Possible unrecognised liver injury is associated with mortality in critically ill COVID-19 patients

Mario Romero-Cristóbal*(iD), **Ana Clemente-Sánchez***, **Patricia Piñeiro, Jamil Cedeño, Laura Rayón, Julia del Río, Clara Ramos, Diego-Andrés Hernández, Miguel Cova, Aranzazu Caballero, Ignacio Garutti, Pablo García-Olivares, Javier Hortal, Jose-Eugenio Guerrero, Rita García, Rafael Bañares†** and **Diego Rincón†**

Abstract

Background: Coronavirus disease (COVID-19) with acute respiratory distress syndrome is a life-threatening condition. A previous diagnosis of chronic liver disease is associated with poorer outcomes. Nevertheless, the impact of silent liver injury has not been investigated. We aimed to explore the association of pre-admission liver fibrosis indices with the prognosis of critically ill COVID-19 patients.

Methods: The work presented was an observational study in 214 patients with COVID-19 consecutively admitted to the intensive care unit (ICU). Pre-admission liver fibrosis indices were calculated. In-hospital mortality and predictive factors were explored with Kaplan–Meier and Cox regression analysis.

Results: The mean age was 59.58 (13.79) years; 16 patients (7.48%) had previously recognised chronic liver disease. Up to 78.84% of patients according to Forns, and 45.76% according to FIB-4, had more than minimal fibrosis. Fibrosis indices were higher in non-survivors [Forns: 6.04 (1.42) *versus* 4.99 (1.58), $p < 0.001$; FIB-4: 1.77 (1.17) *versus* 1.41 (0.91), $p = 0.020$)], but no differences were found in liver biochemistry parameters. Patients with any degree of fibrosis either by Forns or FIB-4 had a higher mortality, which increased according to the severity of fibrosis ($p < 0.05$ for both indexes). Both Forns [HR 1.41 (1.11–1.81); $p = 0.006$] and FIB-4 [HR 1.31 (0.99–1.72); $p = 0.051$] were independently related to survival after adjusting for the Charlson comorbidity index, APACHE II, and ferritin.

Conclusion: Unrecognised liver fibrosis, assessed by serological tests prior to admission, is independently associated with a higher risk of death in patients with severe COVID-19 admitted to the ICU.

Keywords: biomarkers, coronavirus, critical care, liver diseases, survival analysis

Correspondence to:
Rafael Bañares Cañizares
Hospital General
Universitario Gregorio
Marañón, Liver Unit,
Doctor Esquerdo 46,
Madrid 28007, Spain

School of Medicine,
Complutense University,
Madrid, Spain

CIBEREHD, Instituto de
Salud Carlos III, Madrid,
Spain
rbanares@ucm.es

Mario Romero-Cristóbal
Liver Unit and Digestive
Department, H.G.U.
Gregorio Marañón, Madrid,
Spain

Ana Clemente-Sánchez
Liver Unit and Digestive
Department, H.G.U.
Gregorio Marañón, Madrid,
Spain

CIBEREHD, Instituto de
Salud Carlos III, Madrid,
Spain

Patricia Piñeiro
Ignacio Garutti
Department of
Anesthesiology, H.G.U.
Gregorio Marañón, Madrid,
Spain

Jamil Cedeño
Pablo García-Olivares
Jose-Eugenio Guerrero
Intensive Care Unit, H.G.U.
Gregorio Marañón, Madrid,
Spain

Laura Rayón
Julia del Río
Clara Ramos
Diego-Andrés Hernández
Miguel Cova
Aranzazu Caballero
Liver Unit and Digestive
Department, H.G.U.
Gregorio Marañón, Madrid,
Spain

Javier Hortal
Department of
Anesthesiology, H.G.U.
Gregorio Marañón, Madrid,
Spain

CIBERES, Instituto de
Salud Carlos III, Madrid,
Spain

School of Medicine,
Complutense University,
Madrid, Spain

Introduction

The pandemic outbreak of the novel coronavirus (SARS-CoV-2) has become a serious public health emergency worldwide, and the identification of factors associated with unfavourable outcomes is particularly important. The severity of the disease increases with older age, male gender and the presence of comorbidities, especially cardiovascular disease, hypertension, type-2 diabetes, chronic renal failure and obesity.[1] Thus, in patients hospitalised with coronavirus disease 2019 (COVID-19) and no relevant comorbidities, mortality is less than 4%, while in patients suffering from one, two or three of these comorbidities, mortality increases to 14%, 21% and 60%, respectively.[2]

Rita García
CIBEREHD, Instituto de Salud Carlos III, Madrid, Spain

Department of Internal Medicine, H.G.U. Gregorio Marañón, Madrid, Spain.

Diego Rincón
Liver Unit and Digestive Department, H.G.U. Gregorio Marañón, Madrid, Spain

School of Medicine, Complutense University, Madrid, Spain

CIBEREHD, Instituto de Salud Carlos III, Madrid, Spain

*These authors share first authorship.

†These authors share senior authorship.

Cirrhotic patients with acute respiratory distress syndrome (ARDS) of any aetiology have a worse prognosis than patients without cirrhosis.[3] Preliminary data also suggest that the prognosis of COVID-19 is worse in patients with chronic liver disease, mainly in the cirrhotic stage.[4–6] Importantly, the comorbidity pattern of patients with severe COVID-19 is similar to that observed in metabolic dysfunction-associated fatty liver disease (MAFLD). In fact, a recent study has reported a higher prevalence of MAFLD in severe COVID-19 patients than in those with milder forms of the disease.[7] Moreover, the risk of developing severe COVID-19 in patients with MAFLD seems to be independently associated with the FIB-4 score: MAFLD patients with intermediate or high FIB-4 scores at admission, as an estimate of the existence of relevant liver injury, have a poorer prognosis than patients with MAFLD with a low FIB-4 score or those without MAFLD.[8] Furthermore, a recent study described the independent association between the FIB-4 score, also obtained at the time of admission, and the need for mechanical ventilation.[9] However, there is no information about the potential impact of the presence of unrecognised liver disease on mortality in severe COVID-19 patients requiring critical care.

Therefore, the aim of the present study was to explore the possible contribution of underlying chronic liver disease, estimated by liver fibrosis indices obtained before COVID-19 admission, to the prognosis of patients requiring intensive care management.

