Inflammatory cytokines, T lymphocyte subsets, and ritonavir involved in liver injury of COVID-19 patients

Signal Transduction and Targeted Therapy (2020) 5:255

Dear Editor,

Coronavirus disease 2019 (COVID-19) is a type of novel coronavirus and no specific treatment is currently available. Apart from damages to the lung, COVID-19 is also able to trigger liver injury. Numerous observational studies have revealed that elevation of liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), was detected in some COVID-19 patients, especially in severe cases.1 Unfortunately, the underlying mechanisms and risk factors of COVID-19-induced liver damage have not been completely elucidated. Therefore, identification of novel risk factors in liver injury of COVID-19 patients is essential for the prevention and treatment of liver damage. In this study, 192 patients with COVID-19 hospitalized in Chongqing Public Health Center were recruited to identify the risk factors in COVID-19 patients with liver injury.

The characteristics of 163 mild and 29 severe patients with COVID-19 were analyzed in this study. The results indicated that age and hospitalization time of severe patients were significantly higher compared to mild cases (P < 0.05, Supplementary Table S1). Additionally, the respiratory (fever, shortness of breath, and chest tightness) and gastrointestinal symptoms (anorexia) in severe patients were more obvious (P < 0.05, Supplementary Table S1). Furthermore, liver injury was more commonly detected in the severe group, in whom the expression levels of inflammatory cytokines (procalcitonin, C-reactive protein, erythrocyte sedimentation rate, interleukin (IL)-6, IL-10, and IL-17A) were remarkably elevated (P < 0.05, Supplementary Table S1). The numbers of T lymphocyte subsets (CD3+, CD4+ and CD8+ T cells) in patients with severe COVID-19 were notably decreased compared to mild patients (P < 0.05, Supplementary Table S1).

Further studies on liver function alterations in patients with mild and severe COVID-19 suggested that the levels of albumin were significantly reduced, and the production of ALT, AST, and lactate dehydrogenase (LDH) was remarkably elevated in patients with severe COVID-19 at admission. Furthermore, the severity of liver injury in patients was aggravated during hospitalization, and the levels of ALT, AST, alkaline phosphatase and LDH in severe patients with COVID-19 were significantly increased compared to the mild group (Supplementary Fig. S2). When the patients tested negative for COVID-19 and reached the discharge standard, liver function of both the experimental groups was significantly improved. These findings suggested that, during the development of COVID-19, liver injury could be aggravated. Taken all together, age, respiratory and gastrointestinal symptoms, the levels of inflammatory cytokines, numbers of T lymphocyte subsets, and degree of liver injury were associated with COVID-19 severity.

To further analyze the risk factors in liver injury of COVID-19 patients, 75 patients (39.06%) with liver injury and 117 patients without liver injury were recruited at admission. More male participants were found in the liver injury group compared to patients without liver injury (P < 0.05, Supplementary Table S2). In addition, more obvious gastrointestinal symptoms (anorexia), reduced number of T lymphocyte subsets (CD3+ and CD4+ T cells), and upregulation of inflammatory cytokines (r-interferon, tumor necrosis factor (TNF)-α, IL-2, IL-4, IL-10, and IL-17A) were detected in patients with severe COVID-19. Logistic regression was performed to identify potential risk factors in COVID-19 patients with liver injury at admission. Univariate analysis revealed that gender, TNF-α, IL-2, IL-4, IL-17A, CD3+ T, and CD4+ T cells were novel risk factors in COVID-19 patients with liver injury (P < 0.05, Supplementary Table S3). Multivariate analysis indicated that gender (odds ratio (OR) = 0.31, 95% confidence interval (CI) = 0.11–0.80, P = 0.007), IL-2 (OR = 4.07, 95% CI = 1.25–14.20, P < 0.001), IL-17A (OR = 1.63, 95% CI = 1.23–2.40, P = 0.004), and CD4+ T (OR = 3.90, 95% CI = 1.41–12.17, P < 0.001) cells were independent risk factors in COVID-19 patients with liver injury (P < 0.05, Supplementary Table S3).

