New Microbiologica, 46, 3, 296-302, 2023, ISSN 1121-7138

FULL PAPER

Risk of mortality in people with chronic liver diseases hospitalized for Coronavirus disease of 2019 (COVID-19) in a tertiary hospital in Lombardy, Italy

Martina Ranzenigo[1,2], Daniele Ceccarelli[2], Laura Galli[2], Costanza Bertoni[1,2], Alessia Siribelli[1,2], Emanuela Messina[2], Hamid Hasson[2], Riccardo Lolatto[2], Antonella Castagna[1,2], Caterina Uberti-Foppa[1,2], Giulia Morsica[2]

[1]*Vita-Salute San Raffaele University, Milan, Italy;*
[2]*Department of Infectious Diseases, San Raffaele Hospital, Milan, Italy*

SUMMARY

The impact of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection on patients with pre-existing chronic liver diseases (CLD) remains elusive. The aim of this study was to investigate the in-hospital mortality in patients hospitalized for Coronavirus disease of 2019 (COVID-19) with CLD (CLD group) compared to those without CLD (non-CLD group). We performed a retrospective cohort study including patients with confirmed SARS-CoV-2 infection, hospitalized at San Raffaele Hospital (Milan), stratified according to the presence or absence of CLD. A propensity score was estimated and used to match the two groups by age, gender, body mass index, type 2 diabetes mellitus, and hypertension. Predictors of mortality were assessed using univariate and multivariate logistic regression model. Among 1210 patients with COVID-19, 41 (3.4%) were included in the CLD group and 1169 (96.6%) in the non-CLD group. Using a propensity score, we matched 41 patients in the CLD group with 123 in the non-CLD group. At admission, patients in the CLD group had worse liver function, lower platelets count, and lower c-reactive protein levels. By multivariate analysis, the CLD group showed a higher risk of death: OR 4.04 (95% CI 1.29-12.70; p= 0.017). Our study showed that COVID-19 with chronic liver diseases has a higher risk of mortality during hospitalization.

Received June 30, 2023 *Accepted* August 30, 2023

INTRODUCTION

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection, causing Coronavirus disease of 2019 (COVID-19), has been associated with a mortality rate that may vary from 1% to 3% worldwide (World Health Organization, available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports). Studies show that mortality is higher with older age and in individuals with comorbidities like diabetes, renal diseases, obesity, and cardiovascular or respiratory diseases (Wang *et al*., 2020; Zhou *et al*., 2020; Namayandeh *et al*., 2023). Nowadays, with the advent of antivirals and monoclonal antibodies, the selection of people who can benefit from treatment is of pivotal importance; therefore, the early identification of predictors for mortality may modify the outcome of COVID-19. Although the main target of SARS-CoV-2 is the lung, multi-organ involvement has been reported. In this context, a transient increase of transaminases or, less frequently, acute liver failure may occur in patients with COVID-19 (Portincasa *et al*., 2020). The mechanism of liver involvement by SARS-CoV-2 remains unclear, although a direct cytopathic effect, ischemic liver damage, immune-mediated or drug-induced liver failure have been postulated (Iavarone *et al*., 2020; Napodano *et al*., 2021). While a consistent increase in mortality is expected in cirrhotic patients due to concomitant lower functional reserve of the liver, and in a few cases acute or chronic liver failure, it is not well established whether mild chronic liver disease is associated with a worse outcome in patients with COVID-19 (Kovalic *et al*., 2020; Mantovani *et al*., 2020; Vaishnav *et al*., 2022). The present study aims to investigate the effect of chronic liver diseases on COVID-19 in-hospital mortality.

Key words:
COVID-19, SARS-CoV-2, Chronic Liver Diseases, Cirrhosis.

Corresponding author:
Giulia Morsica
E-mail: morsica.giulia@hsr.it

METHODS

Study Group

San Raffaele Hospital (Milan, Italy) is a tertiary health-care centre which was designed as a SARS-

COV-2 hub by Italian health authorities. It was actively involved in the cure of patients affected by COVID-19 from the initial outbreak of the pandemic caused by SARS-COV-2. In this retrospective study, we included all the patients hospitalized at San Raffaele Hospital from 25th February 2020 to 12th June 2021 with a diagnosis of SARS-CoV-2 infection, defined as a positive real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assay result from a nasopharyngeal swab and compatible signs, symptoms, and/or radiological findings.

Demographic and clinical data of these patients were extracted and anonymized from electronic hospital records and recorded in a dedicated database (COVID-BioB clinical database) of San Raffaele Hospital.

The study was approved by the Ethics Committee of San Raffaele Hospital (protocol No. 34/int/2020) and was registered on ClinicalTrials.gov (NCT04318366). All patients signed an informed consent form. Our research was in accordance with the Declaration of Helsinki.