Patients and methods

Study design and source of data
This is an observational study including all patients admitted consecutively to the intensive care unit (ICU) of the Hospital General Universitario Gregorio Marañón (Madrid, Spain) with a diagnosis of COVID-19, up to 30 April 2020. All patients had a positive result for SARS-CoV-2 on a reverse-transcriptase–polymerase chain reaction (RT-PCR) assay of a nasopharyngeal swab.

Demographic, clinical and laboratory data were obtained from electronic medical records and included in a database within a secure framework. Antiviral therapies during COVID-19 were also recorded. The researchers analysed only de-identified data. To evaluate previous chronic comorbidities and to estimate the severity of the patient's condition at the time of ICU admission, the Charlson Comorbidity Index (CCI) and the Acute Physiology and Chronic Health Evaluation II (APACHE II) score were calculated as previously described.[10,11] The primary study endpoint was time to in-hospital mortality.

Calculation of fibrosis scores
The biochemical parameters were determined by spectrophotometry (ADVIA Chemistry XPT, Siemens, Erlangen, Germany) and the platelet count by flow cytometry (Unicel DxH 800, Beckman Coulter, High Wycombe, UK).

The FIB-4 index was calculated as: [age (years) $\times$ aspartate aminotransferase (AST) (IU/l)]/[platelet count (10^9/l) $\times \sqrt{}$ alanine aminotransferase (ALT) (IU/l)]. The FIB-4 index has an area under the receiver operator characteristics (ROC) curve of 0.85 for the prediction of significant fibrosis in chronic hepatitis C,[12,13] and has also been validated for the estimation of fibrosis in MAFLD.[14]

The Forns index was calculated as: $7.811 - 3.131 \times \ln$ [platelet count (10^9/l)] $+ 0.781 \times \ln$ [gamma glutamyl-transferase (IU/l)] $+ 3.467 \times \ln$ [age (years)] $- 0.014 \times$ [cholesterol (mg/dl)]. The Forns index has an area under the ROC curve of 0.81 for the prediction of advanced fibrosis in chronic hepatitis C.[15] Similar to the FIB-4 index, the Forns index has been accepted for the stratification of liver injury in MAFLD patients.[16]

The nonalcoholic fatty liver disease (NAFLD) fibrosis score (NFS) was calculated as follows: $[0.037 \times$ age (years)] $+ [0.094 \times$ body mass index (BMI) (kg/m^2)] $+ \{1.13 \times$ [impaired fasting glucose or diabetes (yes $= 1$, no $= 0$)]$\} + \{0.99 \times$ [AST (IU/l)/ALT (IU/l) ratio]$\} - [0.013 \times$ platelet count (10^9/l)] $- [0.66 \times$ albumin (g/dl)] $- 1.675$. This test was specifically developed for MAFLD patients, with an area under the ROC curve of 0.82 for the prediction of significant fibrosis.[17]

The indices were calculated using laboratory data obtained before hospitalisation for COVID-19 that were retrieved from electronic records. When more than one dataset was available, we selected the one closest to the admission. Only determinations

obtained up to 1 year before COVID-19 admission were considered valid for the purpose of this study.

Statistics

Continuous variables are expressed as means [standard deviation (SD)] or medians [interquartile range (IQR)] as appropriate. The assumption of normality was tested by constructing normal probability plots. Categorical variables are expressed as proportions (percent). To compare the differences in continuous variables between groups, Student's t test, analysis of variance (ANOVA) or the Mann–Whitney U test were used when appropriate. Chi-squared or Fisher's exact tests were applied to compare categorical variables between groups.

The evolution of the different laboratory parameters during the hospital stay and its relationship with survival were analysed using kernel density estimation. Observed survival rates for the previously described FIB-4 and Forns index categories were calculated according to Kaplan–Meier analysis and compared with the log-rank test.[13,15] Univariate and multivariate Cox regression models were developed to evaluate the independent prognostic contribution of the liver fibrosis scores. We have chosen a time-dependent multivariate model because hospital and ICU stay of patients with severe COVID-19 are often long-lasting.[18] To evaluate the independent influence of each non-invasive index on mortality, we constructed two different multivariate models that included, alternatively, the FIB-4 or Forns index. To simplify the interpretation of results and to avoid overfitting, we included in the models CCI (as a summary variable for age and comorbidity), the APACHE II score (as a standard prognostic index in ICU patients) and ferritin values (as a surrogate of the intensity of the inflammatory response). Variables that showed p values $\leqslant 0.10$ in univariate analyses were included in the multivariate model.

To avoid the issues associated with missing values in the FIB-4 and Forns indices, we performed an additional analysis after multiple imputation procedures. First, 20 new datasets were generated by imputing the missing values for each fibrosis score with linear regression, using as covariates age, sex, height and weight. Next, we calculated the hazards ratio (HR) for each variable of interest by averaging the values obtained for each filled-in dataset.

All reported p values are two-tailed. The level of statistical significance was set at $p < 0.05$. All the calculations were performed with the software Stata version 14 (StataCorp, College Station, TX, USA). Plots were created with R software version 3.6.3.

Results

Baseline characteristics of the included patients are shown in Table 1. A total of 214 patients were admitted to the ICU during the study period, 154 of them (71.96%) men. The mean age at hospital admission was 59.58 (13.79) years. The most frequently observed comorbidities were hypertension and dyslipidaemia (52.8% and 43.93%, respectively). Diabetes was present in 21.96% of the cases, while only 7.48% of the patients had chronic obstructive pulmonary disease (COPD); 16 patients (7.48%) had a previous diagnosis of chronic liver disease, but only two of them had advanced fibrosis. Specific treatments for COVID-19 are detailed in Table 1.

The median follow-up time (starting at the day of hospital admission) was 38 (IQR 22–54) days. At the end of the study, 84 patients were dead (39.25%), 69 (32.24%) had been discharged, 34 (15.89%) were alive and had been transferred to conventional wards and 27 (12.62%) were still in the ICU.

Liver fibrosis scores

The Forns and FIB-4 indices prior to hospital admission could be calculated in 156 (72.90%) and 177 (82.71%) cases, respectively. Mean values for the two scores were 5.37 (1.60) and 1.55 (1.03), respectively. Estimation of liver fibrosis before COVID-19 admission showed that only 21.15% of patients, according to the Forns index, and 54.24%, according to the FIB-4 index, had no or minimal fibrosis (F0-F1) (Figure 1a). As expected, those patients in whom liver fibrosis scores were available had a greater proportion of comorbidities. However, previously diagnosed liver disease had a similar frequency in both groups (Supplemental Table S1).

