ORIGINAL ARTICLE

Follow-up evaluation of patients with liver test abnormalities detected during SARS-CoV2 infection

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Abstract

Abnormal liver function tests (A-LFTs) during admission for coronavirus disease-19 (COVID-19) are frequent, but its evolution after COVID-19 resolution remains unexplored. We evaluated factors related to A-LFTs during COVID-19 and assessed the liver outcome after patients' discharge. This is a observational study including: (1) retrospective analysis of variables related to A-LFTs during COVID-19; and (2) follow-up evaluation with blood test, transient elastography and liver biopsy in those with persistent A-LFTs. A-LFTs were defined according to CTCAEv4.0. Among 595 patients, 366 (61.5%) showed A-LFTs. The ratio of partial pressure of oxygen and inspired oxygen fraction (P/F) below 200, ferritin ≥1000 ng/mL, male gender and antibiotic and immunomodulatory treatments were related to A-LFTs. Follow-up evaluation was performed in 153 individuals. Persistent A-LFTs at follow-up was similar in patients with/without A-LFTs during admission (14.1% vs. 4.9%, $p =0.104$). Fifteen (93%) and 58 (39%) patients with/without A-LFTs at follow-up showed metabolic fatty liver disease criteria ($p <0.001$), which were histologically confirmed. In conclusion, A-LFTs during COVID-19 were related to infection severity. Abnormalities remitted at follow-up in >80% of patients, and no correlation between A-LFTs at admission and at follow-up was found. Most patients with A-LFTs at follow-up had non-invasive and histologically proven fatty liver disease.

KEYWORDS
COVID-19, follow-up, liver function tests, metabolic-associated fatty liver disease (MAFLD), SARS-CoV2

1 | INTRODUCTION

The first cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) were reported in December 2019 in Wuhan, China. In Barcelona, the first cases of SARS-CoV2 infection were diagnosed at the end of February 2020. As of January 1, 2022, there have been 6.29 million cases of infection and 89,405 deaths by SARS-CoV-2 in Spain,[1] and the pandemic is having a huge damaging impact socially and economically.

Severe acute respiratory syndrome coronavirus 2 is a ribonucleic acid (RNA) virus usually transmitted among humans through the respiratory route with respiratory droplets or small particles. Angiotensin I converting enzyme 2 (ACE2) and serine proteases, such as transmembrane serine protease 2 (TMPRSS2), are key determinants for cell tropism of the virus.[2] Consequently, organs with high ACE2 and TMPRSS2 levels are the most damaged, including the respiratory tract and lungs causing acute respiratory distress syndrome (ARDS), and the digestive tract, inducing nausea, vomiting and diarrhoea.[3,4] However, COVID-19 can also affect other organs such as the brain, heart, circulatory vessels, kidneys and liver.[5]

From the beginning of the pandemic, independent cohorts of patients with SARS-CoV2 infection described liver function tests (LFTs) abnormalities, especially in transaminases levels. Increased transaminases were reported in 21%–35.5% of hospitalized patients in China,[6–8] but meta-analysis of these series estimated lower pooled prevalence rates of 15%[9] and 19%.[10] In the second half of 2020, subsequent large US series reported abnormal aspartate-aminotransferase (AST) in 74%–83.4%, and alanine-aminotransferase (ALT) in 45%–61.6% of COVID-19 patients. However, less than 20% of patients had AST or ALT more than five times the upper limit of normality (ULN).[11,12] Importantly, severe LFTs abnormalities were related to a more severe respiratory infection in all published series.[8,11–13]

Persistent symptomatology several weeks after SARS-CoV2 infection has been reported, and this new medical entity has been named post-acute COVID-19 syndrome (PACS). PACS comprises two sub-groups: first, long-COVID is the presence of symptoms after 4 weeks of infection onset; and second, the presence of sequelae is diagnosed when symptoms persist after 12 weeks of the acute infection, associated to the evidence of irreversible tissue damage.[14,15] Thus far, liver involvement in the course of PACS remains underexplored. In this regard, no data about the evolution of abnormal LFTs (A-LFTs) and liver damage after resolution of the acute infection have been reported so far.

2 | PATIENTS AND METHODS

2.1 | Study aims and design

We aimed at evaluating clinical factors of SARS-CoV2-associated liver impairment and at assessing the liver outcome after resolution of the acute infection. The study was designed as two independent phases. First, in a retrospective step, the first 600 patients admitted with acute SARS-CoV2 infection in our university hospital (Hospital del Mar, Barcelona, Spain) were evaluated. Patients were identified through a retrospective review of medical records from 28th February 2020, to 8th April 2020. Individuals were included if SARS-CoV2 infection was the main admission diagnosis. Patients with biliary diseases, hepatic metastasis or previously confirmed hepatic toxicity were excluded. During admission, oral consent to provide a serum sample and participate in further investigation studies was provided by all patients. Secondly, a follow-up phase was started 6 months after patient's discharge. Patients with known chronic liver disease were excluded of this phase of the study, and the remaining patients included in the retrospective phase were consecutively contacted by phone to undergo a liver evaluation consisting of a blood test, a transient elastography and a medical consultation, whether they presented or not with A-LFTs during admission. All participants provided written informed consent the day of the first visit. The study protocol was approved by the Ethical Committee of our institution 'Comitè Ètic d'Investigació Clínica - Parc de Salut Mar', study reference (2020/9371/I) in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

2.2 | Data collection. Definition of liver test abnormalities and COVID-19 severity

Sociodemographic data, comorbidities (hypertension, diabetes, body mass index) and acute SARS-CoV2 infection data were obtained from medical records and the hospital discharge report. An admission laboratory test to evaluate liver function and inflammatory parameters was performed in all patients. Also, hepatotropic viruses were screened (hepatitis C virus (HCV) antibodies and hepatitis B virus (HBV) surface antigen), and treatments received during admission were reviewed.