During hospitalization, the number of patients with liver injury was increased significantly, as liver injury was detected in 133 COVID-19 patients (69.27%), while 59 patients exhibited no liver damage. Interestingly, liver injury was detected in 25 out of 29 (86.21%) severe COVID-19 patients. The results suggested that the proportion of male patients, nausea, and ritonavir/antibiotics treatment were significantly higher in participants with liver injury (P < 0.05, Supplementary Table S4). In addition, patients with liver injury exhibited reduced number of T lymphocyte subsets (CD3+, CD4+ and CD8+ T cells), and increased levels of inflammatory cytokines (TNF-α, IL-2, IL-6, and IL-10) during hospitalization (P < 0.05, Supplementary Table S4). Logistic regression was carried out to identify putative risk factors in COVID-19 patients with liver injury during hospitalization. Univariate analysis revealed that ritonavir, IL-2, IL-6, IL-10, CD4+ T, and CD8+ T cells were novel risk factors of COVID-19 patients with liver injury during hospitalization (P < 0.05, Table 1). Multivariate analysis suggested that ritonavir (OR = 4.75, 95% CI = 1.89–16.55, P < 0.001), IL-6 (OR = 3.27, 95% CI = 2.09–5.60, P < 0.001), CD4+ (OR = 0.99, 95% CI = 0.95–1.23, P = 0.010) and CD8+ T cells (OR = 1.38, 95% CI = 0.97–1.96, P < 0.001) were independent risk factors in COVID-19 patients with liver injury (P < 0.05, Table 1).

Dysregulation of immune response was observed in COVID-19 patients.2 During the development of COVID-19, the expression of programmed cell death receptor 1 and Tim-3 in T lymphocytes was increased, suggesting the depletion of T cells.3 In accordance with these findings, the results of this study suggested that the numbers of T lymphocyte subsets (CD3+, CD4+ and CD8+ T cells) were significantly reduced in patients with liver injury during hospitalization. In addition, CD4+ T cells were an independent risk factor in COVID-19 patients with liver injury at admission. Furthermore, CD4+ and CD8+ T cells were potential independent risk factors in COVID-19 patients with liver injury during hospitalization. Taken all together, depletion of T lymphocytes was detected to serve essential roles on liver injury in COVID-19 patients.

Received: 31 July 2020 Revised: 16 September 2020 Accepted: 23 September 2020

© The Author(s) 2020

https://doi.org/10.1038/s41392-020-00363-9
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-mediated production of inflammatory cytokines could contribute to liver injury. In this study, the expression levels of r-interferon, TNF-α, IL-2, IL-4, IL-10, and IL-17A were remarkably elevated in patients with liver injury at admission. Multivariate analysis indicated that IL-2 and IL-17A were independent risk factors in COVID-19 patients with liver injury at admission, suggesting IL-2 and IL-17A were key inflammatory cytokines that could lead to liver injury. However, the inflammatory factors continued to change during the development of COVID-19. The results of this study revealed that COVID-19 patients with liver injury exhibited increased levels of inflammatory cytokines (TNF-α, IL-2, IL-6, and IL-10) compared to participants without liver injury. Furthermore, multivariate analysis revealed that IL-6 was an independent risk factor in COVID-19 patients with liver injury during hospitalization. In summary, an increased production of inflammatory cytokines was detected in COVID-19 patients, which was associated with liver injury. In clinical practice, alteration of inflammatory cytokines should be closely monitored during the treatment.

At present, no specific treatment is available for COVID-19 patients. The commonly used antiviral drugs lopinavir/ritonavir are mainly metabolized in the liver, and the side effects include liver dysfunction. Previous studies have suggested that use of lopinavir/ritonavir was associated with significantly aggravated liver injury. In this study, the proportion of ritonavir- and antibiotics-treated cases was significantly increased in COVID-19 patients with liver injury. Multivariate analysis indicated that ritonavir treatment was an independent risk factor in COVID-19 patients with liver injury in clinical practice, alteration of inflammatory cytokines should be closely monitored during the treatment.

In conclusion, liver injury was found in many COVID-19 patients, especially those with severe symptoms. The underlying mechanisms of liver injury in COVID-19 patients mainly involve SARS-CoV-2-mediated liver damage, immune response, and cytokine production, as well as drug-induced liver injury. Therefore, during the treatment of COVID-19, intervention targeting CD4+ and CD8+ T cells, antagonist of inflammatory cytokines, could be a promising therapeutic strategy for the treatment of liver injury in COVID-19 patients, and drug-induced liver damage should also be avoided.

ACKNOWLEDGEMENTS
This work was supported by the Chongqing Special Research Project for Prevention and Control of Novel Coronavirus Pneumonia (No. cstc2020jsx-fyzx0103).

AUTHOR CONTRIBUTIONS
Z.M., S.H., and A.Z. conceived and designed the study. S.L., K.Z., L.G., J.L., G.Y., and Y.C. collected the data. K.Z., L.G., and Y.B. analyzed the data. S.L., K.Z., L.G., and Y.B. wrote the paper. Z.M., S.H., and A.Z. reviewed and edited the manuscript. All authors read and approved the manuscript.

