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Liver involvement is not associated with mortality: results from a large cohort of SARS-CoV-2-positive patients

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Summary

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is frequently associated with liver test abnormalities.

Aims: To describe the evolution of liver involvement during SARS-CoV-2 infection and its effect on clinical course and mortality.

Methods: Data of 515 SARS-CoV-2-positive patients were collected at baseline and during follow-up, last evaluation or death. Stratification based on need for hospitalisation, severe disease and admission to intensive care unit (ICU) was performed. The association between liver test abnormalities (baseline and peak values) and ICU admission or death was also explored.

Results: Liver test abnormalities were found in 161 (31.3%) patients. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were increased in 20.4%, 19% and 13.6% of patients, respectively. Baseline liver test abnormalities were associated with increased risk of ICU admission (OR 2.19 [95% CI 1.24-3.89], $P = 0.007$) but not with mortality (OR 0.84 [95% CI 0.49-1.41], $P = 0.51$). Alkaline phosphatase (ALP) peak values were correlated with risk of death (OR 1.007 [95% CI 1.002-1.01], $P = 0.005$) along with age, multiple comorbidities, acute respiratory distress syndrome, ICU admission and C-reactive protein. Alterations of liver tests worsened within 15 days of hospitalisation; however, in patients with the longest median follow-up, the prevalence of liver test alterations decreased over time, returning to around baseline levels.

Conclusions: In SARS-CoV-2-positive patients without pre-existing severe chronic liver disease, baseline liver test abnormalities are associated with the risk of ICU admission and tend to normalise over time. The ALP peak value may be predictive of a worse prognosis.

Maurizio Pompili and Antonio Gasbarrini equally contributed to this paper.

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1 | INTRODUCTION

On December 31, 2019, Chinese authorities reported a group of pneumonia cases in Wuhan. A zoonotic infection was suspected, and a novel pathogenic human coronavirus (CoV) with a certain homology with respect to severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV was sequenced and identified as SARS-CoV-2.

The human-to-human transmission of SARS-CoV-2 was rapid, and due to the increase in the number of cases outside China, the World Health Organization (WHO) defined the infection as a pandemic on March 12, 2020. Italy was the second country to be hit after China, but Spain, Germany, France and other European countries, as well as the United States, are also facing heavy consequences of this pandemic.

During the past SARS outbreak, hepatic impairment was described in up to 60% of patients 1, and was associated with elevation of serum transaminases, hypoproteinaemia and prolongation of prothrombin time.

Chinese data on patients with SARS-CoV-2 infection report a prevalence of abnormal liver test as high as 76.3%, while the prevalence in Western patients seems to be lower.[1-4] However, these studies report baseline or short-term follow-up evaluations. Therefore, the evolution of liver involvement, its correlation with patients' mortality or resolution of SARS-CoV-2 infection is still unknown.

In this paper, we report the experience of a tertiary care centre in Italy facing the emergency of the SARS-CoV-2 infection, describing the prevalence and the evolution of liver involvement over time and its impact on patients' clinical course and mortality.

2 | PATIENTS AND METHODS

2.1 | Study design and data collection

All patients aged >18 years tested positive for SARS-CoV-2 infection at the Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome from March 6th to April 16th, 2020 were included in this retrospective study.

Nasopharyngeal swabs for SARS-CoV-2 diagnostic testing were obtained according to the WHO guidelines[5] and analysed in the Microbiology Laboratory of our Hospital (Real-time PCR, *AllplexTM 2019-nCoV Assay* [Seegene]). To exclude the infection or to confirm its resolution, two negative samples must be obtained at least 48 hours apart.

According to the Italian Society for Infectious and Tropical Diseases (SIMIT) guidelines,[6] patients were treated with antivirals (lopinavir/ritonavir or darunavir/ritonavir) plus hydroxychloroquine whereas the anti-interleukin 6 (IL-6) agent tocilizumab was used in selected patients. Acute respiratory distress syndrome (ARDS) was defined as the ratio of arterial oxygen partial pressure (PaO2) to fractional inspired oxygen (FiO2) ≤100 mm Hg.[7]

To investigate the prevalence of liver damage in our cohort of patients, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin and albumin were collected at baseline, then on the date closest to 15 days from admission. At the same timepoints, lactate dehydrogenase (LDH), C-reactive protein (CRP), fibrinogen, D-dimer as inflammatory markers and international normalised ratio (INR), platelets (PLTS), white blood cells (WBC), neutrophils and lymphocytes counts were also recorded. In a subgroup of 53 patients with a longer follow-up (>1 month), a third data timepoint was recorded at the last available evaluation. Peak values of AST, ALT, GGT, ALP and bilirubin achieved during the hospitalisation were recorded. History of liver disease and comorbidities were also assessed.

The study was approved by the Institutional Ethics Committee of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome (ID number: 3042) and was conducted according to the principles of the Declaration of Helsinki.