Liver function tests abnormalities were defined using peak AST, ALT and total bilirubin (TBIL) during admission, according the Common Terminology Criteria for Adverse Events (CTCAE) 4.0[16] as: (i) grade 1 if AST/ALT 1–3 xULN and TBIL ≤1.5 mg/dL; (ii) grade 2 if AST/ALT 3–5 xULN and/or TBIL 1.5–3 mg/dL; (iii) grade 3 if AST/ALT 5–20 xULN and/or TBIL 3–10 mg/dL; (iv) grade 4 if AST/ALT >20 xULN and/or TBIL >10 mg/dL. Patients were further categorized regarding the absence or presence of A-LFTs. The ratio of partial pressure of oxygen (PaO$_2$) and inspired oxygen fraction (FiO$_2$) (P/F ratio) was used for the evaluation of COVID-19 severity. Severe COVID-19 was defined as P/F ratio < 200 or organ dysfunction leading to patient admission in an intensive care unit (ICU).

2.3 | Follow-up non-invasive and histological liver evaluation

Follow-up evaluation consisted of a blood test, a transient elastography and a medical consultation to evaluate chronic liver diseases.

Transient elastography was performed using FibroScan® (Echosens, Paris, France) according to manufacturer's recommendations and in fasting conditions.[17] Liver stiffness measurement (LSM; kPa) and Controlled Attenuation Parameter (CAP; dB/m) were collected. Metabolic-associated fatty liver disease (MAFLD) was defined with non-invasive methods as obesity (body mass index [BMI] ≥30 kg/m^2) or diabetes or metabolic syndrome associated to CAP >300 db/m or CAP 250–300 db/m with radiologic evidence of steatosis.[18,19] Harmful alcohol intake was considered ≥30 g per day in men and ≥20 g per day in women. Subsequently, patients showing A-LFTs and/or LSM ≥8 kPa at follow-up were offered a liver biopsy.[20] Percutaneous liver biopsy was performed using a 14-gauge Tru-Cut needle. Samples were paraffin-embedded and stained with haematoxylin-eosin and Masson's trichromic. SARS-CoV2 immuno-histochemical staining with rabbit polyclonal nucleoprotein antibody (SinoBiological, 40.143-T62) was also performed. A single expert pathologist blinded to clinical data and transient elastography values evaluated the liver tissue. Steatosis, steatohepatitis and fibrosis were evaluated using the NASH CRN scoring system and its histo-pathological algorithm.[21,22] We retrospectively reviewed if patients required follow-up by the Post-Acute COVID-19 Unit of our center and if they have been diagnosed with PACS according to the definition above.[14]

2.4 | Statistical analysis

Categorical variables were described as frequencies and percentages, and continuous variables as medians and interquartile ranges. Baseline characteristics were compared between groups using χ^2, Fisher's exact test or Mann–Whitney U test as appropriate. Covariates that were significant with $p < 0.05$ in univariate analysis were included in multivariate models. Binary logistic regression was used to explore the association between A-LFTs and COVID-19 severity with clinical variables. The area under the receiver operating characteristics (ROC) curve (AUC) was used to quantify discriminative ability of the evaluated variables. Mortality analysis was performed using Cox regression, and Kaplan–Meier curves and log-rank test were used to compare patients with and without A-LFTs. Survival time was calculated using the date of death or 1st October 2020, whichever occurred first, as last follow-up.

Considering an A-LFTs prevalence of ~10% in the general population in Spain, and a minimum prevalence in our cohort ~40%, assuming a dropout rate ~40%, accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 50 subjects per group were required to detect statistically significant differences in patients with or without A-LFTs during follow-up. All the analyses in the present study were two-tailed. Statistical analysis and graphs were performed using IBP SPSS statistics V 24.0 (IBP Corp) and STATA V14.2 (StataCorp).

3 | RESULTS

3.1 | Characteristics of admitted patients with COVID-19

The study flowchart is shown in Figure 1. Baseline characteristics of the 595 included patients, according to presence or absence of A-LFTs, are depicted in Table 1. Median age of our study cohort was 62 years (50–73), and 360 (60.5%) were male. Hypertension and diabetes mellitus were present in 244 (41.0%) and 99 (16.6%) of patients, respectively. Median BMI was 28.3 kg/m^2, and 131 (38%) patients had a BMI ≥30 kg/m^2. Harmful alcohol use was reported in 24 (5.7%) patients, and five (0.8%) had cirrhosis.

Median time from symptoms onset to hospital admission was 8 (5–10) days. Gastrointestinal symptoms were reported by 193 patients (32.4%), mostly diarrhoea. Azithromycin, hydroxy-chloroquine, and ceftriaxone were the most prescribed drugs. Immunomodulatory treatment was administered to 141 (23.7%) patients, mostly tocilizumab.

3.2 | Factors related to abnormal LFTs during COVID-19 hospitalization

During hospitalization, A-LFTs were found in 366 (61.5%) patients: 228 (38.3%) grade 1, 70 (11.8%) grade 2, 62 (10.4%) grade 3 and 6 (1.0%) grade 4 (Table S1). An increased in AST and/or ALT was the predominant LFTs abnormality, being present in 363 (99.2%) patients, while 25 (6.8%) patients had TBIL increase (with three patients showing increased TBIL alone). Median time from symptoms onset to the maximum peak of LFTs was 12 (9–16) days. Patients with A-LFTs had a variable liver profile at admission (Table 1): 113 (30.9%) and 253 (69.1%) patients showed absent and present A-LFTs at the time of admission, respectively. Among the 113 patients without A-LFTs at admission, median time to A-LFTs appearance was 6 (3–8) days. In multivariate analysis [aOR (CI 95%)], A-LFTs were independently related with P/F ratio < 200 [1.98 (1.16–3.40), $p = 0.012$], ferritin ≥1000 ng/mL [3.50 (2.13–5.75), $p < 0.001$], male gender [2.08 (1.35–3.20), $p = 0.001$] and the use of antibiotic [1.86 (1.14–3.04), $p = 0.014$] and immunomodulatory [2.10 (1.09–4.03), $p = 0.026$] treatment (Table 1). No patients showed clinical or analytical symptoms of liver failure during admission. The low number of patients with increased TBIL does not allow to explore differences based on the type of alteration in the liver profile.

