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Abnormal liver tests and non-alcoholic fatty liver disease predict disease progression and outcome of patients with COVID-19

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Abbreviations: ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; COVID-19, coronavirus disease; FIB-4, fibrosis-4; GGT, gamma-glutamyl transferase; HSI, hepatic steatosis index; LFTs, liver function tests; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; SaO2, capillary oxygen saturation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TBIL, total bilirubin; TP, prothrombin time; ULN, upper limit of the normal.

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Introduction

Coronaviruses are enveloped viruses that display the largest genome of all RNA viruses known to cause respiratory infections in humans [1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the novel coronavirus disease that first occurred in 2019 (COVID-19) and became a serious global public health problem, declared by World Health Organization (WHO) as a global public health emergency. It can cause respiratory, intestinal, hepatic, and neuronal disease and may lead to acute respiratory distress syndrome, multiple organ failure, and death in severe cases [2].

As this infectious disease continues to spread, more information is needed to better identify the patients who require hospitalization or those who are likely to develop severe COVID-19 disease. The identification of risk factors associated with outcomes should allow for patient stratification in view of personalized clinical care, in order to improve the outcome.

Liver impairment has been commonly reported in COVID-19 patients [3]. Abnormal liver function tests (LFTs) are present on admission, prior to initiating treatment with hepatotoxic drugs that may induce liver injury [4]. However, the association between LFTs and different degrees of COVID-19 severity is still poorly described.

The features of metabolic syndrome, including obesity, diabetes, and hypertension, are associated with a severe COVID-19 course [5]. Non-alcoholic fatty liver disease (NAFLD) is associated with these metabolic comorbidities. The association and evolution of SARS-CoV-2 infection in patients with a preexisting liver disease like NAFLD are still incompletely understood.

The current study aimed to investigate liver injury, clinical features, and risk factors in patients with associated mild, moderate, and severe COVID-19.

Patients and methods

Study design and participants

We retrospectively included all consecutive patients hospitalized for SARS-CoV-2 infection between February, 22 and May, 16, 2020 at the emergency rooms (ERs) of two French tertiary hospitals (Nouvel Hôpital Civil and Hôpital de Hautepierrre – University Hospital of Strasbourg). The study protocol was approved by the Ethics Committee of Strasbourg University Hospital (CE-2020–138–26/08/2020).

Data collection

The data collected for the hospitalized patients suffering from SARS-CoV-2 infection included their epidemiological and medical history, clinical signs, and biological and imaging data only at admission.
Three groups of patients were defined according to the hospitalization: [1] mild disease: patients hospitalized at the emergency room (ER) and discharged from the hospital without any indication of admission to a conventional hospitalization unit (CHU) or intensive care unit (ICU); [2] moderate disease: patients admitted to the ER and hospitalized thereafter in a CHU without signs of severe disease that would need intensive care support; [3] severe disease: patients needing intensive care support on admission or during the hospitalization in a CHU, patients hospitalized in the ICU, and patients who died during hospitalization in a CHU.

Liver tests were considered abnormal if the liver enzyme values, i.e., aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and total bilirubin (TBIL), were elevated according to our laboratory upper limit of normal (ULN). Different patterns were defined: hepatocellular, cholestatic, and mixed patterns. The hepatocellular pattern was considered if AST or ALT were elevated higher than ULN; the cholestatic pattern if GGT or PAL were elevated up to ULN; the mixed pattern was a combination of the first two ones.

The exclusion criteria were described in the Fig. 1, as follows: patients with multiple hospitalizations for COVID-19, hospitalization for a pathology that may disturb LFTs (liver metastasis, cholangitis, cholecystitis, pancreatitis), patients transferred to other hospitals in Strasbourg or elsewhere, lost to follow-up or with missing clinical and biological data, patients with underlying chronic liver disease (viral, alcohol, autoimmune). Patients with diagnosed NAFLD were included in the study.

