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ORIGINAL ARTICLE

Implications of liver injury in risk-stratification and management of patients with COVID-19

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Abstract

Background Infection with SARS-CoV-2 has been associated with liver dysfunction, aggravation of liver burden, and liver injury. This study aimed to assess the effects of liver injuries on the clinical outcomes of patients with COVID-19.

Methods A total of 1520 patients with severe or critical COVID-19 from Huoshenshan Hospital, Wuhan, were enrolled. Chronic liver disease (CLD) was confirmed by consensus diagnostic criteria. Laboratory test results were compared between different groups. scRNA-seq data and bulk gene expression profiles were used to identify cell types associated with liver injury.

Results A total of 10.98% of patients with severe or critical COVID-19 developed liver injury after admission that was associated with significantly higher rates of mortality (21.74%, $p<0.001$) and intensive care unit admission (26.71%, $p<0.001$). Pre-existing CLDs were not associated with a higher risk. However, fatty liver disease and cirrhosis were associated with higher risks, supported by evidences from single cell and bulk transcriptome analysis that showed more TMPRSS2^{+} cells in these tissues. By generating a model, we were able to predict the risk and severity of liver injury during hospitalization.

Conclusion We demonstrate that liver injury occurring during therapy as well as pre-existing CLDs like fatty liver disease and cirrhosis in patients with COVID-19 is significantly associated with the severity of disease and mortality, but the presence of other CLD is not associated. We provide a risk-score model that can predict whether patients with COVID-19 will develop liver injury or proceed to higher-risk stages during subsequent hospitalizations.

Keywords Fatty liver disease · Viral hepatitis · Cirrhosis · Hospitalization · Disease progression · Prognosis · ICU admission · scRNA-seq analysis · TMPRSS2 · Clinical prediction model

Abbreviations

ACE2	Angiotensin-converting enzyme 2
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
LDH	Lactate dehydrogenase
CHB	Chronic hepatitis B
CHC	Chronic hepatitis C
CI	Confidence interval
CLD	Chronic liver disease
CRP	C-reactive protein
FLD	Fatty liver disease
GGT	γ-Glutamyl transpeptidase
HBV	Hepatitis B virus
HCV	Hepatitis C virus
MAFLD	Metabolic dysfunction-associated fatty liver disease

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scRNA-seq Single-cell RNA sequencing
TMPRSS2 Transmembrane protease serine 2

Introduction

A novel coronavirus named SARS-CoV-2 began to rapidly spread across the world in December 2019 and was declared a global pandemic by the World Health Organization. The attachment of SARS-CoV-2 to the target cell is initiated by interactions between the spike glycoprotein (S) of the virus and its receptor of the host cell, angiotensin-converting enzyme 2 (ACE2). Subsequently, SARS-CoV-2 S protein is cleaved by a plasma membrane-associated type II transmembrane serine protease (TMPRSS2), leading to membrane fusion which is essential to release the viral contents into the infected cell cytosol. Both ACE2 and TMPRSS2 are essential for viral spreading to the host cell [1].

Several recent studies have shown that more than 50% of patients with COVID-19 develop liver abnormalities, of whom 20% have liver injury [2–5]. Furthermore, a pathological study based on patients who had died from severe COVID-19 showed that their liver tissue had moderate microvesicular steatosis and mild lobular and portal activity indicating that SARS-CoV-2 might cause liver injury [6]. Previous research has also examined the association between markers of liver injury and mortality rates in patients with COVID-19, and has reported that aspartate transaminase (AST) levels display the highest correlation with mortality compared to other indicators of liver injury [7].

Chronic liver disease (CLD), such as chronic viral hepatitis, metabolic dysfunction-associated fatty liver disease (MAFLD) that was previously termed non-alcoholic fatty liver disease, and alcohol-related liver disease, affects approximately 1.5 billion people throughout the world and causes 2 million deaths each year [8]. Previous studies have shown that 2–11% of patients with COVID-19 have a pre-existing CLD [2, 4, 9]. Recent studies have found that obese patients with MAFLD have higher risks of severe COVID-19 symptoms [10]. However, research on early risk stratification and management is limited. Thus, the purpose of this study was to explore the implication of liver injury and CLD in patients with COVID-19.

Methods

Study design and data collection

We collected electronic health records, including medical history and all laboratory results, from February 4 to April 10 for 1520 patients diagnosed with severe or critical COVID-19 and admitted to Huoshenshan hospital from February 4 to March 30, 2020, of which 1466 performed liver function tests. Written informed consent was waived due to the urgency of the COVID-19 pandemic. The diagnosis and severity of COVID-19 were based on practice guidelines issued by The Chinese National Health Commission (http://en.nhc.gov.cn/2020-03/29/c_78469.htm).

We used a six-category scale score to describe the clinical status of COVID-19: (1) discharged; (2) hospitalized, not requiring oxygen therapy; (3) hospitalized, requiring low-flow oxygen therapy; (4) hospitalized, requiring high-flow oxygen therapy, noninvasive mechanical ventilation, or both; (5) hospitalized, requiring extracorporeal membrane oxygenation, invasive mechanical ventilation, or both; (6) death. Higher scores indicated higher risks (Table 1).

Liver injury definition and chronic liver disease classification

The upper limit unit of normal of liver function tests was as follows: alanine aminotransferase (ALT), 40 IU/L; AST, 40 IU/L; total bilirubin, 17.1 μmol/L; total bile acid, 10 μmol/L; gamma-glutamyl transferase (GGT), 50 IU/L; and alkaline phosphatase (ALP), 125 IU/L. Patients whose ALT and/or AST, ALP, and/or GGT levels were higher than twice the upper limit unit of normal were considered as having hepatic injury [3], which were further classified by hepatocellular type (elevation of AST/ALT) and cholestatic type (elevation of ALP/GGT) (Table 2).