3.3 | Severity and mortality of COVID-19 patients and liver impairment

Severe SARS-CoV2 infection was diagnosed in 249 (41.8%) patients: 241 (40.5%) patients showed a P/F ratio < 200 during
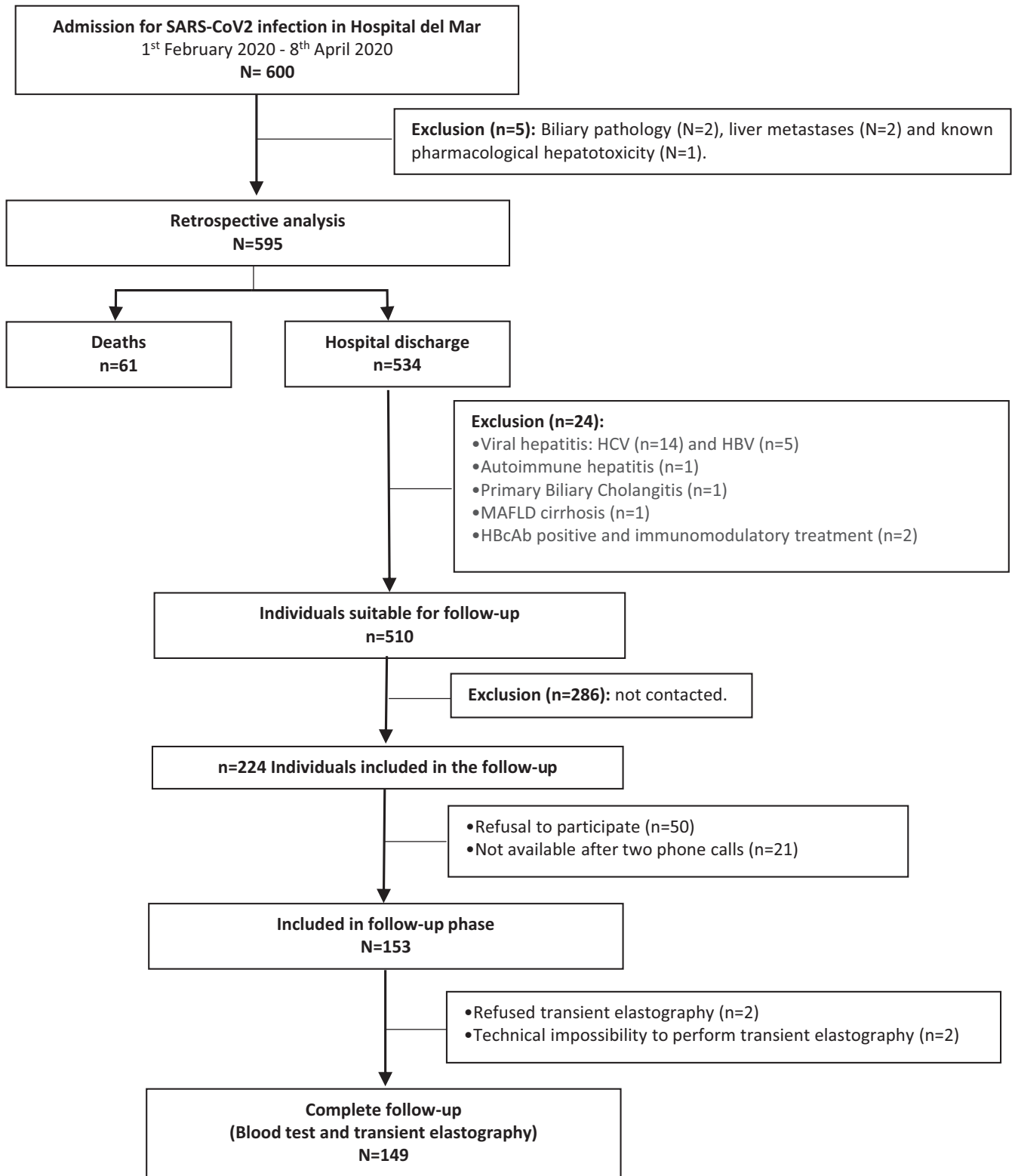
FIGURE 1 Study flow-chart

hospitalization and 96 (16.1%) required ICU admission. A-LFTs were found in 171 (49.4%) and 195 (78.3%) patients with mild and severe COVID-19, respectively ($p < 0.001$). In a multivariate analysis, variables independently related to SARS-CoV2 severity were [aOR (95% CI); p]: BMI ≥30 kg/m² [1.88 (1.07–3.31), $p = 0.029$],

ferritin ≥1000 ng/mL [1.81 (1.01–3.26); $p = 0.048$], IL-6 ≥40 pg/mL [3.43 (1.97–5.97); $p < 0.001$], lymphocytes <1000/µL [3.24 (1.87–5.60); $p < 0.001$] and A-LFTs [2.81 (1.51–5.23); $p = 0.001$] (Table S2). The ability to discriminate severe SARS-CoV2 infection was evaluated using ROC curves of the continuous variables. IL-6

TABLE 1 Baseline characteristics of COVID-19 hospitalized patients according to the presence or absence of abnormal liver function tests (LFTs)