Relevant data retrieved from the COVID-BioB clinical database were sex, age, body mass index (BMI), past medical history, laboratory data, and clinical outcome. Relevant laboratory liver tests included alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), total bilirubin, prothrombin time in seconds (PT in seconds), partial thromboplastin time in seconds (PTT in seconds), PT-international normalized ratio (PT-INR); c-reactive protein (CRP) was included as an unspecific inflammatory marker and neutrophil-to-lymphocyte (N/L) ratio was included as a marker of stress used in critically ill patients (Salciccioli *et al*., 2015). Laboratory tests were performed at admission (baseline) before any supportive and/or curative treatment and at the discretion of the treating physician until the time of discharge/death.

Patients were classified into two groups: the first group consisted of patients with pre-existing CLD (CLD group) and the second group consisted of patients without pre-existing CLD (non-CLD group).

Diagnosis of CLD included mild liver diseases (chronic liver diseases without cirrhosis) and cirrhosis, and was based on past medical history and on a combination of clinical features, laboratory parameters, and radiological evaluation. The fibrosis-4 index (Fib-4) was included as a non-invasive estimate of liver fibrosis (Sterling *et al*., 2006).

Statistical analysis

Descriptive statistics were used to summarize the data; results were reported as median (interquartile range, IQR) or frequency (%), as appropriate.

Characteristics were compared by the chi-square or Fisher's exact test (categorical variables) or by the Wilcoxon rank-sum test (continuous variables).

A logistic regression analysis was applied to estimate the propensity of CLD, conditioned on a pre-specified list of baseline covariates (age, gender, body mass index (BMI), type 2 diabetes mellitus (DM2), hypertension); the predicted probabilities of having CLD (propensity-score) were used to match CLD with non-CLD patients in a 1:3 ratio (41 patients with CLD and 123 without CLD).

A multivariable unconditional logistic regression model was performed to identify factors associated with the risk of in-hospital mortality.

Variables known to have a potential effect on the outcome death were considered to obtain the multivariable model and included: age, gender, presence of comorbidities, presence of CLD, neutrophil to lymphocyte ratio, creatinine, and c-reactive protein. A stepwise variable selection algorithm with entry and stay criteria of 0.10 and 0.05, respectively, was applied; adjusted odds ratios (aOR) of mortality were reported with the corresponding 95% CI for significant covariates.

A 2-sides alpha value <0.5 was used for statistical significance.

All analyses were conducted using SAS statistical software version 9.4 (Statistical Analyses System Inc, Cary, NC, USA).

RESULTS

Clinical and laboratory characteristics of patients

The present study included 1210 patients with a confirmed diagnosis of SARS-CoV-2 infection: 41 patients (3.4%) were assigned to the CLD group and 1169 (96.6%) to the non-CLD group.

Among the 41 patients with CLD, 16 (39%) had a diagnosis of cirrhosis; the model for end-stage liver disease (MELD) score was ≤15 in 11/15 (73%) patients with cirrhosis, with a median value of 11 (IQR 9-16), suggesting relatively preserved liver function.

The most common aetiologies of CLD were fatty liver disease (10 cases, 24.3%), hepatitis C virus infection (9 cases, 22%), hepatitis B virus infection (4 cases, 9.7%), hepatocellular carcinoma (4 cases, 9.7%), and alcoholic hepatitis (3 cases, 7.3%).

Comparison of characteristics at hospital admission was performed after matching 41 patients in the CLD group with 123 patients in the non-CLD group. The 2 groups were generally well balanced with clinical variables similarly distributed in the two groups.

Median time to symptoms in the CLD and non-CLD group was similar [6 days (IQR 3-9) vs. 7 days (3-10); p= 0.377].

Characteristics of patients with or without CLD are described in *Table 1*.

Liver-related laboratory chemistry was worse in the

CLD group**.** In detail: higher GGT [71 (IQR 42-145) vs 39 (IQR 25-73) U/L; p=0.002] and ALP [74 (63-117) vs 62 (52-79) U/L, p=0.007] levels, more prolonged PT in seconds [14.4 (IQR 13.7-16.0) vs 13.8 (IQR 13.1-14.7); p=0.005], PT-INR [1.10 (IQR 1.05-1.22) vs. 1.06 (IQR 1.00-1.12); p=0.005] and PTT in seconds [30.4 (IQR 28.6-32.8) vs 29.3 (IQR 27.6-30.8); p=0.033]. PLT count [175 (IQR 115-214) vs 199 (IQR 156-250) 10^9/L; p=0.004] and CRP levels were lower in the CLD group [35 (IQR 9-94) vs 58 (IQR 26-112) mg/L; p=0.023]. Finally, the non-invasive biomarker of liver fibrosis, Fib-4, was higher in CLD patients, with values >3.25 (fibrosis degree F3-F4 according to metavir score) in 22/41 (53.7%) patients with CLD.