ADDITIONAL INFORMATION
The online version of this article (https://doi.org/10.1038/s41392-020-00363-9) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Shengtao Liao1, Ke Zhan1, Li Gan2, Yang Bai3, Jinfang Li4, Guodan Yuan5, Ying Cai6, An Zhang6, Song He1 and Zhechuan Mei2

1Department of Gastroenterology, The Second Affiliated Hospital of Chongqing Medical University, 400010 Chongqing, China; 2Laboratory of Forensic Medicine and Biomedical Informatics, College of Basic Medicine, Chongqing Medical University, 400016 Chongqing, China; 3Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Chongqing Medical University, 400010 Chongqing, China; 4Department of Neurology, The Second Affiliated Hospital of Chongqing Medical University, 400010 Chongqing, China; 5Department of Critical Care Medicine, Chongqing Public Health Medical Center, 400036 Chongqing, China and 6Department of Critical Care Medicine, The Second Affiliated Hospital of Chongqing Medical University, 400010 Chongqing, China. These authors contributed equally: Shengtao Liao, Ke Zhan, Li Gan, Yang Bai

Correspondence: An Zhang (zhangan@hospital.cqmu.edu.cn) or Song He (hedoctor65@cqmu.edu.cn) or Zhechuan Mei (meizhechuan@cqmu.edu.cn)

| Table 1. Univariate and multivariate analyses of potential risk factors in COVID-19 patients with liver injury during hospitalization |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Univariable OR (95% CI) | P value       | Multivariable OR (95% CI) | P value       |
| Sex             | 0.46 (0.17–1.2)  | 0.117          | 4.75 (1.89–16.55)         | <0.001        |
| Nausea          | 2.21 (0.79–7.19) | 0.152          | 0.99 (0.95–1.23)          | 0.010         |
| Platelet count, x10^9/L | 1 (1–1.01)   | 0.670          | 1.38 (0.97–1.96)          | <0.001        |
| Hemoglobin, g/L | 1.02 (1–1.05)   | 0.127          | 0.74 (0.63–0.88)          | <0.001        |
| Monocyte count, x10^9/L | 3.86 (0.67–49.3) | 0.247          | 3.27 (2.09–5.60)          | <0.001        |
| Ritonavir       | 4.15 (2.11–15.83) | <0.001        | 4.75 (1.89–16.55)         | <0.001        |
| Antibiotics     | 2.7 (80.86–12.47) | 0.122          | 0.99 (0.95–1.23)          | 0.010         |
| CD4+ T cell, per μL | 0.92 (0.86–0.97) | 0.018          | 1.38 (0.97–1.96)          | <0.001        |
| CD8+ T cell, per μL | 1.43 (1.01–2.4) | 0.007          | 1.38 (0.97–1.96)          | <0.001        |
| CD4+ T/CD8+ T cell | 1.08 (1.03–1.15) | 0.057          | 0.99 (0.95–1.23)          | 0.010         |
| CD3+ T cell, per μL | 0.41 (0.22–0.74) | 0.052          | 1.38 (0.97–1.96)          | <0.001        |
| TNF-α, pg/mL    | 1.53 (1.05–2.49) | 0.051          | 3.27 (2.09–5.60)          | <0.001        |
| IL-10, pg/mL    | 1.78 (1.34–2.51) | 0.001          | 3.27 (2.09–5.60)          | <0.001        |
| IL-6, pg/mL     | 2.63 (1.18–7.66) | <0.001        | 3.27 (2.09–5.60)          | <0.001        |
| IL-2, pg/mL     | 1.33 (1.15–1.62) | 0.001          | 3.27 (2.09–5.60)          | <0.001        |
REFERENCES
1. Carvalho T. COVID-19 research in brief: 24 April to 1 May, 2020. Nat. Med. https://doi.org/10.1038/d41591-020-00016-y (2020).
2. Haberman, R. et al. Covid-19 in immune-mediated inflammatory diseases - case series from New York. N. Engl. J. Med. 383, 85–88 (2020).
3. Giamarellos-Bourboulis, E. J. et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe 27, 992.e3–1000.e3 (2020).
4. Yao, Z., Zheng, Z., Wu, K. & Junhua, Z. Immune environment modulation in pneumonia patients caused by coronavirus: SARS-CoV, MERS-CoV and SARS-CoV-2. Aging 12, 7639–7651 (2020).
5. Zhang, Y. et al. Liver impairment in COVID-19 patients: a retrospective analysis of 115 cases from a single centre in Wuhan city, China. Liver Int. https://doi.org/10.1111/liv.14455 (2020).