2.2 | Statistical analysis

Patients' characteristics and laboratory examinations were reported as median and interquartile range (continuous variables) or as frequencies and percentages (categorical variables). Baseline serum levels of liver function test (ie AST, ALT, GGT), which were the main object of our study, were also stratified according to three cut-offs (>ULN; >ULN but up to 3 × ULN) for a better definition of possible alterations. Missing data for each variable in the database accounted for <5% except for AST (12.1%).

Descriptive and inferential statistics were initially carried out on the overall population, then on three different subgroups identified on the basis of (a) need for hospitalisation (b) severity of the disease (c) admission to intensive care unit (ICU). Wilcoxon test for continuous variables and chi-squared test or Fisher exact test for categorical ones were used for group comparisons. The strength of the correlation between liver function test and markers of inflammation was explored using Spearman coefficient, adjusting *P*-values for multiple comparisons (Bonferroni correction). Wilcoxon test for paired samples was used to compare baseline laboratory examinations with those detected during follow-up. Univariate analysis considering ICU admission or death as outcomes was performed, including variables of clinical significance; those with a P-value $< .05$ were then included in the multivariate regression model. In 75 out of the 77 patients admitted to ICU (97.4%) liver tests peak values occurred after ICU admission. For this reason, liver tests peak values were included only in the predictive model for mortality.

The analyses were performed using R statistics program version 3.6.2.

3 | RESULTS

During the study period, 515 patients who tested positive for SARS-CoV-2 infection were admitted to the Emergency Department of our Hospital and included in the following analysis (Table 1).

TABLE 1 Demographic characteristics and laboratory examinations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients. Continuous variables are reported as median (interquartile range), categorical ones as frequency (percentage). Statistically significant comparisons are highlighted in bold

	Overall (515)	Hospitalisation (448)	No-hospitalisation (67)	*P*-value	ARDS (146)	No ARDS (369)	*P*-value	ICU (77)	No-ICU (438)	*P*-value
Age	65 (53-77)	68 (55-78)	52 (43.5-59)	**<0.0001**	69 (58-77)	62 (52-77)	**0.009**	70 (61-77)	63 (52-77)	**0.019**
Sex										**0.003**
Male	323 (62.7)	286 (88.5)	37 (11.5)	0.178	116 (35.9)	207 (64.1)	**<0.0001**	60 (18.6)	263 (81.4)	
Female	192 (37.3)	162 (84.4)	30 (15.6)		30 (15.6)	162 (84.4)		17 (8.9)	175 (91.1)	
BMI (kg/m^2)	25.3 (23.4-27.6)	25.35 (24.43-27.54)	24.83 (24.66-25.04)	0.66	25.46 (24.22-27.71)	25.24 (23.43-27.34)	0.07	25.95 (24.07-28.68)	25.3 (23.43-27.34)	0.08
Comorbidities	350 (68)	321 (71.7)	-	-	109 (74.7)	-	-	56 (72.3)	-	-
1	144 (28.0)	127 (28.3)			42 (28.8)			24 (31.2)		
2	90 (17.5)	79 (17.6)			27 (18.5)			14 (18.2)		
3	59 (11.5)	58 (12.9)			26 (17.8)			12 (15.6)		
>3	57 (11.1)	57 (12.7)			14 (9.6)			6 (7.8)		
WBC (×10^9/L)	6.12 (4.78-8.14)	6.17 (4.76-8.28)	5.91 (4.80-7.19)	0.26	6.37 (4.76-8.68)	6.07 (4.79-7.85)	0.27	6.89 (5.10-9.44)	6.02 (4.76-7.89)	**0.010**
Neutrophils (×10^9/L)	4.54 (3.30-6.32)	4.69 (3.38-6.54)	4.22 (3.14-5.11)	**0.017**	4.82 (3.58-7.23)	4.40 (3.21-6.10)	**0.016**	5.77 (4.03-8.03)	4.40 (3.25-6.06)	**0.0002**
Lymphocytes (×10^9/L)	1.03 (0.76-1.36)	1.01 (0.75-1.33)	1.18 (0.85-1.54)	**0.017**	0.87 (0.65-1.18)	1.09 (0.79-1.46)	**<0.0001**	0.80 (0.64-1.14)	1.07 (0.79-1.42)	**<0.0001**
Platelets (×10^9/L)	196 (156-247)	195 (155-247)	198 (162-236)	0.68	184 (151-241)	199 (158-249)	0.17	182 (143-246.5)	196 (158-247)	0.27
Abnormal liver test	161 (31.3)	154 (34.4)	7 (10.4)	**<0.0001**	62 (42.5)	99 (26.8)	**<0.0001**	41 (53.2)	120 (27.4)	**<0.0001**
AST (IU/L)	31 (22-48)	31 (23-48)	31 (19-38)	0.67	33 (23-55)	31 (23-46)	0.60	37 (24-60)	31 (22-44)	0.244
AST > ULN	105 (20.4)	98 (21.9)	7 (10.4)	-	44 (30.1)	61 (16.5)	-	27 (35.1)	78 (17.8)	-
AST up to 3 × ULN	96 (18.6)	89 (19.9)	7 (10.4)		41 (28.1)	55 (14.9)		24 (31.2)	72 (16.4)	
AST >3 × ULN	9 (1.7)	9 (2.0)	0 (0.0)		3 (2.1)	6 (1.6)		3 (3.9)	6 (1.4)	
ALT (IU/L)	27 (16-40)	27 (16-40)	25 (16-35)	0.22	32 (21-48)	24 (15-37)	**<0.0001**	35 (21-53)	25 (16-38)	**0.0004**
ALT > ULN	98 (19)	96 (21.4)	6 (9.0)	-	42 (28.8)	60 (16.3)	-	26 (33.8)	76 (17.4)	-
ALT up to 3 × ULN	88 (18)	86 (19.2)	6 (9.0)		40 (27.4)	52 (14.1)		23 (29.9)	69 (15.8)	
ALT >3 × ULN	10 (2)	10 (2.2)	0 (0.0)		2 (1.4)	8 (2.2)		3 (3.9)	7 (1.6)	
GGT (IU/L)	40 (23-62)	40 (25-63)	22 (14-26)	**0.025**	45 (30-65)	33 (20-61)	**0.026**	47 (33-71)	36 (20-60)	**0.001**
GGT > ULN	70 (13.6)	70 (15.6)	0 (0.0)	-	25 (17.1)	45 (12.2)	-	20 (26.0)	50 (11.4)	-
GGT up to 3 × ULN	60 (11.7)	60 (13.4)	0 (0.0)		21 (14.4)	39 (10.6)		18 (23.4)	42 (9.6)	
GGT >3 × ULN	10 (1.9)	10 (2.2)	0 (0.0)		4 (2.7)	6 (1.6)		2 (2.6)	8 (1.8)	
ALP (IU/L)	62 (51-79)	62 (51-79)	80 (70-88)	0.22	58 (48-74)	65 (52-83)	**0.024**	60 (49-82)	63 (51-78)	0.60
Bilirubin (mg/dL)	0.6 (0.4-0.8)	0.6 (0.4-0.8)	0.6 (0.5-0.9)	0.92	0.7 (0.5-0.9)	0.6 (0.4-0.8)	**0.007**	0.7 (0.5-0.9)	0.6 (0.4-0.8)	**0.019**
Albumin (g/L)	33 (29-36)	32 (29-36)	43 (41-46)	**<0.0001**	31 (28-35)	34 (30-36)	**0.009**	30 (27-32)	33 (30-36)	**<0.0001**
CPK (IU/L)	111 (68-197)	117 (67-218)	86 (73-112)	**0.009**	141 (90-299)	103 (60-188)	**<0.0001**	160.5 (83.25-349.75)	112 (63-195)	**0.0004**
LDH (IU/L)	305 (234-415)	317 (244-430)	217 (186-309)	**<0.0001**	371 (293-467)	278 (217-371)	**<0.0001**	396 (305-638)	288 (223-392)	**<0.0001**
CRP (mg/L)	62.8 (22.2-134.7)	73.8 (25.9-143.4)	13.9 (5.1-42.3)	**<0.0001**	104.7 (39.6-161.5)	46.0 (16.4-116.7)	**<0.0001**	134.4 (74.1-177.5)	52.5 (17.9-121.2)	**<0.0001**