Based on the above criterion together with a categorization of when the abnormal liver function value occurred for the first-time relative to hospitalization time, the patients with liver injury were grouped as: a pre-admission injured group that had patients who had already presented with liver injury on admission, and post-admission injured group that had patients who developed liver injury during hospitalization.

Pre-existing CLDs, including chronic hepatitis B (CHB), chronic hepatitis C (CHC), and fatty liver disease (FLD), were diagnosed by consensus diagnostic criteria.

Statistical analysis

The statistical analyses in this study were performed using R (version 3.6.0). Fisher's exact test was applied for categorical variables. We utilized the Mann–Whitney *U* test or Kruskal–Wallis *H* test for continuous variables, and the results were presented as the median (25%–75% interquartile range, IQR). A p value < 0.05 was considered statistically significant.

Table 1 Characteristics of 121 COVID-19 patients with different types of chronic liver disease

	Total ($n=121$)	CHB ($n=64$)	CHC ($n=20$)	FLD ($n=37$)	p value
Age, median (IQR)	62.00 (51.00–69.00)	63.00 (54.75–70.00)	66.50 (58.75–70.25)	57.00 (38.00–66.00)	**0.015**
Sex, *n* (%)					0.163
Female	43 (35.54)	22 (34.38)	4 (20.00)	17 (45.95)	
Male	78 (64.46)	42 (65.63)	16 (80.00)	20 (54.05)	
Comorbidities, *n* (%)					
Hypertension	33 (27.27)	15 (23.43)	7 (35.00)	11 (29.73)	0.506
Diabetes	16 (13.22)	11 (17.19)	3 (15.00)	2 (5.41)	0.264
Cardiovascular disease	10 (8.26)	5 (7.81)	2 (10.00)	3 (8.11)	0.908
Chronic nephrosis	2 (1.65)	2 (3.13)	0 (0.00)	0 (0.00)	0.674
Treatment, *n* (%)					
Oxygen	63 (52.07)	36 (56.25)	8 (40.00)	19 (51.35)	0.455
Convalescent plasma transfusion	15 (12.40)	12 (18.75)	2 (10.00)	1 (2.70)	0.051
Antivirus	103 (85.12)	52 (81.25)	19 (95.00)	32 (86.49)	0.354
Steroid	18 (14.88)	11 (17.19)	1 (5.00)	6 (16.22)	0.502
Liver function tests					
ALT (IU/L)	28.75 (18.15–46.18)	27.10 (15.69–43.00)	25.29 (14.88–46.19)	36.70 (24.69–61.47)	**0.038**
ALT > 40, *n* (%)	51 (42.15)	25 (39.06)	7 (35.00)	19 (51.35)	0.391
AST (IU/L)	23.70 (18.23–36.8)	23.70 (17.30–37.94)	21.38 (18.30–34.27)	25.35 (20.50–35.55)	0.526
AST > 40, *n* (%)	34 (28.10)	19 (29.69)	4 (20.00)	11 (29.73)	0.734
TBIL (μmol/L)	10.05 (8.40–13.00)	10.6 (8.87–13.95)	10.07 (8.16–13.57)	9.25 (8.14–10.81)	**0.034**
TBIL > 17.1, *n* (%)	23 (19.01)	16 (25.00)	4 (20.00)	3 (8.11)	0.100
TBA (μmol/L)	5.10 (3.50–9.46)	6.00 (4.07–11.61)	4.15 (3.03–6.53)	4.67 (3.24–7.06)	0.090
TBA > 10, *n* (%)	41 (33.88)	28 (43.75)	4 (20.00)	9 (24.32)	0.052
ALP (IU/L)	73.70 (59.70–91.93)	71.05 (57.38–82.58)	83.90 (65.75–105.35)	73.09 (61.59–100.39)	0.146
ALP > 125, n (%)	15 (12.40)	7 (34.38)	2 (20.00)	6 (16.22)	0.749
GGT (IU/L)	35.90 (24.45–59.28)	30.48 (22.70–52.30)	33.90 (22.90–38.34)	54.15 (34.75–79.26)	**< 0.001**
GGT > 50, *n* (%)	43 (35.54)	22 (34.38)	2 (10.00)	19 (51.35)	**0.006**
Laboratory parameters, median (IQR)					
C-reactive protein (mg/L)	35.9 (24.45–59.28)	30.48 (22.70–52.30)	33.90 (22.90–38.34)	54.15 (34.75–79.26)	**< 0.001**
IL-6 (pg/mL)	2.98 (1.50–14.250)	3.46 (1.50–16.38)	2.35 (1.64–3.85)	2.75 (1.50–6.85)	0.550
D-dimer (mg/L)	0.61 (0.25–1.59)	0.64 (0.296–1.857)	0.88 (0.33–3.57)	0.38 (0.13–0.78)	**0.042**
PT (s)	93.90 (90.00–97.45)	93.80 (89.61–96.50)	91.60 (87.60–96.30)	96.90 (93.58–100.99)	**0.003**
INR	0.61 (0.25–1.59)	0.64 (0.30–1.86)	0.88 (0.33–3.57)	0.38 (0.13–0.78)	**0.042**
LYM# ($\times 10^{9/L}$)	1.6 (1.17–1.95)	1.59 (1.16–1.89)	1.28 (1.07–1.76)	1.86 (1.62–2.11)	**0.003**
Clinical outcome, *n* (%)					0.535
Discharged	111 (91.74)	57 (89.06)	19 (95.00)	35 (94.59)	
Remained in hospital	5 (4.13)	3 (4.69)	0 (0.00)	2 (5.41)	
Death	5 (4.13)	4 (6.25)	1 (5.00)	0 (0.00)	

Liver scRNA-seq data processing and estimation of the abundance of liver cell type

The healthy liver and cirrhotic liver 10× scRNA-seq processed matrices were downloaded from GSE136103 [11], and the HBV-infected liver 10× scRNA-seq processed matrix was obtained from Ido Amit Lab [12]. The unsupervised clustering and visualization were performed in the *Seurat* R package v3.1.1 [13], and the liver progenitor clusters were determined by *TROP2*, *ALB*, *AFP*, *KRT8*, *KRT19*, *THY1*, and *KIT* [14]. The cells expressing *ACE2* and *TMPRSS2* were counted, and Fisher's exact test was then conducted.