Moreover, NAFLD was diagnosed using the hepatic steatosis index (HSI = 8 × [ALT/AST] + body mass index [BMI] [+ 2 if type 2 diabetes yes, + 2 if female]) > 36 points [6] and the severity related to the stage fibrosis with NAFLD fibrosis score (NFS) [7]. This HSI has been validated in COVID-19 patients to evaluate NAFLD [8–10]. The fibrosis was also evaluated using fibrosis-4 index (FIB-4): low (< 1.30), intermediate (1.30–2.67), and high (> 2.67) [11]. To evaluate the severity of the metabolic liver disease, we analyzed the different FIB-4 scores in patients diagnosed with NAFLD according to the HSI score.

Quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) tests for SARS-CoV-2 nucleic acid were performed on admission with nasopharyngeal swabs. The primer and probe sequences targeted two regions on the RdRp gene and were specific to SARS-CoV-2. The assay sensitivity was around 10 copies/reaction (in-house method, Institut Pasteur, Paris, France) [12].

Chest computer tomography (CT) imaging was performed on admission for each patient. The images were classified based on the percentage of the whole lung parenchyma affected by COVID-19 in the following six groups: normal (no lesion), minimal (0%–10%), moderate (11%–25%), important (26%–50%), severe (51%–75%), and critical (> 75%) [13]. To simplify clinical data analysis, the patients were divided into three subgroups: ≤ 25%, 26%–50%, and > 50%, according to their lesion extension.

