## Conflict of interest

The authors declare no competing interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

All authors contributed to the literature research and writing of the letter. All authors have read and agreed to the published version of the letter.

## Supplementary data

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## References

*Author names in bold designate shared co-first authorship*

[1] Heinrich S, Craig AJ, Ma L, Heinrich B, Greten TF, Wang XW. Understanding tumour cell heterogeneity and its implication for immunotherapy in liver cancer using single-cell analysis. J Hepatol 2021;74:700–715.
[2] Cramer T, Vaupel P. Is tissue hypoxia the principal mechanism for immune evasion and malignant progression in Hepatocellular Carcinoma? J Hepatol 2021;75:735–736.
[3] **Ma L**, **Hernandez MO**, Zhao Y, Mehta M, Tran B, Kelly M, et al. Tumor cell biodiversity drives microenvironmental reprogramming in liver cancer. Cancer Cell 2019;36. 418-430.e416.
[4] Wilson GK, Tennant DA, McKeating JA. Hypoxia inducible factors in liver disease and hepatocellular carcinoma: current understanding and future directions. J Hepatol 2014;61:1397–1406.
[5] Schito L, Semenza GL. Hypoxia-inducible factors: master regulators of cancer progression. Trends Cancer 2016;2:758–770.
[6] Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Canc Res 1998;58:1408–1416.
[7] Bhandari V, Hoey C, Liu LY, Lalonde E, Ray J, Livingstone J, et al. Molecular landmarks of tumor hypoxia across cancer types. Nat Genet 2019;51:308–318.
[8] Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 2020;382:1894–1905.

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# Letter regarding "Association of liver abnormalities with in-hospital mortality in patients with COVID-19"

*To the Editor:*

With great enthusiasm, we read a breakthrough study demonstrating the association of liver abnormalities with in-hospital mortality in patients with COVID-19.[1] Integrating 10 candidate predictor parameters determined by multivariate regression analyses, Ding and colleagues formulated a novel prognostic nomogram to predict the survival of patients with COVID-19. However, several crucial pitfalls should be taken into account.

In the study of Ding *et al.*,[1] a total of 2,073 patients with COVID-19 were involved. Subsequently, 10 candidate predictor parameters (including age, severe pneumonia, lymphocyte count, PLT, CRP, D-dimer, creatinine, cTnI, AST, and D-Bil) were selected to construct the prognostic model and plot the nomogram. However, is the limited sample size large enough to establish the prediction model which can predict the overall survival probability of patients with COVID-19? As a matter of fact, we are skeptical about the reliability of this nomogram. In the authoritative methodological research published in the journal of *BMJ*, Riley *et al.*[2] pointed out that a robust prediction model should be constructed based on the required sample size that is large enough to sufficiently target precious model prediction and minimize model overfitting. Thus, the required sample size of Ding *et al.* was calculated according to formulas of Riley *et al.*[2,3] Firstly, $\ln L_{null}$ is $-5.605(0.010 \times 100 \times ln(0.010) - 0.010 \times 100)$, and $\max(R^2_{CS})$ is $0.106\left(1 - \exp\left(\frac{2\times(-5.605)}{100}\right)\right)$. Secondly, the conservative value of $R^2_{cs}$ is $0.106 \times 8\%=0.008$. Thirdly, we inputed a key time point to predict the overall survival probability (14 days = 0.038 year), alongside the number of candidate predictor parameters (n = 10), the anticipated mean follow-up (37.81 days = 0.104 year), the mortality rate (0.096), and the conservative value of $R^2_{cs}$ (0.008). Finally, the minimum required sample size of 14-day survival prediction for Ding *et al.*'s study was calculated in Stata software with the following codes:

pmsampsize, type(s) rsquared(0.008) parameters(10) rate(0.096) timepoint(0.038) meanfup(0.104)

The result is shown in Fig. 1A, indicating that at least 11,200 patients are required for 14-day survival prediction, corresponding to 1,164.8 deaths and an EPP (events per candidate predictor parameter) of 11.18. In addition, the minimum required sample sizes of 21-day and 28-day survival prediction were also calculated (Fig. 1B and C). Therefore, the minimum required sample size to construct the prognostic nomogram should be 11,200 patients, which is much larger than the sample size (2,073 patients) of Ding *et al.*'s study.

