catalytic subunit, depletes hepatic glutathione to an extent similar to APAP *in vivo* but is unable to activate Nrf2.[3]

Next, in our study, immunohistochemistry analysis showed slightly stronger staining of phosphorylated (P-)Nrf2 in cells surrounding the necrotic areas in most liver sections from mice treated with APAP, although the contrast of the staining in some sections was not pronounced, possibly because of the low level of P-Nrf2 protein expression. However, in the same liver sections, we observed substantial staining of Nqo1, Gstα3, Gstm1, Gstm5, and AKR1C in the hepatocytes surrounding necrotic areas, probably because of the strong hepatic expression level of these enzymes, suggesting that the malfunction of the Nrf2/ARE system is zone-specific. These data will be submitted for publication in the near future.

Last, we agree that regulation of mitochondrial oxidant stress by c-Jun N-terminal kinase is the critical downstream event in APAP-induced liver injury.[4] Nrf2 has been recently recognized as a prominent player in mitochondria function. Interestingly, Barzegari et al. (2020) reported that the mitochondria-targeted antioxidant mito-TEMPO activates Nrf2.[5] However, the contribution of Nrf2 to the regulation of mitochondrial oxidant stress in APAP-induced liver injury remains unclear and warrants further study.

Xiuwen Tang, Ph.D.[1]
Xiu Jun Wang, Ph.D.[2]
[1]**Department of Biochemistry and Department of Thoracic Surgery**
The First Affiliated Hospital
Zhejiang University, Hangzhou, China
[2]**Department of Pharmacology and Cancer Institute**
The Second Affiliated Hospital Zhejiang University School of Medicine
Zhejiang University
Hangzhou, China

REFERENCES

1) **Chen Y**, **Liu K**, **Zhang J**, Hai Y, Wang P, Wang H, et al. c-Jun NH2 -Terminal protein kinase phosphorylates the Nrf2-ECH homology 6 domain of nuclear factor erythroid 2-related factor 2 and downregulates cytoprotective genes in acetaminophen-induced liver injury in mice. Hepatology 2020;71:1787-801.
2) Kaplowitz N, Than TA, Win S. Acetaminophen hepatotoxicity: strong offense and weakened defense. Hepatology 2020;71:1530-1532.
3) Goldring CE, Kitteringham NR, Elsby R, Randle LE, Clement YN, Williams DP, et al. Activation of hepatic Nrf2 in vivo by acetaminophen in CD-1 mice. Hepatology 2004;39:1267-1276.
4) Jaeschke H, Ramachandran A. The role of oxidant stress in acetaminophe-induced liver injury. Curr Opin Toxicol 2020;20-21:9-14.
5) Barzegari A, Nouri M, Gueguen V, Saeedi N, Pavon-Djavid G, Omidi Y. Mitochondria-targeted antioxidant mito-TEMPO alleviate oxidative stress induced by antimycin A in human mesenchymal stem cells. J Cell Physiol 2020;235:5628-5636.

Author names in bold designate shared co-first authorship.

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Potential conflict of interest: Nothing to report.

Letter to the Editor: Coronavirus Disease 2019–Related Liver Injury and Clinical Outcomes: Does It Really Exist?

TO THE EDITOR:

We read with interest the study by Lei and colleagues on the association between markers of liver injury and mortality in coronavirus disease 2019 (COVID-19) in China.[1] The authors have painstakingly collated data from a large number of patients with COVID-19 from multiple centers across Wuhan. They found that an increase in aspartate aminotransferase (AST) and its dynamicity correlated with COVID-19-related liver injury and patient outcomes. They concluded that the dynamic patterns of liver injury indicators, represented by AST, correspond with COVID-19-related liver injury. A basic understanding of enzymes that form part of the liver test is fundamental to the interpretation of