Statistics

The variables collected were expressed as the numbers and frequencies for qualitative variables (ordinal and categorical) and minimum, maximum, median, mean, and standard deviation for quantitative variables (discrete and continuous). These data were summarized in the descriptive phase.
| Variable                           | Mild COVID-19 (n = 101) | Moderate COVID-19 (n = 393) | p-value |
|-----------------------------------|-------------------------|----------------------------|---------|
| Age (years, SD*)                  | 57.7 (18.4)             | 65.7 (7)                   | < 0.001 |
| Gender (male, %)                  | 52 (51.4)               | 198 (50.4)                | 0.468   |
| BMI (kg/m², SD)                   | 26.9 (4.9)              | 28.8 (5)                  | 0.004   |
| Comorbidity                       |                         |                            |         |
| Diabetes (n, %)                   | 21 (20.8)               | 105 (26.7)                | 0.251   |
| Hypertension (n, %)               | 38 (37.6)               | 217 (55.2)                | 0.002   |
| Dyslipidemia (n, %)               | 17 (16.8)               | 129 (32.8)                | 0.001   |
| Chronic pulmonary disease (n, %)  | 19 (18.8)               | 94 (24)                   | 0.291   |
| Chronic cardiac disease (n, %)    | 19 (18.8)               | 106 (27)                  | 0.097   |
| Chronic kidney disease (n, %)     | 4 (4)                   | 26 (6.6)                  | 0.482   |
| Cancer (n, %)                     | 2 (2)                   | 20 (5.1)                  | 0.277   |
| Symptoms onset                    |                         |                            |         |
| “Flu-like” symptoms (n, %)        | 87 (86.1)               | 366 (93.1)                | 0.041   |
| Respiratory symptoms (n, %)       | 87 (86.1)               | 351 (89.3)                | 0.014   |
| Digestive symptoms (n, %)         | 40 (39.6)               | 208 (52.9)                | 0.019   |
| Neurological symptoms (n, %)      | 45 (44.6)               | 184 (46.8)                | 0.738   |
| Clinical features                 |                         |                            |         |
| Oxygen saturation SaO2 (% SD)      | 96 (3.9)                | 92.1 (5.9)                | < 0.001 |
| Debit of oxygen at admission (L/mn, sd) | 0.4 (1.8)          | 2.3 (7.4)                 | < 0.001 |
| Systolic arterial pressure (mmHg, SD) | 132.4 (17.9)     | 133.1 (25.8)              | 0.683   |
| Laboratory tests                  |                         |                            |         |
| AST U/L (reference range 11–34)   | 30.4 (20.4)             | 45.5 (36.2)               | < 0.001 |
| ALT U/L (reference range 8–41)    | 35.7 (27.5)             | 42.3 (34)                 | 0.05    |
| ALP U/L (reference range 41–117)  | 66.1 (132.2)            | 76.1 (40.6)               | 0.938   |
| GGT U/L (reference range 6–40)    | 30.2 (23.7)             | 87.6 (119.2)              | 0.004   |
| Bilirubin µmol/L (reference range 1.7–21.0) | 9.1 (4.9)          | 11.3 (18.3)               | 0.279   |
| Albumin g/L (reference range 35–50) | 43.1 (3.9)            | 39.2 (5)                  | < 0.001 |
| Abnormality type (n, %)           |                         |                            |         |
| Hepatocellular > ULN              | 39 (38.6)               | 221 (56.2)                | 0.002   |
| Cholestatic > ULN                 | 2 (16.7)                | 112 (61.2)                | 0.004   |
| Mixed > ULN                       | 0 (0)                   | 72 (39.3)                 | 0.004   |
| PT (normal range > 70%) (%, SD)   | 92.6 (8)                | 87.9 (15.7)               | 0.042   |
| CRP mg/L (reference range < 4)    | 36.1 (54.1)             | 87.9 (70.5)               | < 0.001 |
| Leucocytes *10⁹/L (reference range 4.5–6.5) | 6.2 (2.6)          | 7(3.4)                     | 0.02    |
| Neutrophil count *10⁹/L (reference range 1.3–7.7) | 4.2 (2.2)          | 5.3 (3)                    | < 0.001 |
| Lymphocytes *10⁹/L (reference range 1.0–4.0) | 1.5 (0.8)          | 1.2 (1.3)                  | < 0.001 |
| ≤ 1.0 (n, %)                      | 31 (30.7)               | 200 (50.9)                | 0.001   |
| Fibrinogen g/L (reference range: 2–4) | 5(1.4)                   | 5.9 (1.5)                 | 0.016   |
| Platelets *10⁹/L (reference range: 150–400) | 236.2 (104.6) | 226.3 (99.8)              | 0.331   |
| D-Dimers (reference range: 150–400) | 765 (698)             | 2452.9 (4111.5)           | 0.05    |
| Metabolic and fibrosis characteristics |                         |                            |         |
| FIB-4 status (n, %)               |                         |                            | < 0.001 |
| ≤ 1.3                             | 59 (58.4)               | 111 (28.3)                |         |
| 1.3–2.67                          | 29 (28.7)               | 142 (36.2)                |         |
| > 2.67                            | 13 (12.9)               | 139 (35.5)                |         |
| NAFLD (n, %)                      | 62 (61.4)               | 249 (63.4)                | 0.73    |
| NAFLD+ and FIB-4 (n, %)           |                         |                            | < 0.001 |
| NAFLD + FIB-4 < 1.3               | 33 (32.7)               | 62 (15.8)                 |         |
| NAFLD + FIB-4 1.3–2.67            | 19 (18.8)               | 94 (23.9)                 |         |
| NAFLD + FIB-4 > 2.67              | 49 (48.5)               | 237 (60.3)                |         |
| NFS (mean, SD)                    | -2 (1.5)                | -0.7 (1.7)                 | < 0.001 |
| Radiologic findings               |                         |                            | < 0.001 |
| Severity of pneumonia (CT scan)   |                         |                            |         |
Table 1 (Continued)

| Variable                | Mild COVID-19 (n = 101) | Moderate COVID-19 (n = 393) | p-value |
|-------------------------|-------------------------|-----------------------------|---------|
| -absence                | 25 (26%)                | 24 (6.8%)                   |         |
| 50%                     | 58 (60.4%)              | 185 (52.6%)                 |         |
| 25%                     | 10 (10.4%)              | 116 (33%)                   |         |
| 25%                     | 3 (3.1%)                | 27 (7.7%)                   |         |
| Delays from symptom onset to hospitalization (days, SD) | 7.2 (6.8) | 7.6 (6.8) | 0.592 |
| Time of hospitalization (days, SD) | 1 (0.9) | 10.6 (8.5) | < 0.001 |

BMI: body mass index; SD: standard deviation; COVID-19: coronavirus disease 2019; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; ULN: upper limit of the normal; GGT: gamma-glutamyl transferase; LFTs: liver function tests; PT: prothrombin time; FIB-4: fibrosis-4; CRP: C-reactive protein; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CT: computed tomography; HSI: hepatic steatosis index; NAFLD: non-alcoholic fatty liver disease; NFS: FIB-4 fibrosis score; SaO2: capillary oxygen saturation.

