of these patients will be hospitalised with confusion, disorientation or coma (known as overt HE -OHE-) each year (3-4). Importantly, when patients with cirrhosis develop OHE, they are prone to repeated episodes and the chance of complete reversibility decreases, their health-related quality of life declines and the risk of death is abruptly increased up to 20-30% at 1-year (5-6). Ammonia is a product of amino acid metabolism that is known to accumulate in the blood of patients with cirrhosis due to the reduced function of the urea cycle, which is uniquely located in the liver. In health, its circulating levels are tightly controlled but in liver disease, hyperammonemia is commonly observed and recognized to play a pivotal role in the pathogenesis of HE (7). It is involved in several pathophysiological processes such as astrocytic swelling, astrocytic senescence, neuronal cell death, neuroinflammation, mitochondrial dysfunction, altered cerebral bioenergetics impaired glioneuronal communication resulting in neurological dysfunction (8-9). Despite being one of the main drivers of brain dysfunction, for many years the European and the American Association for the Study of the Liver (EASL and AASLD) stated that increased blood ammonia alone does

not add any diagnostic, staging, or prognostic value for HE in patients with chronic liver disease, but in case an ammonia level is checked in a patient with OHE and its levels are normal, the diagnosis of HE is not clear (1). In relation to this, the new EASL clinical guidelines recommended that in patients with delirium/encephalopathy and liver disease, plasma ammonia measurement should be performed, as a normal value brings the diagnosis of HE into question (2). In the last decades, evidence is emerging that supports the utility of ammonia for risk stratification both in acutely decompensated patients and clinically stable outpatients with cirrhosis (10-11). Nonetheless, its role in guiding HE treatment is unclear and there is equipoise in its use in clinical practice. The aim of the present narrative review is to provide the latest evidence on the value of ammonia as a biomarker in patients with cirrhosis.

**Ammonia for HE diagnosis**

The value of ammonia for OHE diagnosis has been a matter of debate for many years. EASL and AASLD recommend that patients with suspected HE should be subjected to the same standard diagnostic evaluation as other patients with altered consciousness to identify alternative or additional causes of neuropsychiatric impairment to improve diagnostic accuracy and the results of treatment (1-2). Comprehensive assessment of the state of consciousness might include imaging modalities, neurophysiological and neuropsychological tests and, biochemical measurements, in particular ammonia levels. Many years ago, several studies showed that a normal blood ammonia level has a high negative predictive value (12, 13). Therefore, a normal ammonia in a patient with cirrhosis and delirium should prompt renewed or further differential diagnostic work-up for other causes of delirium. A study performed in 42 patients with cirrhosis, 27 with HE and 15 without HE, and 9 controls without liver disease showed that all venous ammonia (AUC: 0.90 ± 0.03), arterial ammonia (AUC: 0.93 ± 0.04) and $pNH_3$ (AUC: 0.92 ± 0.04) present an excellent accuracy in identifying patients with HE (12). Nonetheless, some patients presented high ammonia levels with a normal mental status. This discordance between ammonia levels and the neurological symptoms suggests the possibility that factors other than ammonia play an important role in the pathogenesis of HE and that blood ammonia concentration may not always be representative of the ammonia levels to which the brain is directly exposed. Given that HE is a diagnosis of exclusion, the latest EASL guideline recommends measuring plasma ammonia levels for HE diagnosis with a high level of evidence and a 95% consensus (2). The role of

ammonia in identifying patients that have minimal HE (mHE) o grade 1 HE has not been assessed. Figure 1 shows a proposed algorithm for assessment of OHE in hospitalized patients with cirrhosis and optimize their management.

**Ammonia for staging severity of HE**

Several studies showed a positive correlation between ammonia and the severity of HE. In a prospective single-center cohort study with 121 hospitalized patients with cirrhosis, the authors measured arterial and venous total ammonia, as well as arterial and venous partial pressure of ammonia. They found that each of the four measures increased with the severity of HE: arterial total ammonia ($r_s$=0.61, p≤0.001), venous total ammonia ($r_s$=0.56, p ≤0.001), arterial partial pressure of ammonia ($r_s$=0.55, p≤0.001), and venous partial pressure of ammonia ($r_s$=0.52, p ≤0.001) (14). The previously mentioned study that included 42 patients with cirrhosis and 9 without liver disease also showed that venous ammonia (*r*=0.56), arterial ammonia (*r*=0.76), $pNH_3$ (*r*=0.75) were all correlated with the severity of HE, concluding that there is little to be gained by measuring pNH3 or arterial ammonia over venous ammonia. Nonetheless, there were considerable overlap of the values observed in patients with differing degrees of HE (12). In a cohort study that included 101 patients with acute-on-chronic liver failure (ACLF) requiring admission to the intensive care unit, ammonia levels (μmol/L) were 73±32, 91±26, 90±24 in patients with HE grade 0-1, HE 2 and HE 3-4, respectively (p=0.02), indicating that ammonia was elevated in OHE patients, but was not corelated with the severity of HE (15). Therefore, although ammonia is generally elevated in patients with OHE, its levels do not really correlate with the severity of HE and an isolated high ammonia level does not always determine OHE diagnosis. Therefore, it is important to note that the effect of ammonia on the brain may be conditioned by other factors such as age, sodium levels, inflammation, infection or the use of neurotropic drugs (16).

**Ammonia for staging severity of the underlying liver disease**

Beyond its connection with HE, ammonia has been linked to the severity of liver disease in several aspects. In the outpatient clinic, a prospective multicenter study including 754 patients with cirrhosis, ammonia levels standardized to the upper-limit of normal for the reference laboratory (AMM-ULN) exhibited higher levels in patients with more advanced stages of cirrhosis (0.9, 1.5, and 1.6 in Child-Turcotte-Pugh -CTP- groups A, B, and C, respectively, and 1.0, 1.4, and 1.6 in MELD score groups ≤9, 9–12,

and ≥12, respectively, p<0.001), with a positive correlation with both the MELD (r=0.22, p<0.001) and MELD-Na score (r=0.25, p<0.001). Moreover, AMM-ULN level was significantly higher in decompensated cirrhosis (1.6) compared with the compensated group (0.9) (p<0.001) (17). Similar results were reported in various studies (14, 18-19). In a retrospective, single-centre cohort study including 549 clinically stable outpatients with compensated advanced chronic liver disease, ammonia also exhibited a progressive increase across different clinical stages of cirrhosis (p<0.001) (18).

