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Letter to the Editor - Reply to ‘Ammonia - an old friend with a new area of application

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Conflicts of Interest: 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 Discovery, a spin out company from University College London, Hepyx Limited and Cyberliver. He had research collaborations with Yaqrit Discovery. The other authors have no conflicts of interest to declare.

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To the editor,

We are very appreciative of the interest garnered by our recently published article entitled ‘Plasma ammonia levels predict hospitalisation with liver-related complications and mortality in clinically stable outpatients with cirrhosis’ and were pleased to receive further validating data from Gairing et al. in response to our findings. [1, 2] In our manuscript, we demonstrated that ammonia levels, expressed as a ratio of the local laboratory upper limit of normal (AMM-ULN), are an independent predictor of hospitalisation with liver-related complications and mortality in stable outpatients with cirrhosis and that an AMM-ULN cut-off value of 1.4 defines the risk of liver-related complications and consequent mortality. In order to address overfitting, we collected data between 3 independent units for utilisation in our training and test models and furthermore validated findings in an external patient cohort of a further 130 patients demonstrating the validity of AMM-ULN as a marker of adverse outcomes in these patients.

In their study, Gairing et al. have evaluated our findings in a further external cohort of 147 patients where venous ammonia measurements were sampled and patients prospectively followed up. [3] 17% of patients from this cohort were hospitalised during a median follow up of 569 days and the authors confirmed that ammonia measurements were able to identify patients at risk of developing liver-related complications (AUROC 0.74 and 0.68 at 6- and 12-months, respectively). Patients with ammonia levels >ULN suffered an increased frequency of hospitalisation (47.4% vs. 12.5%, p<0.001). We would like to thank for authors for their interest in our work and congratulate the authors on this relevant and important data.
Firstly, as the authors highlight, this validation of our findings in a further external dataset adds weight to our assertion that measurement of ammonia carries prognostic utility as a biomarker in identifying patients at risk of liver-related complications and consequent mortality. We would comment that there are differences in statistical methodology in that we performed time-dependent competing risk AUROC analyses as opposed to AUROC analyses at 6- and 12- months and therefore these results cannot be directly compared between the studies.

Notably in the Gairing study, most patients had compensated cirrhosis (73% Child Pugh A, median MELD score 9, IQR 7-12) and there was a low frequency of patients with AMM-ULN values ≥1.4 which limited the value of this as a proposed threshold for identifying high-risk patients at earlier disease stages. [3] These data do highlight the utility of an AMM-ULN threshold ≥1.4 for defining a high-risk population, when applied to a population at a relatively earlier disease stage and lower ammonia levels.

We therefore revisited our dataset and performed a sub-analysis evaluating the discriminatory capability of AMM-ULN to define patients at high risk of developing cirrhotic complications in progressive disease stages. We separated the patients from our test cohort by Child Pugh class and constructed Kaplan-Meier curves utilising AMM-ULN ≥1.4 as a cut off for the high-risk population. AMM-ULN ≥1.4 was able to discriminate patients at high risk of developing liver-related complications across all groups (log-rank p values 0.048, 0.0021 and 0.0058 for patients with Child Pugh A, B and C cirrhosis, respectively). However, the absolute number of high-risk patients in the Child Pugh A group was low at 7 of 51 patients [13.7%, number needed to screen (NNS): 7], limiting the utility of AMM-ULN measurement in this group. In patients with
Child Pugh B and C cirrhosis, 43 of 87 (49.4%, NNS 2) and 29 of 47 (61.7%, NNS 2) were identified in the high-risk group utilising the 1.4 threshold, respectively.

In conclusion, Gairing et al. provide important data that validate our findings that ammonia measurement is an independent variable in predicting liver-related complications in clinically stable outpatients with cirrhosis. We would further agree with the findings of Gairing et al. that, whilst AMM-ULN ≥1.4 maintains a discriminatory capability in patients with compensated Child Pugh A cirrhosis, a relatively low proportion of patients in this disease stage have elevated AMM-ULN levels; therefore, the timing of ammonia measurement demonstrates wider utility in later stages of Child Pugh B and C cirrhosis.
REFERENCES


Figure 1: Kaplan-Meier analyses demonstrating cumulative probability of hospitalisation due to liver-related complications in the validation cohort separated into Child Pugh class A, B and C respectively. Patients with AMM-ULN ≥1.4 were allocated to the high-risk group. Differences in overall survival were assessed by log rank test.
p = 0.048
Complications probability

A-ULN

High Low

Number at risk

Days

High

Low

Days

0 500 1000 1500 2000 2500

0 500 1000 1500 2000 2500

p = 0.0021
The Kaplan-Meier survival analysis shows a significant difference in complications probability between the high and low A-ULN groups, with a p-value of 0.0058. The graph indicates a higher risk of complications in the high A-ULN group compared to the low A-ULN group. The table below provides the number at risk for each group at different time points:

<table>
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