TGH TRANSLATIONAL GASTROENTEROLOGY AND HEPATOLOGY
A RISING JOURNAL FOR HIGH-QUALITY RESEARCH IN GASTROENTEROLOGY AND HEPATOLOGY

Peer Review File

Article information: https://dx.doi.org/10.21037/tgh-21-94

Reviewer A

This is a retrospective analysis of recors of laboratory data from patients at admission in the hospital because of COVID-19.The laboratory data of the first 24 hours were correlated with the risk for mortality, mechanical ventilation and length of hospital stay.Special attention was payed to some of liver parameters:AST,ALT,ALP and T,Bilirrubin..Albumin serum level was mentioned.Among the drugs used only PPI use was recorded.A correlation between AST-serum level and the risk of mortality, mechanical ventilation and length of hospital stay was found.

Comments:

1. a definition of liver injury is mandatory to determine the severity of liver injury.INR-value is one of the most important markers of liver function

Reply 1: We agree that the INR is a very important marker for the liver's synthetic function and is used as part of clinical calculators as well. However, our study was not directed towards the evaluation of degree/severity of liver injury. It was to evaluate for the presence (or absence) of liver chemistry abnormalities noted on admission and to assess if there was any link to adverse outcomes (such as mortality, intubation risk and length of hospitalization). Due to the design of our study being a retrospective analysis and being conducted with the use of a large cohort of patients across multiple centers, it was not possible for us to exclude those with elevations of INR from being on medications such as anticoagulants and also elevations in cases of sepsis/DIC. Hence, we did not include the INR as part of our study, though initially we had envisioned to do so but due to limitations we could not.

2. no values for albumin serum level is reported. Hypoalbuminemia is the most important prognostic marker, which is independent of age and of comorbidities.

Reply 2: We agree that albumin is a very important maker for prognostication and hence we did incorporate it into our study. We did include a value for serum albumin as we recognized it as a marker for synthetic function as well (page 9, lines 201-203). In our study, hypoalbuminemia was associated with increased risk of mortality, intubation and greater length of hospital stay (page 14, lines 300-311).

3. liver damage is a seldom complication of COVID-19 and replicating virus particles have not been found in the liver (Dorward DA et al. Am J resp critical Care Medicine 2021;203:192-201.

Reply 3: We agree that cases of acute fulminant liver damage due to COVID-19 are

not commonly encountered. However, liver injury of varying degrees manifesting as even slight derangements of liver chemistries does occur and the virus is noted to be in the liver though predominantly in the cholangiocytes and the virus exerts hepatotoxic effects in other ways as well (page 16, lines 349-356). There are a multitude of studies that have shown how COVID affects the liver and the degree of injury is variable and many of them only report mild injury as a manifestation (pages 18-19, lines 408-426; page 20, lines 435-443). The aim of our study was to evaluate abnormalities in liver chemistries that were present on admission and to evaluate their presence to any links to worse outcomes. Our study was not designed to investigate the spectrum of injury and the range of degrees of injury based on the dataset that we had available. However further studies can be performed to evaluate that further.

4. some "liver" enzymes may arise from other sources and should not cause concerns about liver function (Bougash M et al.Lancet 2020;5:March 20)

Reply 4: In our manuscript, we note on page 20, lines 450-454, that elevated AST may occur in the presence of muscle injury and that liver injury may also result from hepatotoxic agents such as medication.

5. the introduction begins with "A year has lapsed"....the pandemic started in January 2020 20 months ago

Reply 5: We acknowledge this comment.
Changes in the text: We have modified our text as advised by removing the reference to time in the first sentence of the Introduction (see page 7, lines 138-139).

Reviewer B

1. The background needs to be a little more engaging. It is well known that COVID-19 is associated with elevation of liver biochemistries in 14% to 53% of patients and occurs more frequently in patients with severe illness. Also it is well known that male patients are at risk of developing severe illness and increased mortality due to COVID-19 compared to female patients.

Reply 1: We modified parts of the introduction to make it more engaging without disclosing our results (page 7, lines 141-143, 147-151, and 153-154). Some of the results such as male gender being linked to worse outcomes we have included in our discussion portion.
Changes in text: Described above (page 7, lines 141-143, 147-151, and 153-154)

2. Line 118/119 :An abundance of literature on the impact of the virus on the liver is also available.Here are a few examples
Bloom, P.P., Meyerowitz, E.A., Reinus, Z., Daidone, M., Gustafson, J., Kim, A.Y., Schaefer, E. and Chung, R.T., 2021. Liver biochemistries in hospitalized patients with

COVID−19. Hepatology, 73(3), pp.890-900

Li, J. and Fan, J.G., 2020. Characteristics and mechanism of liver injury in 2019 coronavirus disease. Journal of clinical and translational hepatology, 8(1), p.13.

Jothimani D, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. Journal of hepatology. 2020 Nov 1;73(5):1231-40.

Zhang, C., Shi, L. and Wang, F.S., 2020. Liver injury in COVID-19: management and challenges. The lancet Gastroenterology & hepatology, 5(5), pp.428-430

Reply 2: We agree that there is an abundance of literature available since the onset of the pandemic, however certain aspects are still not clear and organs such as the lungs have gathered more attention. For example, the ACE2 receptor is present predominantly on cholangiocytes, the pattern of liver chemistry derangement in many studies was noted to be hepatocellular and this is not entirely clear as to why this occurs though other factors are thought to be involved in the liver injury. So even though there is much more literature available now as compared to a year or so ago, some aspects still need further investigation. We did make some changes to our statement to clarify our viewpoint (page 7, lines 145-149).