(Continues)

TABLE 1 (Continued)

	Overall (515)	Hospitalisation (448)	No-hospitalisation (67)	*P*-value	ARDS (146)	No ARDS (369)	*P*-value	ICU (77)	No-ICU (438)	*P*-value
INR	1.04 (1.00-1.09)	1.05 (1.00-1.09)	1.02 (0.98-1.07)	**0.003**	1.06 (1.02-1.10)	1.04 (1.00-1.08)	**<0.001**	1.07 (1.03-1.09)	1.04 (1.00-1.09)	**0.008**
Fibrinogen (mg/dL)	486 (401-605)	497 (411-613)	402 (330-485)	**<0.0001**	530 (439-656)	467 (387-589)	**<0.0001**	594 (462-703)	475 (394-588)	**<0.0001**
D-dimer (ng/mL)	1040 (587-2539)	1091 (618-2682)	425 (258-541)	**<0.0001**	1417 (762-3359)	959 (522-1824)	**<0.001**	1393 (914-3693)	970 (522-2153)	**0.003**

Note: Laboratory examinations reference range: WBC 4-10 × 10^9/L; Neutrophils 2-7 × 10^9/L; Lymphocytes 1-3 × 10^9/L; PLTS 150-450 × 10^9/L; AST 7-45 IU/L; ALT 7-45 IU/L; GGT 8-61 IU/L; ALP 40-129 IU/L; Bilirubin 0.3-1.2 mg/dL; Albumin 34-48 g/L; CPK 30-170 IU/L; LDH <250 IU/L; CRP <5 mg/L; INR 0.8-1.2; Fibrinogen 200-400 mg/dL; D-dimer <500 ng/mL.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CPK, creatine phosphokinase; CRP, C-reactive protein; GGT, gamma glutamyl transferase; INR, international normalised ratio; LDH, lactate dehydrogenase; PLTS, platelets; WBC, white blood cells.