Because the NFS was available in only 44% of the cases (due mainly to the lack of an albumin value), this index was not considered for statistical comparisons. However, the distribution of the available NFS values was consistent with the fibrosis

Table 1. Baseline and COVID-19 related characteristics (*N* = 214).

|	
Age (years)	59.58 (13.79)
Sex (male)	154/214 (71.96)
Race	
Caucasian	161/214 (75.23)
Hispanics	48/214 (22.43)
Others	5/214 (2.34)
Cardiovascular risk factors	
Hypertension	113/214 (52.80)
Treatment with ACEI or ARB	77/214 (35.98)
Dyslipidaemia	94/214 (43.93)
Diabetes	47/214 (21.96)
BMI (Kg/m^2)	30.88 (5.74)
Harmful alcohol intake	21/214 (9.81)
Previous or active smoking	49/214 (22.90)
Other comorbidities	
COPD	16/214 (7.48)
Chronic kidney disease	28/214 (13.08)
Charlson index	1.90 (1.98)
Known history of liver disease	16/214 (7.48)
Etiology	
Alcohol	2/16 (12.50)
Hepatitis C	2/16 (12.50)
MAFLD	9/16 (56.25)
Others	3/16 (18.75)
Severity of liver disease	
Mild fibrosis	14/16 (87.50)
cACLD	1/16 (6.25)
Decompensated liver disease	1/16 (6.25)
Specific treatment for COVID-19	
Lopinavir/ritonavir	207/214 (96.73)
Hydroxychloroquine	211/214 (98.60)
Remdesivir	35/214 (16.36)

(Continued)

Table 1. (continued)

Beta-interferon	96/214 (44.86)
Azithromycin	97/214 (45.33)
Ceftriaxone	167/214 (78.37)
Corticosteroids	182/214 (85.05)
Tocilizumab	166/214 (77.57)
Laboratory tests	
Before admission	
AST (U/L)	29.43 (24.46)
ALT (U/L)	31.51 (22.73)
Bilirubin (mg/dl)	1.45 (10.41)
Albumin (g/dl)	4.20 (0.58)
Platelet count (10^9/l)	224.05 (66.07)
At admission	
AST (U/L)	84.5 (80.86)
ALT (U/L)	51.21 (36.28)
Bilirubin (mg/dl)	0.78 (1.37)
Albumin (g/dl)	3.85 (0.64)
Platelet count (10^9/l)	199.82 (89.11)

Data are expressed as mean (SD) or *n* (%).
ACEI, angiotensin converting enzyme inhibitors; ALT, alanine aminotransferase; ARB, angiotensin receptor blockers; AST, aspartate aminotransferase; BMI, body mass index; cACLD, compensated advanced chronic liver disease; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; MAFLD, metabolic associated liver disease; SD, standard deviation.

categories determined by the baseline FIB-4 or Forns index (Figure 1b).

Baseline factors related to survival

As shown in Table 2, age, history of past or active smoking and different comorbidities (hypertension, diabetes, dyslipidaemia, cardiovascular disease, COPD and chronic kidney disease) were associated with death. The CCI was also significantly higher in patients who died. Treatment with angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) was related to death in the overall cohort [38 (45.24%) *versus*
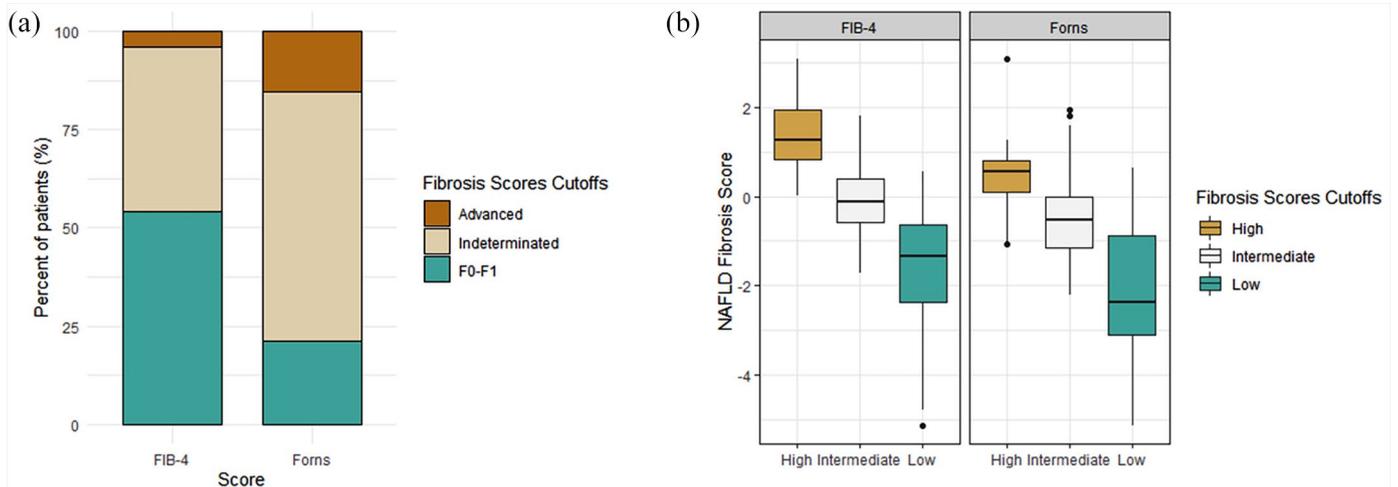
Figure 1. (a) Distribution of patients according to the different fibrosis categories established by baseline Forns and FIB-4 indices. (b) Stratification of the NAFLD fibrosis score values (*n* = 95) according to the fibrosis severity determined by the Forns and FIB-4 indices. *p*-value for mean comparison between categories <0.01 for both indices.
NAFLD, nonalcoholic fatty liver disease.

39 (30%), *p* = 0.023] but not in the subgroup of patients with hypertension [37 (66.07%) *versus* 38 (66.67%), *p* = 0.947]. Importantly, known chronic liver disease was not associated with worse outcomes.