For the bivariate analysis between groups, Fisher’s exact test or Chi-squared tests were applied to compare qualitative variables, and Wilcoxon or Student t-tests were employed to compare quantitative variables. To analyze the primary outcome, logistic regression was performed after adjusting for confounding factors that might disturb the LFTs on admission, such as hypoxemia (capillary oxygen saturation [SaO2] < 93%, related to severe pneumopathy) and hepatotoxic drugs (acetaminophen; antibiotics) administered prior to hospitalization. Hypotension, defined by systolic blood pressure < 90 mmHg, was not considered because of the low number of cases concerned (< 10).

### Results

Between February, 22 and May, 15, 2020, a total of 1381 patients with SARS-CoV-2 infection were admitted to the ERs. After excluding 662 patients according to our exclusion criteria, 719 were included in the subsequent analyses, as presented in the study flowchart (Fig. 1).

According to COVID-19 severity, 101 (14%) patients displayed mild disease, 393 (54.7%) presented with moderate disease, and 225 (31.3%) developed severe disease. The mean hospitalization length was 11.7 ± 12.8 days.

The mean age was 64.7 (19–97) years old, and 387 (58.8%) patients were men. Comorbidities were present in 513 (71.3%) patients, and the most common were BMI > 25 Kg/m² in 479 (70.6%) and BMI > 30 Kg/m² in 240 (33.3%), hypertension in 386 (53.7%), dyslipidemia in 226 (31.5%), diabetes in 201 (28%), chronic cardiac disease in 232 (32.3%), chronic respiratory disease in 176 (24.5%), chronic renal disease in 59 (8.2%), and cancer in 32 (4.5%).

Liver tests were performed on admission. The frequency of different abnormal LFTs, such as AST, ALT, ALP, GGT, TBIL, albumin, and prothrombin time (PT), was 57%, 35.9%, 11.4%, 56.5%, 5.8%, 18.4%, and 9.1%, respectively, while the mean values for these parameters were 53.8 (±83.1) u/L, 46.2 (±67.6) u/L, 74.2 (±39.1) u/L, 84.4 (±104.4) u/L, 11.3 (±14.5) µmol/L, 38.2 (±5.5) g/dL, and 87.8% (±14.7%), respectively.

The LFTs exhibited a hepatocellular pattern in 60.9%, cholestatic pattern in 63.1%, and mixed-type pattern in 46.8% of patients.

### Comparison between mild and moderate COVID-19 on admission

Table 1 summarizes the clinical and biological characteristics on admission for patients with mild and moderate disease.

LFT laboratory data showed higher elevations of AST (p < 0.001), ALT (p = 0.05), and GGT (p = 0.004) in addition to lower albumin (p < 0.001) and PT (p = 0.042) in patients with moderate versus mild COVID-19 (Table 1). AST levels were higher in patients hospitalized in a CHU compared to those in ER: 1–2xULN (p = 0.001 and >2xULN (p = 0.001).

Patients with moderate COVID-19 exhibited elevated GGT levels compared to mild disease, 53.6% vs 16.7% (p = 0.016), respectively and more disturbed hepatocellular (p = 0.002), cholestatic (p = 0.004) and mixed-type (0.004) patterns. These patients had also higher NFS (p < 0.001) and more severe liver fibrosis than patients with mild COVID-19: 71.7% had an intermediate/high FIB-4 score compared to 41.6% with mild disease (p < 0.001). Patients with moderate disease had more NAFLD with an intermediate/high FIB-4 score compared to patients with mild disease (p < 0.001) (Table 1).

As can be seen in Table 2, after adjusting for confounding factors that could disturb LFTs, such as hypoxemia and hepatotoxic drugs, patients with AST 2xULN and hepatocellular pattern 2xULN had a 5.5-fold higher risk of being hospitalized (OR 5.599; 95% CI: 1.29–24.25, p = 0.021 and OR 5.415; 95% CI: 1.27–23.86, p = 0.023, respectively). Patients with metabolic syndrome (the association of 3 metabolic risk factors (MRF)) had more than 2.5-fold higher risk of hospitalization (OR 2.56; 95% CI: 1.29–5.09, p = 0.007).