Median age was 65 years (53-77), with a prevalence of men (62.7%). All patients reported flu-like symptoms, such as fever (487 [94.6%]), cough (327 [63.5%]), fatigue (46 [8.9%]) and muscle pain (11 [2.1%]); gastrointestinal symptoms were observed in 49 patients (9.5%) and included diarrhoea (34), vomiting (8), abdominal pain (3), rectal bleeding (3). Ageusia and/or anosmia (7 [1.4%]) and conjunctivitis (6 [1.2%]) were also reported. Dyspnoea was present in 229 (44.5%) patients. In 415 patients (80.6%) radiological signs of pneumonia were observed at the first clinical evaluation.

A clear epidemiological link could be identified in up to 213 (41.4%) patients; contacts with positive subjects (102 [19.8%]), stay in long-term care facilities (49 [9.5%]) and recent travel in highly endemic areas (33 [6.4%]) were the most common ones. The prevalence of healthcare workers who tested positive was 2.9%.

At least one comorbidity could be recognised in about 68% of SARS-CoV-2-positive patients included in this cohort, and 58.8% of them presented more than one pre-existing pathologic condition. The most commonly associated ones were: arterial hypertension (168 [48%]), cardiovascular disease (100 [28.6%]), diabetes mellitus (60 [17.1%]), neurological diseases (57 [16.3%]), obesity (42 [12%]), chronic obstructive pulmonary disease (39 [11.1%]), tumours (38 [10.8%]), chronic renal disease (16 [4.6%]), psychiatric disorders (12 [3.4%]). Previously known chronic liver disease without clinical and blood chemistry signs of decompensated cirrhosis and including chronic hepatitis B virus (HBV) and human immunodeficiency virus (HIV) coinfection in two cases, chronic hepatitis C virus (HCV) infection in other two cases, autoimmune hepatitis in one case and non-alcoholic fatty liver disease (NAFLD) in another case, represented a minor comorbid condition (1.2%).

Four-hundred-forty-eight (87%) patients required hospitalisation, and, among them, 146 (32.6%) presented with ARDS and 77 (15%) required ICU admission. Overall, liver test abnormalities were found in 161 (31.3%) patients. Increase in AST, ALT and GGT above ULN was found in 20.4%, 19% and 13.6%, respectively; these alterations were mild/moderate (lower than 3 × ULN), and in only 5% of cases a more severe increase, above 3 × ULN, was observed. Relevant alterations in ALP or bilirubin serum levels were observed in a minority of patients (Table 1).

The prevalence of liver test alteration was higher in patients with ARDS (62 [42.5%]) and in those requiring admission to ICU (41 [53.2%]). AST or ALT elevation was more frequent than GGT elevation, being present in up to 30% of those with ARDS and 35% of those admitted to ICU. Conversely, only 10.4% of the patients who were not hospitalised presented liver involvement (Table 1).

Peak values that occurred during hospitalization were AST 40.5 (25-66) IU/L, ALT 47 (24-104) IU/L, GGT 48 (26-88) IU/L, ALP 74 (57-100) IU/L and bilirubin 0.8 (0.6-1.1) mg/dL. No cases of severe liver injury were reported; antiviral therapy with lopinavir/ritonavir was stopped in one patient aged 87 due to the severe increase of ALT serum levels (ALT 537 IU/L), without evidence of significant cholestatic damage (GGT 169 IU/L, ALP 58 IU/L, bilirubin 0.6 mg/dL). This patient died 6 days later due to severe respiratory dysfunction.

Indirect markers of systemic inflammation such as CRP, fibrinogen and D-dimer were increased in all patients at baseline and were higher in patients requiring hospitalisation, those with ARDS or admitted to ICU. Albumin serum levels were decreased (33 [29-36] g/L), especially in patients with ARDS (31 [28-35] g/L) and in those admitted to ICU (30 [27-32] g/L). AST, ALT and GGT were weakly associated with inflammatory parameters at baseline (Tables S1-S4).

After a median follow-up of 16 (10-26) days, 77 (15%) of the hospitalised patients died, 410 (79.6%) were discharged and 28 (5.4%) were still hospitalised. Liver test alterations at baseline were associated with higher risk of ICU admission (OR 2.19 [95% CI 1.24-3.89], $P = 0.007$; Table 2) but not with death (OR 0.84 [95% CI 0.49-1.41], $P = 0.51$; Table 3). As shown in Figure 1, the cumulative incidence of death or discharge was similar between patients with or without liver test abnormalities at baseline. Older age, the presence of multiple comorbidities, ICU admission, high CRP and ALP peak values were, instead, associated with an increased risk of mortality in our multivariate regression model (Table 3).