During hospitalization, 11 of 41 (26.8%) patients in the CLD group and 16 of 123 (13%) in the non-CLD group died (OR 2.06; 95% CI 1.04-4.08; p=0.037). Median time to death was similarly distributed between CLD group and non-CLD group (9 days (IQR 6-46) vs 9 days (IQR 6-19); p=0.656).

We also compared characteristics of patients who died or remained alive during hospitalization. At admission, patients who died were older and had a higher frequency of CVD than those with a favourable outcome; they had a worse liver function profile with higher ALP levels (p=0.042), more prolonged PT in seconds (p=0.004) and mildly increased PT-INR (p=0.004); they also had a lower PLT count (p=0.002), higher creatinine levels (p<0.001), lower lymphocytes (p=0.002), higher N/L ratio (p = 0.008)

Table 1 - *Baseline characteristics of patients with COVID-19 stratified in CLD-group and non-CLD group, after a propensity score matching.*

Characteristics	*CLD group N=41*	*Non-CLD group N=123*	*P-value*
Age, years	72 (63 - 81)	72 (61 - 81)	0.737
Sex, male	25 (61.0)	80 (65.0)	0.778
BMI, kg/m²			0.712
Normal <=25	3 (15.0%)	6 (11.8%)	
Overweight >25	17 (85.0%)	45 (88.2%)	
Unknown	*21*	*72*	
Type 2 diabetes, yes	10 (24.4%)	21 (17.1%)	0.420
Hypertension, yes	21 (51.2%)	621 (54.4%)	1.000
CVD, yes	20 (48.8%)	40 (32.5%)	0.092
Chronic lung diseases, yes	2 (4.9%)	1 (0.8%)	0.155
Malignancies, yes	2 (4.8%)	4 (3.2%)	0.640
CKD, yes	3 (7.3%)	4 (3.2%)	0.368
*MELD**	11 (9-16)	-	
Neutrophils, 10^9/L	3.7 (2.5 - 6.6)	4.9 (3.3 - 7.0)	0.115
Lymphocytes, 10^9/L	0.9 (0.7 - 1.2)	0.9 (0.6 - 1.2)	0.703
N/L ratio	4.00 (2.62 - 10.50)	5.15 (3.43 - 8.56)	0.214
ALP, IU/L	74 (63 - 117)	62 (52 - 79)	0.007
ALT, IU/L	28 (17 - 71)	36 (22 - 58)	0.681
AST, IU/L	48 (27 - 80)	46 (29 - 63)	0.560
Bilirubin, mg/dL	0.54 (0.36 - 0.73)	0.47 (0.35 - 0.70)	0.205
GGT, IU/L	71 (42 -145)	39 (25 - 73)	0.002
Platelets count, 10^9/L	175 (115 - 214)	199 (156 - 250)	0.004
PT, seconds	14.4 (13.7 - 16.0)	13.8 (13.1 - 14.7)	0.005
PT-INR	1.10 (1.05 - 1.22)	1.06 (1.00 - 1.12)	0.005
PTT, seconds	30.4 (28.6 - 32.8)	29.3 (27.6 - 30.8)	0.033
Creatinine, mg/dL	0.96 (0.79 - 1.46)	1.02 (0.86 - 1.18)	0.974
CRP, mg/L	35 (9 - 94)	58 (26 - 112)	0.023
Fib-4 score	3.52 (2.48 - 6.54)	2.71 (1.73 - 4.05)	0.003
Time to symptoms, days	6 (3-9)	7 (3-10)	0.377

*Only in cirrhotic patients (n=16), 1 patient missing.

Abbreviations: BMI= body mass index; CVD=cardiovascular diseases; CKD=chronic kidney diseases; MELD=Model for End-stage Liver Disease; N/L=-neutrophils/lymphocytes ratio; ALP=alkaline phosphatase; ALT=alanine aminotransferase (normal value <59 IU/L); AST=aspartate aminotransferase (normal values <35 IU/L); GGT=gamma glutamyl transpeptidase (normal value <68 IU/L); PT=prothrombin time; INR=International Normalized Ratio; PTT=partial thromboplastin time; CRP=c-reactive protein (normal values <6 mg/L).