Both liver fibrosis scores calculated before COVID-19 admission were significantly greater in non-survivors than in survivors (Table 2). Moreover, the probability of survival was significantly lower in patients who had any degree of fibrosis estimated by either the FIB-4 or Forns index. In addition, mortality increased according to the severity of fibrosis estimated by the FIB-4 or Forns index (Figure 2; *p* value for trend <0.05 for both indices). The mortality rate of patients in whom liver fibrosis indices could not be calculated was similar to that in the rest of the cohort (Supplemental Table S1).

Figure 3 depicts the comparison of laboratory values between survivors and non-survivors at different time points. There were no relevant differences in ALT, AST or bilirubin levels between the two groups at hospitalisation, at the time of ICU admission or considering the highest value of each parameter during evolution.

Multivariate regression analysis
In univariate analysis, a history of previous or active smoking, the CCI, creatinine, serum albumin, the APACHE II score, serum ferritin and both fibrosis indices were associated with mortality. Interestingly, the FIB-4 and Forns indices were independently associated with survival after adjustment for the CCI, the APACHE II score and serum ferritin (Table 3).

These results were reproduced when a multiple imputation method was used for the management of missing values for the FIB-4 and Forns indices (Supplemental Table S2).

Discussion
Several studies have suggested that alterations in liver function tests may play a relevant role in the natural history of COVID-19.[1,4,8,9,19] However, the contribution of pre-established liver disease to the prognosis of COVID-19 has not been fully assessed. Importantly, the vast majority of available information has evaluated the influence of altered liver function tests on different outcomes when COVID-19 is already established, and it is difficult to ascertain whether they represent real markers of pre-existing liver disease or are manifestations of SARS-CoV-2 infection. Here, we describe that higher scores of liver fibrosis indices commonly used in clinical practice (the FIB-4 and Forns indices) obtained before COVID-19 initiation, and therefore indicative of a possible pre-existing chronic liver disease, are related to survival of patients with COVID-19 hospitalised in the

Table 2. Comparison of baseline characteristics between survivors and non-survivors.

|	Non-survivors (*n* = 84)	Survivors (*n* = 130)	*p* value
Age (years)	64.36 (9.80)	56.49 (15.09)	<0.001
Sex (male)	65 (77.38)	89 (68.46)	0.156
Race			
Caucasian	63 (75)	98 (73.38)	
Hispanics	19 (22.62)	29 (22.31)	
Others	2 (2.38)	3 (2.31)	0.998
Hypertension	56 (66.67)	57 (43.85)	0.001
Diabetes	25 (29.76)	22 (16.97)	0.027
Dyslipidemia	44 (52.34)	50 (38.46)	0.045
BMI (kg/m^2)	30.79 (5.69)	30.95 (5.79)	0.844
Active or previous smoking	28 (33.33)	22 (16.92)	0.006
Harmful alcohol intake	8 (9.52)	13 (10.08)	0.895
Cardiovascular disease	55 (65.48)	60 (46.15)	0.006
COPD	12 (14.29)	4 (3.08)	0.002
Chronic kidney disease	19 (22.28)	9 (6.92)	0.001
Known chronic liver disease	8 (9.52)	8 (6.15)	0.360
Charlson index	2.59 (2.20)	1.46 (1.70)	<0.001
Treatment with ACEI or ARB	38 (45.24)	39 (30)	0.023
Forns index[a] continuous score	6.04 (1.42)	4.99 (1.58)	<0.001
Forns index categories			
<4.2	5 (8.20)	28 (29.47)	
4.2–6.9	40 (65.57)	59 (62.11)	
>6.9	16 (26.23)	8 (8.42)	<0.001
FIB-4[b] continuous score	1.77 (1.17)	1.41 (0.91)	0.020
FIB-4 categories			
<1.45	29 (41.43)	67 (62.62)	
1.45–3.25	37 (52.86)	37 (34.58)	
>3.25	4 (5.71)	3 (2.80)	0.017
Patients with previous known liver disease (*n* = 16)			
Forns index[c] continuous score	5.91 (1.62)	6.24 (1.96)	0.743
FIB-4[c] continuous score	2.23 (2.00)	2.04 (2.66)	0.889

Continuous variables are shown as mean (SD) and categorical variables as *n* (%).Student *t* test was used for comparisons between means and Fisher's exact test for comparison between categorical variables.
[a]Forns Index was available in 156 patients of the total sample.
[b]FIB-4 was available in 177 patients of the total sample.
[c]Forns index and FIB-4 were available in 13 patients in the subgroup of those with previous known liver disease.
ACEI, ACE inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; COPD, chronic obstructive pulmonary disease; SD, standard deviation.
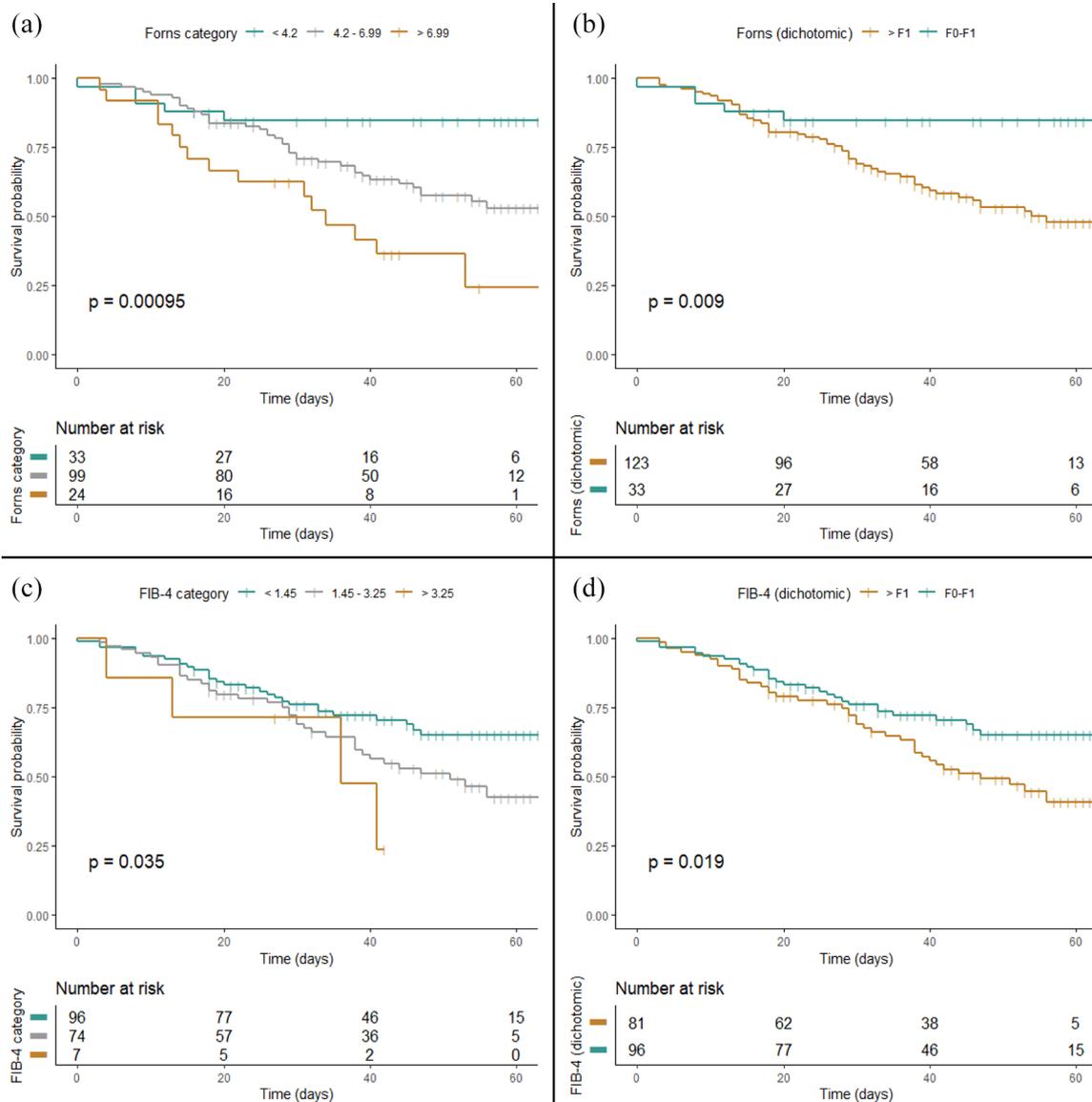
Figure 2. (a–d) Kaplan–Meier survival curves according to pre-existing fibrosis. Survival curves according to the severity of fibrosis (a,c) or the presence or absence of fibrosis (b,d) estimated by the Forns index (a,b) or the FIB-4 index (c,d).