	All patients	LFTs abnormalities during admission				
		Present				
	(N = 595)	(N = 366)	Absent (N = 229)	p-Value	aOR (CI 95%)	p-Value
Sociodemographic data and comorbidities						
Age (years)	62 (50–73)	62 (51–72)	61 (49–75)	0.835		
Gender, male (%)	360 (60.5)	254 (69.4)	106 (46.3)	<0.001	2.08 (1.35–3.20)	0.001
Origin, n (%) (n = 518)				0.418		
Caucasian	363 (70.1)	219 (69.1)	144 (71.6)			
South and Central America	79 (15.3)	29 (15.8)	29 (14.4)			
Others	76 (12.8)	48 (13.1)	28 (12.2)			
Hypertension, n (%)	244 (41.0)	144 (39.3)	100 (43.7)	0.297		
Diabetes, n (%)	99 (16.6)	55 (15.0)	44 (19.2)	0.182		
Body mass index (kg/m^2) (n = 345)	28.3 (25.5–32.0)	28.3 (25.4–32.4)	28.2 (25.6–31.6)	0.901		
BMI < 25 kg/m^2, n (%)	69 (20.0)	46 (20.4)	23 (19.3)	0.901		
BMI 25–30 kg/m^2, n (%)	145 (42.0)	93 (41.2)	52 (43.7)			
BMI > 30 kg/m^2, n (%)	131 (38.0)	87 (38.5)	44 (37.0)			
Harmful alcohol intake, n (%) (n = 423)	24 (5.7)	19 (7.5)	5 (3.0)	0.055		
COVID-19 information						
Peak Total bilirubin (mg/dL)	0.5 (0.3–0.6)	0.5 (0.3–0.6)	0.4 (0.2–0.6)	0.088		
Peak AST (U/L)	53 (38–84)	57 (43–91)	24 (20–32)	<0.001		
Peak ALT (U/L)	61 (44–103)	65 (50–112)	25 (17–31)	<0.001		
Ferritin (ng/mL) (n = 518)	771 (394–1308)	1025 (599–1661)	469 (255–828)	<0.001		
Ferritin ≥1000 mg/dL, n (%)	197 (38.0)	167 (52.0)	30 (15.2)	<0.001	3.50 (2.13–5.75)	<0.001
Interleukin-6 (pg/mL) (n = 523)	28.4 (9.7–64.8)	35.7 (13.1–84.8)	19.9 (7.1–46.5)	<0.001		
Interleukin-6 ≥ 40 pg/mL, n (%)	214 (40.9)	154 (47.5)	60 (30.2)	<0.001		0.177
Lymphocytes (/μL) (n = 590)	1020 (738–1423)	950 (680–1270)	1140 (830–1540)	<0.001		
Lymphocytes <1000/μL	279 (47.3)	195 (53.7)	84 (37.0)	<0.001		0.809
D-Dimer (mcg/L) (n = 540)	670 (450–1240)	730 (470–1373)	640 (400–1110)	0.024		
D-Dimer ≥500 mcg/L	370 (68.5)	238 (71.3)	132 (64.1)	0.087		
Treatments prescribed, n (%)						
Azithromycin and hydroxychloroquine	572 (96.1)	353 (96.4)	219 (95.6)	0.803		
Antibiotics	453 (76.1)	312 (85.2)	141 (61.6)	<0.001	1.86 (1.14–3.04)	0.014
Antivirals	89 (15.0)	58 (15.8)	31 (13.5)	0.480		
Immunomodulatory	141 (23.7)	120 (32.8)	21 (9.2)	<0.001	2.10 (1.09–4.03)	0.026
Worst P/F ratio	236 (130–324)	196 (106–285)	292 (219–360)	<0.001		
Worst P/F ratio < 200, n (%)	241 (40.5)	190 (51.9)	51 (22.3)	<0.001	1.98 (1.16–3.40)	0.012
ICU admission, n (%)	96 (16.1)	84 (23.0)	11 (4.8)	<0.001		0.070
Severe COVID-19, n (%)	249 (41.8)	195 (53.3)	54 (23.6)	<0.001		
Hospital stay (days)	9 (5–15)	10 (6–18)	6 (4–10)	<0.001		
Death, n (%)	61 (10.3)	41 (11.2)	20 (8.7)	0.334		

Note: Univariate and multivariate analysis of factors related to the presence of abnormal liver function tests during COVID-19.

Abbreviations: ICU, Intensive Care Unit; LFTs, Liver Function Tests; P/F ratio, partial pressure of oxygen (PaO$_2$) and inspired oxygen fraction (FiO2) ratio.

and lymphocytes had the better performance (AUC_{IL-6} 0.730 and AUC_{Lymph} 1–0.287, respectively), whereas AST (AUC_{AST} 0.569), ALT (AUC_{ALT} 0.522) and TBIL (AUC_{TBIL} 0.566) performance was poor (Figure S1).

Median surveillance time was 6.1 (5.9–6.3) months in both study groups, irrespective of the presence of A-LFTs during COVID-19 hospitalization. Global mortality was 10.3%: 11.2% and 8.7% in patients with or without A-LFTs, respectively ($p = 0.334$). No mortality differences were found when comparing patients with or without A-LFTs (Log-Rank = 0.292) (Figure S2). In our cohort, mortality was independently related to age [aHR 1.15 (1.10–1.20); $p < 0.001$], P/F ratio [aHR 0.98 (0.97–0.99); $p < 0.001$] and ICU admission [aHR 3.37 (1.51–7.51); $p = 0.003$] (Table S3).

3.4 | Screening for HCV and HBV

HCV antibodies (HCV-Ab) and HBV surface antigen (HBs-Ag) testing was performed in 508 (85.7%) and 509 (85.5%) patients, respectively. HCV-Ab were positive in 13 (2.6%) patients, all of them previously diagnosed with chronic hepatitis C (10 with sustained virological response, and three without). Furthermore, positive HBsAg was found in five (1.0%), four of whom new diagnoses, and one patient who already was under antiviral treatment with entecavir. All four newly diagnosed patients had HBV-DNA <100 U/L. None of them received immunomodulatory treatment, but one required high-dose corticosteroid treatment. No HBV-DNA or transaminases flare was detected in the 6-month follow-up of COVID-19 in the four HBV-infected patients who accepted the follow-up.

3.5 | Liver evaluation during follow-up after SARS-CoV2 infection

The first 224 discharged patients were consecutively contacted by phone and 153 (68.3%) accepted a follow-up evaluation (Figure 1). Median time between discharge and outpatient evaluation was 9.8 months (8.3–11.0). Baseline characteristics of patients evaluated at follow-up ($n = 153$) resembled the whole study cohort ($n = 595$) (Tables 1 and 2). At follow-up, median age was 64 years (52–72), and 91 (59.5%) were male. Hypertension and diabetes mellitus were present in 61 (39.9%) and 24 (15.7%) of patients, respectively. Median BMI at admission and at follow-up was 27.8 kg/m^2 and 29.3 kg/m^2 respectively. At follow-up, 64 (42.1%) patients had obesity and six (3.9%) presented harmful alcohol intake.