In hospitalized patients, an elevated ammonia level is also associated with a higher frequency of organ failures. In a cohort study including 312 patients with acute decompensation (AD) or ACLF, the median level of arterial ammonia at admission was higher in ACLF compared to AD patients (103 vs 86μmol/L, p<0.001). In addition, those with baseline ammonia levels ≥79.5μmol/L presented 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 with patients without hyperammonemia (20). In a study including 174 ACLF patients based on the Asian Pacific Association for the Study of the Liver (APASL) definition, an ammonia level ≥89μmol/L was associated with a higher frequency of cerebral failure (36.2% vs 5.2%, p< 0.001) and coagulation failure (39.7% vs 16.4%, p< 0.001). This study also showed a positive correlation between serum ammonia and ACLF grade (r=0.342, P<0.001) (21).

In terms of portal hypertension, higher ammonia levels have been associated with increasing hepatic venous pressure gradients (HVPG); 28.3μmol/L (24.0-37.1), 30.7 (22.0-35.8), 35.5 (26.8-46.0), and 44.8 (33.8-59.6) in patients with HVPG of 0-5 mmHg, 6-10 mmHg, 10-15 mmHg, and higher than 16 mmHg, respectively (18). A single-center cross-section study from Egypt including 359 patients with cirrhosis also demonstrated a correlation between serum ammonia and esophageal varices (EV). The optimal cutoff for the presence of EV was >123 μg/dL (72.23μmol/L), yielding a sensitivity and specificity of 70% and 92%, respectively, and an accuracy of 83.4%. An ammonia cutoff value of >156 μg/dL (91.6μmol/L) was associated with high-risk EV with a sensitivity, specificity and accuracy of 52.38%, 75.68%, and 64.2%, respectively (22). Similarly, the value of serum ammonia as a non-invasive method for early prediction of EV was confirmed in a more recent study, although with different crude

ammonia cutoffs (23). Additionally, a single-center cross-sectional study involving 381 patients with HCV or hepatitis B virus (HBV)-related cirrhosis revealed that plasma ammonia correlates with the presence and severity of portal hypertensive gastropathy (PHG). Cases with severe PHG had significantly higher levels of blood ammonia (192.69 ± 59.87 vs.151.39 ± 54.37 μg/dL, p=0.026) (24).

Despite all the evidence correlating ammonia levels with severity of liver disease (Table 1), there are significant overlaps between different stages that challenges its role as a biomarker for disease staging. However, the associations described here suggest that ammonia may be more than a biomarker and may independently drive organ dysfunction as it is known to be cause of immune dysfunction, sarcopenia and liver injury in patients with chronic liver disease.

**Ammonia for guiding HE treatment**

The first and most important aspect in the treatment of HE is the identification and correction of precipitating causes, but, notably, in 30% of cases of HE, precipitating factors are not identified (25). Specific treatment for HE has little prospect of success without management of precipitating factors, and it remains unclear whether HE specific treatment alone improves prognosis (2). It is generally accepted that hyperammonemia is the cardinal pathogenic factor in the pathogenesis of HE. Therefore, specific treatment strategies for HE aim to reduce ammonia levels by either decreasing ammonia production or increasing its elimination. Nonabsorbable disaccharides (lactulose, lactitol), rifaximin, probiotics, fecal microbiota transplantation aim to decrease ammonia production, while Zinc, Carnitine, L-ornithine-L-aspartate, metabolic ammonia scavengers (sodium benzoate, glycerol phenylbutyrate, ornithine phenylacetate) aim to increase elimination (26-27) (Figure 2). Nonetheless, in clinical trials, patients are usually not categorized by hyperammonemia, which probably impacts negatively in the development of new drugs considering that most of the therapies are directed to reduce ammonia levels. An example of this is the failure of a Phase 2b study of AST-120, which used R-BANS, a neuropsychometric test to select patients for inclusion, which failed most likely due to the lack of an accurate biomarker for patient selection (28). The failure of ornithine phenylacetate in a Phase 2b study was due to the inclusion of patients in whom ammonia was normal. Removal of these patients from the

subsequent post-hoc analyses, allowed the trial to demonstrate significant effect of the drug on time to HE improvement (29).

A similar situation is seen in clinical practice. Whereas a recent study demonstrated that ammonia testing during hospitalization has increased significantly from 2007 to 2015, and particularly in 2014 and 2015, these levels are not used for guiding HE treatment (30-31). This was illustrated by a study performed on 1202 patients admitted to the hospital with HE, that aimed to evaluate the impact of ammonia levels on HE management. The authors showed that ammonia levels were only measured in 46% of patients, and that there was no difference in lactulose dose in patients with and without elevated ammonia levels ($p=0.42$) (32).

Based on the current evidence, ammonia is a useful companion and response biomarker for personalized prevention and treatment of HE but further validation would add to how ammonia can be used for this purpose in the real world.

**Ammonia as a prognostic biomarker in the outpatient setting**

Until recently there were no data evaluating the role of ammonia as a prognostic biomarker in the outpatient population with cirrhosis. Most of the studies have been developed during the last 2-years. A prospective multicentre study including 754 clinically stable outpatients with cirrhosis from 3 liver units demonstrated that ammonia was an independent predictor of hospitalisation due to not only HE but also other liver-related complications and mortality. Multivariable analysis revealed that AMM-ULN carried greater weight than any other prognostic variable, with higher predictive accuracy than traditional severity scores such as Child-Pugh or MELD. An AMM-ULN of 1.4 times was identified as the optimal cut-off to define the risk of hospitalization and mortality (17). These results were validated in three external cohorts of 130 (17), 147 (33) and in 549 patients (18). In the latter study, the authors also demonstrated that venous ammonia predicts hepatic decompensation, non-elective liver-related hospitalisation, ACLF and liver-related deaths, independently of established prognostic indicators including C-reactive protein and HVPG (18). Therefore, although venous ammonia is linked with several key disease-driving mechanisms, its prognostic value is not explained by associated hepatic dysfunction, systemic inflammation, or portal hypertension severity, suggesting direct toxicity. In relation to the prognostic value of

ammonia, a recent study from China evaluated the discrimination and calibration of a new prognostic model based on plasma ammonia replacing HE in the CTP score. The authors demonstrated the feasibility and potential for plasma ammonia to enhance the prognostication of transplant-free survival (34).