The bulk gene expression profiles from mixed cell types for HBV-infected, HCV-infected, MAFLD, cirrhotic, and healthy livers were downloaded from GSE83148, GSE149601, GSE130970, GSE112221, and GSE83148, respectively. CIBERSORTx, a web-based tool used for estimation of cell type abundances from bulk transcriptomes [15], was applied to estimate the abundance of

Table 2 Clinical features of patients who had chronic liver disease with and without cirrhosis

	Total ($n=127$)	Chronic liver disease with cirrhosis ($n=13$)	Chronic liver disease without cirrhosis ($n=114$)	p value
Sex, *n* (%)				1.000
Male	77 (60.63)	8 (61.54)	69 (60.53)	
Female	50 (39.37)	5 (38.46)	45 (39.47)	
Age, median (IQR)	63.00 (51.50–69.00)	68.00 (60.00–74.00)	62 (51.25–68.75)	**0.038**
Hospital stays, median (IQR)	14.00 (8.00–23.00)	15.00 (8.00–25.00)	14.00 (8.00–22.75)	0.774
ICU admission, *n* (%)	9 (7.08)	2 (15.38)	7 (6.14)	0.231
Highest six-category scale score			0.045	
2	58 (45.67)	2 (15.38)	56 (49.12)	
3	45 (35.43)	6 (46.15)	39 (34.21)	
4	18 (14.17)	5 (38.46)	13 (11.40)	
5	1 (0.79)	0 (0.00)	1 (0.87)	
6	5 (3.94)	0 (0.00)	5 (4.39)	
Laboratory parameters, median (IQR)				
ALT (IU/L)	28.75 (18.15–46.17)	25.49 (14.90–34.40)	29.62 (18.30–47.73)	0.318
AST (IU/L)	23.70 (18.23–36.8)	29.50 (17.10–45.40)	22.90 (18.38–35.55)	0.342
ALB (g/L)	37.70 (34.3–40.9)	33.15 (32.00–34.50)	38.1 (35.22–40.96)	**0.002**
GLB (g/L)	28.00 (25.00–30.50)	32.48 (29.04–34.41)	27.7 (24.70–29.85)	**0.006**
TBIL (μmol/L)	10.05 (8.40–13.00)	21.03 (10.50–27.23)	9.90 (8.30–12.62)	**0.006**
DBIL (μmol/L)	3.55 (2.96–4.80)	10.90 (3.60–15.38)	3.50 (2.84–4.40)	**0.002**
TBA (μmol/L)	5.10 (3.50–9.46)	15.62 (5.70–30.8)	4.94 (3.33–8.71)	**0.007**
ALP (IU/L)	73.70 (59.70–91.92)	91.92 (77.60–116.33)	71.10 (58.60–89.84)	**0.005**
GGT (IU/L)	35.90 (24.45–59.28)	39.35 (25.33–85.78)	35.38 (24.45–57.43)	0.277
LDH (IU/L)	186.65 (164.75–244.06)	255.2 (193.42–267.97)	184.10 (162.55–229.98)	**0.011**
MONO%	7.64 (6.31–9.00)	9.27 (7.97–10.90)	7.44 (6.17–8.70)	**0.007**
NEUT ($\times 10^{9/L}$)	3.62 (2.92–5.28)	2.98 (2.31–3.28)	3.80 (2.93–5.35)	**0.033**
LYM ($\times 10^{9/L}$)	1.60 (1.18–1.95)	1.06 (0.87–1.17)	1.69 (1.26–1.98)	**< 0.001**
CRP (mg/L)	2.74 (1.06–12.5)	12.12 (5.54–19.17)	2.57 (0.93–9.52)	**0.010**
PT (s)	13.19 (12.49–14.04)	15.68 (14.36–17.08)	13.10 (12.44–13.88)	**< 0.001**
D-Dimer (mg/L)	0.61 (0.24–1.59)	3.57 (1.97–5.04)	0.52 (0.22–1.30)	**< 0.001**
IL-6 (pg/mL)	2.98 (1.50–14.25)	22.78 (9.86–27.84)	2.70 (1.50–6.62)	**0.006**

TROP2$^+$*TMPRSS2*$^+$ cells in different livers, using custom signature matrix extracted from scRNA-seq profile and mixture files from bulk RNA-seq as input, and the fractions of *TROP2*$^+$*TMPRSS2*$^+$ cells in each sample were downloaded for visualization and comparison.

Construction of risk score

The significance of each variable was assessed between the non-injury and post-admission injured groups by univariate logistic regression. Indicators with an odds ratio (OR) > 1 and p value < 0.001 were used for the final model to investigate whether the patient would develop liver injury. We also selected significant indicators between patients who stayed at 2–4 scales and who developed into 5–6 scales to predict the highest six-category scale score. The performance of the scoring model was assessed using receiver operating characteristic (ROC) curves created from fivefold cross-validation. The average area under ROC (AUROC) was calculated by the cvAUC R package (version 1.1.0).

