ACKNOWLEDGEMENTS

Dr Yuli Huang receives research fund from the Guangdong Basic and Applied Basic Research Fund (Key project of Guangdong-Foshan Joint Fund; 2019B1515120044), Science and Technology Innovation Project from Foshan, Guangdong (FS0AA-KJ218-1301-0006) and the Clinical Research Startup Program of Shunde Hospital, Southern Medical University (CRSP2019001).

CONFLICT OF INTEREST

The authors do not have any disclosures to report.

Xiaoyan Cai[1]
Sulin Zheng[2]
Yan Zhang[2]
Yuli Huang[2]

[1]*Department of Scientific Research and Education, Shunde Hospital, Southern Medical University (the First People's Hospital of Shunde), Foshan, China*
[2]*Department of Cardiology, Shunde Hospital, Southern Medical University (the First People's Hospital of Shunde), Foshan, China*

Correspondence
Yuli Huang, Department of Cardiology, Shunde Hospital, Southern Medical University (the First People's Hospital of Shunde), Foshan, China.
Email: hyuli821@smu.edu.cn

ORCID

Yuli Huang https://orcid.org/0000-0001-5423-5487

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DOI: 10.1111/liv.14500

SARS-CoV-2 virus and liver expression of host receptors: Putative mechanisms of liver involvement in COVID-19

Zhang et al showed that COVID-19 affected patients' present liver biochemistry abnormalities, including elevation of aminotransferases, gamma-glutamyl transferase and alkaline phosphatase.[1] Hence, several possible clinical scenarios in the setting of liver diseases have been postulated. First, patients with chronic liver disease may be more vulnerable to the severe clinical consequences of COVID-19, including oxygen desaturation and hypoxemia due to severe pneumonia or the cytokine storm.[1,2] Second, liver biochemistry abnormalities are the consequence of drug toxicity.

There is a third potential but poorly explored clinical scenario, which is the possibility that the novel 2019 coronavirus, also known as SARS-CoV-2, may directly or indirectly cause liver injury. In fact, SARS-CoV2 viral load in the stool, which has been detected in about 48% of patients even in stool collected after respiratory samples tested negative,[3] is likely to be associated with portal venous viraemia.

We assessed the gene expression levels of SARS-CoV2-interacting host receptors in the liver tissue and their distribution across cell types according to single-cell transcriptomic experiments retrieved from the Single Cell Portal. We focused on angiotensin-converting enzyme 2 (*ACE2*), transmembrane serine protease 2 (*TMPRSS2*) and paired basic amino acid cleaving enzyme (*FURIN*) gene expression levels. Our analysis shows that the three human host receptors are expressed in the liver tissue; however, expression levels extensively vary across cell types. *ACE2* presents the highest expression levels in cholangiocytes, followed by hepatocytes (Figure 1C). *TMPRSS2* is

SS and CJP should be considered joint senior authors.