Moreover, though the calibration curve showed good consistency, the ROC (receiver operating characteristic) curve and

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**A**

. pmsampsize, type(s) rsquared(0.008) parameters(10) rate(0.096) timepoint(0.038) meanfup(0.104)
NB: Assuming 0.05 acceptable difference in apparent and adjusted R-squared
NB: Assuming 0.05 margin of error in estimation of overall risk at time point = 0.038
NB: Events per Predictor Parameter (EPP) assumes overall event rate = 0.096

| | Samp_size | Shrinkage | Parameter | Rsq | Max_Rsq | EPP |
|---|---|---|---|---|---|---|
| Criteria 1 | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |
| Criteria 2 | 1,809 | 0.593 | 10 | 0.008 | 0.11 | 1.81 |
| Criteria 3* | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |
| Final | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |

Minimum sample size required for new model development based on user inputs = 11,200,
corresponding to 1,164.8 person-time** of follow-up, with 112 outcome events
assuming an overall event rate = 0.096, and therefore an EPP = 11.18

**B**

. pmsampsize, type(s) rsquared(0.008) parameters(10) rate(0.096) timepoint(0.058) meanfup(0.104)
NB: Assuming 0.05 acceptable difference in apparent and adjusted R-squared
NB: Assuming 0.05 margin of error in estimation of overall risk at time point = 0.058
NB: Events per Predictor Parameter (EPP) assumes overall event rate = 0.096

| | Samp_size | Shrinkage | Parameter | Rsq | Max_Rsq | EPP |
|---|---|---|---|---|---|---|
| Criteria 1 | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |
| Criteria 2 | 1,809 | 0.593 | 10 | 0.008 | 0.11 | 1.81 |
| Criteria 3* | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |
| Final | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |

Minimum sample size required for new model development based on user inputs = 11,200,
corresponding to 1,164.8 person-time** of follow-up, with 112 outcome events
assuming an overall event rate = 0.096, and therefore an EPP = 11.18

**C**

. pmsampsize, type(s) rsquared(0.008) parameters(10) rate(0.096) timepoint(0.077) meanfup(0.104)
NB: Assuming 0.05 acceptable difference in apparent and adjusted R-squared
NB: Assuming 0.05 margin of error in estimation of overall risk at time point = 0.077
NB: Events per Predictor Parameter (EPP) assumes overall event rate = 0.096

| | Samp_size | Shrinkage | Parameter | Rsq | Max_Rsq | EPP |
|---|---|---|---|---|---|---|
| Criteria 1 | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |
| Criteria 2 | 1,809 | 0.593 | 10 | 0.008 | 0.11 | 1.81 |
| Criteria 3* | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |
| Final | 11,200 | 0.9 | 10 | 0.008 | 0.11 | 11.18 |

Minimum sample size required for new model development based on user inputs = 11,200,
corresponding to 1,164. 8 person-time** of follow-up, with 112 outcome events
assuming an overall event rate = 0.096, and therefore an EPP = 11. 18

**Fig. 1. The minimum required sample sizes for survival predictions in Ding *et al.*'s nomogram calculated by the pmsampsize package in Stata software.** (A) 14-day survival prediction. (B) 21-day survival prediction. (C) 28-day survival prediction. The results indicated that at least 11,200 patients are required for 14-day, 21-day, and 28-day survival predictions, corresponding to 1,164.8 deaths and an EPP of 11.18. EPP, events per candidate predictor parameter. (This figure appears in color on the web.)

DCA (decision curve analysis) curves were eagerly warranted.[4,5] The ROC curve can evaluate the discriminative ability of the nomogram, while the DCA curve can evaluate the clinical usefulness of the nomogram. Furthermore, Ding *et al.*'s nomogram would be more valuable if they applied the disease-specific survival to establish the prognostic nomogram.[6] After all, the overall survival may be impacted by other factors except for COVID-19.

We appreciate Ding *et al.* for their vital thoughts on the association between liver abnormalities and COVID-19, which has paved a novel way to predict the overall survival probability of patients with COVID-19. However, the minimum required sample size to construct the prognostic nomogram in Ding *et al.*'s study should be 11,200 patients. The discriminative ability and the clinical usefulness of the nomogram were definitely necessary. Consequently, these above issues may strongly impact the reliability and applicability of Ding *et al.*'s nomogram.