Results

Liver injury is associated with a poor prognosis in patients with COVID-19

We identified 263 (17.9%) patients with liver injury (Table S1) to explore the impact of liver injury on severe or critical cases of COVID-19. Amongst these patients, 102 (38.78%) had presented with liver injury on admission (pre-admission injured group) and 161 (61.22%) patients developed liver injury during their hospitalization (post-admission injured group). As shown in Fig. 1a, hypertension was

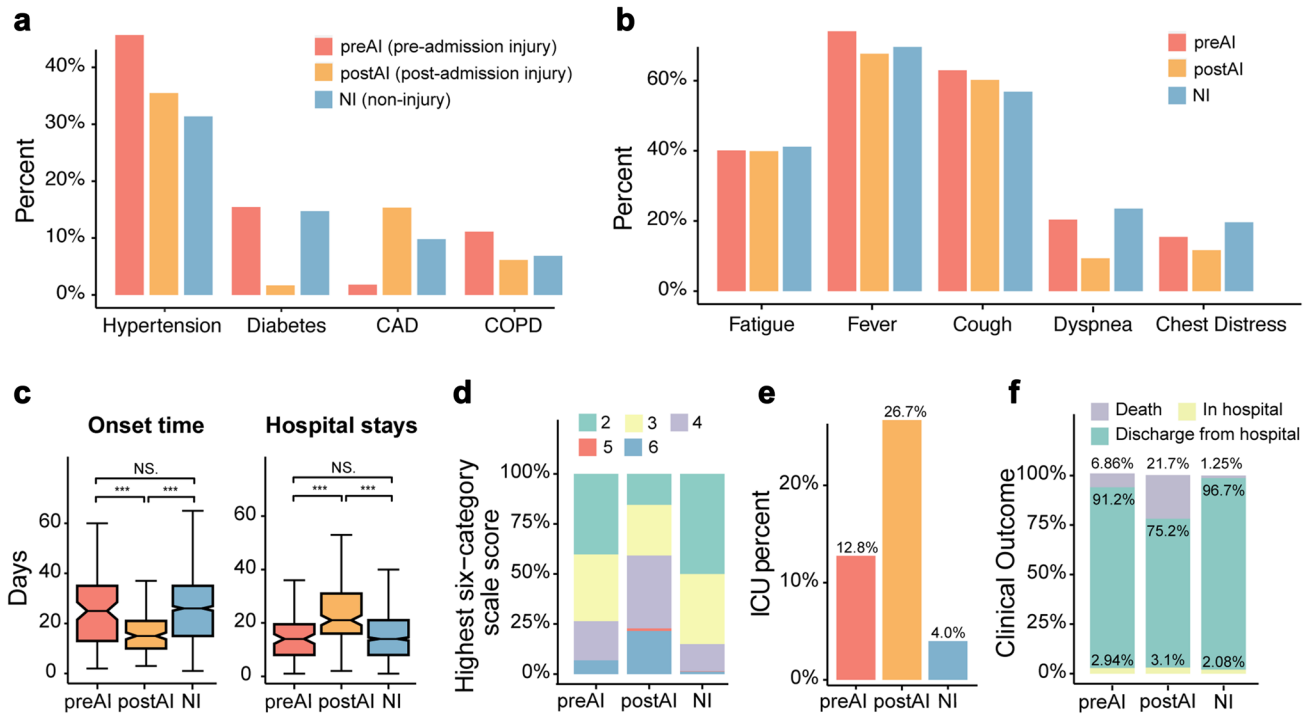


Fig. 1 Characteristics of patients with or without liver injury during hospitalization. **a** Common comorbidities. **b** Common COVID-19 symptoms. **c** Days from COVID-19 symptom onset to hospitalization and hospital stays. **d** Highest six-category scale scores during hospitalization. **e** ICU admission during hospitalization. **f** Final clinical outcome for patients in the three groups. The *y*-axis is percentage of patients with the corresponding characteristics out of the total patients in that groups. *preAI* pre-admission injury, liver injury identified upon admission, *postAI* post-admission injury, liver injury identified after admission, *NI* non-injury, liver injury not found during the disease course

