Letter to the Editor

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Reply to: "Ammonia - an old friend with a new area of application"

To the Editor:

We are very appreciative of the interest garnered by our recently published article 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) is 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 from three independent units for utilisation in our training and test models and furthermore validated findings in an external cohort of 130 individuals, demonstrating the validity of AMM-ULN as a marker of adverse outcomes.

In their study, Gairing *et al.* have evaluated our findings in a further external cohort of 147 individuals where venous ammonia measurements were sampled and patients prospectively followed up.[3] 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 the 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 results cannot be directly compared between the studies.

Notably in the study by Gairing *et al.*, most individuals had compensated cirrhosis (73% Child-Pugh A, median MELD score 9, IQR 7-12) and few participants had 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 early disease stage with lower ammonia levels.

We therefore revisited our dataset and performed a subanalysis 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 (Fig. 1). 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) participants 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.

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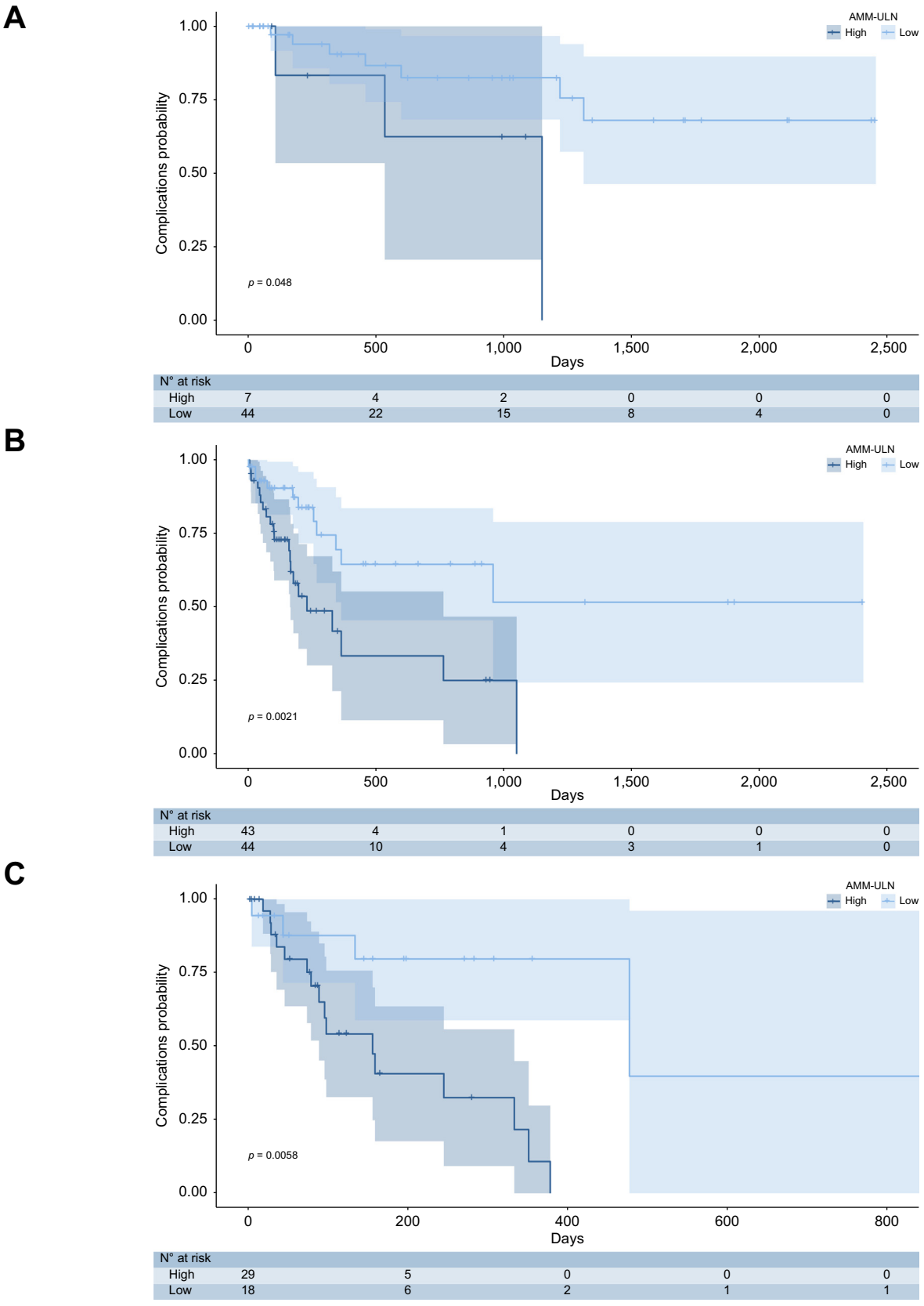

Fig. 1. Kaplan-Meier analyses demonstrating cumulative probability of hospitalisation due to liver-related complications in the validation cohort separated into Child Pugh class A, B and C respectively. Patients with AMM-ULN ≥1.4 were allocated to the high-risk group. Differences in overall survival were assessed by log-rank test. AMM-ULN, ammonia levels as a ratio of the local laboratory upper limit of normal.

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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.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Statistical analyses were performed by JA C-A. The manuscript was prepared and written by THT and MPB and revised by RJ and DLS. All authors have reviewed and approved the final submitted manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.09.023.

References

Author names in bold designate shared co-first authorship

[1] Gairing SJ, Kaps L, Schleicher EM, Galle PR, Labenz C. Ammonia - an old friend with a new area of application. J Hepatol 2022. Online ahead of print.

[2] **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. Online ahead of print.

[3] Gairing SJ, Anders J, Kaps L, Nagel M, Michel M, Kremer WM, et al. Evaluation of IL-6 for stepwise diagnosis of minimal hepatic encephalopathy in patients with liver cirrhosis. Hepatol Commun 2022;6:1113–1122.