We then analysed the evolution of liver involvement during hospitalisation. Liver test recorded within 15 days after admission or at

TABLE 2 Univariate analysis and multivariate logistic regression model considering intensive care unit (ICU) admission as outcome. Statistically significant comparisons are highlighted in bold

	Univariate		Multivariate	
Variable	**Odds ratio (95% CI)**	***P*-value**	**Odds ratio (95% CI)**	***P*-value**
Age	1.01 (0.99-1.03)	0.195	-	-
Sex (male vs female)	2.26 (1.29-4.14)	**0.005**	1.34 (0.68-2.72)	0.402
Comorbidities				
One vs none	1.18 (0.62-2.26)	0.62	-	-
Two vs none	1.09 (0.51-2.27)	0.83	-	-
Three vs none	1.32 (0.58-2.87)	0.49	-	-
More than three vs none	0.59 (0.21-1.48)	0.29	-	-
ARDS	7.12 (4.19-12.44)	**<0.0001**	6.09 (3.42-11.17)	**<0.0001**
Baseline abnormal liver test (yes vs no)	2.60 (1.58-4.30)	**0.0002**	2.19 (1.24-3.89)	**0.007**
CRP	1.006 (1.003-1.009)	**<0.0001**	1.005 (1.001-1.008)	**0.002**
Albumin	1.004 (0.99-1.01)	0.26	-	-
WBC	1.00 (0.99-1.00)	0.08	-	-
Lymphocytes	0.99 (0.998-0.999)	**0.004**	0.99 (0.99-1.00)	0.29
D-dimer	1.00 (0.99-1.00)	0.45	-	-

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, gamma glutamyl transferase; WBC, white blood cells.

TABLE 3 Univariate analysis and multivariate logistic regression model considering patients' death as outcome. Statistically significant comparisons are highlighted in bold

	Univariate		Multivariate	
Variable	**Odds ratio (95% CI)**	***P*-value**	**Odds ratio (95% CI)**	***P*-value**
Age	1.11 (1.08-1.14)	**<0.0001**	1.18 (1.12-1.26)	**<0.0001**
Sex (male vs female)	1.21 (0.73-2.08)	0.46	-	-
Comorbidities				
One vs none	2.49 (0.97-7.26)	0.07	2.51 (0.52-14.1)	0.26
Two vs none	5.12 (1.99-14.87)	**0.002**	3.81 (0.74-23.20)	0.12
Three vs none	8.36 (1.99-14.87)	**<0.0001**	5.91 (1.14-37.09)	**0.04**
More than three vs none	14.66 (5.86-42.33)	**<0.0001**	10.19 (1.93-66.57)	**0.009**
ARDS	2.53 (1.53-4.18)	**0.0003**	1.77 (0.68-4.63)	0.23
ICU admission	5.15 (2.97-8.94)	**<0.0001**	6.68 (2.44-19.72)	**0.0003**
Baseline Abnormal liver test (yes vs no)	0.84 (0.49-1.41)	0.51	-	-
AST peak value	1.002 (0.99-1.004)	0.17	-	-
ALT peak value	1.00 (0.99-1.002)	0.75	-	-
GGT peak value	1.001 (0.99-1.003)	0.15	-	-
ALP peak value	1.004 (1.001-1.007)	**0.007**	1.007 (1.002-1.01)	**0.005**
Bilirubin peak value	1.39 (1.03-1.96)	**0.04**	-	-
CRP	1.009 (1.006-1.01)	**<0.0001**	1.007 (1.001-1.01)	**0.008**
Albumin	0.87 (0.82-0.92)	**<0.0001**	0.96 (0.87-1.005)	0.494
WBC	1.00 (1.00-1.00)	**0.0002**	-	-
Lymphocytes	1.00 (0.99-1.00)	0.52	-	-
D-dimer	1.00 (1.00-1.00)	**0.02**	-	-

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, gamma glutamyl transferase; ICU, intensive care unit; WBC, white blood cells.