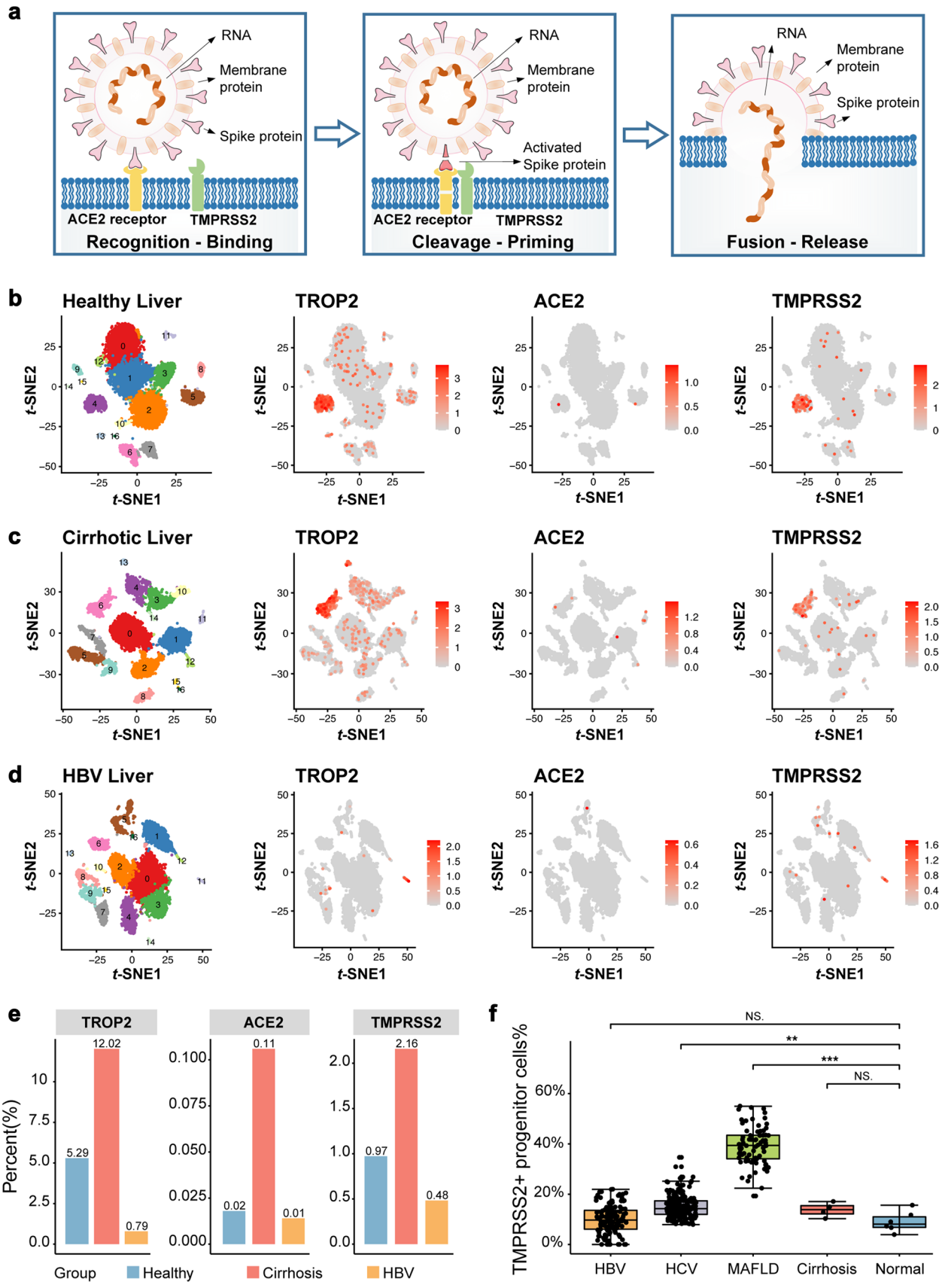
more commonly seen in the post-admission injured group (45.68%, $p=0.025$). The median time from symptom onset to admission was significantly shorter in the post-admission injured group than that in the pre-admission injured and non-injured groups (Fig. 1c, Fig. S1D, median: 15 vs. 25 or 26 days, $p<0.001$), suggesting that disease progression was faster in the post-admission injured patients. The length of hospital stay was significantly longer in the post-admission injured group (Fig. 1c, Fig. S1E, median 21 days) than that in the other two groups (median 14 days for each). Furthermore, the six-category scale scores for the post-admission injured group were significantly enriched in the 3–6 range (Fig. 1d), indicating a higher risk. Conversely, over 50% of patients without liver injury remained at levels 2 and 3. The post-admission injured group also had significantly higher mortality rates than the pre-admission injured and non-injured groups during hospitalization (Fig. 1f, 21.74% vs. 6.86% or 1.25%, $p<0.001$), as well as increased intensive care unit (ICU) admission rates (Fig. 1e, 26.71% vs. 12.75% or 3.99%, $p<0.001$).

There was no significant difference in age, sex and treatment strategy among the three groups (Fig. S1A, Fig. S1B, Fig. S1C). Among the 161 COVID-19 patients with liver injury during hospitalization, 59 were hepatocellular type (elevation of AST/ALT), 61 cholestatic type (elevation of ALP/GGT), and 41 mixed type (Fig. S2A), but there was no significant difference in prognosis (Fig. S2B, Fig. S2C) and treatments (Fig. S2D).

CLD is not significantly associated with a poor prognosis in patients with COVID-19

We compared the differences between severe or critical COVID-19 patients with and without CLD to evaluate the influence of SARS-CoV-2 on patients with pre-existing CLD. As shown in Table S2, 127 (8.35%) of the 1520 patients with severe or critical cases of COVID-19 had CLD, including 64 patients with CHB, 20 with CHC, 37 with FLD, and 6 with liver cirrhosis but without documented etiological factors. Among all the comorbidities tested in this study, hypertension was the only one that showed a significant difference between the groups (27.56% with CLD vs. 37.19% without CLD, $p=0.034$).

Laboratory test results were also compared between the two groups. The median platelet count was significantly lower in the patients with CLD than that in those without CLD (206.00 $\times 10^{9/L}$ vs. 220.00 $\times 10^{9/L}$, $p=0.008$). Interferon gamma was significantly decreased in patients with pre-existing CLD


a
RNA
Membrane protein
Spike protein
ACE2 receptor
TMPRSS2
Recognition - Binding
Activated Spike protein
Cleavage - Priming
Fusion - Release
b
Healthy Liver
TROP2
ACE2
TMPRSS2
c
Cirrhotic Liver
d
HBV Liver
t-SNE1
t-SNE2
e
TROP2
ACE2
TMPRSS2
Percent(%)
12.02
5.29
0.79
0.11
0.02
0.01
2.16
0.97
0.48
Group
Healthy
Cirrhosis
HBV
f
TMPRSS2+ progenitor cells%
NS.
**
***
NS.
HBV
HCV
MAFLD
Cirrhosis
Normal