Table 2 - *Characteristics of patients with COVID-19, including baseline and last laboratory tests, stratified according to the outcome (dead or alive).*

Characteristics	*[ALL] N=164*	*Dead N=27*	*Alive N=137*	*p-value*
Age, years	72 [62 - 81]	84 [76 - 87]	70 [60 - 79]	<0.001
Sex, male	105 (64.0%)	21 (77.8%)	84 (61.3%)	0.159
BMI, kg/m²				0.343
Normal <=25	9 (12.7%)	2 (33.3%)	7 (10.8%)	
Overweight >25	62 (87.3%)	4 (66.7%)	58 (89.2%)	
Unknown	*93*	*21*	*72*	
CLD, yes	41 (25.0%)	11 (40.7%)	30 (21.9%)	0.068
Type 2 diabetes, yes	31 (18.9%)	7 (25.9%)	24 (17.5%)	0.453
Hypertension, yes	83 (50.6%)	15 (55.6%)	68 (49.6%)	0.725
CVD, yes	60 (36.6%)	17 (63.0%)	43 (31.4%)	0.004
Chronic lung diseases, yes	3 (1.8%)	0 (0.0%)	3 (2.2%)	1.000
Malignancies, yes	6 (3.7%)	2 (7.4%)	4 (2.9%)	0.257
CKD, yes	7 (4.3%)	2 (7.4%)	5 (3.7%)	0.324
ALP-bl, IU/L	65 [53 - 83]	72 [60 - 128]	64 [52 - 80]	0.042
ALP-last, IU/L	66 [54 - 97]	88 [63 - 127]	64 [54 - 90]	0.014
ALT-bl, IU/L	36 [20 - 60]	31 [17 - 50]	37 [22 - 60]	0.329
ALT-last, IU/L	33 [20 - 64]	40 [23 - 67]	32 [20 - 63]	0.286
AST-bl, IU/L	46 [29 - 67]	54 [30 - 99]	45 [29 - 61]	0.061
AST-last, IU/L	29 [21 - 56]	62 [29 - 118]	28 [21 - 41]	<0.001
BIlirubin-bl, mg/dL	0.50 [0.36 - 0.71]	0.54 [0.36 - 0.83]	0.50 [0.36 - 0.70]	0.733
Bilirubin-last, mg/dL	0.49 [0.37 - 0.77]	0.76 [0.52 - 1.81]	0.47 [0.36 - 0.65]	0.001
Creatinine-bl, mg/dL	1.01 [0.83 - 1.25]	1.43 [1.04 - 2.34]	0.97 [0.82 - 1.16]	<0.001
Creatinine-last, mg/dL	1.01 [0.84 - 1.23]	1.57 [0.98 - 2.45]	0.98 [0.83 - 1.16]	0.001
GGT-bl, IU/L	46 [27 - 91]	39 [21 - 110]	46 [29 - 87]	0.602
GGT-last, IU/L	38 [23 - 81]	49 [25 - 101]	37 [23 - 76]	0.266
Lymphocytes-bl, 10^9/L	0.9 [0.6 - 1.2]	0.6 [0.4 - 0.9]	0.9 [0.7 - 1.2]	0.002
Lymphocytes-last, 10^9/L	1.5 [1.0 - 2.1]	0.7 [0.4 - 1.2]	1.7 [1.2 - 2.3]	<0.001
Neutrophils-bl, 10^9/L	4.7 [3.0 - 6.8]	5.9 [2.4 - 8.8]	4.6 [3.1 - 6.4]	0.411
Neutrophils-last, 10^9/L	4.1 [2.9 - 6.6]	8.4 [4.2 - 12.9]	4.0 [2.8 - 5.9]	<0.001
N/L ratio-bl	4.86 [3.08 - 9.00]	7.92 [3.98 - 14.00]	4.67 [3.00 - 8.29]	0.008
N/L ratio-last	2.73 [1.58 - 5.78]	9.67 [5.08 - 16.20]	2.31 [1.50 - 3.92]	<0.001
CRP-bl, mg/L	54 [21 - 107]	54 [26 - 120]	54 [20 - 105]	0.266
CRP-last, mg/L	8 [2 - 37]	94 [51 - 135]	6 [1 - 19]	<0.001
Platelets count-bl, 10^9/L	188 [147 - 236]	137 [92 - 216]	192 [156 - 242]	0.002
Platelets count-last, 10^9/L	236 [166 - 314]	155 [80 - 250]	242 [186 - 330]	<0.001
PT INR-bl	1.07 [1.01 - 1.14]	1.11 [1.06 - 1.43]	1.06 [1.00 - 1.12]	0.004
PT INR-last	1.09 [1.03 - 1.15]	1.17 [1.08 - 1.42]	1.08 [1.03 - 1.14]	0.001
PT-bl, seconds	14.0 [13.2 - 14.9]	14.5 [13.9 - 18.7]	13.9 [13.1 - 14.7]	0.004
PT-last, seconds	14.2 [13.5 - 15.1]	15.4 [14.2 - 18.6]	14.1 [13.4 - 14.9]	0.001
PTT-bl, seconds	29.4 [27.8 - 31.2]	30.0 [28.8 - 33.6]	29.4 [27.6 - 31.0]	0.058
PTT-last, seconds	29.3 [27.6 - 31.2]	29.9 [27.9 - 32.1]	29.2 [27.4 - 31.0]	0.169
Fib-4-bl	2.95 [1.87 - 4.47]	7.05 [5.11 - 11.10]	2.59 [1.73 - 3.59]	<0.001
Fib-4-last	1.51 [0.99 - 3.03]	7.09 [2.85 - 15.80]	1.31 [0.94 - 2.18]	<0.001