Among 153 patients with a follow-up evaluation, 92 (60.1%) patients had A-LFTs during hospitalization: 55 (59.8%) grade 1, 16 (17.4%) grade 2, 20 (21.7%) grade 3 and 1 (1.1%) grade 4; and LFTs were persistently abnormal in 13 (14.1%). Additionally, three patients without A-LFTs at admission showed A-LFTs at follow-up. Indeed, 16 (10.5%) showed A-LFTs at follow-up, and LFTs normalization was observed in 79 (85.9%) (Figure 2A,B). The proportion of patients with A-LFTs at follow-up was similar irrespectively of the presence of A-LFTs at admission. Herein, 13 (14.1%) out of 92 patients with A-LFTs during admission showed A-LFTs at follow-up, as compared with three (4.9%) of 61 patients without A-LFTs during admission ($p = 0.104$). In addition, among the 16 patients with A-LFTs at follow-up, all but one showed grade 1 alteration. Patients with A-LFTs at follow-up were younger (median age 53 vs. 65 years; $p = 0.053$), and a higher proportion of individuals from Central and South America was evidenced in contrast to patients with normal LFTs (50.0% vs. 18.2%; $p = 0.008$). Hypertension, diabetes mellitus, BMI and alcohol intake were similar between groups. Similarly, inflammatory parameters, severity of COVID-19 and use of antimicrobial and immunomodulatory drugs during admission were similar between patients with and without A-LFTs at follow-up (Table 2).

At follow-up, transient elastography was performed in 149 individuals. In four patients, LSM was unavailable due to technique failure, but was included in subsequent analyses. Patients with A-LFTs at follow-up showed higher LSM (6.0 vs. 4.4 kPa, $p < 0.001$) and CAP (334 vs. 276 db/m, $p = 0.002$) values than patients with normal LFTs (Figure 2C,D). MAFLD criteria were met by 58 (38.9%) patients at follow-up, of which 10 (17.2%) had A-LFTs, and eight (13.8%) showed an LSM ≥8 kPa. Alcohol-associated fatty liver disease (ALD) and mixed MAFLD/ALD were diagnosed in 1 (0.7%) and 6 (4.0%) patients. Importantly, fatty liver disease was diagnosed in 15 (93.8%) and 50 (37.6%) patients with A-LFTs and normal LFTs ($p < 0.001$). Primary biliary cholangitis and alpha-1-antitrypsin deficit were diagnosed in one (0.7%) and two (1.3%) patients. No patient showed features suggesting autoimmune hepatitis, and we also did not diagnose any unknown HBV and HCV infection (Figure 3). Besides, among the 153 patients evaluated, 61 (39.9%) required medical assistance for persisting symptoms, and 13 (8.5%) have been diagnosed with PACS. The prevalence of PACS was similar in patients with/without A-LFTs at follow-up, being 12.5% and 8%, respectively ($p = 0.544$).

3.6 | Liver biopsy after SARS-CoV2 infection

A liver biopsy was performed in 14 patients with A-LFTs at follow-up ($n = 6$), an LSM >8 kPa ($n = 5$) or both ($n = 3$) (Table 3). All samples met quality criteria based on the length and number of portal spaces.[23] SARS-CoV2 immunohistochemistry stained negative in all liver biopsies. Steatosis and steatohepatitis were described in one (7.1%) and 10 (71.4%) patients. Significant fibrosis was reported in eight (57.1%) patients: six (75%) METAVIR F2 and two (25%) METAVIR F3. All patients with significant fibrosis had steatohepatitis. No patient had histological signs of autoimmune hepatitis or other aetiologies.

4 | DISCUSSION

Abnormal liver tests are common in hospitalized patients with SARS-CoV2 infection. In our study, encompassing 600 admitted COVID-19 patients, more than 60% showed increased bilirubin or transaminases. In line with previous data, less than 20% had grade 3 or 4

TABLE 2 Characteristics of patients with follow-up after COVID-19 and non-invasive liver evaluation of them after SARS-CoV2 infection

| | | LFTs abnormalities at follow-up | | |
| | All patients | Present | Absent | |
	N = 153	N = 16	N = 137	p-Value
Age (years)	64 (52–72)	53 (51–65)	65 (53–73)	0.053
Male gender, n (%)	91 (59.5)	11 (68.8)	80 (58.4)	0.592
Origin, n (%)				0.008
Caucasian	112 (73.2)	6 (37.5)	106 (77.4)	
South and central America	33 (21.6)	8 (50.0)	25 (18.2)	
Others	8 (5.2)	2 (12.6)	6 (4.3)	
Hypertension, n (%)	61 (39.9)	6 (37.5)	55 (40.1)	>0.9
Diabetes, n (%)	24 (15.7)	5 (31.3)	19 (13.9)	0.136
BMI at admission (kg/m^2) (n = 142)	27.8 (25.3–31.7)	27.6 (24.5–32.5)	28.1 (25.3–31.6)	0.549
BMI < 25 kg/m^2, n (%)	33 (23.2)	4 (26.7)	29 (22.8)	0.618
BMI 25–30 kg/m^2, n (%)	64 (45.1)	5 (33.3)	59 (46.5)	
BMI > 30 kg/m^2, n (%)	45 (31.7)	6 (40.0)	39 (30.7)	
BMI at follow-up (kg/m^2) (n = 152)	29.3 (27.1–32.8)	31.5 (26.4–34.1)	29.3 (27.1–32.3)	0.506
BMI < 25 kg/m^2, n (%)	21 (13.8)	1 (6.3)	20 (14.7)	0.413
BMI 25–30 kg/m^2, n (%)	67 (44.1)	6 (37.5)	61 (44.9)	
BMI > 30 kg/m^2, n (%)	64 (42.1)	9 (56.3)	55 (40.4)	
Abdominal circumference (cm)	102 (95–111)	105 (97–116)	102 (95–111)	0.518
Delta BMI (%) (n = 142)	5.4 (1.4–8.3)	6.6 (1.2–13.2)	5.4 (1.4–8.1)	0.166
Harmful alcohol intake, n (%)	12 (7.8)	3 (18.8)	9 (6.6)	0.115
COVID-19 severity and treatment received during admission				
Worst P/F ratio < 200, n (%)	57 (37.3)	7 (43.8)	50 (36.5)	0.593
ICU admission, n (%)	25 (16.3)	3 (18.8)	22 (16.1)	0.727
Severe COVID-19, n (%)	60 (39.2)	7 (43.8)	53 (38.7)	0.789
Antibiotic treatment, n (%)	118 (77.1)	11 (68.8)	107 (78.1)	0.528
Immunomodulatory treatment, n (%)	46 (30.1)	3 (18.8)	43 (31.4)	0.394
Blood test at follow-up				
Total Bilirubin (mg/dL)	0.4 (0.3–0.6)	0.5 (0.4–0.6)	0.4 (0.3–0.6)	0.321
AST (U/L) (n = 147)	20 (17–24)	37 (30–46)	19 (17–22)	<0.001
ALT (U/L) (n = 152)	18 (14–26)	53 (45–69)	17 (13–23)	<0.001
GGT (U/L)	22 (16–34)	53 (41–101)	20 (15–30)	<0.001
ALP (U/L) (n = 151)	74 (62–95)	96 (74–114)	72 (61–93)	0.010
Albumin (g/dL) (n = 150)	4.5 (4.3–4.7)	4.6 (4.5–4.8)	4.5 (4.3–4.7)	0.061
Prothrombin time (%) (n = 151)	110 (101–121)	113 (102–121)	110 (101–120)	0.429
Platelets (/L)	230 (202–273)	258 (213–298)	230 (201–270)	0.217
Transient elastography (N = 149)				
LSM (kPa)	4.5 (4.1–5.6)	6.0 (5.1–7.2)	4.4 (4.0–5.3)	<0.001
LSM ≥8 kPa, n (%)	9 (6.0)	3 (18.8)	6 (4.5)	0.057
CAP (db/m)	280 (241–327)	334 (286–359)	276 (239–319)	0.003
CAP ≥250 db/m, n (%)	106 (71.1)	15 (93.8)	91 (68.4)	0.040
CAP ≥300 db/m, n (%)	61 (40.9)	12 (75.0)	49 (36.8)	0.006
PACS, n (%)	13 (8.5)	2 (12.5)	11 (8.0)	0.544