◂**Fig. 2** *TMPRSS2*$^+$ cells fraction in different liver tissues. **a** Schematic of SARS-CoV-2 virus infection process. SARS-CoV-2 utilizes ACE2 for entry into cells and TMPRSS2 for spike (S) protein priming. Following membrane fusion, the virus RNA will be released into the host cell. tSNE representation of scRNA-seq expression matrix and expression maps for genes *TROP2*, *ACE2* and *TMPRSS2* in **b** healthy liver ($n=11{,}106$ cells), **c** cirrhotic liver ($n=6620$ cells), **d** HBV liver ($n=7244$ cells). Color bars indicate log2 normalized expression. **e** Fractions of cells expressing *TROP2*, *ACE2* and *TMPRSS2* in the three liver scRNA-seq datasets. **f** Fractions of *TROP2*$^+$*TMPRSS2*$^+$ cells in the bulk datasets from HBV, HCV, MAFLD, cirrhotic and healthy livers

than that in those without pre-existing CLD (median: 2.68 vs.5.32 pg/mL, $p=0.032$). A similar trend was also observed for interleukin-2 and CD3$^+$/CD4$^+$ T-helper cell fractions, but these changes were not statistically significant.

No significant evidence of CLD being a risk factor for the severity or mortality of COVID-19 was found. This result may be due to the consistent and targeted delivery of liver protection treatments in patients with CLD. In addition, this result implies that liver injury occurring during the course of COVID-19 is associated with a poorer prognosis but pre-existing CLD is not.

Patients with FLD are at a higher risk of liver injury compared to patients with viral hepatitis

We conducted a comprehensive analysis of 121 patients with both COVID-19 and chronic liver comorbidities. Of these 121 patients, 64 (52.89%) had CHB, 20 (16.53%) had CHC, and 37 (30.58%) had FLD (Table S3). The clinical outcomes were not significantly different among the different types of CLD ($p=0.535$). However, all 5 recorded deaths occurred in patients with viral hepatitis. Patients with FLD had higher levels of ALT (median: 36.70 IU/L, $p=0.038$) and GGT (median: 54.15 IU/L, $p<0.001$) than those with CHB or CHC. Furthermore, over 50% of patients with FLD had abnormal levels of ALT ($p=0.391$) and GGT ($p=0.006$).

C-reactive protein (CRP) (median: 54.15 mg/L, $p<0.001$) and the absolute lymphocyte count (lymphocyte#; median: 1.86, $p=0.003$) were higher in the FLD patients. Prothrombin time was also significantly prolonged (median: 96.90 s, $p=0.003$) and the international normalized ratio was significantly lower in patients with FLD (median: 0.38, $p=0.042$), suggesting that coagulation disorders and dysfunction of the liver occurred concurrently in patients with pre-existing FLD. These results imply that patients with FLD suffer more severe liver damage.

Patients with both COVID-19 and cirrhosis are at a higher risk of disease progression

Cirrhosis is a complication of many liver diseases. Therefore, we analyzed the clinical characteristics and laboratory features of patients with CLD and with and without cirrhosis. As shown in Table S4, 13 (10.24%) patients had CLD with cirrhosis, of which 4 had CHB, 2 had CHC, 1 had MAFLD, and 6 had cryptogenic cirrhosis. No significant differences in terms of hospital stays ($p=0.774$) or ICU admission rates ($p=0.231$) were observed. However, the highest six-category scale scores for patients with both CLD and cirrhosis were significantly enriched at 3 and 4, while for those without cirrhosis were mainly at 2 and 3 ($p=0.045$). This result indicated that patients with both CLD and cirrhosis were at a higher risk of disease progression.

We confirmed by examining laboratory results that most liver enzymes were significantly higher in patients with cirrhosis, except ALT, AST, and GGT. Moreover, the levels of D-dimer and two well-known pro-inflammatory biomarkers (interleukin-6 and CRP) were found to be higher in patients with cirrhosis. All evidence mentioned above showed that patients with both COVID-19 and cirrhosis were at an elevated risk of disease progression compared with the patients who had CLD without cirrhosis.

Cirrhotic and fatty livers generate more TMPRSS2$^+$ *cells*

We studied the liver scRNA-seq data from recent publications to investigate why patients with cirrhosis are more affected by SARS-CoV-2 [11, 12]. ACE2 and TMPRSS2 were shown to be necessary for the virus entry and infection (Fig. 2a). Consistent with many recent reports, the level of the SARS-CoV-2 entry-receptor *ACE2* was low in liver tissue. However, a small population of *TROP2*$^+$ liver epithelial progenitors expressed *ACE2* and *TMPRSS2* (Fig. 2b–e). Of the 11,106 cells detected in healthy livers, only 2 cells expressed *ACE2* and 108 expressed *TMPRSS2* (Fig. 2b). Of the 6620 cells analyzed from cirrhotic livers, 7 cells expressed *ACE2* and 143 expressed *TMPRSS2* (Fig. 2c). This result represents a significant increase in the number of *TMPRSS2*$^+$ cells in the cirrhotic livers ($p<0.001$, Fisher's exact test). Of 7244 cells analyzed from untreated HBV-infected livers, only 1 cell expressed *ACE2* and 35 expressed *TMPRSS2* (Fig. 2d). The *TMPRSS2*$^+$ cells were significantly fewer in HBV-infected liver than those in both healthy and cirrhotic livers ($p<0.001$, Fisher's exact test) (Fig. 2e).