ICU. Moreover, this association remains significant after adjustment for two main components of prognosis in ICU patients – chronic comorbidity (estimated in our study by the CCI) and severity of the clinical condition at the time of ICU admission (evaluated by the APACHE II) – as well as for the intensity of the inflammatory response (estimated by serum ferritin values). Thus, our findings suggest that a careful evaluation of pre-existing liver injury may be relevant in the clinical work-up of COVID-19 patients since, when indirect signs of fibrosis are already present before the

disease onset, clinical evolution may be worse. Furthermore, and according to our results, the excess of risk conferred by the elevation of pre-admission liver fibrosis markers suggests that a prioritization strategy for early vaccination should be considered in these patients. Importantly, the CCI has been identified as a robust predictor of mortality in ICU patients with acute lung injury and severe ARDS, independently of its aetiology,[20,21] and has been associated with poorer outcomes in chronic liver disease.[22] Furthermore, the APACHE II score is a widely validated instrument
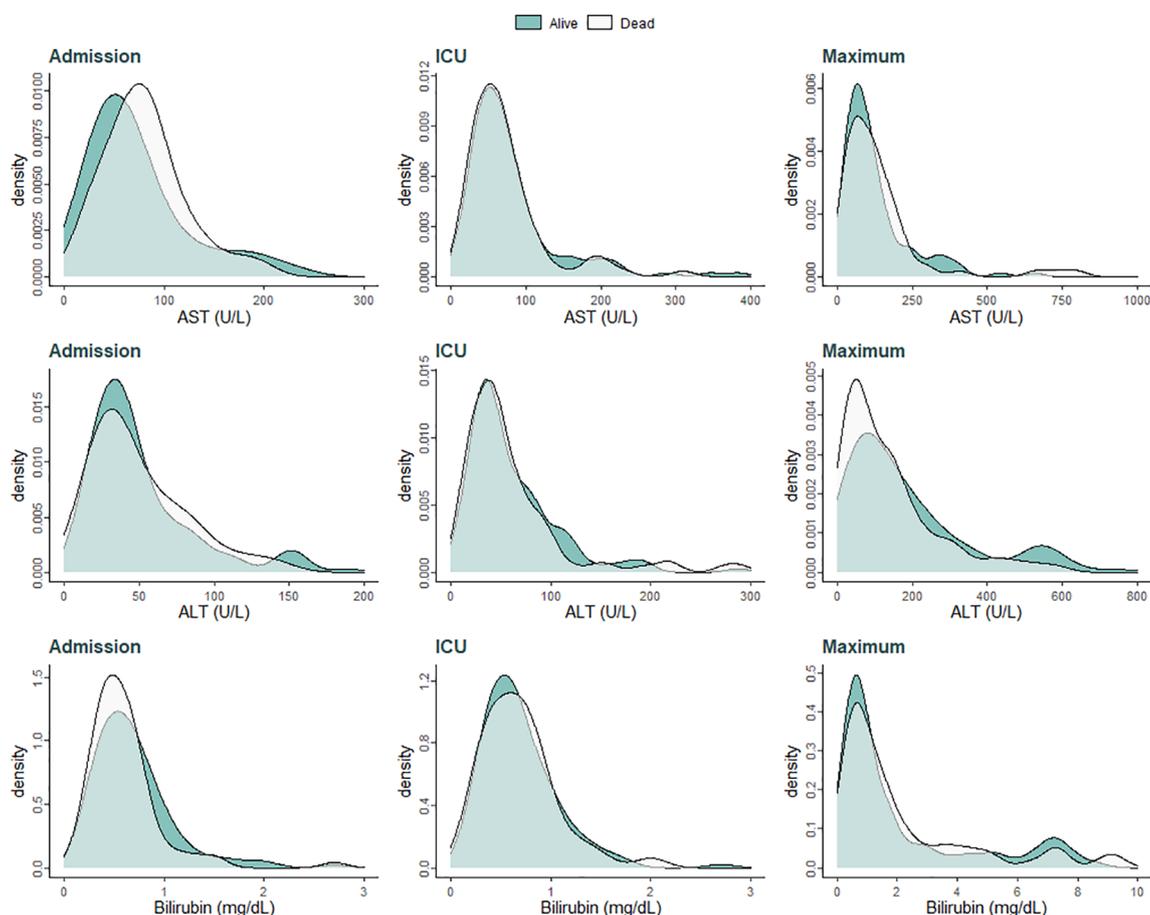
Figure 3. Distribution of the liver laboratory tests in survivors and non-survivors at different time points during hospitalisation. Kernel density estimates were used for graphical representation; *p* values were nonsignificant in all cases.