Note: Data of all patients (N = 153), and according to the presence or absence of abnormal liver function tests (LFTs) at follow-up.

Abbreviations: BMI, Body Mass Index; CAP, Controlled Attenuation Parameter; ICU, Intensive Care Unit; LFTs, Liver Function Tests; LSM, Liver Stiffness Measurement; P/F ratio, partial pressure of oxygen (PaO_2) and inspired oxygen fraction (FiO_2) ratio; PACS, Post-Acute COVID-19 Syndrome.
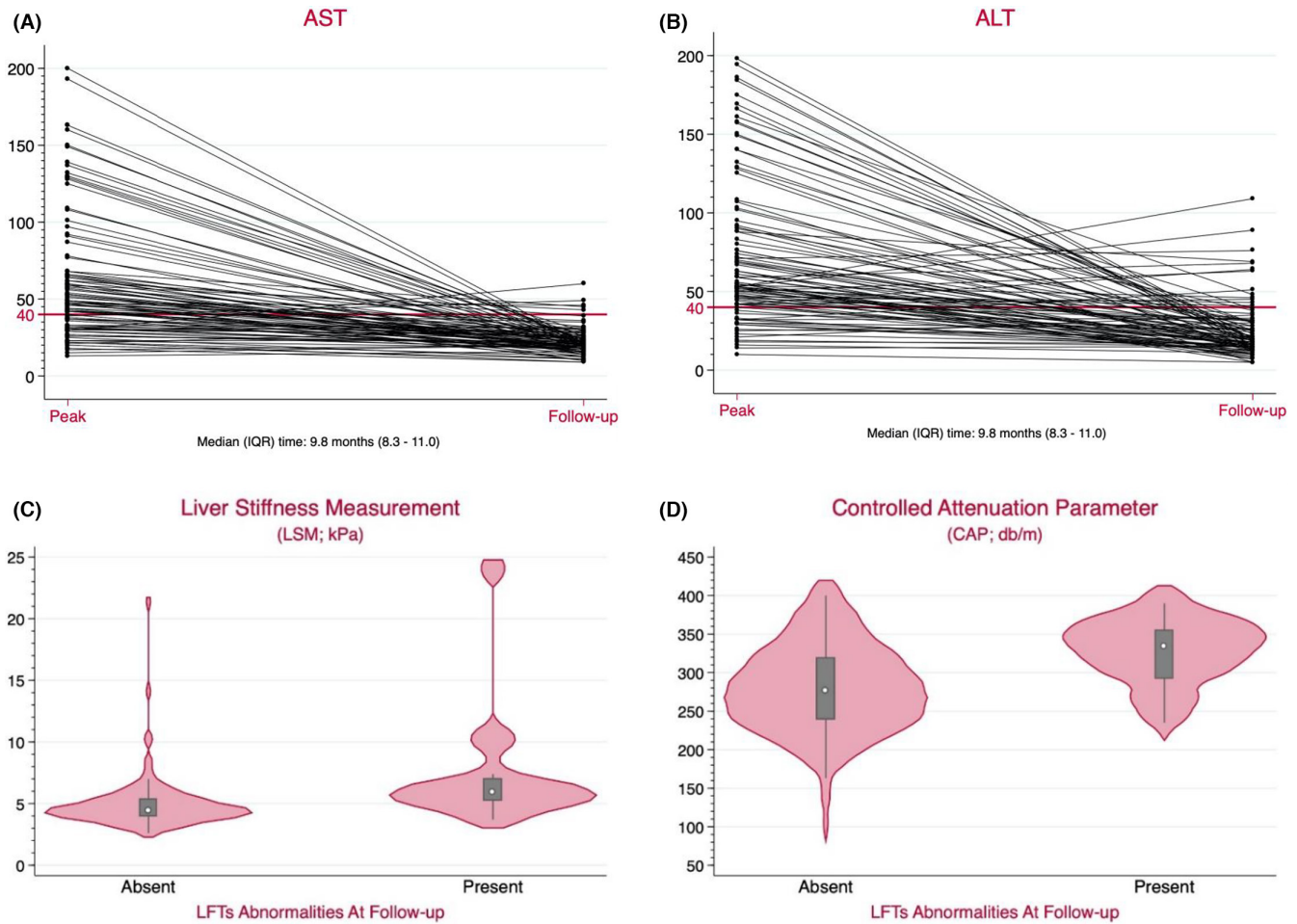
FIGURE 2 Liver assessment at follow-up. (A, B) Evolution of AST and ALT from peak value during COVID-19 to follow-up (median time 10 months). Median (IQR) peak AST and ALT was 52 (37–93) U/L and 60 (45–128) U/L. Median (IQR) AST and ALT at follow-up was 20 (17–24) and 18 (14–26). (C, D) Distribution of liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) values according to the presence or absence of LFTs abnormalities at follow-up. Each violin plot represents the density curves, the median (white point), the interquartile range (box) and 95% confidence intervals (lines)

A-LFTs, and no cases of liver failure were documented.[8,11,12] Also, we confirmed that A-LFTs during COVID-19 hospitalization were related to the severity of SARS-CoV2 infection and were independently related to severe respiratory failure, inflammatory markers and the use of specific drugs. Importantly, A-LFTs during admission were unrelated to the presence of A-LFTs during follow-up, and alteration in liver enzymes remitted in most patients. Finally, A-LFTs at follow-up were related to fatty liver disease, caused by previous risk factors such as hypertension, diabetes and obesity, but were unrelated to SARS-CoV2.