Despite all the new evidence supporting the role of ammonia in the prognosis of HE and other-liver related complications in outpatients with cirrhosis, it is not routinely measured, nor is its measurements recommended in the current clinical guidelines. Rather, for prediction purposes, the EASL and AASLD recommend that patients with cirrhosis should be evaluated for the presence of mHE using neurophysiological, neuropsychological or psychophysical tests, given that mHE has been described as a condition that predisposes to OHE (1-2). However, a recent prospective-observational study of 132 patients with cirrhosis compared six tests including psychometric hepatic encephalopathy score (PHES), Animal Naming Test, Critical Flicker Frequency (CFF), Inhibitory Control Test, EncephalApp and Continuous Reaction Time Test for their predictive ability showing an overall limited value of these tests in predicting OHE and no ability to predict rehospitalization or mortality (35). In addition, most clinicians do not routinely use them because performance of the tests is time-consuming, requires training, are not reimbursed and the tools are not widely available (36). A recent prospective study aimed to determine the role of neuropsychological or psychophysical tests and ammonia for risk stratification regarding subsequent OHE development demonstrated that, on multivariable analysis, AMM-ULN but not PHES or CFF was an independent predictor for development of OHE. Using data from 3 European liver units and including 426 outpatients with cirrhosis without previous OHE episodes, the authors developed the AMMON-OHE model that included sex, diabetes, albumin, creatinine and AMM-ULN. This new model performed better than PHES, CFF and their combination with other clinical and biochemical variables and showed a C-index of 0.844 and 0.728 in two external validation cohorts of 267 and 381 patients. Further analyses of a subgroup of patients with previous OHE showed that the AMMON-OHE model was also useful for the prediction of additional episodes of OHE. An online App is available (https://ammon-ohe.shinyapps.io/ammon-ohe/) for clinical use (19). Therefore, the AMMON-OHE model may be useful not only for prognosis but also for the selection of patients for clinical trials or as a companion biomarker for the better use of currently available drugs and development of future therapies. Figure 3 includes an

algorithm based on the AMMON-OHE model to stratify the risk of OHE in clinically stable outpatients with cirrhosis and optimize their management.

**Ammonia as a prognostic biomarker in the inpatient setting**

In last decades, several studies have evaluated the prognostic role of ammonia in patients admitted to the hospital with AD or ACLF with controversial results. In a retrospective single centre cohort study of 494 consecutive hospitalized patients (265 with HE), the authors demonstrated that an elevated ammonia on admission was associated with reduced 90-day transplant-free survival after adjusting for established predictors and that patients with >60μmol/L had higher 90- and 30-day risk of death or transplantation (45.2% vs. 31.2%, p=0.001; and 31.6% vs 15.7%, p<0.0001, respectively) (10). Similarly, a prospective multicentre study including 498 patients admitted with an AD showed that ammonia was an independent predictor of 28-day mortality (HR=1.009, p<0.001) and that an ammonia level ≥79.5 μmol/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]). In addition, lack of improvement in baseline ammonia at day 5 was associated with high mortality (70.6%) (11). In another study evaluating the dynamics of ammonia levels during hospitalization of 229 consecutive patients with ACLF and 83 patients with AD, persistent or incident hyperammonemia was associated with new-onset organ failure 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) (20). A more recent study including 492 and 600 patients admitted to the intensive care unit (ICU) in the derivation and validation cohorts, respectively, showed that a model of serial ammonia between days 1 and 3 predicted hospital mortality with a c-index of 0.73 and a Brier score=0.17 in the validation cohort (37). In addition, a study of 101 ACLF patients admitted to the ICU with serial ammonia levels, jugular venous oxygen saturation and white cell count at days 0, 1, 3 and 7, demonstrated that not only deterioration in ammonia but also in inflammation and cerebral oxygenation are predictive of mortality and that improvement in these parameters is associated with a reduction HE grade (15). Four studies conducted using the APASL-ACLF definition also confirmed that ammonia is correlated with organ failure and mortality, regardless of the presence of HE (21, 38-40). The results of these studies highlight the importance of ammonia measurement in the inpatient setting, not only as a prognostic biomarker, but

also as a therapeutic target. Table 2 summarizes the most recent evidence for the importance of ammonia as a prognostic biomarker.

**Ammonia in extrahepatic complications associated with cirrhosis**

Ammonia has been implicated in the pathogenesis of other liver-related complications. Sarcopenia is one of the most frequent, with a prevalence of 40%-70%. It is associated with poor prognosis and increased mortality both before and after liver transplantation (LT) (41-42). In addition, a recent meta-analysis showed that sarcopenia is positively associated with the presence of HE (OR 2.74; 95% CI 1.87-4.01) (43). In liver disease, muscle plays a significant compensatory role in ammonia detoxification. This process involves the enzyme glutamine synthetase, which converts glutamate to glutamine in the presence of ammonia. Muscle loss may thereby contribute to the development of hyperammonemic encephalopathy (44-46). On the anther hand, increasing evidence shows that high serum and muscle ammonia levels contribute to sarcopenia in cirrhosis. The possible mechanism includes mediating myostatin transcription and expression which inhibits skeletal muscle protein synthesis, mitochondrial dysfunction and autophagy (47-49). Thus, hyperammonemia leads to severe muscle damage and sarcopenia, which in turn restricts the ability of muscle to eliminate excess blood-borne ammonia. Ammonia-lowering therapies are implicated in breaking this vicious cycle and therefore might be a promising treatment target for sarcopenia (50-53).

Immune dysfunction is another important feature of patients with cirrhosis, characterized by systemic inflammation and immune deficiency (54). In patients with cirrhosis, neutrophils exhibit increased oxidative burst and reduced phagocytic capacity (55). It has been shown that hyperammonemia induces neutrophil swelling, further impairing their phagocytic activity (56-57). Increased spontaneous oxidative burst was also observed in canine polymorphonuclear cells incubated with ammonia (58). A previous study also demonstrated the inhibitory effect of ammonia on neutrophil chemotaxis (59). Ammonia induced dendritic cell dysfunction was reported as well. In vitro experiments showed that ammonia diminished dendritic cells (DC) count, phagocytosis and lymphocyte stimulation. Ammonia also induced DC swelling and mitochondrial dysfunction. The reduced phagocytosis function of DC cells was confirmed in a hyperammonemia mice model (60). More recently, a clinical study showed that patients with OHE had increased risk of developing a new infection.

Although the authors hypothesized that this may be the result of brain dysfunction on immune function, the role of ammonia cannot be excluded. Taken together, these results suggest that treating hyperammonemia may improve immune dysfunction in patients with cirrhosis (61).

**Importance of correct ammonia measurements**

It is important to highlight that ammonia measurement is complex and it is only clinically useful if measured meticulously using standardized protocols. Ammonia levels can be altered by exercise and diet. Therefore, it should not be measured for at least 2-hours after strenuous exercise and 4 hours of a meal. Furthermore, it requires careful sample handling in cooled EDTA tubes, rapid transport to the laboratory in refrigerated conditions and standard laboratory assays. The values obtained are highly variable making it difficult to compare across laboratories. To account for different 'normal ranges' in ammonia levels in different laboratories, a new calibrated ammonia level has been validated using the formula: *serum ammonia / reference laboratory upper limit of normal* (AMM-ULN), to ensure uniformity and generalisability across hospitals (62).