We estimated the abundance of SARS-CoV-2 vulnerable cells for more liver bulk expression profiles with CIBERSORTx using the signatures built from the same healthy liver scRNA-seq dataset. To obtain a better reference signature, we limited *TROP2*$^+$*TMPRSS2*$^+$ cells to a subset of the cell population in "cluster 4" marked by progenitor markers ALB, KRT8 and KRT19 (Fig. S3). As *ACE2* expression was too low to be confidently identified with general scRNA-seq depth, we did not restrict the signature to *ACE2*$^+$ cells. As shown in Fig. S4, CIBERSORTx was run for each bulk

transcriptome from different patients to impute *TMPRSS2*$^+$ cell fractions, with all the signature and mixture matrices listed in Table S7. Compared to HBV- and HCV-infected livers, MAFLD livers had much higher *TMPRSS2*$^+$ progenitor cells (Fig. 2f) indicating that MAFLD livers might be more susceptible to the SARS-CoV-2 virus. Similarly, the cirrhotic livers also had higher *TMPRSS2*$^+$ progenitor cells than healthy livers, which was comparable to the scRNA-seq results.

Hypertension may increase the risk of liver injury for patients without pre-existing CLD

A logistical regression model was used to identify the clinical characteristics, comorbidities, and symptoms that could increase the risk of liver injury among patients without pre-existing CLD. As shown in Fig. S5, male sex was highly associated with the risk of liver injury suggesting that male patients are more likely to develop liver injury (see Table S5 for details). Furthermore, the association of hypertension and liver injury was significant for patients without pre-existing CLD but not for patients with pre-existing CLD (Table S6).

Risk scoring model for assessing liver injury and clinical outcomes for COVID-19 patients

We built a risk scoring system based on 22 routine laboratory tests performed within 3 days after admission, such as liver function and routine blood tests. This system was used to evaluate the risk of liver injury in patients with COVID-19 as early as possible and provide guidance for the management of these patients. First, the univariate logistic regression model was applied to identify potential laboratory parameters with liver injury, and only those with an OR > 1 and p value < 0.001 were retained. Next, the multivariate logistic regression models were used to determine the effect of those factors identified from the univariate logistic regression analysis. Finally, we identified 3 indicators at admission, including ALT (OR 1.07, 95% CI 1.02–1.12), CRP (OR 1.02, 95% CI 1.01–1.04), and LDH (OR 1.29, 95% CI 1.20–1.39). Figure 3a showed the distribution of tested values for selected indicators. To determine the robustness of this model, a fivefold cross-validation method was employed. In this procedure, the original training data set is randomly partitioned into five subsets with the same sample size, and each subset is called one fold. 4 subsets are cross-validation training set, and 1 subset is cross-validation testing set. We trained our model on the cross-validation training set and test the model's predictions against the validation set. The average AUC of fivefold cross-validation was 85% (Fig. 3c). Similarly, we selected 6 indicators (Fig. 3b) to predict whether patients would proceed to six-category scale scores of 5 or 6. The average AUC reached 92% (Fig. 3d). Since patients with pre-existing diseases including FLD, cirrhosis and hypertension were shown to be associated with higher risks, we also added these variables in our model, but the average AUC was not increased, being 85% and 91%, respectively (Fig. S6). Therefore, we did not include the medical history in our final models. An R package provides all operations required for the clinical outcome prediction of new patients (https://github.com/liangyuan-njmu/PredictModel).

Discussion

Liver dysfunction has frequently been observed in patients with COVID-19 [16] who require intensive care [17]. We found that patients who developed liver injuries during hospitalization had higher mortality and ICU admission rates than those without liver injury and with liver injury upon admission. In addition, the patients with post-admission liver damage had significantly prolonged hospital stays.

No significant differences in mortality or ICU admission rates between patients with and without CLD were observed, suggesting that liver injury but not CLD is associated with disease severity and clinical outcomes in patients with COVID-19. 30.58% of patients with FLD developed liver injury, which was higher than the overall percentage of liver injury in this COVID-19 cohort (17.9%); this result suggests that patients with FLD may be at a higher risk of liver injury. MAFLD is strongly associated with obesity, and one recent study showed that COVID-19 more severely affected younger adults with obesity [18]. Given the known association between obesity and MAFLD [19], our observation of higher risks for patients with FLD and the higher abundance of *TMPRSS2*$^+$ progenitor cells in MAFLD livers may provide a possible explanation for why obese patients suffer more from COVID-19.

Both proteomics and transcriptomics data confirmed that small population cells in liver tissues were ACE2 positive [20], causing livers susceptible to SARS-CoV-2. Fan et al. and Lin et al. revealed that SARS-CoV-2 could directly bind to *ACE2*$^+$ cholangiocytes and damage bile duct tissue, suggesting a possible mechanism for SARS-CoV-2-induced liver injury [21, 22]. A more recent study found that *ACE2* and *TMPRSS2* are expressed in *TROP2*$^+$ liver progenitor cells, a cholangiocyte-biased progenitor subpopulation, highlighting another potential cause of liver damage [23]. ACE2 has also been shown to be up-regulated in cirrhotic livers [24], indicating that patients with pre-existing cirrhosis may suffer from severe liver injury and faster disease progression. In this study, the six-category scale scores for patients with cirrhosis were higher. By analyzing public scRNA-seq data, we