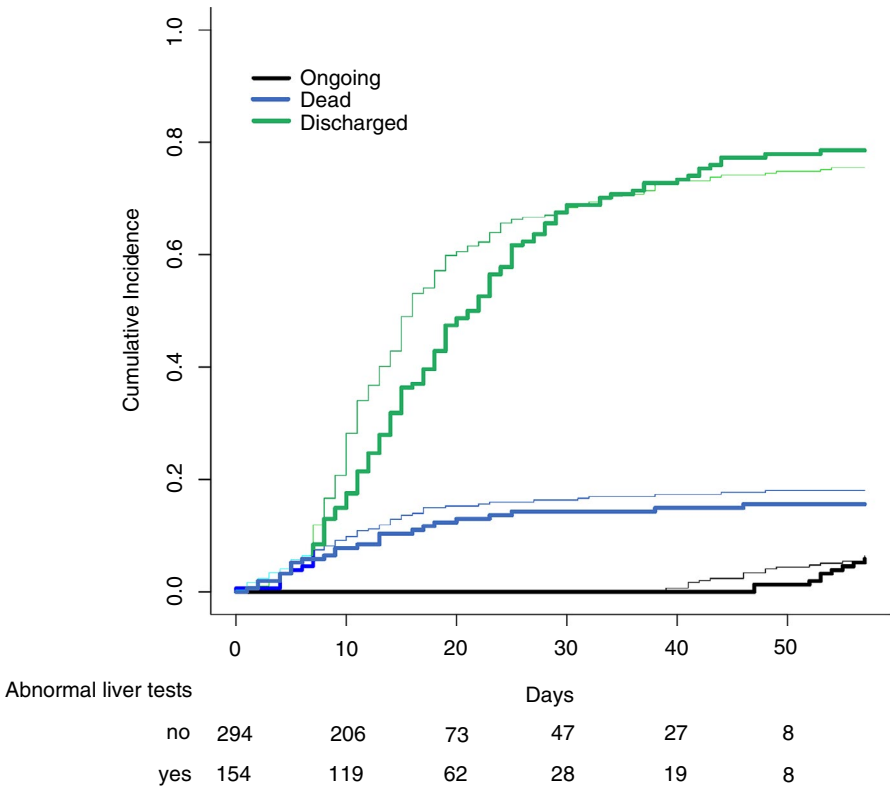

FIGURE 1 Cumulative incidence of events in hospitalised patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Green line: discharged patients; blue line: deceased patients; black line: patients who were still hospitalised. Bold lines and thin lines represent patients with abnormal or normal liver test respectively

the last evaluation for patients who were discharged or died, showed a worsening trend in all groups (Table 4). Conversely, inflammatory parameters decreased significantly, except for D-dimer. In the 53 patients with the longest median follow-up (36 [25-53] days) the last available data were also acquired. Interestingly, although the proportion of patients with liver function test abnormalities was higher at the first timepoint (within 15 days after admission), it returned similar to baseline at the last evaluation, suggesting a progressive normalisation (Figure 2).

One hundred and six (20.6%) patients had a negative swab at the time of the last evaluation. At that time, the prevalence of liver test abnormalities between patients with positive or negative nasopharyngeal swabs was not significantly different (150/343 [43.7%] vs 41/105 [39%], respectively; $P = 0.43$).

4 | DISCUSSION

This study demonstrates that in patients without severe chronic liver disease liver involvement during SARS-CoV-2 infection is usually mild, is associated with increased risk of ICU admission but not with mortality and tends to resolve over time.

We found a 31.3% prevalence of liver test abnormalities in patients with SARS-CoV-2 infection, which was slightly lower than reported in previous Western[2,3] and Chinese studies .[4] Pure cholestatic alterations characterised by the increase of both ALP and GGT were extremely rare, whereas GGT elevation was present in 13.6% of patients. Noteworthy, all the recorded alterations were mild, did not require any intervention, except withdrawal of antiviral therapy in a single case.

While previous studies mainly addressed liver test abnormalities at baseline or few days after the admission,[2,4] this is the first analysis to include patients with a longer follow-up and to evaluate liver involvement at more than 1 month after the admission. Our study revealed that liver test initially increase during hospitalisation and then improve over time, finally reaching values similar to baseline levels or even lower, as shown in Figure 2 by the trend of the proportion of patients with liver test abnormalities.

This was to be expected in a population of patients affected by a viral disease, but the reasons for liver involvement in patients with SARS-COV-2 infection can be multiple.

The virus itself may probably exert a direct damage to the liver. Post-mortem liver tissue examination from patients affected by SARS-CoV-2 infection showed mild and nonspecific inflammatory infiltration.[4,8,9] Viral cytopathic effect is exerted on both liver cells and cholangiocytes; indeed, SARS-CoV-2 binds the angiotensin-converting enzyme 2 (ACE2) receptor to enter the cells,[10] which is mainly expressed on cholangiocytes, vascular endothelium and smooth muscle cells.[11,12] Therefore, both vascular and cholangiocellular damage may represent the reasons for the increase in transaminases and cholestasis parameters in infected patients. However, we demonstrated that liver test tend to normalise regardless of the positive or negative result of the final nasopharyngeal swabs. This suggests that liver injury due to direct viral cytopathic effect is usually of mild entity, and is involved in the development of liver tests abnormalities mostly in the early phases of the infection. On the other hand, histological features

TABLE 4 Laboratory examinations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients at baseline (T0) and within 15 days after admission or at last evaluation or death (T1). Continuous variables are reported as median (interquartile range), categorical ones as frequency (percentage). Statistically significant comparisons are highlighted in bold