It has been previously suggested that liver involvement during SARS-CoV2 infection has a multifactorial aetiology, including severe inflammatory response, drug induced liver injury (DILI) and hypoxic injury.[24,25] Moreover, the evidence of ACE2 and TMPRSS2 expression in liver cells (more frequent in cholangiocytes than hepatocytes), the presence of SARS-CoV2 genome and virions in hepatocytes and the presence of mitochondrial swelling in hepatocytes raised the hypothesis of a direct cytopathic effect.[4,26] In our study, liver impairment during COVID-19 was independently related

to P/F ratio < 200, ferritin ≥1000 ng/mL and the use of antibiotic and immunomodulatory treatments. In this same line, Chew et al. evaluated the interrelationship between three COVID-19 disease states (ischemic, hyperinflammatory and hypercoagulable), and the use of tocilizumab and steroids, with the presence of AST increase in 854 patients. They found that an ischemic state defined as the need of vasopressors for at least 2 days and the use of tocilizumab were independently related to AST >5 times the ULN.[27] Moreover, Phipps et al. showed that patients with higher ALT abnormality required more frequent ICU admission, mechanical ventilation and renal-replacement treatment.[11] However, previous studies did not evaluate if alteration in live enzymes occurred at or during admission and, importantly, neither its relationship with respiratory symptoms onset. In our cohort, more than 75% of patients developed LFTs abnormalities the first 2 weeks since symptoms onset, a similar period than reported between the onset of symptoms and respiratory deterioration.[13] However, in our study, the absence of liver tissue evaluation during admission does not allow to rule out a cytopathic effect of SARS-CoV2 during the acute infection. Our analysis

FIGURE 3 Non-invasive diagnosis of liver disease in patients after the COVID-19. Metabolic associated fatty liver disease (MAFLD) was defined as obesity (body mass index [BMI] ≥30 kg/m^2) or diabetes or metabolic syndrome associated to CAP >300 db/m or CAP 250–300 db/m with radiologic evidence of steatosis. Alcohol-related liver disease was considered if harmful alcohol consumption in the absence of metabolic dysfunction. Other liver chronic liver disease category includes primary biliary cholangitis, alpha-1 antitrypsin deficiency and liver stasis associated to cardiac failure
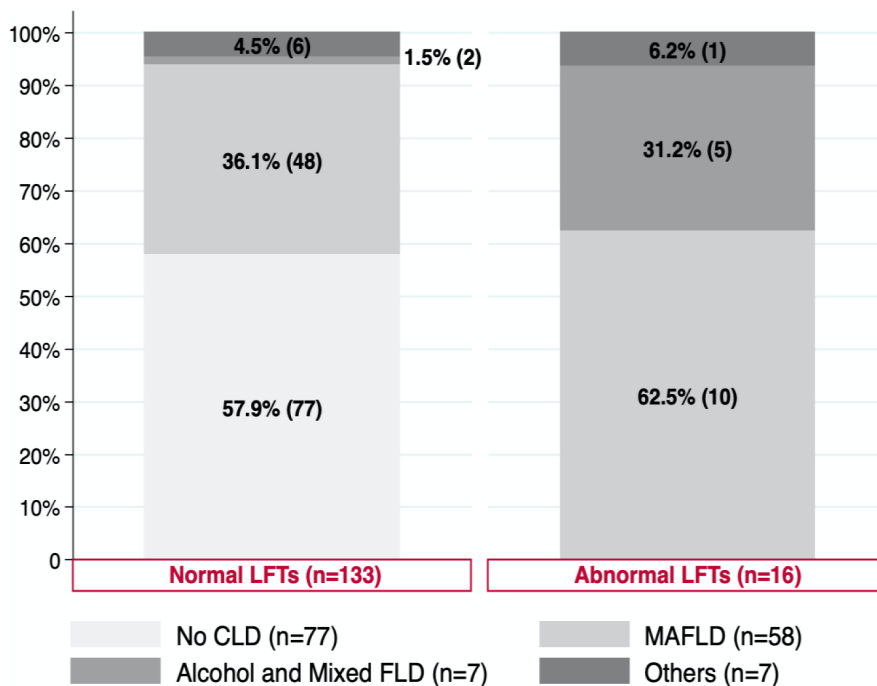
confirms a relationship between abnormal LFTs and COVID-19 severity, especially AST. Therefore, patients with liver impairment during SARS-CoV2 infection should be closely monitored due to the risk of respiratory complications. In this regard, the severity of COVID-19 was higher as high the degree of LFTs abnormality was. However, we show a low ability of transaminases alone to detect severe SARS-CoV2 (AUC$_{AST}$ and AUC$_{ALT}$ <0.6). Thus, a recommendation for a closest monitoring based on a specific AST/ALT value cannot be done with our data. The prevalence of HBV infection is similar to the general population in our area. Accordingly, serological diagnosis of hepatitis B using surface antigen and core antibodies (HBs-Ag and HBc-Ac) should be performed on all patients admitted with a SARS-CoV2 infection, given the potential need of immunosuppressive treatment and, therefore, prophylaxis of reactivation based on current clinical guidelines. Similarly, the scenario of admitted patients, in the context of a global pursue of HCV eradication, makes HCV screening recommendable.