Abbreviations: CLD=chronic liver diseases; CVD=cardiovascular diseases; CKD=chronic kidney diseases; ALP=alkaline phosphatase; bl=baseline; ALT=alanine aminotransferase (normal value <59 IU/L); AST=aspartate aminotransferase (normal values <35 IU/L); GGT=gamma glutamyl transferase (normal value <68 IU/L); N/L=neutrophils/ lymphocytes ratio; PT=prothrombin time; INR=International Normalized Ratio; PTT=partial thromboplastin time; CRP=c-reactive protein (normal values <6 mg/L).
Results described by median (IQR) or frequency (%); p-values was performed by chi-square or Fishers' exact test and Wilcoxon rank-sum test, as appropriate.

Table 3 - *Risk of mortality in the cohort of patients with COVID-19.*

Variable	Univariable Logistic Regression				Multivariable Logistic Regression			
	Crude Odds Ratio	Lower limit	Upper limit	Pvalue	Adjusted Odds Ratio	Lower limit	Upper limit	Pvalue
CLD (Yes vs. No)	2.45	1.03	5.84	0.043	4.04	1.29	12.70	0.017
Age (per 1-year older)	1.12	1.07	1.19	<.0001	1.15	1.07	1.23	<.0001
Sex (Males vs Females)	2.21	0.84	5.83	0.109	6.40	1.64	24.90	0.007
Creatinine (per 0.3 mg/dL higher)	6.85	3.08	15.26	<.0001	1.56	1.20	2.04	0.001

Abbreviation: CLD=Chronic Liver Diseases.

and higher Fib-4 index (p<0.001). Last available laboratory tests at discharge or death showed significantly higher liver enzymes (ALP, p=0.014; AST, p<0.001), worse functional liver tests (bilirubin, p= 0.001; PT in seconds, p=0.004; PT-INR, p=0.001), higher creatinine levels (p=0.001), higher direct or indirect markers of inflammation (CRP, p<0.001; neutrophils count, p<0.001; N/L ratio, p<0.001), lower lymphocytes (p<0.001) and PLT (p<0.001) count and highly increased Fib-4 score (7.09 (IQR 2.85 - 15.80) vs. 1.31 (IQR 0.94 - 2.18); p<0.001) in patients who died compared to the counterpart of alive patients (*Table 2*).

Analysis of potential predictive markers of mortality

At univariable logistic regression analyses, mortality was associated with CLD (OR 2.45; 95% CI 1.03-5.84; p=0.043), age (per 1-year older) (OR 1.12; 95% CI 1.07-1.19; p<.0001) and creatinine levels (per 0.3 mg/dL increase) (OR 6.85; 95%CI 3.08-15.26; p<.0001) (*Table 3*).

The multivariable logistic regression model confirmed that pre-existing liver diseases were associated with a higher risk of mortality, with an OR of 4.04 (95% CI 1.29-12.70, p= 0.017). Other variables associated with a higher risk of mortality were age (OR 1.15, 95% CI=1.07-1.23; p<.0001), male sex (OR 6.40, 95% CI=1.64-24.90; p=0.007) and creatinine levels x 0.3 mg/dL increase (OR 1.56, 95% CI=1.20-2.04; p=0.001) (*Table 3*).

DISCUSSION

We investigated the outcome of patients with pre-existing chronic liver diseases and without liver diseases belonging to a cohort of 1210 patients with COVID-19 in a region of North Italy (Lombardy) with high incidence of SARS-CoV-2 infection. We found 3.4% of patients with concomitant CLD in our COVID-19 cohort, which is similar to the worldwide prevalence already reported in two different meta-analysis studies (Kovalic et al., 2020; Mantovani *et al.*, 2020).

