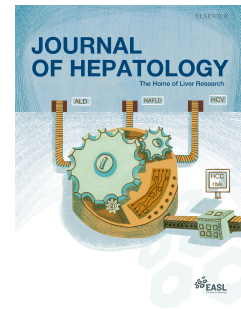


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Reply to ‘The possible link between higher ammonia levels associated with non-alcoholic fatty liver related cirrhosis and diabetes: are we missing chronic kidney disease?’

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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 would like to thank Elfeki *et al.* for their interest in our recently published article[1, 2]. Our study of 754 patients demonstrated that ammonia was an independent predictor of mortality and when expressed as a ratio of the local laboratory upper limit of normal (AMM-ULN), defines a high-risk group for the development of liver-related complications. This was confirmed in an independent validation cohort of 130 stable outpatients with cirrhosis.

Elfeki *et al.* comment on an observation in our data that whilst there were predictable changes in ammonia levels which were more pronounced with progressive severity of liver disease, differences were also seen between aetiologies of liver disease, particularly with respect to non-alcoholic fatty liver disease (NAFLD [mean AMM-ULN 1.6, SD 0.8, $p = 0.011$]), and in patients with type II diabetes mellitus (T2DM [mean AMM-ULN 1.5, SD 0.9, $p = 0.010$]), however patients with both NAFLD and T2DM did not have significantly higher AMM-ULN than in patients with either condition alone ($p = 0.540$, figure 1a). Elfeki *et al.* rightly point out that both NAFLD and T2DM are risk factors for chronic kidney disease (CKD) and that reduced renal clearance in this group of patients may have contributed to the higher levels of ammonia in these patient groups [3, 4]. We therefore examined the relationship between AMM-ULN, CKD status and patient outcomes in our cohort of 754 patients.

Patients with CKD, defined as stage 3A or above, did have a slightly higher distribution of AMM-ULN (1.53 versus 1.37, $p = 0.030$; figure 1b), however there was neither a correlation observed between estimated glomerular filtration (eGFR) and AMM-ULN

(Pearson correlation coefficient, $r = -0.051$, $p = 0.166$), nor was AMM-ULN different between CKD stages (figure 1c). Furthermore, when considering high and low levels of AMM-ULN (greater or less than 1.4), there was no association with CKD ($p=0.066$, Fisher's exact test). In addition, in patients with NAFLD and/or T2DM, no differences were found in AMM-ULN according to the presence or absence of CKD ($p = 0.130$) (figure 1d), suggesting that underlying kidney dysfunction is not the pathophysiological mechanism leading to higher ammonia levels in these groups of patients.

In order to study further the role of CKD in prognostication in cirrhosis we performed a frailty risk analysis for the development of liver-related complications, considering transplantation as a competing risk. CKD was associated with an increased risk of this primary endpoint (HR: 1.47, 95% CI 1.09-2.01, $p=0.013$) and we therefore included AMM-ULN within the model to establish whether CKD is a risk factor for the development of liver-related complications independently of ammonia. Both CKD and AMM-ULN remained significant risk factors independently of the other (CKD: HR 1.42, 95% CI 1.05-1.93, $p = 0.024$; AMM-ULN: HR 4.72, 95% CI 3.57-6.26, $p < 0.001$) with the inclusion of both covariates in the model.

We conclude that whilst CKD is associated with higher AMM-ULN levels and is a risk factor for the development of liver-related complications, renal dysfunction is not the mechanism leading to higher ammonia levels in patients with NAFLD and/or T2DM. When considering AMM-ULN as a predictive variable for the development of liver-related complications, we show that it exerts its effect independently of the presence of CKD.

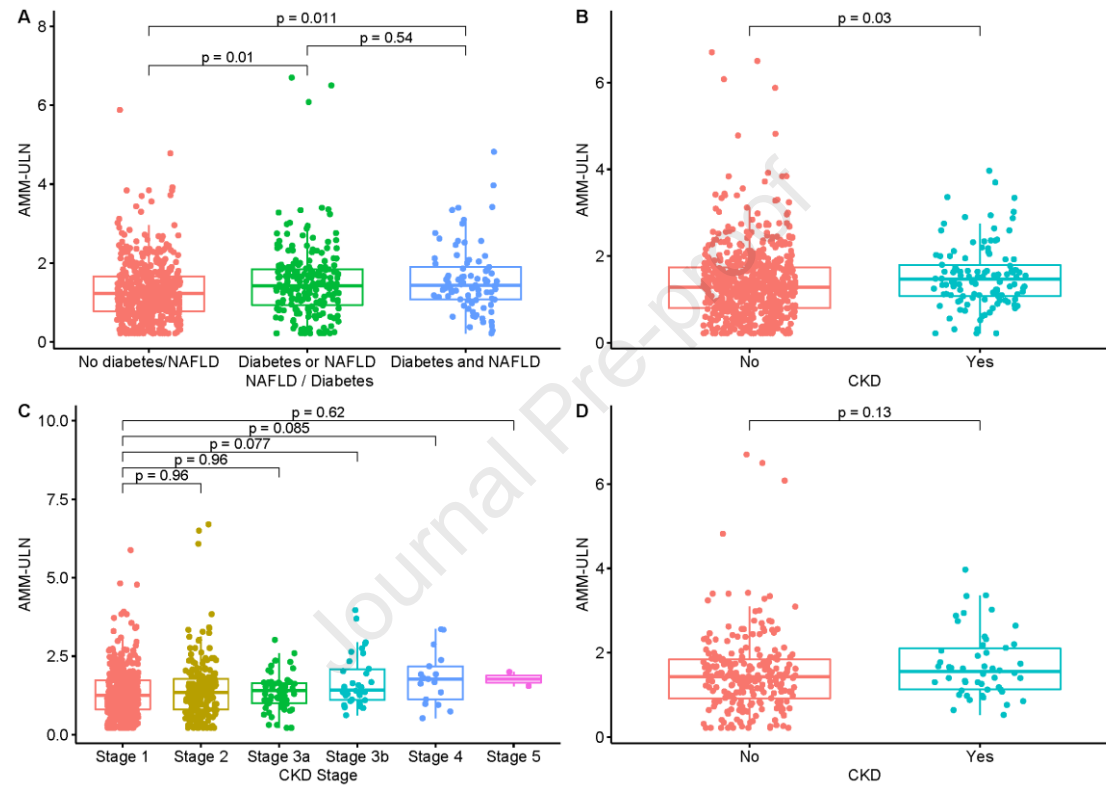
Figure 1:

Figure 1: Distribution of AMM-ULN according to presence and absence of NAFLD and type 2 diabetes (a), presence of CKD (b) by CKD stage (c) and presence of CKD in patients with NAFLD and/or T2DM. t-test for single comparisons and post-hoc analyses for multiple comparisons were performed with significance values corrected using Holm's method.

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