Author’s response to reviews

Title: SERUM BTP CONCENTRATIONS ARE NOT AFFECTED BY HEPATIC DYSFUNCTION

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Dear Dr. Henderson,

Thank you for providing the reviewers comments for the manuscript “Serum BTP Concentrations are not Affected by Hepatic Dysfunction”. Each has been addressed and the manuscript revised accordingly.

Reviewer #1

1. “Basically, the number of patients with advanced cirrhosis is not sufficient. Only 2 patients had moderate to severe ascites and nine patients only exhibited an INR larger than 1.7. “ Indeed,
there are relatively fewer patients with advanced cirrhosis which reflects the composition of the KGH cirrhosis clinics. Nevertheless, there is a clear signal that creatinine concentrations are affected in this “less advanced” cohort while there is no impact on BTP. The small numbers of advanced cirrhotics and resulting difficulty in extrapolation of study findings to them has been included in the limitations section of the manuscript (page 8).

2. “I strongly suggest to provide original data of the analytes in mg/l and µmol/l presented in conventional scatterplots and tables comprising means + 95% confidence intervals or medians+ranges. Table 2 does include the mean ± SD of the Cr, CysC and BTP for both control group and the patient group. I have added the 95% CI for these. (Table 2)

3. “The patient and the control group need to be characterized more in detail to understand potential differences between the cohorts (e.g. elevated BTP, cystatin C and creatinine levels in the control cohort, what were the characteristics of those with advanced cirrhosis))” We do present important demographic data and some limited important clinical and laboratory data for the control group in Table 1. Importantly we include diabetes, age and gender as these are known to impact on serum cystatin C or BTP levels independently of GFR. We do not have any further clinical data on this group. More detailed characteristics of the patient group (demographic, diabetes status, etiology of cirrhosis, Child Pugh and MELD scores and other biochemistry) are also found in Table 2.

4. “By the way, what are control child-pugh A, B, C?”. None of the control group had cirrhosis and cannot be assigned a Child Pugh classification.

5. “And finally the approach to establish ratios of BTP/cystatin C and creatinine/cystatin C is not suitable to adjust for renal function.” In the absence of a gold standard measure of kidney function and where the two groups being compared have clearly different degrees of kidney dysfunction, the ratio is preferable to assess for the presence of non-GFR determinants then a serum concentration alone. The use of kidney function ratios has been utilized in other studies.
For example, in one of the first papers examining the role of cystatin C in cirrhotics, ratios were also to adjust for kidney function (Orlando Clin Chem 48:6, 2002). In the current manuscript, the ratio clearly demonstrates the impact of hepatic dysfunction on Cr (btw controls and patients AND importantly across Child Pugh groups) which has been described using other statistical techniques and gold standard measures. I should note that measuring GFR in cirrhotic patients is extremely difficult and almost never done. Plasma clearance which is much simpler technique to measure GFR is not appropriate in cirrhosis due to tracer clearance in the extracellular fluid compartments and therefore the much more difficult urinary clearance technique is required in the setting of liver disease. Unfortunately, in the absence of urinary catheterization, urinary clearance is imprecise. It would be likely impossible to recruit cirrhotic patients for the lengthy and invasive urinary clearance.

Reviewer #2

1. "This paper is very clearly and well written. My only critique is that it fails to acknowledge a recent and very relevant publication: "Estimation of glomerular filtration rate in patients with cirrhosis by using new and conventional filtration markers and dimethylarginines" published by Ayse Mindikoglu et al. in Clinical Gastroenterology and Hepatology 2016;14:624-632. One of the conclusions of Mindikoglu's article (p. 626) is that there is no strong evidence that beta-trace protein was helpful for predicting mGFR in cirrhosis. They actually found that the estimating equation that had the best performance in cirrhosis patients (when looking at a variety of candidate filtration markers) was an equation that relied on creatinine and cystatin C only. Therefore I think it important that your manuscript be re-written and its importance placed in the context of this work by Mindikoglu." This current manuscript did not include the study by Mindikoglu et al as the latter was not published at the time our manuscript submission (Feb 2016). Thank you however for bringing it to our attention. We have summarized the key findings of this study in the revised manuscript along with some of the difficulties with drawing firm conclusions about BTP in cirrhosis to the manuscript.(page 7) The major difficulty with this study (and most other studies in cirrhosis) is their use of PLASMA clearance. As described in the response 3 to reviewer #1 and in the manuscript, PLASMA clearance (which was done in the Mindikoglu study) is not appropriate in cirrhosis due to tracer clearance in the extracellular fluid compartments leading to variable overestimation of GFR. Urinary clearance techniques are recommended instead. In addition, it is not known when the samples were assayed for BTP; all at once or over time (the GFRs were measured over a 4 year period 2010-2014)? We have recently shown that the BTP assay has changed over time (AJKD 2017, April 12). I did contact the corresponding author to clarify this but I have received no response.
Further, I do not feel that the study by Mindikoglu detracts from the importance of the current study findings. The purpose of this study was to investigate whether hepatic dysfunction impacts on serum BTP concentrations. It was not intended to validate or develop GFR equations based on various serum analytes.

2. “Additionally as an aside this paper by Mindikoglu states that beta-trace protein (as well as B2M, cystatin C and SDMA) is "unaffected by hepatic dysfunction" (p. 625); I did not find any data to support this statement in the references listed by Mindikoglu's paper, but since this is the primary hypothesis of your manuscript and your main conclusion, I would make doubly sure there has not been previously proven or published data demonstrating this, since it is stated in papers such as this as an already established fact.” I reviewed all publications related to non-GFR determinants of BTP in a review of BTP in 2015 published in AJKD (2015;65 (1):131-146). There are no studies (other than the current one) looking at the effect of hepatic dysfunction on serum BTP. I have performed another literature search this week confirming this.