for the assessment of prognosis in critically ill patients, including those with acute respiratory failure, and ARDS as a primary cause of admission.[23,24] Our findings are in line with a recent report that indicates that the severity of COVID-19 was significantly less intense in patients with a low risk fibrosis category – based on the FIB-4 index – as compared with those with intermediate or high fibrosis categories.[8]

One advantage of our study is that it is focussed on the most severe patients (i.e. patients admitted to the ICU). Although the selection of this population increases the statistical power of the analysis due to the number of events, it also limits the generalisation of our results to other relevant less severe settings. However, a recent multicentre study including a large population of unselected in-hospital Spanish patients identified cirrhosis as an independent predictive factor, with a risk

similar to that of other independent predictors of mortality such as chronic heart disease or cancer, indicating that advanced fibrosis could be a relevant player in the COVID-19 prognosis.[25] Another strength of our study is that it is based on calculations of indices before SARS-CoV-2 infection, without possible interferences in liver test changes intrinsically associated with COVID-19. Although we expected that this approach would generate a large amount of missing data, we chose it to avoid the potential bias if we calculated the index values once the disease was initiated (acute-phase platelet rise, hepatocellular liver injury patterns often normalise the AST/ALT ratio to the mean). Importantly, our results were similar after handling missing data by multiple imputation procedures.

It could be argued that the association between these indices and mortality does not really reflect the influence of an unrecognised liver disease as a

Table 3. Univariate and multivariate Cox models with Forns and FIB-4 indices respectively.

Variable	Univariate		Multivariate (Forns) (*n* = 110)		Multivariate (FIB-4) (*n* = 122)	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Sex (male as reference)	0.75 (0.45–1.25)	0.269				
Previous or active smoking	1.73 (1.09–2.74)	0.019	1.35 (0.67–2.73)	0.403	1.29 (0.65–2.56)	0.463
Harmful alcohol intake	0.93 (0.45–1.94)	0.856				
BMI (kg/m^2)	1.00 (0.96–1.04)	0.852				
Charlson	1.20 (1.09–1.32)	<0.001	1.22 (1.00–1.48)	0.046	1.25 (1.08–1.44)	0.003
Race (Caucasian as reference)						
Hispanics	1.17 (0.69–1.97)	0.568				
Others	1.07 (0.25–4.56)	0.925				
Platelets (10^3/ml)	1.00 (0.99–1.00)	0.312				
ALT (IU/l)	1.00 (0.99–1.00)	0.356				
AST (IU/l)	1.00 (0.99–1.00)	0.797				
Bilirubin (mg/dl)	1.08 (0.80–1.47)	0.609				
INR	0.93 (0.51–1.72)	0.826				
Creatinine (mg/dl)	1.37 (0.96–1.97)	0.084	1.65 (0.70–3.90)	0.254	1.12 (0.59–2.12)	0.737
Albumin (g/dl)	0.65 (0.41–1.04)	0.076	0.86 (0.37–1.97)	0.714	0.85 (0.41–1.77)	0.670
Fibrinogen (mg/dl)	1.00 (1.00–1.01)	0.566				
C-reactive protein (mg/dl)	1.01 (0.98–1.02)	0.762				
Ferritin (µg/l)	1.00 (1.00–1.00)	0.000	1.00 (1.00–1.00)	0.027	1.00 (0.99–1.01)	0.132
APACHE II	1.06 (1.03–1.09)	<0.001	1.05 (0.99–1.10)	0.080	1.05 (1.00–1.10)	0.044
Baseline Forns	1.40 (1.17–1.67)	<0.001	1.41 (1.11–1.81)	0.006		
Baseline FIB-4	1.31 (1.08–1.60)	0.007			1.31 (0.99–1.72)	0.051

Baseline characteristics, laboratory data at ICU admission and APACHE II score were included. Variables with $p < 0.10$ in the univariate analysis were included in the multivariate models.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; ICU, intensive care unit; INR, international normalized ratio.

risk factor. In fact, we cannot definitively establish the presence of liver disease based only on fibrosis indices. However, much of the data from our study suggest the true presence of liver disease. First, the relatively high prevalence of pre-existing fibrosis estimated in the study is not surprising considering the high representation of metabolic risk factors present in our cohort; in fact, the estimated fibrosis rate is similar to that reported in studies examining similar populations.[26,27] Unfortunately, a more specific assessment of the underlying aetiology was not possible due to the retrospective characteristic of the study and, especially, to the epidemiological context. Nevertheless, according to the clinical data collected, and taking into account the epidemiology of liver disease in our clinical setting, a reasonable assumption regarding the predominant aetiology of the potential underlying liver disease can be done. First of all, it is important to clarify that all patients admitted to the ICU in the context

of the COVID-19 pandemic in our centre were screened for viral hepatitis. None of the patients without prior history of liver disease tested positive for either hepatitis C virus (HCV) or hepatitis B virus (HBV). After excluding the patients with confirmed previous chronic liver disease, the cohort consisted of 198 patients. Only 9% ($n = 18$) were classified as having active alcohol consumption. Of them, nearly 72% and 40% had intermediate or high risk of fibrosis according to Forns and FIB-4 index, respectively. Among the remaining cohort ($n = 180$), 94.4% of the patients had one or more MAFLD risk factors according to the current definition of the disease[28] (1 risk factor: 33%; 2 risk factors 33.5%; 3 risk factors: 21%; 4 risk factors: 12.5%). Moreover, among those patients without previous diagnosis of liver disease and active drinking ($n = 18$), 16.6%, 77.8% and 5.6% had either 1, 2 or 3 and 4 MAFLD risk factors, respectively. This suggests that, even if alcohol had a relevant contribution to the presumably underlying liver disease according to the non-invasive fibrosis scores, a dual aetiology might be assumed in most cases. In summary, all these data support that the predominant aetiology of the possible unrecognised liver injury in our cohort was MAFLD. Second, both indices are similarly related to survival, also indicating the consistency of our results; in fact, the mortality rate of patients in which the Forns or FIB-4 index estimated an intermediate or high risk of fibrosis was very similar (46% and 50%, respectively). Furthermore, although it was only available in less than half of the cases, the NFS (specifically developed in patients with non-alcoholic fatty liver disease)[17] also discriminated the two populations with a clearly different risk of death [29% in the group with presumably healthy livers (cut-off point < -1.455) *versus* 48% in the remaining patients, data not shown]. The distribution of the available NFS values was consistent with the fibrosis categories determined by the baseline FIB-4 or Forns index. Of note, among the patients with the three liver fibrosis scores available, 88.6% of patients classified as intermediate or high risk according to Forns, were also classified in such categories by the NFS index. Accordingly, 93.8% of the patients in the intermediate or high risk group according to FIB-4, had the same risk category when estimated by NFS index. None of the patients within the low risk group according to NFS were classified as high risk either by Forns or FIB-4 index and vice versa. Finally, we found that the mortality risk parallels the fibrosis estimation obtained by the three