In accordance with several reports (Berenguer *et al.*, 2020; Lumish *et al.*, 2022; Surendra *et al.*, 2023), we showed that older age, male sex, and kidney diseases were predictors of poor outcome in our in-hospital cohort of patients with COVID-19. Most importantly, we found that patients in the CLD group had a significantly higher risk of mortality compared to matched controls. This finding was consistent in our CLD group, which included patients with mild chronic liver diseases and those with cirrhosis with relatively preserved liver function, and was robust in the analysis calculated after propensity score-matching for comorbidities known to be associated with an increased risk of mortality. Reports exploring the outcome of COVID-19 in people with pre-existing liver diseases are limited and conflicting, with some studies indicating a worse outcome in CLD patients with COVID-19 (Gao *et al.*, 2020; Oyelade *et al.*, 2020; Singh et Khan, 2020; Mitrovic *et al.*, 2023) while others have found no association (Kulkarni *et al.*, 2020; Simon *et al.*, 2021). Direct damage of the liver by the virus, inflammatory response, and respiratory distress syndrome-induced hypoxia are the main causative factors of liver damage during COVID-19 (Sharma *et al.*, 2021; D'Ardes *et al.*, 2021). Direct liver injury is likely consequent to expression of angiotensin-converting enzyme 2 (ACE2) on hepatocytes, cholangiocytes, and sinusoidal endothelial cells.

In our CLD-group we showed worse liver function tests at admission in comparison to patients in the non-CLD group. We found abnormal values of hepatic enzymes ALP and GGT. Notably, 32% of cases in our series of CLD patients had fatty liver disease or alcoholic liver disease in which higher levels of these enzymes and in particular GGT may be prevalent compared with those with viral aetiology in CLD compared to non-CLD.

It has recently been postulated (Idalsoaga *et al.*, 2021; Planès *et al.*, 2022) that SARS-CoV-2 infection could determine indirect damage via a sudden systemic inflammatory response. The increase in pro-inflammatory cytokine has a prominent role in seriously ill patients.

We evaluated CRP as an unspecific inflammatory marker that was found lower in the CLD group compared to the non-CLD group. Therefore, this modality of indirect liver damage seemed not to be prominent in our CLD group. However, we did not specifically evaluate pro-inflammatory cytokines and inflammatory biomarkers such as interleukin-2 (IL-

2), interleukin 6 (IL-6), interferon-γ, ferritin and tumour necrosis factor, which may be involved in liver injury during COVID-19 (Huang *et al.*, 2020). Given that immune dysfunction resulting in an immunodeficiency phenotype (Albillos *et al.*, 2014) is a frequent event in cirrhotic patients, it is possible that the lower levels of inflammatory biomarker CRP found in our CLD group were consistent with the presence of cirrhosis in the CLD group (39% of patients).

We also evaluated clinical and laboratory findings at admission and at discharge or death: we found that at admission (baseline evaluation) patients who died had worse liver and kidney function and higher CRP, with a significantly higher median N/L ratio; these two latter parameters represent markers of elevated systemic inflammation. A similar chemistry profile was observed at last laboratory tests available before death. A previous report (Salciccioli *et al.*, 2015) showed that N/L ratio is important in multiple patient populations, including non-critically ill populations. In our group of patients with COVID-19 who died, N/L ratio elevation was seen concomitantly with CRP increase at last evaluation but not at baseline, supporting the hypothesis that these inflammatory biomarkers reflect the increasing severity of the underlying systemic disturbance (Gurusamy *et al.*, 2021; Rathod *et al.*, 2022).

Notably, respiratory failure was the main cause of death in our cohort. Therefore, we cannot exclude that the worse biochemical profile at death was consequent to multiple-organ-failure, caused by respiratory distress syndrome-induced hypoxia. However, liver and kidney biochemistry was also found abnormal at baseline evaluation, possibly before severe hypoxic damage. Recent reports (Liu *et al.*, 2022; Mendizabal *et al.*, 2021) showed that high liver fibrosis scores and in particular Fib-4 index, composed of age, AST, ALT and PLT count, are associated with worse prognosis in patients with COVID-19, suggesting that assessment of this indirect and non-invasive liver fibrosis marker might be useful for early identification of patients at high risk of developing severe COVID-19. Another interesting study (Sterling *et al.*, 2022) suggested that, in the general population with COVID-19, the Fib-4 index may reflect systemic inflammation rather than a measure of hepatic fibrosis and may be associated with poor outcome. Interestingly, in patients who died we found Fib-4 values significantly higher at baseline as well as at death compared with patients with a favourable outcome, suggesting a possible role of this indirect marker of liver fibrosis on the adverse outcome. However, Fib-4 was developed in a cohort of subjects that did not include very old patients; therefore, it may not perform as well in this population. Therefore, confounding factors such as age, which was markedly higher in the group of deceased patients, may have contributed to high Fib-4 values in patients with adverse clinical outcome. Additionally, 11/27 patients in the matched group of dead patients had CLD; therefore this condition may have influenced Fib-4 values.