**Conclusion**

In conclusion, although correct measurement of ammonia requires disciplined sample collection and rapid transport to the laboratory, the measurement is widely available, relatively straightforward, and cheap. As discussed in this review, ammonia offers extremely useful prognostic information in both outpatients and acutely decompensated patients with cirrhosis, provides clinical guidance for the diagnosis of HE and, more importantly, it defines a therapeutic target for personalized medicine approaches (Table 3). It is important to note that like every other biomarker used in clinical practice, ammonia needs to be measured properly and interpretation of a given ammonia level needs to be contextualised to the patient and the clinical situation.

**REFERENCES**

1. Vilstrup H, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American

Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology*. 2014 Aug;60(2):715-35.

2. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the management of hepatic encephalopathy. *J Hepatol*. 2022 Jun 7:S0168-8278(22)00346-4.

3. Tapper EB, Henderson JB, Parikh ND, Ioannou GN, Lok AS. Incidence of and Risk Factors for Hepatic Encephalopathy in a Population-Based Cohort of Americans With Cirrhosis. Hepatol Commun. 2019 Sep 6;3(11):1510-1519.

4. Casadaban LC, Parvinian A, Minocha J, Lakhoo J, Grant CW, Ray CE Jr, et al. Clearing the Confusion over Hepatic Encephalopathy After TIPS Creation: Incidence, Prognostic Factors, and Clinical Outcomes. Dig Dis Sci. 2015 Apr;60(4):1059-66.

5. Arguedas MR, DeLawrence TG, McGuire BM. Influence of hepatic encephalopathy on health-related quality of life in patients with cirrhosis. Dig Dis Sci. 2003 Aug;48(8):1622-6.

6. Tapper EB, Halbert B, Mellinger J. Rates of and Reasons for Hospital Readmissions in Patients With Cirrhosis: A Multistate Population-based Cohort Study. Clin Gastroenterol Hepatol. 2016 Aug;14(8):1181-1188.e2.

7. Bosoi CR, Rose CF. Identifying the direct effects of ammonia on the brain. Metab *Brain Dis*. 2009 Mar;24(1):95-102.

8. **Angelova PR, Kerbert AJC,** Habtesion A, Hall A, Abramov AY, Jalan R. Hyperammonaemia induces mitochondrial dysfunction and neuronal cell death. *JHEP Rep*. 2022 May 23;4(8):100510.

9. Häussinger D, Dhiman RK, Felipo V, Görg B, Jalan R, Kircheis G, et al. Hepatic encephalopathy. Nat Rev Dis Primers. 2022 Jun 23;8(1):43.

10. Patwardhan VR, Jiang ZG, Risech-Neiman Y, Piatkowski G, Afdhal NH, Mukamal K, et al. Serum Ammonia is Associated With Transplant-free Survival in Hospitalized Patients With Acutely Decompensated Cirrhosis [corrected]. J Clin Gastroenterol. 2016 Apr;50(4):345-50.

11. Shalimar, Sheikh MF, Mookerjee RP, Agarwal B, Acharya SK, Jalan R. Prognostic role of ammonia in patients with cirrhosis. Hepatology 2019;70:982–994.

12. Nicolao F, Efrati C, Masini A, Merli M, Attili AF, Riggio O. Role of determination of partial pressure of ammonia in cirrhotic patients with and without hepatic encephalopathy. J Hepatol 2003;38:441–446

13. Gundling F, Zelihic E, Seidl H, Haller B, Umgelter A, Schepp W, et al. How to diagnose hepatic encephalopathy in the emergency department. Ann Hepatol 2013;12:108–114.

14. Ong JP, Aggarwal A, Krieger D, Easley KA, Karafa MT, Van Lente F, et al. Correlation between ammonia levels and the severity of hepatic encephalopathy. Am J Med. Feb 15 2003;114(3):188-93.

15. 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 Jun;22(6):732-42.

16. Rose CF, Amodio P, Bajaj JS, Dhiman RK, Montagnese S, Taylor-Robinson SD, et al. Hepatic encephalopathy: Novel insights into classification, pathophysiology and therapy. J Hepatol. 2020 Dec;73(6):1526-1547.

17. **Tranah TH, Ballester MP, Carbonell-Asins JA**, Ampuero J, Alexandrino G, Caracostea A, et al. Plasma ammonia levels predict hospitalisation with liver-related complications and mortality in clinically stable outpatients with cirrhosis. *J Hepatol.* 2022 Jul 22:S0168-8278(22)02947-6.

18. Balcar L, Krawanja J, Scheiner B, Paternostro R, Simbrunner B, Semmler G, et al. Impact of ammonia levels on outcome in clinically stable outpatients with advanced chronic liver disease. JHEP Rep. 2023 Jan 23;5(4):100682.

19. **Ballester MP, Tranah TH, Balcar L**, Fiorillo A, Ampuero J, Kerbert AJC, et al. Development and validation of the AMMON-OHE model to predict risk of overt hepatic encephalopathy occurrence in outpatients with cirrhosis. J Hepatol. 2023 Oct;79(4):967-976.

20. Shalimar, Rout G, Kumar R, Singh AD, Sharma S, Gunjan D, et al. Persistent or incident hyperammonemia is associated with poor outcomes in acute decompensation and acute-on-chronic liver failure. JGH Open. 2020 Feb 28;4(5):843-850.

21. Hu C, Huang K, Zhao L, Zhang F, Wu Z, Li L. Serum ammonia is a strong prognostic factor for patients with acute-on-chronic liver failure. Sci Rep. 2020 Oct 12;10(1):16970.

22. Elzeftawy A, Mansour L, Kobtan A, Mourad H, El-Kalla F. Evaluation of the blood ammonia level as a non-invasive predictor for the presence of esophageal varices and the risk of bleeding. Turk J Gastroenterol. 2019 Jan;30(1):59-65.

23. Darweesh SK, Elsabaawy MA, Eltahawy MA, Ghanem HS, Abdel-Razek W. Serum ammonia as a non-invasive marker for early prediction of esophageal varices. Eur J Gastroenterol Hepatol. 2021 Feb 1;32(2):230-236.

24. El-Kalla F, Mansour L, Kobtan A, Elzeftawy A, Abo Ali L, Abd-Elsalam S, et al. Blood Ammonia Level Correlates with Severity of Cirrhotic Portal Hypertensive Gastropathy. Gastroenterol Res Pract. 2018 Jul 29;2018:9067583.

25. Bellafante D, Gioia S, Faccioli J, Riggio O, Ridola L, Nardelli S. Old and New Precipitants in Hepatic Encephalopathy: A New Look at a Field in Continuous Evolution. J Clin Med. 2023 Feb 2;12(3):1187.

26. Rahimi RS, Brown KA, Flamm SL, Brown RS Jr. Overt Hepatic Encephalopathy: Current Pharmacologic Treatments and Improving Clinical Outcomes. Am J Med. 2021 Nov;134(11):1330-1338.