categories of the FIB-4 or Forns index, suggesting a biologically plausible association. On the other hand, it is widely recognised that serological indices predict with reasonable accuracy the stage of liver fibrosis, indicating that they are useful screening tools in clinical practice. This seems to be also true for the estimation of fibrosis when the aetiology of liver disease is different from those in which the original indices were derived,[29–31] or when applied to evaluate the contribution of unrecognized liver disease to non-liver related mortality in unselected populations.[32,33] The described association between pre-existing fibrosis and the risk of death in COVID-19 patients should not be undervalued, because there are many pieces of evidence emphasising the prognostic relevance of liver fibrosis in several situations. Moreover, different population-based studies have shown that pathological scores based on the FIB-4, Forns index and NFS are associated with an increased risk of liver-related mortality.[16,34] Finally, our data are consistent with the observations of recent studies showing worse progression of COVID-19 in patients with previously known cirrhosis or in those with high FIB-4 values.[4–6,8,9] Abnormal serum liver enzymes are frequent in COVID-19, especially in severely ill patients.[35] The underlying mechanisms explaining liver tests abnormalities induced by SARS-Cov2 have not been definitively elucidated. Although some studies have considered the possibility of a direct cytopathic effect,[36,37] the alterations in liver tests could also be attributed to non-directly liver-related issues (e.g. systemic inflammatory response syndrome, cytokine storm, ischemic hepatitis/shock, sepsis and drug hepatotoxicity).[38] Importantly, our results indicating a role of previous liver disease in COVID-19 outcomes is specifically related with the liver condition previous to COVID-19 initiation, thus minimising the influence of the acute infection in liver tests. However, the existence of an association between such alterations and mortality is not well documented. While the impairment of liver tests (aminotransferases, bilirubin) has sometimes been suggested as a risk factor of death,[19] other studies have yielded different results.[39] Therefore, we also explored the possible influence of liver test evolution during the disease course on survival. In our population of critically ill patients, liver test impairment throughout COVID-19 progression was mild and not significantly associated with mortality. This is in accordance with the hypothesis that an uncontrolled production of pro-inflammatory mediators leads to the development of ARDS and cytokine

storm syndrome, which is probably responsible for the majority of alterations in liver biochemistry.[39] On the other hand, it is possible to speculate that previous unrecognised fibrosis and liver steatosis may increase the risk of developing an exacerbated inflammatory response and subsequent immune dysfunction.[9] Moreover, our findings emphasise that the evaluation of liver involvement in COVID-19 patients should rely on the estimation of pre-existing liver disease with non-invasive indices rather than on the analysis of isolated values of liver tests.

Our study has some limitations. First, in the context of the COVID-19 pandemic, a significant number of patients with severe COVID-19 did not have access to the ICU mainly because of their age and the existence of comorbidities. Consequently, there could be a potential selection bias because older patients or those with more severe comorbidities (especially advanced liver disease) would be underrepresented in our series. However, our study was aimed mainly at analysing the influence of previously unknown liver disease on the evolution of severe COVID-19 because the prognostic impact of cirrhosis in patients with different severe acute diseases is well known. Second, we were not able to validate our data in an independent series of patients. In this regard, it is noteworthy that our study included a very large cohort of unselected patients consecutively admitted to the ICU at our hospital during the first 2 months of the COVID-19 pandemic, supporting the validity of our conclusions. On the other hand, our aim was to develop an explicative model to assess the influence of pre-existing liver disease on the prognosis of these patients, not to develop a prognostic model that necessarily needs to be validated. Third, we lack additional data to determine more accurately the severity of liver damage or to evaluate the aetiology of the liver disease. Nevertheless, in the epidemiological context of the overwhelming COVID-19 pandemic, the assessment of underlying liver injury and fibrosis severity, either by non-invasive methods such as liver stiffness measurement or ultrasonography, or by invasive procedures such as liver biopsy, was practically impossible. Regarding the aetiology, the approach followed to infer the cause of the liver disease with the available information allows us to reasonably accept that MAFLD predominates in our series. Notably, there is a discrepancy in the proportion of patients with an estimation of non-significant liver fibrosis between the FIB-4 and Forns indices. Nonetheless, the accuracy of an individual outcome prediction with a particular index did not affect the validity of our overall explanatory model. Finally, data regarding the association between fibrosis scores and the outcomes of the overall population of patients admitted with COVID-19, and not only ICU cases, are not available.

In conclusion, pre-existing liver fibrosis, as estimated by serological non-invasive measurements, is independently associated with a significantly higher risk of death in patients with severe COVID-19 admitted to the ICU.

ORCID iD
Mario Romero-Cristóbal [iD] https://orcid.org/0000-0003-1633-2862

Supplemental material
Supplemental material for this article is available online.

References
1. Guan W, Ni Z, Hu Y, *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020; 382: 1708–1720.

2. Epidemiology for Public Health, Istituto Superiore di Sanità. Characteristics of SARS-CoV-2 patients dying in Italy. *Report based on available data on* April 29th, 2020, https://www.epicentro.iss.it/en/coronavirus/bollettino/Report-COVID-2019_23_april_2020.pdf.

3. Gacouin A, Locufier M, Uhel F, *et al.* Liver cirrhosis is independently associated with 90-day mortality in ARDS patients. *Shock* 2016; 45: 16–21.