clinical events. Alanine aminotransferase (ALT) produced in the hepatocytes is a very specific marker of liver cell injury, with relatively lower concentrations in other organ tissues. The rise in ALT may occur with the use of specific drugs, such as antibiotics and glucocorticoids. AST, on the other hand, occurs in two isoforms, indistinguishable on standard assays. It is the mitochondrial isoenzyme, which is produced by the hepatocyte, that reacts to membrane damage similar to ALT, whereas the cytosolic isoenzyme is produced by cells of skeletal muscles, cardiac myocytes, and renal tissue. The use of AST in isolation is not recommended as a marker for hepatocellular injury.[2] The conclusion inculpating AST with COVID-19 liver injury is inaccurate and factually related to the evolving multiorgan dysfunction (MOD). This is underscored by the fact that most patients had already developed cardiac and renal injury before the development of proposed acute liver injury (ALI) at 10-15 days after admission, further exacerbated by antibiotics and glucocorticoids. AST and ALT levels were significantly high in those with lymphocytopenia, a marker of severe COVID-19.[3] Scoring systems for MOD were not used by the authors, adding to the confounding. The definition of ALI in the current study is flawed. An important criterion for diagnosing ALI in those without preexisting liver disease is an international normalized ratio >2.[4] The current study does not incorporate appropriate methods to clearly identify synthetic and metabolic hepatocellular dysfunction. The term "COVID-19-related liver injury" is perchance misleading, akin to the recently described COVID-19 involvement of the pancreas.[5]

Cyriac Abby Philips, M.D., D. M.[1]
Rizwan Ahamed, M.D., D.M.[2]
Philip Augustine, M.D., D.M.[2]
[1]**The Liver Unit and Monarch Liver Lab**
Cochin Gastroenterology Group
Ernakulam Medical Center Hospital
Kochi, India
[2]**Gastroenterology and Advanced GI Endoscopy**
Cochin Gastroenterology Group
Ernakulam Medical Center Hospital
Kochi, India

REFERENCES

1) Lei F, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, et al. Longitudinal association between markers of liver injury and mortality in COVID-19 in China. Hepatology 2020 May 2. https://doi.org/10.1002/hep.31301. [Epub ahead of print]
2) Kwo PY, Cohen SM, Lim JK. ACG clinical guideline: evaluation of abnormal liver chemistries. Am J Gastroenterol 2017;112:18-35.
3) Wang T, Du Z, Zhu F, Cao Z, An Y, Gao Y, et al. Comorbidities and multi-organ injuries in the treatment of COVID-19. Lancet 2020;395:921-1010, e52-e53.
4) Koch DG, Speiser JL, Durkalski V, Fontana RJ, Davern T, McGuire B, et al. The natural history of severe acute liver injury. Am J Gastroenterol 2017;112:1389-1396.
5) de-Madaria E, Siau K, Cárdenas-Jaén K. Increased amylase and lipase in patients with COVID-19 pneumonia: don't blame the pancreas just yet! Gastroenterology 2020 Apr 21. https://doi.org/10.1053/j.gastro.2020.04.044. [Epub ahead of print]

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Potential conflict of interest: Dr. Philips advises Cipla.

REPLY:

We appreciate Philips and colleagues' interest in our report on the associations between liver injury markers and coronavirus disease 2019 (COVID-19) mortality.[1] They raised the question regarding the application of liver injury markers. However, several of their interpretations of our results are misleading or incorrect.

We understand that the use of aspartate aminotransferase (AST) in isolation cannot comprehensively characterize liver injury. To fully explore the associations of liver injury with COVID-19, we investigated the dynamic patterns of four respective and extensively applied markers of liver injury: alanine aminotransferase (ALT), AST, alkaline phosphatase, and total bilirubin. Their associations with COVID-19 mortality were clearly demonstrated. Importantly, we found that the increased levels of all four indicators are consistently associated with higher risk of COVID-19 death, with AST having the largest hazard ratio. Therefore, in our study, liver injury was characterized by four markers, rather than only AST as mentioned by Philips et al.

Until recently, the definition of acute liver injury (ALI) has been inconsistent in the literature.[2] In our study, ALI was defined by ALT, which has been extensively applied in more than 1,000 studies. However, the international normalized ratio (INR), proposed by Philips et al., is a marker of coagulopathy, which can occur in many pathological conditions.[3,4] Although prolonged prothrombin time/INR can occur in chronic liver disease, this condition is more likely to happen at