Post-acute COVID-19 syndrome (PACS) encompasses long-COVID and sequelae. As in the acute infection, a wide heterogeneity of symptoms has been described after COVID-19. The most frequent reported symptoms in adults with PACS were fatigue, dyspnoea and neurological symptoms.[14,15] Although LFTs alteration is frequent during COVID-19, no previous data about the liver outcome after SARS-CoV2 infection had been reported. In our study we show that most patients showed a resolution of A-LFTs after a median follow-up of 10 months, with only 10% of included individuals showing abnormal LFTs according to CTCAE criteria,[16] almost all of them with a mild alteration. More importantly, we show that the presence of abnormal LFTs at follow-up was not related to the presence or severity of liver impairment during admission for SARS-CoV2 infection. In this regard, patients showing A-LFTs at follow-up presented non-invasive and/or histological diagnostic of MAFLD. Remarkably, an

elevated CAP over 300 db/m was found in 41% of patients and up to 75% of patients with A-LFTs. This high prevalence of fatty liver was related to high prevalence of pre-existing risk factors such as diabetes, obesity and metabolic syndrome. Hepatic steatosis has been described in liver biopsies of COVID-19 acute patients.[26] However, we retrospectively reviewed the medical records of the 16 patients with abnormal LFTs at follow-up and showed that 62% and 50% of them already had altered transaminases and ultrasound evidenced steatosis before COVID-19, respectively. In addition, similar prevalence of PACS was observed in patients with/without A-LFTs at follow. Thus, our findings highlight the lack of relationship between COVID-19-related LFTs abnormalities and those observed after follow-up. Finally, we confirmed hepatic steatosis and steatohepatitis in most of the 14 patients undergoing a liver biopsy, excluding the presence of SARS-CoV2 in liver tissue. Our results confirm that patients with abnormal LFTs during admission for SARS-CoV2 infection do not require specific liver follow-up. On the other hand, considering the high incidence of MAFLD criteria, even in those with normal LFTs at follow-up, our results highlight the need to identify and treat MAFLD risk factors in cohorts with high prevalence of obesity, hypertension and diabetes.

Our study has strengths and limitations. The study cohort encompassed a large number of patients, and despite the retrospective design, the cohort is highly homogeneous and provides a thorough characterization of liver impairment during SARS-CoV2 hospitalization. Herein, the exclusion of mild patients not requiring hospital admission limits its applicability to the general population with SARS-CoV2 infection. In addition, lack of information about A-LFTs prior to admission, and the unavailability of steatosis evaluation using liver image tests or liver biopsies during hospitalization, does not allow us to rule out confounding factors regarding previous liver abnormalities. However, careful revision

TABLE 3 Sociodemographic, comorbidities, blood test, elastographic and histologic data of each patient with liver biopsy at follow-up

	Sociodemographic data and comorbidities					At admission		Follow-up evaluation								
	Age (years)	Gender	T2DM	Hypertension	BMI (kg/m²)	AST (U/L)	ALT (U/L)	AST (U/L)	ALT (U/L)	TE (kPa)	CAP (db/m)	Histologic steatosis	Lobular inflammation	Balloning	METAVIR	SARS-CoV2
1	70	F	Yes	Yes	42.4	23	26	37	54	10.6	329	S3	<2 foci	Evident	F3	Negative
7	69	F	No	Yes	32.9	68	47	45	43	24.1	330	S1	2–4 foci	Evident	F3	Negative
2	50	M	Yes	No	23.3	33	31	47	47	9.9	255	S1	2–4 foci	Evident	F2	Negative
3	51	F	Yes	No	30.2	39	32	49	51	5.8	341	S1	<2 foci	Evident	F2	Negative
6	65	F	No	Yes	29.7	30	53	32	89	5.6	273	S2	<2 foci	Moderate	F2	Negative
8	59	F	Yes	Yes	40.0	25	55	11	8	14.1	400	S3	<2 foci	Evident	F2	Negative
12	72	M	No	Yes	32.9	47	50	46	63	6.6	364	S3	2–4 foci	Evident	F2	Negative
14	60	M	No	No	35.5	51	52	28	36	10.3	400	S2	2–4 foci	Evident	F2	Negative
10	54	M	No	No	27.7	59	95	43	28	6.1	280	No	No	No	F1	Negative
11	63	M	Yes	Yes	25.9	49	42	30	45	5.6	332	S2	<2 foci	No	F1	Negative
4	70	F	No	Yes	46.1	20	19	14	17	10.2	351	S1	<2 foci	Moderate	F0	Negative
5	39	F	No	No	39.2	19	24	16	19	8.3	382	S1	<2 foci	Moderate	F0	Negative
9	51	F	No	No	48.9	78	175	14	19	21.4	400	No	No	No	F0	Negative
13	52	M	No	No	27.9	38	59	19	68	4.5	390	No	No	No	F0	Negative

Note: Altered transaminases and transition elastography (TE) > 8 kPa have been highlighted.

Abbreviations: ALT, Alanine-aminotransferase; AST, Aspartate-aminotransferase; CAP, Controlled Attenuation Parameter; F, Female; M, Male; TE, Transient Elastography.

of patients with A-LFTs at follow-up showed previous diagnosis of steatosis in most of them. Additionally, other causes of liver impairment during admission (e.g. other hepatotropic viruses, ultrasound evaluation) were not performed due to the emergent nature of medical attention during SARS-CoV2 first wave. Finally, the low number of patients with cirrhosis does not allow us to evaluate factors associated with liver impairment in patients with previous chronic liver diseases.

In conclusion, we show that abnormal liver function tests during SARS-CoV2 infection are frequent, transient and related to the severity of the infection and the use of medications. The few patients with liver abnormalities during follow-up have pre-existing fatty liver disease risk factors, unrelated to SARS-CoV2. Because of that, although no specific follow-up for patients with abnormal liver function during SARS-CoV2 infection should be recommended, COVID-19 hospitalization is an opportunity to identify and treat risk factors for metabolic dysfunction.

AUTHOR CONTRIBUTIONS

Study concept and design: LC, TB, MGR, MP. *Acquisition of data and technical support*: LC, GP, AS, EG, RF, AV, JR, GD. *Analysis and interpretation of data*: LC, TB, JAC, LN, MP. *Drafting of the manuscript*: LC, MP. *Critical revision of the manuscript for important intellectual content*: TB, JAC, NC, SC, XB, MGR, MP. *Statistical analysis*: LC, JAC, MP. *Study supervision*: MP.

CONFLICTS OF INTEREST

The authors declare no conflict of interest relevant to this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

GUARANTOR OF THE ARTICLE AND FINAL VERSION OF THE MANUSCRIPT

Marc Puigvehí certifies that all the authors have approved the final version of the manuscript.

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SUPPORTING INFORMATION

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