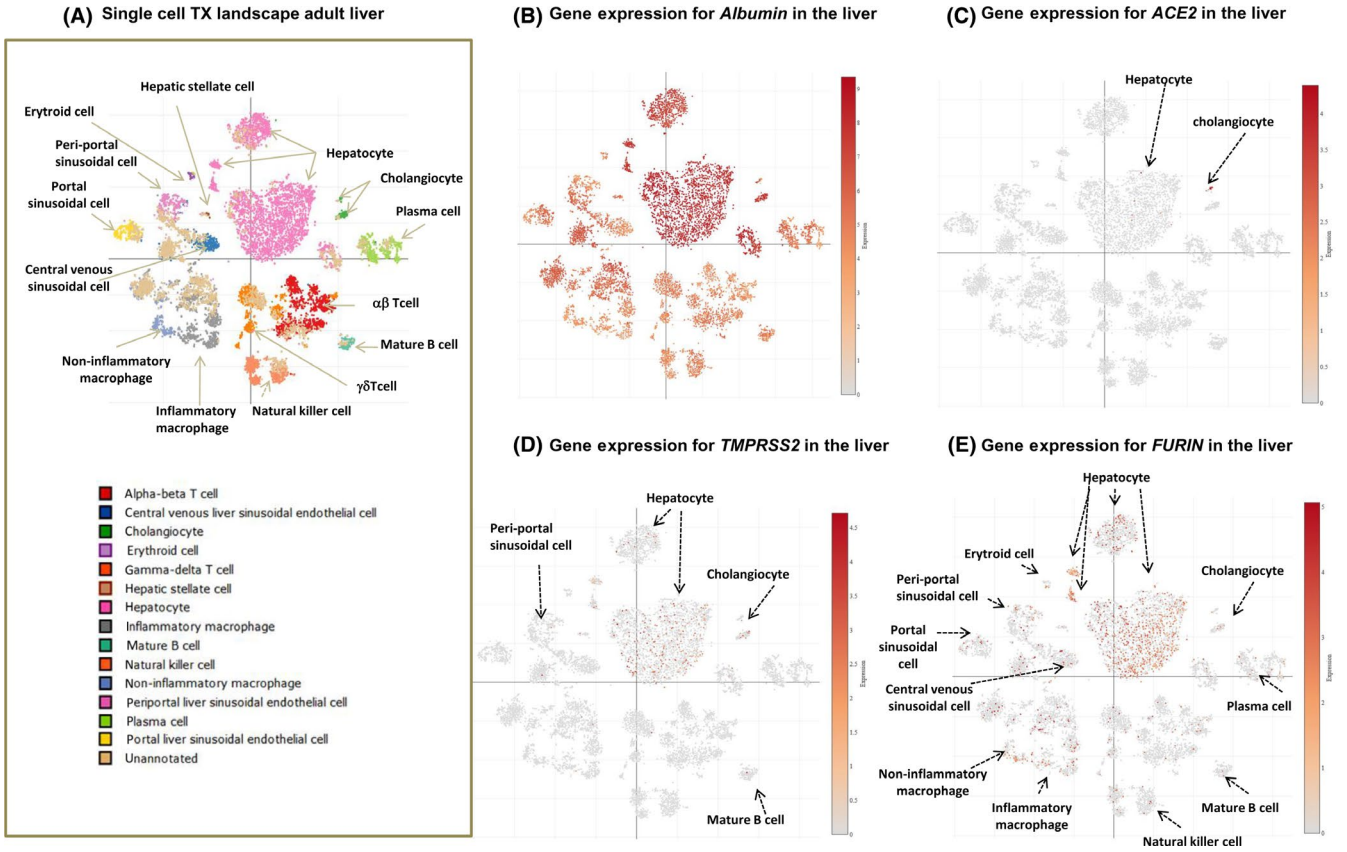

FIGURE 1 Liver gene expression profiling across cell types of host receptors implicated in SARS-CoV-2 infection. Profiling of gene expression was retrieved from the Single Cell Portal available at https://singlecell.broadinstitute.org/single_cell. The analysis was focused on the adult liver dataset from the Human Cell Atlas March 2020 Release, a collection of 23 human single-cell datasets. The human liver cellular landscape analysis by single cell RNA-seq is based on the study of MacParland et al[5] Human liver tissue was obtained from livers procured from deceased donors deemed acceptable for liver transplantation. A, Annotation of liver whole transcriptome involved 15 clusters, including hepatocytes, alpha-beta T cells, central liver sinusoidal endothelial cells, cholangiocytes, erythroid cells, gamma-delta T cells, hepatic stellate cells, inflammatory macrophage, mature B cells, natural killer cells, non-inflammatory macrophages, periportal liver sinusoidal endothelial cells, plasma cells, portal liver sinusoidal endothelial cells and unannotated cells (A). B, To illustrate the pattern and magnitude of differential gene expression levels at different cells in the liver, we assessed the pattern of gene expression of albumin (*ALB*)—the most abundant protein in human blood that is highly expressed in the liver. C-E, Exploration of *ACE2*, *TMPRSS* and *FURIN* expression in the liver

expressed in cholangiocytes, hepatocytes, periportal liver sinusoidal endothelial cells, erytroid cells, and in a much lesser extent in non-inflammatory macrophages and alpha-beta T cells (Figure 1D). *FURIN* shows expression levels across all cell types, from hepatocytes to all populations of liver resident cells (Figure 1E).

Together, these findings support the possibility that SARS-CoV-2 may cause direct liver injury by viral cytopathic effect (directly by lysis and/or by inducing necrotic/apoptotic effect/s). Furthermore, the expression pattern in cell clusters associated with numerous active immune pathways, for example, inflammatory macrophages, natural killer cells, plasma cells, mature B cells and cells of the liver endothelial microenvironment, opens the possibility of SARS-CoV-2 -immune-mediated liver damage.