27. Eriksen PL, Djernes L, Vilstrup H, Ott P. Clearance and production of ammonia quantified in humans by constant ammonia infusion - the effects of cirrhosis and ammonia-targeting treatments. J Hepatol. 2023 Aug;79(2):340-348.

28. Pockros P, Hassanein T, Vierling J, Heuman D, Hillebrand D, Chojkier M, et al. Phase 2, multicenter, randomized study of AST-120 (spherical carbon adsorbent) vs. Lactulose in the treatment of low-grade hepatic encephalopathy (HE). J Hep 2009:50.

29. Rahimi RS, Safadi R, Thabut D, Bhamidimarri KR, Pyrsopoulos N, Potthoff A, et al. Efficacy and Safety of Ornithine Phenylacetate for Treating Overt Hepatic Encephalopathy in a Randomized Trial. Clin Gastroenterol Hepatol. 2021 Dec;19(12):2626-2635.e7.

30. Deutsch-Link S, Moon AM, Jiang Y, Barritt AS, Tapper EB. Serum Ammonia in Cirrhosis: Clinical Impact of Hyperammonemia, Utility of Testing, and National Testing Trends. Clin Ther. 2022 Mar;44(3):e45-e57.

31. Hannah N, Vasic D, Kansal A, Al-Ani A, Hebbard G, Sood S. Serum ammonia does not guide management and is overutilised in patients with cirrhosis in hospital settings. Intern Med J. 2023 Nov;53(11):2057-2064.

32. Haj M, Rockey DC. Ammonia Levels Do Not Guide Clinical Management of Patients With Hepatic Encephalopathy Caused by Cirrhosis. Am J Gastroenterol. 2020 May;115(5):723-728.

33. Gairing SJ, Kaps L, Schleicher EM, Galle PR, Labenz C. Ammonia - an old friend with a new area of application. J Hepatol. 2023 Jan;78(1):e22-e23.

34. Wang X, Zhang M, Xiao J, Zhang W, Wang Y, Zhang S, et al. A modified Child-Turcotte-Pugh score based on plasma ammonia predicts survival for patients with decompensated cirrhosis. QJM. 2023 Jun 8;116(6):436-442.

35. Ehrenbauer AF, Egge JFM, Gabriel MM, Tiede A, Dirks M, Witt J, et al. Comparison of six tests for diagnosing minimal hepatic encephalopathy and predicting clinical outcome - A prospective, observational study. Hepatology. 2024 Feb 13.

36. Hansen MKG, Kjærgaard K, Eriksen LL, Grønkjær LL, Mikkelsen ACD, Sandahl TD, et al. Psychometric methods for diagnosing and monitoring minimal hepatic encephalopathy -current validation level and practical use. Metab Brain Dis. 2022 Mar;37(3):589-605.

37. Cardoso FS, Kim M, Pereira R, Bagulho L, Fidalgo P, Pawlowski A, et al. Early serum ammonia variation in critically ill patients with cirrhosis: A multicentre cohort study. Aliment Pharmacol Ther. 2023 Oct;58(7):715-724.

38. Verma N, Dhiman RK, Choudhury A, Taneja S, Duseja A, Singh V, et al; APASL ACLF Research Consortium (AARC) for APASL ACLF working Party. Dynamic assessments of hepatic encephalopathy and ammonia levels predict mortality in acute-on-chronic liver failure. Hepatol Int. 2021 Aug;15(4):970-982.

39. Chiriac S, Stanciu C, Cojocariu C, Singeap AM, Sfarti C, Cuciureanu T, et al. Role of ammonia in predicting the outcome of patients with acute-on-chronic liver failure. World J Clin Cases. 2021 Jan 26;9(3):552-564.

40. Thanapirom K, Treeprasertsuk S, Choudhury A, Verma N, Dhiman RK, Al Mahtab M, et al. Ammonia is associated with liver-related complications and predicts mortality in acute-on-chronic liver failure patients. Sci Rep. 2024 Mar 9;14(1):5796.

41. Tandon P, Montano-Loza AJ, Lai JC, Dasarathy S, Merli M. Sarcopenia and frailty in decompensated cirrhosis. J Hepatol. Jul 2021;75 Suppl 1(Suppl 1):S147-S162.

42. Ebadi M, Bhanji RA, Mazurak VC, Montano-Loza AJ. Sarcopenia in cirrhosis: from pathogenesis to interventions. J Gastroenterol. Oct 2019;54(10):845-859.

43. Chang KV, Chen JD, Wu WT, Huang KC, Lin HY, Han DS. Is sarcopenia associated with hepatic encephalopathy in liver cirrhosis? A systematic review and meta-analysis. J Formos Med Assoc. Apr 2019;118(4):833-842.

44. Lattanzi B, D'Ambrosio D, Merli M. Hepatic Encephalopathy and Sarcopenia: Two Faces of the Same Metabolic Alteration. J Clin Exp Hepatol. Jan-Feb 2019;9(1):125-130.

45. Jindal A, Jagdish RK. Sarcopenia: Ammonia metabolism and hepatic encephalopathy. Clin Mol Hepatol. Sep 2019;25(3):270-279.
46. Di Cola S, Nardelli S, Ridola L, Gioia S, Riggio O, Merli M. Ammonia and the Muscle: An Emerging Point of View on Hepatic Encephalopathy. J Clin Med. Jan 26 2022;11(3)
47. Qiu J, Thapaliya S, Runkana A, Yang Y, Tsien C, Mohan ML, et al. Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-kappaB-mediated mechanism. Proc Natl Acad Sci U S A. Nov 5 2013;110(45):18162-7.
48. Davuluri G, Krokowski D, Guan BJ, Kumar A, Thapaliya S, Singh D, et al. Metabolic adaptation of skeletal muscle to hyperammonemia drives the beneficial effects of l-leucine in cirrhosis. J Hepatol. Nov 2016;65(5):929-937.
49. Davuluri G, Allawy A, Thapaliya S, Rennison JH, Singh D, Kumar A, et al. Hyperammonaemia-induced skeletal muscle mitochondrial dysfunction results in cataplerosis and oxidative stress. J Physiol. Dec 15 2016;594(24):7341-7360.
50. Kumar A, Davuluri G, Silva RNE, Engelen MPKJ, Ten Have GAM, Prayson R, et al. Ammonia lowering reverses sarcopenia of cirrhosis by restoring skeletal muscle proteostasis. Hepatology. Jun 2017;65(6):2045-2058.
51. Butterworth RF. L-Ornithine L-Aspartate for the Treatment of Sarcopenia in Chronic Liver Disease: The Taming of a Vicious Cycle. Can J Gastroenterol Hepatol. 2019;2019:8182195.
52. Hirooka M, Koizumi Y, Tanaka T, Nakamura Y, Sunago K, Yukimoto A, et al. Treatment on the Spleen Prevents the Progression of Secondary Sarcopenia in Patients With Liver Cirrhosis. Hepatol Commun. Dec 2020;4(12):1812-1823.
53. Chen HW, Dunn MA. Muscle at Risk: The Multiple Impacts of Ammonia on Sarcopenia and Frailty in Cirrhosis. Clin Transl Gastroenterol. May 26 2016;7(5):e170.
54. Albillos A, Martin-Mateos R, Van der Merwe S, Wiest R, Jalan R, Alvarez-Mon M. Cirrhosis-associated immune dysfunction. Nat Rev Gastroenterol Hepatol. Feb 2022;19(2):112-134.
55. Mookerjee RP, Stadlbauer V, Lidder S, Wright GA, Hodges SJ, Davies NA, et al. Neutrophil dysfunction in alcoholic hepatitis superimposed on cirrhosis is reversible and predicts the outcome. Hepatology. Sep 2007;46(3):831-40.
56. S Shawcross DL, Wright GA, Stadlbauer V, Hodges SJ, Davies NA, Wheeler-Jones C, et al. Ammonia impairs neutrophil phagocytic function in liver disease. Hepatology. Oct 2008;48(4):1202-12.