Moderate disease was associated with an intermediate (OR 2.405; 95% CI: 1.39–4.15; p = 0.002) and high (OR 5.415; 95% CI: 2.69–10.89; p < 0.001) FIB-4 score. Similarly,
patients with an elevated NFS had a higher risk of hospitalization (OR 1.754 95% CI: 1.27–2.43; p < 0.001).

In multivariate analyses, patients with metabolic syndrome had almost a 2.4-fold risk of being hospitalized (OR 2.37; 95% CI: 1.18–4.75, p = 0.015).

**Comparison between mild/moderate and severe COVID-19**

We compared the clinical and laboratory tests and radiological findings in patients with severe versus those with moderate and mild COVID-19 disease (Table 3).

The laboratory liver tests showed more elevated AST (p < 0.001), ALT (p = 0.002), and TBIL levels (p = 0.01), in addition to lower albumin (p < 0.001) and PT (p < 0.001) in severe disease patients (Table 3).

Concerning abnormal LFT patterns, severe COVID-19 was associated with all types of patterns: hepatocellular (p < 0.001), cholestatic (p = 0.028), and mixed (p < 0.001).

In severe disease patients, 89.3% had intermediate and high FIB-4 scores compared to 65.5% of patients with moderate and mild disease (p < 0.001). More patients with severe COVID-19 had NAFLD associated with a high FIB-4: 72.8% vs. 57.9%, respectively (p < 0.001) (Table 3).

After adjusting for confounding factors, as described, abnormal LFTs at admission such as AST > 2x ULN (OR 3.187; 1.99–5.08; p < 0.001), hepatocellular pattern > 2x ULN (2.265; 95% CI 1.48–3.45; p < 0.001), intermediate and high FIB-4 score (OR 2.006; 95% CI 1.09–3.67; p = 0.024) and OR 4.756; 95% CI 2.68–8.43; p < 0.001, respectively) or metabolic syndrome (OR 2.45, 95% CI 1.07–5.61, p = 0.03) were associated with a worse evolution of the disease (Table 4).

In multivariate analyses, patients with high FIB-4 index had 3-fold risk to develop a severe disease (OR 3.11; 95% CI 1.4–6.91, p = 0.005).

**Discussion**

Coronavirus disease 2019, the pandemic caused by SARS-CoV-2, has been associated with substantial morbidity and mortality [15]. More information concerning SARS-CoV-2 infection is needed to rapidly identify patients likely to develop severe disease, requiring adjusted care. This is the first monocentric study that describes the outcome of a large cohort of COVID-19 patients based on clinical, laboratory, and radiological data collected at admission, enabling us to rapidly identify factors associated with outcomes that predict mild, moderate, or severe disease at the time of hospital admission the hospital.

Recently published studies have shown that patients admitted for COVID-19 often present with abnormal liver test, the incidence of liver injury ranging from 14% to 78% [1,6,16]. Moreover, preexisting liver disease has been shown to be associated with worsening infection prognosis [17]. However, the mechanistic events remain to be determined, and the causes of LFTs may be multifactorial, including causes unrelated to COVID-19. In contrast to previous studies, we analyzed only the laboratory liver tests at baseline in
Table 3  Characteristics of patients with mild and moderate versus severe COVID-19 on admission.