Not surprisingly, reports from the past 2003-SARS (severe acute respiratory syndrome) epidemic showed not only liver impairment in up to 60% of the patients but also confirmed the presence of SARS-coronavirus by RT-PCR in liver biopsies presenting mild to moderate lobular inflammation and apoptosis.[4]

In conclusion, to understand the pathogenesis of SARS-CoV-2–related liver disease, additional research must be guaranteed, including the search for evidence of viral replication in hepatocytes and liver histology characterization.

KEYWORDS

ACE2, COVID-19, FURIN, host, liver, SARS-CoV-2, single-cell transcriptomics, TMPRSS2

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

SS and CJP: study concept and design; data acquisition; data analysis and interpretation; manuscript drafting; securing funding.

Carlos J. Pirola[1,2]
Silvia Sookoian[1,3]

[1]School of Medicine, Institute of Medical Research A Lanari, University of Buenos Aires, Buenos Aires, Argentina
[2]Department of Molecular Genetics and Biology of Complex Diseases, Institute of Medical Research (IDIM), National Scientific and Technical Research Council (CONICET)–University of Buenos Aires, Buenos Aires, Argentina
[3]Department of Clinical and Molecular Hepatology, Institute of Medical Research (IDIM), National Scientific and Technical Research Council (CONICET)–University of Buenos Aires, Buenos Aires, Argentina

Correspondence
Carlos J. Pirola and Silvia Sookoian, Instituto de Investigaciones Médicas, IDIM-CONICET, Combatientes de Malvinas 3150, CABA-1427, Argentina.
Emails: pirola.carlos@conicet.gov.ar (C.J.P.) and ssookoian@intramed.net (S.S.)

ORCID
Carlos J. Pirola https://orcid.org/0000-0001-8234-4058
Silvia Sookoian https://orcid.org/0000-0001-5929-5470

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DOI: 10.1111/liv.14501

Liver injury in COVID-19: Diagnosis and associated factors

We read with interest the study by Xie et al about the liver injury in non-ICU hospitalized COVID-19 patients[1]; the authors found liver injury was prevalent in COVID-19 patients and might associate with CT scores. However, we believe some concerns should be aroused regarding this conclusion.

Liver function abnormalities were frequent in COVID-19 patients, especially the severe cases.[2] However, as a new contagious disease, there is no standardized diagnostic criteria of COVID-19–associated liver injury at present. Some researchers defined liver injury as any liver function parameter above the upper limit of normal (ULN),[3] but others defined it as liver enzymes higher than two or three times of ULN, and even further classified different liver injury patterns.[4] This study defined elevated levels of alanine transaminase (ALT), aspartate aminotransferase (AST) or bilirubin as liver injury without specifying the ULN of laboratory reference, which was ambiguous and made it difficult to replicate their results.

Furthermore, the time point of diagnosing liver injury was vague. Although we can infer this diagnosis of liver injury was made on the initial laboratory tests on the admission throughout the paper, the authors did not directly mention it. Similarly, the time point of post-treatment was unclear. The authors described post-treatment ALT and AST levels, however, the exact day (i.e. the 1st, 3rd or 7th day after treatment) was unknown, and the time interval may affect the level of liver enzymes. Also, it was unclear whether the post-treatment data came from a single test or from the average of multiple post-treatment tests. Efforts should be made to establish a standardized definition and diagnostic time point of liver injury in COVID-19 patients.

Another important finding in this study was that severe lung lesions on CT (i.e. high CT score) might be related to higher incidence of liver injury. However, the CT scores were assigned on the basis of the percentage of involved lung area, which was semi-quantitative and subjective. Quantification of lung involvements with advanced CT post-processing software or AI algorithms may be more accurate and reproducible.[5] Moreover, although CT score was suggested an independent predictor for liver injury in COVID-19 patients, it remains unclear that how many variables were included in the logistic regression and whether the CT score was the only significant predictor.

In summary, this study provided interesting but preliminary findings. Large-sample multicentre studies are needed to validate these results and further explore COVID-19–associated liver injury.

Zheng Ye
Bin Song

Department of Radiology, West China Hospital, Sichuan University, Chengdu, China
Email: songlab_radiology@163.com