	Hospitalisation T0 (448)	Hospitalisation T1 (448)	*P*-value	ARDS T0 (146)	ARDS T1 (146)	*P*-value	ICU patients T0 (77)	ICU patients T1 (77)	*P*-value
AST (IU/L)	31 (23-48)	33 (23-52.7)	0.36	33 (23-55)	39 (31-57.5)	0.12	37 (24-60)	47 (30.2-66)	0.07
ALT (IU/L)	27 (16-40)	32 (19-65.7)	**<0.0001**	32 (21-48)	51 (27-89.75)	**0.04**	35 (21-53)	54 (23.7-94.5)	0.09
GGT (IU/L)	40 (25-63)	40 (26-76)	0.22	45 (30-65)	47.5 (32.2-82.75)	0.55	47 (33-71)	59.5 (43-111)	0.08
ALP (IU/L)	62 (51-79)	65 (52-84)	0.08	58 (48-74)	62 (51-84)	**0.006**	60 (49-82)	69.5 (54.7-90.7)	**0.16**
Bilirubin (mg/dL)	0.6 (0.4-0.8)	0.6 (0.4-0.8)	0.36	0.7 (0.5-0.9)	0.6 (0.4-0.8)	**0.009**	0.7 (0.5-0.9)	0.6 (0.4-0.8)	**0.01**
WBC ($\times 10^9$/L)	6.17 (4.76-8.28)	6.14 (4.57-8.68)	0.83	4.82 (3.58-7.23)	6.56 (4.28-9.88)	0.49	5.77 (4.03-8.03)	9.74 (7.05-14.12)	0.06
Lymphocytes ($\times 10^9$/L)	1.01 (0.75-1.33)	1.17 (0.88-1.59)	**<0.0001**	0.87 (0.65-1.18)	0.98 (0.70-1.35)	**<0.0001**	0.80 (0.64-1.14)	0.97 (0.72-1.36)	**<0.0001**
Albumin (g/L)	32 (29-36)	31 (27-34)	**<0.0001**	31 (28-35)	29 (25-34)	**0.03**	30 (27-32)	26 (23-30)	**0.02**
LDH (IU/L)	317 (244-430)	251 (207.5-341.5)	**<0.0001**	371 (293-467)	303.5 (235-416.8)	**<0.0001**	396 (305-638)	344 (280-419)	**<0.0001**
CRP (mg/L)	73.8 (25.9-143.4)	23.3 (6.3-89.3)	**<0.0001**	104.7 (39.6-161.5)	30.6 (5.6-123.75)	**<0.0001**	134.4 (74.1-177.5)	71.5 (10.5-159.3)	**<0.0001**
Fibrinogen (mg/dL)	497 (411-613)	437 (324-567.5)	**<0.0001**	530 (439-656)	410 (292.8-585.5)	**<0.0001**	594 (462-703)	442 (308-741)	**<0.0001**
D-dimer (ng/mL)	1091 (618-2682)	1585 (750-4232)	**0.001**	1417 (762-3359)	2118 (1060-4929)	0.06	1393 (914-3693)	4353 (2194-8826)	0.09

Note: Laboratory examinations reference range: AST 7-45 IU/L; ALT 7-45 IU/L; GGT 8-61 IU/L; ALP 40-129 IU/L; Bilirubin 0.3-1.2 mg/dL; Albumin 34-48 g/L; LDH <250 IU/L; CRP <5 mg/L; Fibrinogen 200-400 mg/dL; D-dimer <500 ng/mL.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, gamma glutamyl transferase; LDH, lactate dehydrogenase; WBC, white blood cells.

reported by previous studies may be consistent with the occurrence of drug-induced liver injury (DILI) related to antiviral agents or to other drugs used during hospitalisation[4,13]; a possible contribution of these mechanisms to the alteration of liver test also in our cohort of patients should not be disregarded.

Liver damage can also be consequent to inflammation associated with cytokine storm during viral infection. Indeed, SARS-CoV-2-related disease can evolve through different stages, starting as a mild viral infection with few or no symptoms, potentially progressing to pneumonia and to systemic extra-pulmonary hyperinflammation syndrome.[14] At this stage, serum levels of IL-6 and of other inflammatory mediators such as CRP, ferritin and D-dimer are significantly elevated, whereas a parallel decrease in serum albumin is usually observed. Although in our population the association between liver test and indirect markers of inflammation was weak, we recently reported a strict correlation with serum levels of IL-6, with the highest increase recorded within 15 days after patients' admission.[15] Probably, IL-6 serum levels are more sensitive indicators of the persistence of the inflammatory response than nonspecific inflammatory parameters.

In our population of patients, abnormal liver test were correlated with ICU admission, probably reflecting a more severe evolution of the disease, as previously reported.[4] We also demonstrated that age, multiple comorbid conditions, ARDS, ICU admission and CRP serum levels, but not abnormal baseline liver tests, were associated with the risk of death. Therefore, the presence of other negative prognostic factors is crucial to increase the risk of mortality during SARS-CoV-2 inflammatory syndrome, of which abnormal liver test are a collateral manifestation. The prognostic significance of ALP peak value should be underscored, as also other studies reported an association between clinical deterioration and increased ALP serum levels, but not with other liver test.[16,17] As previously discussed, SARS-CoV-2 causes cholangiocytes injury in experimental models[12]; ALP peak value could be a marker of virus-related liver injury and, therefore, this event could be associated with an unfavourable prognosis. Although this hypothesis is intriguing, multiorgan failure and drug-induced cholangiocellular damage could also explain the association between ALP peak values and mortality.