| Variable | Mild and Moderate COVID-19 (n = 494) | Severe COVID-19 (n = 225) | p-value |
|----------|-------------------------------------|--------------------------|---------|
| Age (years, SD*) | 63.1 (17.5) | 68.3 (16.9) | < 0.001 |
| Gender (male,%) | 251 (50.8) | 138 (61.3) | 0.94 |
| BMI (Kg/m²) (mean, SD) | 28.4 (5.9) | 28.9 (5.9) | 0.255 |
| Comorbidity | | | |
| Diabetes (n,%) | 126 (25.5) | 75 (33.3) | 0.032 |
| Hypertension (n,%) | 255 (51.6) | 131 (58.2) | 0.107 |
| Dyslipidemia (n,%) | 146 (29.6) | 80 (35.7) | 0.118 |
| Chronic pulmonary disease (n,%) | 113 (23) | 63 (28) | 0.161 |
| Chronic cardiac disease (n,%) | 125 (25.3) | 72 (32) | 0.071 |
| Chronic kidney disease (n,%) | 30 (6.1) | 29 (12.9) | 0.003 |
| Cancer (n,%) | 22 (4.5) | 10 (4.5) | 1 |
| Symptoms onset | | | |
| “Flu-like” symptoms (n,%) | 453 (91.7) | 210 (93.3) | 0.549 |
| Respiratory symptoms (n,%) | 438 (88.7) | 208 (92.4) | 0.435 |
| Digestive symptoms (n,%) | 248 (50.2) | 96 (42.7) | 0.064 |
| Neurological symptoms (n,%) | 229 (46.4) | 106 (47.1) | 0.872 |
| Clinical features | | | |
| Oxygen saturation SaO2 (%), SD | 92.9 (5.7) | 85.3 (9.1) | < 0.001 |
| Debit of oxygen at admission (l/mn, sd) | 1.9 (6.7) | 6.5 (7.7) | < 0.001 |
| Systolic arterial pressure, mmHg | 132.5 (20.8) | 131.5 (20.9) | 0.375 |
| Laboratory tests | | | |
| AST U/L (reference range, 11–34) | 48 (87.8) | 73.1 (64.6) | < 0.001 |
| ALT U/L (reference range, 8–41) | 40.9 (32.8) | 57.9 (110) | 0.002 |
| ALP U/L (reference range, 41–117) | 75.2 (39) | 72.3 (39.4) | 0.178 |
| GGT U/L (reference range, 6–40) | 84.2 (116.5) | 84.7 (79.6) | 0.087 |
| Total Bilirubin μmol/l (reference range, 1.7–21.0) | 11.1 (17.5) | 11.6 (6.9) | 0.01 |
| Conjugated Bilirubin μmol/l (reference range < 4.3) | 3.7 (2.6) | 4.2 (2.4) | 0.055 |
| Albumin g/, (reference range, 35–50) | 39.7 (5) | 36.4 (5.4) | < 0.001 |
| PT (normal range > 70%) (%, SD) | 88.9 (14.6) | 85.6 (14.7) | < 0.001 |
| Abnormality type (n,%) | | | |
| Hepatocellular > ULN | 260 (52.6) | 178 (79.1) | < 0.001 |
| Cholestatic> ULN | 114 (58.5) | 81 (71.1) | 0.028 |
| Mixed type = ULN | 72 (36.9) | 72 (63.7) | < 0.001 |
| CRP mg/L (reference range < 4) | 77.7 (70.6) | 148.7 (92.6) | < 0.001 |
| Leucocytes *10⁹/l (reference range 4.5–6.5) | 6.9 (3.3) | 8.6 (4.4) | < 0.001 |
| Neutrophil count *10⁹/l (reference range 1.3–7.7) | 5.1 (2.9) | 6.9 (3.9) | < 0.001 |
| Lymphocytes *10⁹/l (reference range: 1.0–4.0) | 1.2 (1.2) | 1 (1.5) | < 0.001 |
| Fibrinogen g/l (reference range 2–4) | 5.8 (1.5) | 6.8 (1.4) | < 0.001 |
| Platelets *10⁹/L (reference range 150–400) | 228.3 (99.8) | 210.6 (5.4) | 0.028 |
| D-Dimers (reference range 150–400) | 2302.9 (3956.9) | 2849.6 (4072.5) | 0.001 |
| Metabolic and fibrosis characteristics | | | |
| FIB-4 score (n,%) | | | |
| ≤ 1.3 | 170 (34.5) | 24 (10.7) | |
| 1.3–2.67 | 171 (34.7) | 67 (29.9) | |
| ≥ 2.67 | 152 (30.8) | 133 (59.4) | |
| NAFLD (n,%) | 311 (63) | 134 (59.8) | 0.455 |
| NAFLD+ and FIB-4 (n,%) | | | |
| NAFLD+ FIB-4 < 1.3 | 95 (19.2) | 20 (8.9) | |
| NAFLD + FIB-4 1.3–2.67 | 113 (22.9) | 41 (18.3) | |
order to avoid the anomalies induced by different drugs administered during the hospitalization.