57. Shawcross DL, Shabbir SS, Taylor NJ, Hughes RD. Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis. Hepatology. Mar 2010;51(3):1062-9.
58. Breheny CR, Mellanby RJ, Hamilton JA, Gow AG. The effect of ammonia on canine polymorphonuclear cells. Vet Res Commun. Sep 2018;42(3):221-225.
59. Coppi M, Niederman R. Effects of ammonia on human neutrophil N-formyl chemotactic peptide receptor-ligand interaction and cytoskeletal association. Biochem Biophys Res Commun. Nov 30 1989;165(1):377-83.
60. Luo C, Shen G, Liu N, Gong F, Wei X, Yao S, et al. Ammonia drives dendritic cells into dysfunction. J Immunol. Aug 1 2014;193(3):1080-9.
61. Alabsawy E, Shalimar, Sheikh MF, Ballester MP, Acharya SK, Agarwal B, et al. Overt hepatic encephalopathy is an independent risk factor for de novo infection in cirrhotic patients with acute decompensation. Aliment Pharmacol Ther. 2022 Mar;55(6):722-732.
62. Ballester MP, Rose CF, Carbonell-Asins JA, Jalan R. Measuring and Interpreting Ammonia Levels in Cirrhosis. Am J Gastroenterol. 2023 Jul 1;118(7):1297.

**Figure 1.** Proposed algorithm for assessment of overt hepatic encephalopathy in hospitalized patients with cirrhosis (adapted from reference 9). Measurement of ammonia levels in patients with neurological symptoms and normal CT scan is based on the latest EASL guideline (2). Measurement of ammonia levels in patients hospitalized without neurological symptoms is based on the current evidence for the importance of ammonia as a prognostic biomarker (Table 2).

**Figure 2.** Summary of the pathophysiological pathways that each therapy targets for the treatment of hepatic encephalopathy.

**Figure 3.** Proposed algorithm based on the AMMON-OHE model to stratify the risk of overt hepatic encephalopathy in clinically stable outpatients with cirrhosis and optimize their management.

**Table 1.** Selected recent studies summarizing the evidence of ammonia and severity of liver disease.

| Study | Study design | Aim | Main results |
|---|---|---|---|
| Tranah TH et al. J Hepatol, 2022 | Prospective observational study of 754 clinically stable outpatients with cirrhosis | To test if severity of hyperammonaemia is a risk factor for liver-related complications in clinically stable outpatients with cirrhosis | The mean value of AMM-ULN were 0.9, 1.5, and 1.6 in CTP groups A, B, and C ($p<0.001$), and 1.0, 1.4, and 1.6 in MELD groups ≤9, 9–12, and ≥12 ($p<0.001$). AMM-ULN correlated with both MELD and MELD-Na score ($r=0.22$, $p<0.001$) ($r=0.25$, $p<0.001$). AMM-ULN was higher in decompensated compared with compensated patients: 1.6 vs. 0.9 ($p<0.001$). |
| Balcar K et al. JHEP Rep, 2023 | Retrospective study of 549 clinically stable outpatients with cirrhosis | To investigate the prognostic value of venous ammonia levels for liver-related outcomes | AMM-ULN/AMM consistently increased with the CTP score ($p<0.001$), UNOS MELD (2016) score ($p<0.001$), the severity of portal hypertension ($p<0.001$) and across clinical stages ($p<0.001$) |
| Ballester MP et al. J Hepatol, 2023. | Multi-center prospective observational study of 426 outpatients with cirrhosis | To determine the role of neuropsychological or psychophysical tests and ammonia, and to develop a model to stratify the risk of OHE development | AMM-ULN correlated with CTP categories ($p<0.001$) and was higher for decompensated compared with compensated patients ($p<0.001$) |
| Shalimar et al. JGH Open, 2020. | Single center, retrospective study of 312 patients with cirrhosis and AD and ACLF | To evaluate the association between the dynamic changes of ammonia over the first 3 days after admission and the development of organ failure, severity of ACLF, and 28-day outcomes | On day 1, the median ammonia levels were higher in ACLF compared with AD patients ($p<0.001$). Patients with hyperammonemia had a higher proportion of brain failure ($p<0.001$) and respiratory failure ($p<0.001$) compared to patients without hyperammonemia |
| Hu C et al. Sci Rep, 2020. | Single center prospective study of 174 patients with APASL-ACLF | To determine the association between ammonia level and short-term prognosis | An ammonia level ≥89μmol/L was associated with a higher frequency of organ failure: cerebral ($p<0.001$) and coagulation ($p<0.001$). Ammonia level was positively associated with ACLF grade ($r=0.342$, $p<0.001$). |

| | | | |
|---|---|---|---|
| Elzeftawy A et al. Turk J Gastroenterol, 2019. | Single center cross-sectional study, including 359 HCV positive cirrhotic patients | To find out if there was a possibility to use ammonia levels to predict the presence of esophageal varices or high risk varices | Blood ammonia was higher in patients with esophageal varices than without ($p<0.001$). The best cutoff for prediction of the presence of varices was >123µg/dL, yielding a sensitivity and specificity of 70% and 92%, and an accuracy of 83.4% |
| Darweesh SK et al. Eur J Gastroenterol 2021 | Prospective study of 204 patients with HCV related cirrhosis | To assess the value of serum ammonia as a non-invasive method for early prediction of esophageal varices | Serum ammonia was higher with esophageal varices than without ($p<0.0001$) and with large esophageal varices than small/without esophageal varices ($p<0.0001$). Serum ammonia could predict the presence of esophageal varices using a cutoff value of 82µmol/L with a sensitivity of 92.3%, specificity 92% |
| El-Kalla F et al. Gastroenterol Res Pract, 2018 | Cross-sectional study of 381 patients with HCV or HBV related cirrhosis | To assess the relation of blood ammonia to the presence and severity of PHG | Blood ammonia was 162µg/dL, 153 and 103 among patients with PHG, patients with esophageal varices and controls ($p=0.001$). Cases with severe PHG had higher levels of blood ammonia (192.69±59.87 vs.151.39±54.37 µg/dL) ($p=0.026$) |

