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

To the Editor:

We read the recent article from Wang *et al.* with great interest.¹ They report that severe acute respiratory syndrome coronavirus 2 (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 2 patients) possibly in keeping with viral-mediated liver injury. Considering that interleukin-6 (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 the result of 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 intrahepatocyte 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 autolysis⁴) and elevated lactate dehydrogenase 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 aspartate aminotransferase) are not specific for 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 hepatic 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 international normalized ratio – 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

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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.

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Conflict of interest

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

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Authors' contributions

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

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2020.05.035>.

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Reply to: Correspondence relating to “SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19”

To the Editor:

We appreciate the thoughtful comments on our paper. Our study focused on coronavirus disease 2019 (COVID-19)-related liver

enzyme abnormalities, rather than acute liver injury. Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could infect the liver, it is not a hepatotropic virus that induces pronounced hepatitis. In keeping with this, abnormal liver function tests during the course of COVID-19 are common and primarily limited to aminotransferases, while clinically severe

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