3. Based on review of the study, it is unclear if this study satisfies the objectives laid out by its authors. Moreover it only reiterates what is already known in published literature.More than 50% of the population in this retrospective study were obese and almost more than 45 % had underlying DM which places them at increased risk of developing severe COVID-19 infection and these comorbidities are also risk factors for NAFLD which likely exacerbated the LFTs in addition to cytotoxicity by the virus. It is well known that patients with underlying medical comorbidities (obesity, cardiovascular disease, diabetes mellitus, chronic lung disease,CKD, tobacco use , cancer, solid organ transplant patients) have an increased risk of developing severe COVID-19 infection. Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, Tie Y, Fullerton KE. Coronavirus Disease 2019 Case Surveillance - United States, January 22-May 30, 2020. MMWR Morb Mortal Wkly Rep. 2020 Jun 19;69(24):759-765. DId the study show patients without comorbid conditions went on to develop severe illness and Grade III- IV hepatoxicity? Also what were the exlusion criteria of this study?

Reply 3: We used a robust statistical method called propensity score matching to match patients with and without abnormalities in liver chemistries on a wide range of covariates that were likely to impact patient outcomes related to mortality, intubation, and increased length of stay. These covariates included age, gender, race/ethnicity, blood type, BMI, history of smoking, diabetes, COPD, coronary artery disease, malignancy, heart failure, chronic kidney disease/end-stage renal disease, liver disease, serum albumin level, and recent prescription for a PPI or H2 blocker. By calculating a propensity score for each patient and then matching patients based on their propensity scores, we controlled for underlying differences in the covariates

across the two patient groups. In so doing, we were able to analyze the independent effect of abnormal liver chemistries on the outcomes of interest (i.e., mortality, intubation, and increased length of stay).
Changes in the text: We have substantially revised the text in the Statistical Analysis section of our manuscript, to better describe the process and rationale for using propensity score matching to balance the patient groups based on covariates likely to impact the outcomes of interest (i.e., mortality, intubation, and hospital length of stay), which allowed us to more accurately estimate the effect of liver chemistry abnormalities on the outcomes of interest (see pages 10-11, lines 217-247). We added a note to specify that patients who did not have documented laboratory results for all four liver chemistries within 24 hours of the start time of the COVID encounter were excluded from analysis (see pages 8-9, lines 181-183). We also clarified that patients with missing data were excluded from analysis (see page 9, lines 203-204).

4. Line 203 - Although the authors mention describing the Demographic and clinical characteristics of these patients are outlined in Table 1. Except for Figures 1-4 and supplementary material with ICD codes the said Table 1 is missing.Also the authors need to describe the baseline variables such as AST,ALT and T.Bilirubin elevation reflected in the form of a range. It could be helpful to classify based on grades of hepatoxicty if present.

Reply 4: We inadvertently omitted Table 1 when we submitted the manuscript.
Changes in the text: We have added Table 1, as well as several other results tables, to the manuscript (see pages 28-38).

5. Line 290 - Although, there is significantly higher ACE2 expression in the cell clusters of cholangiocytes (59.7%) than hepatocytes (2.6%),based on published literature, the liver injury in COVID-19 is primarily hepatocellular as opposed to a cholestatic, as evidenced by elevation in aspartate aminotransferase (AST) and alanine aminotransferase (ALT).Bloom, P.P., Meyerowitz, E.A., Reinus, Z., Daidone, M., Gustafson, J., Kim, A.Y., Schaefer, E. and Chung, R.T., 2021. Liver biochemistries in hospitalized patients with COVID−19. Hepatology, 73(3), pp.890-900

Reply 5: We agree that this is an interesting phenomenon that has been noticed in many studies and it is not entirely clear as to why this occurs. Further basic science studies are needed to investigate this and provide additional insight and clarification as to why this pattern is seen. Our study was not intended or designed to evaluate this unfortunately as to why this pattern is seen despite the ACE2 expression being predominantly noted more on cholangiocytes. Possible explanations include the effect of other factors including medications used in the treatment of COVID-19 along with changes such as microvesicular steatosis and possibly immune mediated effects induced by the virus (page 16, lines 349-356). Our study also showed a predominance of cholestatic pattern (page 19, lines 433-434).

6. Also, the authors mention in their study that "Elevated AST is the liver chemistry

abnormality associated with the highest risk for mortality (RR 2.27), intubation (RR 2.12), and prolonged hospitalization (RR 1.19)" The authors need to clarify in detail; as to how high were the LFTs that were associated with increased risk of mortality, intubation and increased LOS and if these patient had associated risk factors that could have mainly contributed for decompensation? Trivial LFT elevations have been noted in patients with mild to moderate and severe COVID-19 illness leading to increased mortality, mechanical ventilation and prolonged hospitalization.However, this decompensation cannot be attributed to the trivial elevation in LFTs. Was the LFT elevation the main reason for increased LOS?

Reply 6: Following the propensity score analysis which matched patients across a wide range of covariates and then used logistic regression to adjust for the covariates, we conducted a secondary analysis to ascertain which liver chemistry abnormalities were associated with the highest risk of poor outcomes. Although our findings do not suggest that elevated LFTs are the main or the sole reason for poorer outcomes, they do suggest that elevated LFTs may serve as prognostic markers (at the time of presentation/admission with COVID-19 infection) for poorer outcomes. We acknowledge the need to more clearly delineate the LFT thresholds that were associated with increased risk of poor outcomes.
Changes in the text: We have clarified that we used logistic regression to perform secondary analyses after using propensity score matching to determine that elevations in liver chemistries were associated with poor outcomes (see page 11, lines 248-250). To clarify the elevations in LFTs that were analyzed for associations with mortality, intubation, and increased length of stay, we have added the thresholds used to define these abnormalities (see page 15, lines 339-340).