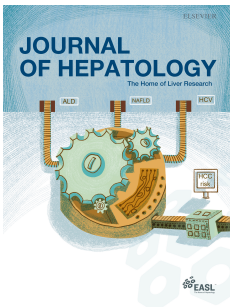

SARS-CoV-2: is the liver merely a bystander to severe disease?

Mansoor N. Bangash, Jaimin M. Patel, Dhruv Parekh, Nicholas Murphy, Rachel M. Brown, Ahmed M. Elsharkawy, Gautam Mehta, Matthew J. Armstrong, Desley Neil

PII: S0168-8278(20)30355-X

DOI: https://doi.org/10.1016/j.jhep.2020.05.035

Reference: JHEPAT 7780

To appear in: *Journal of Hepatology*

Received Date: 20 May 2020

Accepted Date: 23 May 2020

Please cite this article as: Bangash MN, Patel JM, Parekh D, Murphy N, Brown RM, Elsharkawy AM, Mehta G, Armstrong MJ, Neil D, SARS-CoV-2: is the liver merely a bystander to severe disease?, *Journal of Hepatology* (2020), doi: https://doi.org/10.1016/j.jhep.2020.05.035.

Word count: 768 words

SARS-CoV-2: is the liver merely a bystander to severe disease?

Mansoor N Bangash*[1,2,3], Jaimin M Patel[1,2,3], Dhruv Parekh[1,2,3], Nicholas Murphy[1,2,3], Rachel M Brown[2,4,5], Ahmed M Elsharkawy[2,5,6], Gautam Mehta[7,8], Matthew J Armstrong[2,5,6], Desley Neil[2,4,5]

***corresponding author: M Bangash[1]. mansoor.bangash@uhb.nhs.uk, 079500095291**

1. Liver Intensive Care Unit, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Birmingham, UK.

2. Birmingham Liver Failure Research Group, Institute of Inflammation and Ageing, NIHR Biomedical Research Centre, University of Birmingham, Birmingham, UK

3. Birmingham Acute Care Research Group, Institute of Inflammation and Ageing, NIHR Biomedical Research Centre, University of Birmingham, Birmingham, UK

4. Department of Cellular Pathology, QEHB, Mindelsohn Way, Birmingham, UK

5. Institute of Inflammation and Ageing, NIHR Biomedical Research Centre, University of Birmingham, Birmingham, Birmingham, UK

6. Liver Unit, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Birmingham, UK.

7. Institute for Liver and Digestive Health, Royal Free Campus, UCL, London, UK

8. Institute of Hepatology, Foundation for Liver Research, London, UK

Author contributions

All authors contributed equally to the content and production of the letter, all authors checked and approved the final draft of the letter

Conflict of interest statements

Dr. Armstrong reports personal fees from Novo Nordisk, outside the submitted work. All other authors have no conflicts of interest to declare.

Financial support statement

No funding was required to support this work.

Keywords

severe acute respiratory syndrome coronavirus 2, COVID-19, hepatitis, inflammation, cell death

Dear Editor

We read the recent article from Wang et al with great interest.[1] Their study shows SARS-CoV-2 positive patients with ≥1 week history of increased aminotransferases have worse acute pulmonary disease (radiological and physiological) than those without. They also report higher ferritin levels, higher proportions of patients with a low albumin and raised direct bilirubin, and histological features (*albeit in only two patients*) possibly in keeping with a viral-mediated liver injury in this group. Considering that Interleukin (IL)-6 and C-reactive protein (CRP) are similar between patients with normal and prolonged abnormal liver aminotransferases, the authors speculate that liver injury is a direct effect of SARS-CoV-2 viral hepatitis rather than an indirect immune mediated injury. The fact that increases in liver aminotransferases occur and tend to parallel the severity of pulmonary disease remains unquestioned[2], however, whether the liver injury is a true viral hepatitis rather than a bystander to the multi-organ pathophysiology of critical illness requires further discussion.

Wang et al provide evidence for direct viral infection based on electron microscopy where they identified multiple intra-hepatocyte microvesicular structures with "crowns" as SARS-CoV-2 virions. However, normally occurring clathrin-coated vesicles have a similar appearance. Additionally, the tissue is undergoing autolysis, as is usual for post-mortem tissue, and autolysed multi-vesicular bodies (MVBs) are seen in the images. It is therefore possible that the observed cytosolic microvesicles are the intraluminal vesicles of autolysed MVBs. In the context of systemic inflammation, hepatocytes are known to produce MVBs which release the contained vesicles as extracellular vesicles by exocytosis during non-apoptotic cell death (e.g. pyroptosis).[3] Indeed, the authors demonstrate TUNEL-positive hepatocytes (not specific for apoptosis, but also positive in non-apoptotic cell death and autolysis[4]) and elevated LDH levels (a marker of non-apoptotic cell death), supporting pyroptosis and autolysis as alternate explanations for these clinical and tissue findings, respectively. Moreover, as the authors acknowledge, hepatocytes express little to no Angiotensin Converting Enzyme-2 (ACE2) receptors, the cellular entry point for SARS-CoV-2. Taken together, and in the absence of SAR-CoV-2 *in situ* hybridisation, immunohistochemistry/immunoelectron microscopy or demonstration of SARS-CoV-2 RNA or proteins within the liver, we believe the authors, as others, have mislabelled these electron microscopic structures as SARS-CoV-2 virions.[5]