We are aware that the study suffers from some limitations. It is a retrospective analysis that was conducted in a tertiary health-care centre that was designed as a SARS-CoV-2 hub, so it is likely that there is reporting bias with over-representation of cases suffering from other comorbidities or more severe COVID-19. However, we considered two groups of patients (CLD and non-CLD) that were balanced for several covariates (age, gender, BMI, DM2, hypertension) recognized as being associated with a worse outcome of COVID-19. One other limitation is the small number of patients with CLD. Although this is a single-centre study, the prevalence of CLD we found in our hospital is similar to the worldwide prevalence already reported in various meta-analysis studies.

Finally, we had no information on functional reserve of the liver evaluated by Child-Pugh-Turcotte classification in patients with cirrhosis. However, the MELD score was available in our patients with cirrhosis, providing information on the severity of liver disease and prognosis.

CONCLUSION

Our study showed that patients with pre-existing chronic liver diseases have a worse outcome with an increased risk of mortality. Other associated factors were older age, male sex and kidney disfunction. The finding of poor outcome among COVID-19 patients with chronic liver diseases deserves further investigation; however, it appears to be an interplay of local liver injury and increase of systemic inflammation. Physicians and hepatologists should be aware of the potential worse outcome of COVID-19 in patients with mild or moderate liver diseases, in addition to those with advanced liver diseases.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

G.M., M.R. designed the research and wrote the paper; L.G., D.C., R.L. collected and reviewed data and performed statistical analyses; C.B., A.S., E.M., H.H., A.C., C.U.F. reviewed the manuscript.

Acknowledgements

We thank all the doctors, nurses and site personnel.

Patient consent statement

The study was approved by the Ethics Committee of San Raffaele Hospital (protocol No. 34/int/2020) and was registered on ClinicalTrials.gov (NCT04318366).

All patients signed an informed consent form. Our research was in accordance with the Declaration of Helsinki.

References

Albillos A., Lario M., Álvarez-Mon M. (2014). Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol*. **61**, 1385-1396.

Berenguer J., Ryan P., Rodriguez-Bano J., Carratalà J., Jarrín I., et al. (2020). Characteristics and predictors of death among 4035 consecutively hospitalized patients with COVID-19 in Spain. *Clin Microbiol Infect*. **26**, 1525-1536.

D'Ardes D., Boccatonda A., Cocco G., Fabiani S., Rossi I., et al. (2022). Impaired coagulation, liver dysfunction and COVID-19: discovering an intriguing relationship. *World J Gastroenterol*. **28**, 1102-1112.

Gao F., Zheng K.I., Wang X.B., Yan H.D., Sun Q.F., et al. (2020). Metabolic associated fatty liver disease increases coronavirus disease disease severity in nondiabetic patients. *J Gastroenterol Hepatol*. **36**, 204-207.

Gurusamy E., Mahalakshmi S., Kaarthikeyan G., Ramadevi K., Arumugam P., et al. (2021). Biochemical predictors for SARS-CoV-2 severity. *Bioinformation*. **17**, 834-839.

Huang C., Wang Y., Li X., Ren L., Zhao J., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. **395**, 497-506.

Iavarone M., D'Ambrosio R., Soria A., Triolo M., Pugliese N., et al. (2020). High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol*. **73**, 1063-1071.

Idalsoaga F., Ayares G., Arab J.P., Díaz L.A. (2021). COVID-19 and Indirect Liver Injury: a narrative synthesis of the evidence; *J Clin Transl Hepatol*. **9**, 760-768.

Kovalic A.J., Satapathy S.K., Thuluvath P.J. (2020). Prevalence of chronic liver disease in patients with COVID-19 and their clinical outcomes: a systematic review and meta-analysis. *Hepatol Int*. **14**, 612-620.

Kulkarni A.V., Kumar P., Tevethia H.V., Premkumar M., Arab J.P., et al. (2020). Systematic review with meta-analysis: liver manifestations and outcomes in COVID-19. *Aliment Pharmacol Ther*. **52**, 584-599.

Liu M., Mei K., Tan Z., Huang S., Liu F., et al. (2022). Liver fibrosis scores and hospitalization, mechanical ventilation, severity, and death in patients with COVID-19: A systematic review and dose-response meta-analysis. *Can J Gastroenterol Hepatol*. **29**, 7235860.