This study is one of the few large available studies exploring the outcome of liver involvement during SARS-CoV-2 infection in Western patients. Although data were collected in a single centre, being a guarantee of their homogeneity and of treatment approach, this study suffers of the generic limitations related to the retrospective collection of data. In particular, the prevalence of chronic liver disease was low in our study population, but we cannot exclude that some patients (eg those with metabolic comorbidities) could be affected by liver disease. However, patients' records were accurately revised, and based on laboratory examinations, radiological

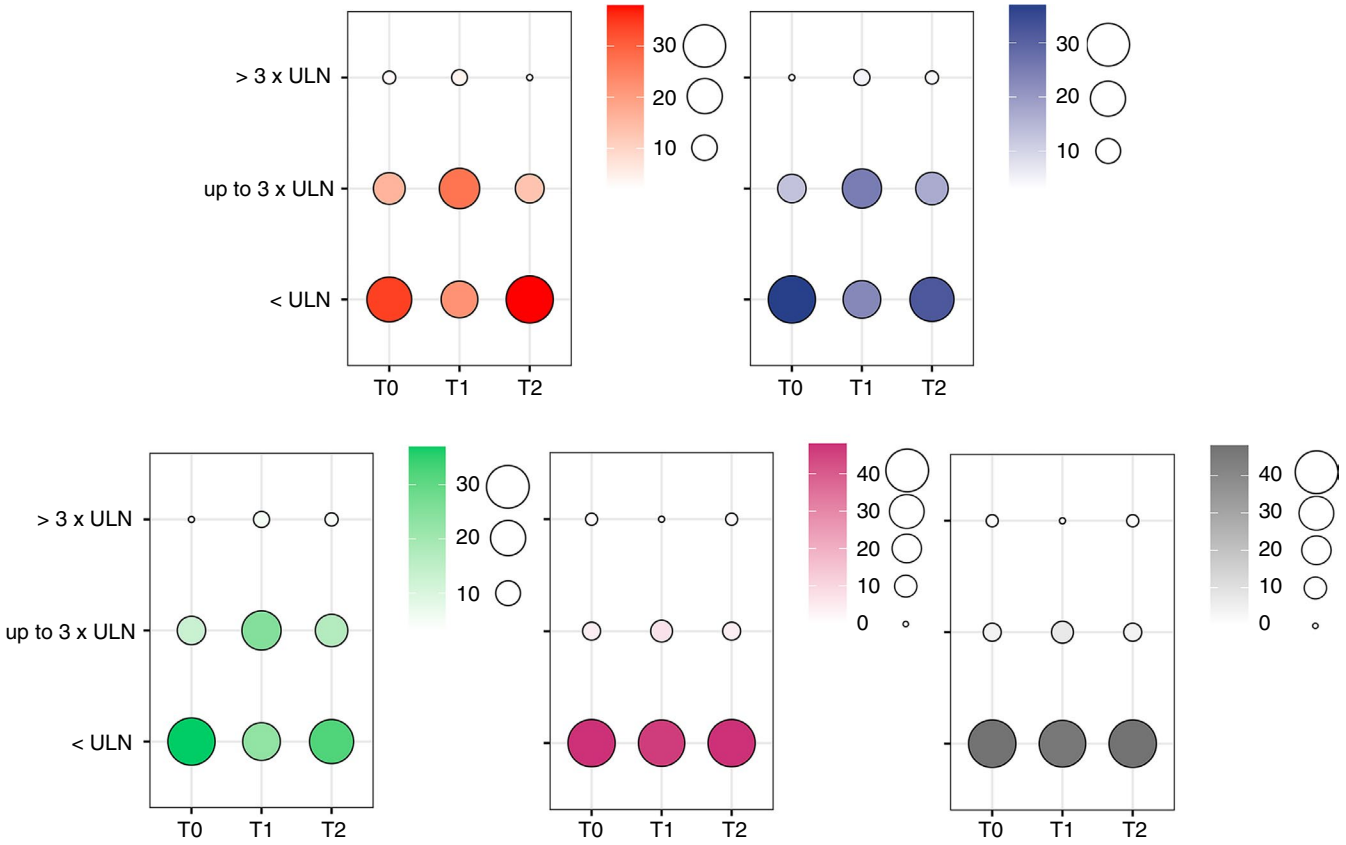

FIGURE 2 Bubble plot showing the evolution of liver test in 53 hospitalised patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and long follow-up. Colour intensity and size of the circles are proportional to the number of patients with or without liver test alterations (aspartate aminotransferase [AST], red; alanine aminotransferase [ALT], blue; gamma glutamyl transferase [GGT], green; alkaline phosphatase [ALP], purple; total bilirubin, grey) at baseline (T0), within 15 days after admission (T1) and at the last evaluation or death (T2)

findings and clinical data, we can reasonably rule out that patients with pre-existing advanced liver disease were included in the study group. Therefore, our conclusions can only be applied to patients with SARS-CoV-2 infection without severe chronic liver disease or cirrhosis, for whom high morbidity and mortality have been reported.[3,18]

In conclusion, SARS-CoV-2 infection is not associated with clinically meaningful liver injury in Western patients without advanced chronic liver disease. Baseline liver test abnormalities can be found in more than 30% of cases, especially in patients with ARDS; these alterations are associated with the risk of ICU admission, being a possible indicator of more severe clinical evolution, but not with mortality, and tend to normalise over time. ALP could be a surrogate marker of virus-related liver injury and its peak value seems to be predictive of a worse prognosis.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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