In this present cohort, on admission, abnormal LFTs were present in 68.9% of patients, being more elevated than those previously reported in China at 14.9%, yet similar to those estimated in the United States at between 40%–67.5%. This controversy could be explained by the higher proportion of obese and diabetic patients in occidental countries [18].

Hepatocellular parameters, such as the AST and ALT, were elevated in 57% and 35.9% of patients, respectively, being beyond 2xULN in 17.4% and 4%, respectively. Patients with severe disease had a higher AST compared with moderate and mild disease patients \((p < 0.001)\). The ALT was also more elevated in severe COVID-19 patients when compared with the other patient groups \((p = 0.02)\). After adjusting for confounding factors, elevated transaminases, such as AST > 2xULN, and hepatocellular pattern type > 2xULN were associated with a 3-fold \((p < 0.001)\) and almost 3.5-fold \((p < 0.001)\) higher risk, respectively, of a poorer clinical outcome.

Recent studies have shown that SARS-CoV-2 binds angiotensin converting enzyme 2 (ACE2) receptors localized both in the liver and bile duct cells. The expression is higher in the cholangiocytes that may be associated with cholestatic abnormalities (elevated ALP and GGT) rather than hepatocellular abnormalities (AST and ALT) [1,20].

In our study, the ALP, GGT, and TBIL were elevated in 11.4%, 56.5%, and 5.8% of patients, respectively. Only an abnormal GGT was associated with moderate disease \((GGT \text{ ULN}, OR 0.108; 95\%CI: 0.013–0.89, p = 0.039)\). Interestingly, ALP > 1–2xULN was more common in moderate and mild disease (91.5%) than in severe disease \((83.9\%); p = 0.045)\). Our data confirm the hypothesis of Hundt et al. reporting that the mechanism of liver damage may be not caused by direct cytopathic effects of the novel coronavirus [19].

Obesity was associated with COVID-19 disease severity [21,22]. Indeed, in our study, 72.5% of patients that required hospitalization were overweight, with 34.6% being obese. The proportion of overweight patients increased with disease severity, as follows: 58.4% in mild, 71.8% in moderate, and 73.3% in severe COVID-19. Moreover, the hospitalized patients had a higher BMI compared to those discharged from an ER: 28.9 vs. 26.9 Kg/m² \((p = 0.001)\).

A strong association between obesity and the severity of COVID-19 was previously reported, even in the absence of other comorbidities, yet the underlying mechanism is unclear [23].

Obesity is characterized by a chronic inflammatory state and disrupted immune response. The main cytokines responsible for chronic inflammation are tumor necrosis factor-alpha (TNF-α), interleukin (II)–6, and IL-1 beta(β) [5]. Moreover, SARS-CoV-2 may cause a hyper-inflammatory reaction through the excessive release of cytokines, a condition known as “cytokine storm,” that may be enhanced by obesity, particularly in patients with a preexisting liver disease like NAFLD [24]. Adipose tissue may also be a viral reservoir and an immunological hub for the inflammatory response [25]. Individuals with severe obesity, diabetes, and hypertension are more likely to be infected and are at a higher risk of complications and death due to COVID-19 [2].

Indeed, in our study, metabolic factors were associated with the severity of COVID-19. Hypertension \((p = 0.002)\) and dyslipidemia \((p = 0.001)\) were associated with moderate disease, and diabetes was associated with a severe course of the disease \((p = 0.032)\). Patients with metabolic syndrome had a 2.5-fold higher risk to develop a moderate \((p = 0.007)\) or a severe disease \((p = 0.033)\).