Regarding the blood parameters in the study, aminotransferases (in particularly AST) are not specific to liver injury and are also released after acute muscle injury. The authors identify higher levels of creatinine kinase in patients with raised aminotransferases raising the possibility of a predominantly muscle rather than the liver source. Acute and chronic infective illnesses drive catabolic processes that involve muscle (protein) breakdown.[6] In keeping with this, patients with severe pulmonary SARS-CoV-2 infection lose weight and we have found them to have a high incidence of critical illness neuromyopathy on recovery from their respiratory failure. Notwithstanding this, the real elephant in the room is the greater degree of respiratory compromise that associates with only modest liver aminotransferase derangement and the complete lack of clinical correlation with clinically significant liver disease. Parameters disturbed in severe acute liver failure are lactate, glucose and INR – these were all well preserved in the data presented by the authors. The patterns of direct bilirubin and albumin are therefore unlikely due to poor synthetic liver function. Reductions in albumin more

likely reflect increased systemic endothelial permeability and albumin loss from the circulation, something which commonly and rapidly occurs in acute systemic illnesses in patients without liver disease.[7]

Despite IL-6 and CRP being similar between patient groups, lymphocyte subset depletion, neutrophil counts, ferritin and markers of fibrinolysis are all significantly increased in patients with prolonged abnormal aminotransferases, clearly suggesting increased immune activation, as we have previously highlighted.[2] Furthermore recent studies have confirmed increased NETosis, a form of non-apoptotic and highly immunogenic cell death causing bystander damage and coagulation changes, accompanies disease severity.[8] Immune-mediated bystander damage then remains a credible mechanism for liver enzyme release and has already been shown to be involved in chimeric antigen receptor T cell-mediated cytokine release syndrome.[9]

In conclusion, we do not believe that the findings of Wang et al conclusively demonstrate a direct cytotoxic effect of SARS-CoV-2 on the liver. Based on the above perspectives, we feel that raised liver aminotransferases associated with SARS-CoV-2 positivity are more likely attributable to illness severity, in which host response and iatrogenic harm (i.e. drugs, ventilation) drive bystander liver injury, thus explaining its association with mortality and in an analogous fashion to patterns seen in sepsis.[10] We still encourage clinicians to remain vigilant for drug-induced liver injury, and for liver damage in high risk groups (i.e. drug/alcohol abusers, family history etc), but not to get overly distracted by raised liver aminotransferases in this context.

References

1. Wang Y, Liu S, Liu H, Li W, Lin F, Jiang L, Li X, Xu P, Zhang L, Zhao L, CaoY, Kang J, Yang J, Li L, Liu X, Li Y, Nie R, Mu J, Lu F, Zhao S, Lu J, Zhao J, SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19, Journal of Hepatology(2020), doi: https://doi.org/10.1016/j.jhep.2020.05.002.

2. Bangash MN, Patel J, Parekh D. COVID-19 and the liver: little cause for concern. Lancet Gastroenterol Hepatol. 2020.

3. Li W, Deng M, Loughran PA, Yang M, Lin M, Yang C, et al. LPS Induces Active HMGB1 Release From Hepatocytes Into Exosomes Through the Coordinated Activities of TLR4 and Caspase-11/GSDMD Signaling. Front Immunol. 2020;11:229.

4. Grasl-Kraupp B, Ruttkay-Nedecky B, Koudelka H, Bukowska K, Bursch W, Schulte-Hermann R. In situ detection of fragmented DNA (TUNEL assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death: a cautionary note. Hepatology. 1995;21(5):1465-8.

5. Miller SE, Brealey JK. Visualization of putative coronavirus in kidney. Kidney International (2020); https://doi.org/10.1016/j.kint.2020.05.004

6. Wang A, Medzhitov R. Not the usual suspect: type I interferon-responsive T cells drive infection-induced cachexia. Nat Immunol. 2019;20(6):666-7.

7. Siddall E, Khatri M, Radhakrishnan J. Capillary leak syndrome: etiologies, pathophysiology, and management. Kidney Int. 2017;92(1):37-46.

8. Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, et al. Neutrophil extracellular traps in COVID-19. JCI Insight. 2020.

9. Liu Y, Fang Y, Chen X, Wang Z, Liang X, Zhang T, et al. Gasdermin E-mediated target cell pyroptosis by CAR T cells triggers cytokine release syndrome. Sci Immunol. 2020;5(43).

10. Kobashi H, Toshimori J, Yamamoto K. Sepsis-associated liver injury: Incidence, classification and the clinical significance. Hepatol Res. 2013;43(3):255-66.