4. Qi X, Liu Y, Wang J, *et al*. Clinical course and risk factors for mortality of COVID-19 patients with pre-existing cirrhosis: a multicentre cohort study. *Gut* 2021; 70: 433–436.

5. Singh S and Khan A. Clinical characteristics and outcomes of COVID-19 among patients with pre-existing liver disease in United States: a multi-center research network study. *Gastroenterology* 2020; 159: 768–771.

6. Iavarone M, Ambrosio RD, Soria A, *et al*. High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol* 2020; 73: 1063–1071.

7. Ji D, Qin E, Xu J, *et al*. Non-alcoholic fatty liver diseases in patients with COVID-19: a retrospective study. *J Hepatol* 2020; 73: 451–453.

8. Targher G, Mantovani A, Byrne CD, *et al*. Risk of severe illness from COVID-19 in patients with metabolic dysfunction-associated fatty liver disease and increased fibrosis scores. *Gut* 2020; 69: 1545–1547.

9. Ibáñez-Samaniego L, Bighelli F, Usón C, *et al*. Elevation of liver fibrosis index FIB-4 is associated with poor clinical outcomes in patients with COVID-19. *J Infect Dis* 2020; 222: 726–733.

10. Charlson ME, Pompei P, Ales K, *et al*. A new method of classifying prognostic in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373–383.

11. Knaus W, Draper E, Wagner D, *et al*. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; 13: 818–829.

12. Vallet-Pichard A, Mallet V, Nalpas B, *et al*. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and Fibrotest. *Hepatology* 2007; 46: 32–36.

13. Sterling RK, Lissen E, Clumeck N, *et al*. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43: 1317–1325.

14. Vilar-Gomez E and Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: clinical prediction rules and blood-based biomarkers. *J Hepatol* 2018; 68: 305–315.

15. Forns X, Ampurdanes S, Llovet JM, *et al*. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; 36: 986–992.

16. Nabi O, Lacombe K, Boursier J, *et al*. Prevalence and risk factors of nonalcoholic fatty liver disease and advanced fibrosis in general population: the French Nationwide NASH-CO Study. *Gastroenterology* 2020; 159: 791–793.

17. Angulo P, Hui JM, Marchesini G, *et al*. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; 45: 846–854.

18. Bhatraju PK, Ghassemieh BJ, Nichols M, *et al*. Covid-19 in critically ill patients in the Seattle Region – case series. *N Engl J Med* 2020; 382: 2012–2022.

19. Lei F, Liu YM, Zhou F, *et al*. Longitudinal association between markers of liver injury and mortality in COVID-19 in China. *Hepatology* 2020; 72: 389–398.

20. Seuransky JE, Martin GS, Mendgz-Tellez P, *et al*. Pulmonary vs nonpulmonary sepsis and mortality in acute lung injury. *Chest* 2008; 134: 534–538.

21. Ando K, Doi T, Moody SY, *et al*. The effect of comorbidity on the prognosis of acute lung injury and acute respiratory distress syndrome. *Intern Med* 2012; 51: 1835–1840.

22. Powell EE, Skoien R, Rahman T, *et al*. Increasing hospitalization rates for cirrhosis: overrepresentation of disadvantaged Australians. *EClinicalMedicine* 2019; 11: 44–53.

23. Larsson J, Itenov TS and Bestle MH. Risk prediction models for mortality in patients with ventilator-associated pneumonia: a systematic review and meta-analysis. *J Crit Care* 2017; 37: 112–118.

24. Auriemma CL, Zhuo H, Delucchi K, *et al*. Acute respiratory distress syndrome-attributable mortality in critically ill patients with sepsis. *Intensive Care Med* 2020; 46: 1222–1231.

25. Berenguer J, Ryan P, Rodríguez-Baño J, *et al*. Characteristics and predictors of death among 4,035 consecutively hospitalized patients with COVID-19 in Spain. *Clin Microbiol Infect* 2020; 26: 1525–1536.

26. Rinella ME. Nonalcoholic fatty liver disease a systematic review. *JAMA* 2015; 313: 2263–2273.

27. Miyaaki H, Ichikawa T, Taura N, *et al*. Predictive value of the fibrosis scores in patients with chronic hepatitis C associated with liver fibrosis and metabolic syndrome. *Intern Med* 2011; 50: 1137–1141.

28. Eslam M, Newsome PN, Sarin SK, *et al*. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020; 73: 202–209.

29. Vilar-Gomez E, Calzadilla-Bertot L, Wong VW, *et al*. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced

nonalcoholic fatty liver disease: a multi-national cohort study. *Gastroenterology* 2018; 155: 443–457.

30. Sumida Y, Yoneda M, Hyogo H, *et al.* Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; 12: 2–9.

31. Kim D, Kim W, Kim H, *et al.* Association between non-invasive fibrosis markers and mortality among adults with non-alcoholic fatty liver disease in the United States. *Hepatology* 2013; 57: 1357–1365.

32. Parikh NS, Kamel H, Navi B, *et al.* Liver fibrosis indices and outcomes after primary intracerebral hemorrhage. *Stroke* 2020; 51: 830–837.

33. Unalp-Arida A and Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. *Hepatology* 2017; 66: 84–95.

34. Angulo P, Bugianesi E and Bjornsson E. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013; 145: 782–789.

35. Bloom PP, Meyerowitz EA, Reinus Z, *et al.* Liver biochemistries in hospitalized patients with COVID-19. *Hepatology* 2021; 73: 890–900.

36. Galanopoulus M, Gkeros F, Doukatas A, *et al.* COVID-19 pandemic: pathophysiology and manifestations from the gastrointestinal tract. *World J Gastroenterol* 2020; 26: 4579–4588.

37. Cabibbo G, Rizzo GEM, Stornello C, *et al.* SARS-CoV-2 infection in patients with a normal or abnormal liver. *J Viral Hepat* 2021; 28: 4–11.

38. Sultan S, Altayar O, Siddique SM, *et al.* AGA institute rapid review of the gastrointestinal and liver manifestations of COVID-19, meta-analysis of international data, and recommendations for the consultative management of patients with COVID-19. *Gastroenterology* 2020; 159: 320–334.

39. Bangash MN, Patel J and Parekh D. COVID-19 and the liver: little cause for concern. *Lancet Gastroenterol Hepatol* 2020; 5: 529–530.

Visit SAGE journals online
journals.sagepub.com/
home/tag

⑤SAGE journals