Lumish H.S., Kim E., Selvaggi C., Cao T., Gupta A., et al. (2022). Biomarkers of cardiac injury, renal injury, and inflammation are strong mediators of sex-associated death in COVID-19. *Front Cardiovasc Med*. **9**, 809997.

Mantovani A., Beatrice G., Dalbeni A. (2020). Coronavirus disease 2019 and prevalence of chronic liver disease: a meta-analysis. *Liver International*. **40**, 1316-1320.

Mendizabal M., Ridruejo E., Piñero F., Anders M., Padilla M., et al. (2021). Comparison of different prognostic scores for patients with cirrhosis hospitalized with SARS-CoV-2 infection. *Ann Hepatol*. **25**, 100350.

Mitrovic N., Sabanovic M., Vujovic A., Jovanovic J., Nikolic N., et al. (2023). Influence of chronic liver diseases on the course and outcome of COVID-19. *Plos One*. **18,** 7: e0288350.

Namayandeh S.M., Dehghan H., Lotfi M.H., Khajehaminian M.R., Hosseini S., et al. (2023). Clinical courses of 24563 hospitalized COVID-19 patients during the first 12 months of the pandemic in the Central City of Iran. *Sci Rep*. **13**, 6521.

Napodano C., Pocino K., Stefanile A., Marino M., Miele L., et al. (2021). COVID-19 and hepatic involvement: The liver as a main actor of the pandemic novel. *Scand J Immunol*. **93**, e12977.

Oyelade T., Alqahtani J., Canciani G. (2020). Prognosis of COVID-19 in patients with liver and kidney diseases: an early systematic review and meta-analysis. *Trop Med Infect Dis*. **5**, 80.

Planès R., Pinilla M., Santoni K., Hessel A., Hessel A., et al. (2022). Human NLRP1 is a sensor of pathogenic coronavirus 3CL proteases in lung epithelial cells. *Mol Cell*. S1097-2765(22)00433-6.

Portincasa P., Krawczyk M., Machill A., Lammert F., Di Ciaula A. (2020). Hepatic consequences of COVID- 19 infection. Lapping or biting? *Eur J Intern Med*. **77**, 18-24.

Rathod B.D., Amle D., Khot R.S., Amle D., Khot R.S., et al. (2022). Neutrophil-to-lymphocyte ratio as a predictor of disease severity and mortality in Coronavirus disease 2019: prospective study from central India. *Cureus*. **14**, e23696.

Salciccioli D.J., Marshall D.C., Pimentel M.A., Santos M.D., Pollard T., et al. (2015). The association between the neutrophil-to-lymphocyte ratio and mortality in critical illness: an observational cohort study. *Critical Care*. **19**, 13.

Sharma P., Kumar A., Anikhindi S., Bansal N., Singla V., et al. (2021). Effect of COVID-19 on pre-existing liver disease: what hepatologist should know? *J Clin Exp Hepatol*. **11**, 484-493.

Simon T.G., Hagström H.., Sharma R, Söderling J., Roelstraete B., et al. (2021). Risk of severe COVID-19 and mortality in patients with established chronic liver disease: a nationwide matched cohort study. *BMC Gastroenterol*. **21**, 439.

Singh S., Khan A. (2020). Clinical characteristics and outcomes of Coronavirus disease 2019 among patients with preexisting liver disease in the United States: a multicenter research network study. *Gastroenterology*. **159**, 768-771.e3.

Sterling R.K., Lissen E., Clumeck N., Sola R., Correa M.C., et al. (2006). Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology*; **43**, 1317-1325.

Sterling R.K., Oakes T., Gal T.S., Stevens M.P., deWit M., et al. (2020). The fibrosis-4 index is associated with need for mechanical ventilation and 30-day mortality in patients admitted with coronavirus disease. *J Infect Dis*. **222**, 1794-1797.

Surendra H., Praptiningsih C.Y., Ersanti A.M., Rahmat M., Noviyanti W., et al. (2023). Clinical characteristics and factors associated with COVID-19-related mortality and hospital admission during the first two epidemic waves in 5 rural provinces in Indonesia: A retrospective cohort study. *PLoS One*. **18**, e0283805.

Vaishnav M., Elhence A., Biswas S., Pathak P., Anand A., et al. (2022). The outcome in cirrhosis after hospital discharge is not worsened with COVID-19 infection: a propensity score-matched analysis. *J Clin Exp Hepatol*. **12**, 830-840.

Wang D., Hu B., Hu C., Zhu F., Liu X., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. *JAMA*. **323**, 1061-1069.

World Health Organization (WHO). Coronavirus disease (COVID-2019) situation reports 2022. Available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. Accessed 4 august 2022.

Zhou F., Yu T., Du R., Fan G., Liu Y., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. **395**, 1054-1062.