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## Conflict of interest

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## Authors' contributions

G-J.H and M-S.L designed the letter; M-S.L wrote the letter; G-J.H revised the letter.

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## Supplementary data

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## References

[1] Ding ZY, Li GX, Chen L, Shu C, Song J, Wang W, et al. Association of liver abnormalities with in-hospital mortality in patients with COVID-19. J Hepatol 2021;74(6):1295–1302.
[2] Riley RD, Ensor J, Snell K, Harrell FJ, Martin GP, Reitsma JB, et al. Calculating the sample size required for developing a clinical prediction model. BMJ 2020;368:m441.
[3] Riley RD, Snell KI, Ensor J, Burke DL, Harrell FJ, Moons KG, et al. Minimum sample size for developing a multivariable prediction model: PART II–binary and time-to-event outcomes. Stat Med 2019;38(7):1276–1296.
[4] Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115(7):928–935.
[5] Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making 2006;26(6):565–574.
[6] Burke HB. Overall survival vs disease-specific survival. JAMA Oncol 2018;4(4):586.

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# Liver injury in COVID-19 – The culprit may not be COVID-19!

*To the Editor:*

We read the study by Ding *et al*[1] with great interest. We congratulate the authors on conducting this large, multicentric study. They have demonstrated that raised levels of aspartate aminotransaminase and direct bilirubin at admission can be used as independent predictors of mortality in patients with coronavirus disease 2019 (COVID-19). They developed a prognostic model with a nomogram that can be used to predict the overall survival probability in patients with COVID-19. Importantly, they have reported that chronic HBV infection is not associated with an increased risk of lethal outcomes in patients with COVID-19. Previous studies have revealed that liver abnormalities occur more frequently in patients with severe COVID-19 and this has been consequential in establishing the association between various liver function abnormalities and their effect on mortality in COVID-19.[2] This is due to the multiplier effect of multiple organ dysfunction, cytokine storm, drug effects and hypoxic liver injury, rather than the direct cytopathic effect of the SARS-CoV-2. Kulkarni *et al.*[3] performed a meta-analysis of 20,874 patients and reported that 3.6% of patients had chronic liver disease (CLD). The odds of developing severe COVID-19 in patients with CLD was 0.81 (95% CI 0.31–2.09; $p$ = 0.67) compared to non-CLD patients. COVID-19 patients with elevated liver chemistries had higher risk of mortality (odds ratio [OR] 3.46 [2.42–4.95, $p$ <0.001]) and severe disease (OR 2.87 [95% CI 2.29–3.6, $p$ <0.001]) compared to patients without elevated liver chemistries. In this study,[1] around 61.8% patients had some abnormal liver chemistry during the course of hospitalization. However, there are some important issues that have not been addressed in the paper.

The study fails to identify underlying CLD as a predictor of worse outcome, possibly due to the small number of patients in the study. Also, the severity of liver disease in terms of various scoring systems has not been corelated with the outcomes. Only 11 individuals had compensated cirrhosis and 3 had decompensated cirrhosis. Standard severity scoring systems like Child-Pugh and model for end-stage liver disease for cirrhosis and Maddrey's discriminant function for alcoholic hepatitis have not been described and correlated with survival.[4,5]

Although most patients developed some transaminitis during admission, there were several confounding factors that blur the direct association of liver dysfunction attributable to COVID-19. Cai *et al.*[6] reported use of drugs, systemic inflammation, secondary sepsis, polypharmacy, shock and hypoxia as contributors to liver injury in COVID-19. The presence of hypoxic injury[7] is also a contributor to liver injury and has not been described in this study. It is evident from the description that the hospitalized patients in this cohort were sick, with most requiring high flow oxygen or invasive ventilation which could have contributed independently to liver injury. Also, the use of various medications during the hospital course should be included in the analysis. The use of hepatotoxic drugs like lopinavir and ritonavir have been shown to be associated with liver injury.[6] Alcohol abuse or use of Chinese herbal medication before admission should also be

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