Abbreviations: acute decompensation (AD), acute-on-chronic liver failure (ACLF), ammonia upper limit of normal (AMM-ULN), Child-Turcotte-Pugh (CTP), clinical stages (CS), compensated advanced chronic liver disease (cACLD), hepatitis B virus (HBV), hepatitis C virus (HCV), overt hepatic encephalopathy (HE), portal hypertensive gastropathy (PHG)

**Table 2.** Selected recent studies summarizing the evidence for the importance of ammonia as a prognostic biomarker

| Study | Aim | Study Design | Number of patients included | Main Results | Conclusion |
|---|---|---|---|---|---|
| **In the outpatient setting** | | | | | |
| Tranah TH et al. J Hepatol, 2022 | To test the hypothesis that severity of hyperammonaemia is a risk factor for hospitalization with liver-related complications | Prospective multicentre study | 754 clinically stable outpatients in the main cohort and 130 patients for validation | AMM-ULN was an independent predictor of both liver-related complications ($p<0.001$) and mortality ($p<0.001$). Statistical differences in survival were found between high and low levels of AMM-ULN both for complications and mortality ($p<0.001$) using 1.4 as the optimal cut-off | Ammonia is an independent predictor of hospitalisation with liver-related complications and mortality |
| Gairing SJ et al. J Hepatol, 2023 | To analyze and validate the findings by Tranah et al | Single centre retrospective study | 147 outpatients | Frequency of liver-related hospitalization was higher in participants with ammonia levels above the ULN (9 of 19, 47.4%) than in those with ammonia levels below the ULN (16 of 128, 12.5%) | Ammonia can identify outpatients with cirrhosis at high-risk of liver-related hospitalization |
| Balcar L et al. JHEP Rep, 2023 | To investigate the prognostic value of venous ammonia levels for liver-related outcomes and its correlation with key disease-driving mechanisms | Single centre retrospective cohort | 549 clinically stable outpatients for aim I and partly overlapping cohort of 193 patients for aim II | Ammonia was associated with liver-related death ($p=0.044$). AMM-ULN ≥1.4 was independently predictive of hepatic decompensation ($p<0.001$), non-elective liver-related hospitalisation ($p=0.008$), and - in those with decompensated cirrhosis ACLF ($p=0.031$). Venous ammonia was correlated with markers of endothelial dysfunction and liver fibrogenesis/matrix remodelling | Venous ammonia predicts hepatic decompensation, non-elective liver-related hospitalisation, ACLF, and liver-related death, independently of C-reactive protein and HVPG |
| Want X et al. QJM, 2023 | To investigate the discrimination and calibration of a new prognostic model (aCTP) based on AMM-ULN replacing HE in the CTP score | Single centre retrospective study, prospective validation | 554 clinically stable outpatients in the main cohort and 185 patients for validation | The aCTP score showed better agreements between predicted and observed events in the validating cohorts than the CTP score (C-statistics: 0.75 and 0.69, $p<0.001$). The aCTP score showed power to predict AD (C-statistics: 0.76) and ACLF (C-statistics: 0.81) | The study demonstrates potential for plasma Amm replacing HE (aCTP) to enhance the prognostication of transplant-free survival |
| Ballester MP et al. J Hepatol, 2023 | To determine the role of neuropsychological or psychophysical tests and ammonia, and to develop a model (AMMON-OHE) to stratify the risk of subsequent OHE development in outpatients with cirrhosis | Prospective multicentre study | 426 patients with cirrhosis without previous OHE for the main cohort and 2 cohorts of 267 and 381 outpatients for validation | Significant differences were found in time-to-OHE with the highest risk in patients with abnormal PHES plus high AMM-ULN ($p<0.001$). AMM-ULN but not PHES or CFF was an independent predictor of OHE occurrence ($p=0.015$). The AMMON-OHE model showed a C-index of 0.844 and 0.728 for the prediction of a first episode of OHE in two external validation cohorts | This study developed and validated the AMMON-OHE model |

| | | | | | |
|---|---|---|---|---|---|
| **In the inpatient setting** | | | | | |
| Patwardhan VR et al. 2016 | To determine whether ammonia is associated with transplant-free survival in patients with AD and ACLF | Retrospective single centre cohort study | 494 consecutive hospitalized patients (265 with HE) | Every doubling of ammonia was associated with hazard ratios of 1.22 and 1.21 for 90- and 30-day transplant or mortality. Patients with >60μmol/L had higher 90- and 30-day risk of death or transplantation (p=0.001 and p<0.0001, respectively) | An elevated ammonia on admission was associated with reduced 90-day transplant-free survival |
| Sawhney R et al. 2016 | To determine the role of ammonia, inflammation, and cerebral oxygenation in ACLF patients with and without HE | Prospective study. Ammonia, JVO2 and WBC were measured at days 0, 1, 3, and 7 | 101 with ACLF admitted to the intensive care unit | Deterioration in inflammation, JVO2 and ammonia were predictive of mortality. JVO2 deviation and hyperammonemia were associated with the presence and severity of HE; improvement in these parameters was associated with a reduction in HE grade | Ammonia, JVO2 and WBC were important prognostic biomarkers and therapeutic targets |
| Shalimar et al. 2019 | To determine the relationship between ammonia and severity of HE and its association with organ dysfunction and short-term mortality | Prospective observational multicentre study | 498 admitted with AD | Ammonia correlated with severity of HE (p<0.001) and was an independent predictor of 28-day mortality (p<0.001). Ammonia ≥79.5 μmol/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 at day 5 was associated with high mortality (70.6%) | Ammonia correlated with severity of HE and failure of other organs and is a risk factor for mortality; lack of improvement in ammonia is associated with high risk of death |
| Shalimar et al. 2020 | To assess the effect of persistent or incident hyperammonemia on organ failure and outcomes in AD and ACLF | Paired ammonia on day 1 and 3 of admission. Hyperammonemia was defined as ≥79.5 μmol/L on day 3 | 229 consecutive admissions with ACLF and 83 with AD | Persistent or incident hyperammonemia was associated with new-onset organ failure 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 (p<0.001) | Persistent or incident hyperammonemia during first 3 days of hospitalization in AD or ACLF was associated with increased risk of organ failure and death |
| Hu C et al. 2020 | To determine the association between ammonia level and short-term prognosis in ACLF | ACLF according to the APASL definition (subanalyses in HBV reactivation) | 174 with ACLF (106 with HBV reactivation) | Ammonia was higher in non survivors (p=0.013) and was related to ACLF grade (p<0.001) and organ failure, including liver (p=0.048), coagulation (p<0.001) and brain (p<0.001). Ammonia was a prognostic factor in HBV induced ACLF | Ammonia correlated with organ failure and was an independent risk factor for mortality in ACLF and HBV related ACLF |
| Verma N et al. 2021 | To evaluate the dynamics of HE and ammonia estimation in ACLF | ACLF patients from the APASL-ACLF Research Consortium | 3009 ACLF, 1315 (43.7%) with HE at presentation; grade I-II in 981 and III-IV in 334 | Ammonia was a predictor of HE occurrence, higher HE grades and 30-day mortality (p<0.05; each). The dynamic increase in ammonia over 7 days could predict non survivors and progression of HE (p<0.05; each) | Ammonia was associated with the presence, severity and progression of HE and mortality in ACLF |
| Chiriac S et al. 2021 | To assess the role of venous ammonia in predicting the | Retrospective observational | 446 with ACLF (34% grade I, | Receiver operating characteristic analysis showed good accuracy for the prediction of IHM for | Ammonia can be used as an inexpensive predictor of |