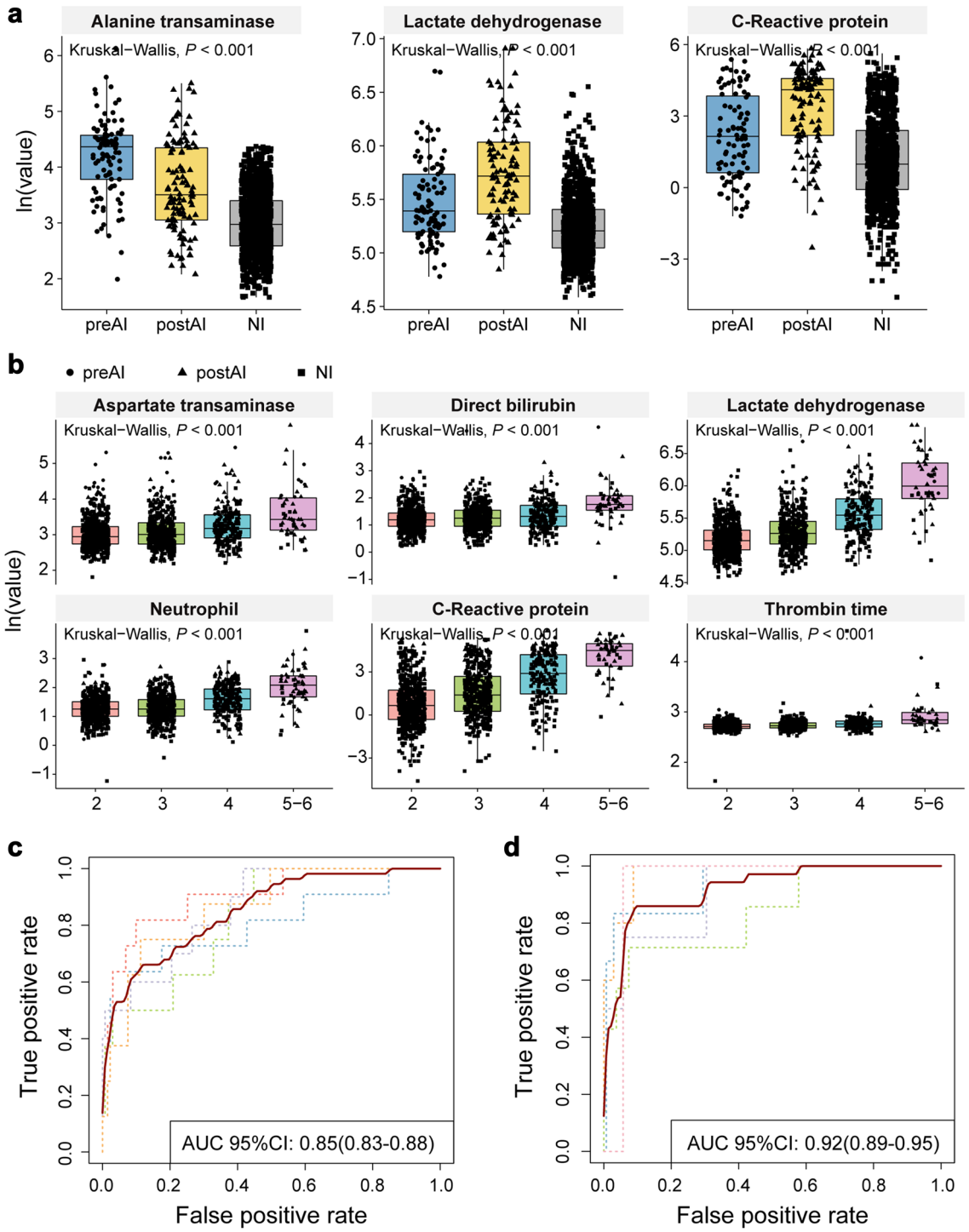


Fig. 3 Risk scoring model. **a** Boxplot for blood test results used in liver injury prediction model. The *p* values were from Wilcoxon test. **b** Boxplot for blood test results used in prognosis prediction model. The *p* values were from Kruskal–Wallis test. **c** The ROC curves of fivefold cross-validation for predicting liver injury during hospitalization. **d** The ROC curves of fivefold cross-validation for predicting prognosis

revealed that the cirrhotic livers generated more $ACE2^+$ and $TMPRSS2^+$ cells than healthy livers, and HBV-infected livers had the fewest $ACE2^+$ and $TMPRSS2^+$ cells among the 3 liver types. CIBERSORTx estimation from bulk RNA-seq also confirmed that there were slightly more $TMPRSS2^+$ progenitor cells in cirrhotic livers than

HBV-infected and healthy livers. This may explain why patients with COVID-19 and cirrhosis had worse clinical outcomes than those with viral hepatitis.

Patient risk must be classified upon admission. Inspired by the MELD score, which is an existing scoring system used to prioritize liver transplantation and predict overall and postoperative outcomes in patients with hepatic and renal dysfunction [25–27], we constructed a similar scoring system to evaluate the liver impairment of patients with severe or critical COVID-19. The levels of ALT, lactate dehydrogenase, and CRP upon admission were used to build a linear regression equation to predict liver injury in subsequent hospital stays that could be used by clinicians to determine whether early liver protection management is required. Our other model can be used to predict those patients with COVID-19 who may have the highest severity of symptoms.

In conclusion, we comprehensively evaluated the clinical characteristics and laboratory parameters of patients with severe or critical COVID-19 symptoms. Patients who developed liver injuries during hospitalization had worse clinical outcomes and longer hospital stays. Our study suggests that performing liver protection treatments within one week of admission is beneficial for these patients. In particular, careful attention should be paid to patients with pre-existing CLD, cirrhosis, or FLD because of their worse liver function. Similarly, the liver function of patients with hypertension but without pre-existing CLD should be monitored. Further, we built a risk scoring system to predict liver injury upon admission. To conclude, we assessed the implication of liver injury and CLD for risk-stratification and management of patients with COVID-19, and we believe that our findings will help to improve clinical outcomes for these patients.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflicts of interest.

Ethical approval It was a retrospective cohort study, approved by institutional ethics boards.

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