| | | | | | |
|---|---|---|---|---|---|
| | outcome of cirrhotic patients with ACLF | study. APASL ACLF criteria | 37.2% grade II, 23.5% grade III, and 5.3% grade IV) | ammonia (AUC=0.812). A cut-off of 152.5μmol/L was identified for mortality (sensitivity=0.706, 1-specificity=0.190) | mortality in patients with ACLF |
| Cardoso F et al. 2023 | To describe and assess the impact of serum ammonia variation in patients' outcomes | Retrospective observational study | 492 and 600 patients ≥18 years old admitted to the intensive care units | On ICU day 1, median ammonia was higher in patients with grade 3/4 HE than those with grade 2 or grade 0/1 HE ($p<0.001$). Higher day 2 ammonia was associated with higher hospital mortality. In the validation cohort, serial ammonia (ICU days 1 and 3) predicted hospital mortality (c-statistic=0.73) | Early serum ammonia variation was independently associated with hospital mortality |
| Thanapirom K et al. 2024 | To evaluate the association between ammonia and liver-related complications and its ability in predicting mortality | Prospective study using the APASL definition | 701 ACLF patients from the ACLF Research Consortium | Ammonia was higher in patients with HE and ascites and was associated with liver-related complications. Baseline arterial ammonia was an independent predictor of 30-day mortality | Baseline arterial ammonia levels were associated with 30-day mortality and liver-related complications |

Abbreviations: acute decompensation (AD), acute-on-chronic liver failure (ACLF), adjusted hazard ratio (aHR), ammonia upper limit of normal (AMM-ULN), alcoholic hepatitis (AH), acute kidney injury (AKI), acute liver failure (ALF), cerebral herniation (CH), cerebral oedema (CE), cerebral blood flow (CBF), Child-Turcotte-Pugh (CTP), chronic liver disease (CLD), hepatic encephalopathy (HE), hepatic venous pressure gradient (HVPG), intracranial hypertension (ICH), jugular venous oxygen saturation (JVO2), white blood cell count (WBC)

**Table 3.** Summary of the key points of ammonia as a biomarker in patients with cirrhosis

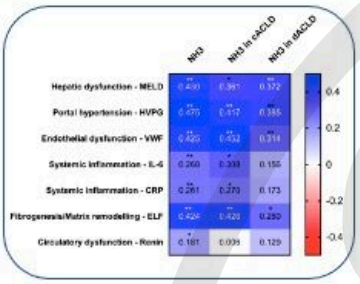
| Type of biomarker | Rationale | Key points |
|---|---|---|
| Diagnostic biomarker | • A normal blood ammonia level has a high negative predictive value for the diagnosis of OHE<br>• Some patients present high ammonia levels with a normal mental status which suggests that other factors play an important role in the pathogenesis of HE | • A normal ammonia in a patient with delirium should prompt renewed or further differential diagnostic work-up for other causes of delirium<br>• A high ammonia level with normal mental status is not diagnostic of OHE |
| Surrogate biomarker | Although with significant overlaps, ammonia correlates with severity of underlying liver disease, number of organ failures, portal hypertension and systemic inflammation | Ammonia may be used as a surrogate biomarker of liver disease severity, organ dysfunction, portal hypertension and systemic inflammation |
| Companion or response biomarker | Ammonia levels are not used for guiding HE treatment in clinical practise | Ammonia may be used as a companion biomarker to evaluate response to treatment |
| Prognostic biomarker | Ammonia offers useful prognostic information in both outpatients and acutely decompensated patients with cirrhosis | Ammonia measurement is useful in both outpatients and inpatients to predict OHE, other liver-related decompensations, organ failures and death |
| Therapeutic target | Specific treatment for HE aims to reduce ammonia levels but patients are usually not categorized by hyperammonemia | Ammonia should be measured as a therapeutic target for personalized prevention and treatment of OHE and for the development of new drugs |

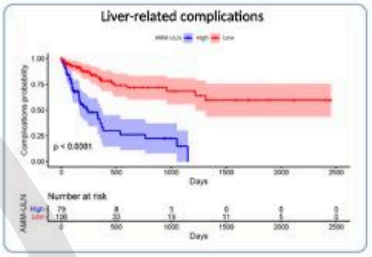
Abbreviations: overt hepatic encephalopathy (OHE).

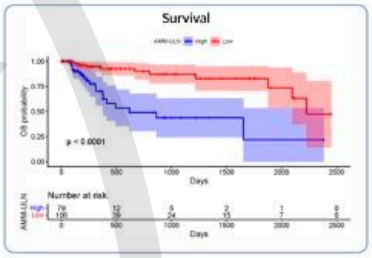
# The value of ammonia as a biomarker in patients with cirrhosis

Ammonia is a **therapeutic target** and can be used for <u>personalized prevention and treatment</u> of hepatic encephalopathy

Ammonia predicts:
1. Hepatic encephalopathy
2. Other liver-related decompensations
3. Organ failures
4. Death




**Prognostic biomarker**

**Companion or response biomarker**

**Susceptibility or risk biomarker**

$NH_3$

AMM-ULN*

**Surrogate biomarker**

**Diagnostic biomarker**

Ammonia correlates with:
1. Severity of underlying liver disease
2. Number of organ failures
3. Portal hypertension
4. Systemic inflammation

*Calibrated ammonia using the upper limit of normal

Ammonia is useful for overt hepatic encephalopathy diagnosis