Abnormal GGT is commonly observed in NAFLD and is a sensitive marker of steatosis [11]. In our study, the ALP was elevated only in 11.4% of patients, and the mean values were not significantly higher in mild disease compared to moderate \((p = 0.938)\) and severe forms \((p = 0.178)\). Moreover, 56.5% of patients had abnormal GGT levels, and this was correlated with the severity of COVID-19 when comparing severe versus moderate \((p = 0.02)\) and mild disease forms \((p = 0.016)\).

| Variable | Mild and Moderate COVID-19 | Severe COVID-19 (\(n = 225\)) | p-value |
|----------|---------------------------|-----------------------------|--------|
| NAFLD + FIB-4 \(> 2.67\) | 286 (57.9) | 163 (72.8) | 0.435 |
| NAFLD fibrosis score (mean, SD) | -0.8 (1.8) | -0.6 (1.6) | 0.435 |
| Radiologic findings | | | |
| Severity of pneumonia (CT scan) | | | |
| - absence (n,%) | 49 (10.9%) | 10 (5%) | < 0.001 |
| - \(< 25\%\) | 243 (54.2%) | 53 (26.6%) | |
| - 25–50\% | 126 (28.1%) | 75 (37.7%) | |
| - \(> 50\%\) | 30 (6.7%) | 61 (30.7%) | |
| Delays from symptoms onset to hospitalization (days, SD) | 6.6 (6.2) | 7.2 (5.3) | 0.062 |
| Time of hospitalization | 8.7 (8.5) | 19.1 (12.8) | < 0.001 |

SD, standard deviation mean values; BMI: body mass index; SD: standard deviation; COVID-19: coronavirus disease 2019; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; ULN: upper limit of the normal; GGT: gamma-glutamyl transferase; LFTs: liver function tests; PT: prothrombin time; FIB-4: fibrosis-4; CRP: C-reactive protein; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CT: computed tomography; HSI: hepatic steatosis index; NAFLD: non-alcoholic fatty liver disease; NFS: NAFLD fibrosis score; SaO2: capillary oxygen saturation; TBIL: total bilirubin.
The prognosis of liver mortality was related to liver fibrosis [26]. Liver fibrosis evaluated with the FIB-4 score was associated with the severity of COVID-19 illness: 71.7% of patients with intermediate/high FIB-4 scores needed hospitalization compared to 41.6% of those with only mild disease (p < 0.001), and it was higher in patients with severe SARS-CoV-2 infection than in those with moderate and mild disease (89.3% compared to 65.5%, respectively) (p < 0.001). Patients who needed hospitalization had a more elevated NFS compared to those who were discharged from an ER (p = 0.001).

We associated the FIB-4 scores in patients diagnosed with NAFLD (Tables 2 and 4). In the hospitalized patients, 84.2% had an intermediate/high FIB-4 compared to 67.3% of those discharged from an ER (p < 0.001). In patients with a high FIB-4, 72.8% had severe disease compared to the 57.9% of those with moderate and mild disease (p < 0.001). Moreover, the patients with NAFLD without fibrosis (FIB-4 < 1.3) were at a lower risk of hospitalization (OR 0.404; 95%CI: 0.20–0.81; p < 0.011).

In the multivariate-adjusted analysis, patients with high FIB-4 scores had an almost 3-fold greater risk of hospitalization (p = 0.011), and a worse course of disease (p < 0.001).

In the context of the COVID-19 pandemic, along with the high prevalence of NAFLD in the general population, it is mandatory to early identify patients at risk of developing a severe disease. Applying non-invasive tests such as the FIB-4 and NFS in patients with metabolic liver disease could enable us to better and more quickly identify these patients that may have a poorer prognosis [27].

Our study had some limitations. First, the data were collected retrospectively, and there was missing information regarding the tobacco and alcohol consumption, several clinical parameters (waist and hip circumferences), and laboratory tests pertaining to metabolic syndrome, including glucose, glycated hemoglobin, cholesterol, and triglycerides levels. Second, while all patients underwent transaminase measurements at admission, only half of them underwent cholestasis enzyme assessment at admission, which could be deemed as a limitation in the statistical analysis. Third, HIS index was used in several publications to define NAFLD in COVID 19 patients [8–10]. It is a simple, disponible score that may help us to easy identify high-risk patients. His-limitation is related to the elevated transaminases levels that could underestimate the index.

In conclusion, obese patients with metabolic syndrome and a preexisting liver disease, including NAFLD and liver fibrosis, display a dysregulated hepatic innate immunity and pro-inflammatory state that is likely to enhance the “cytokine storm” due to SARS CoV-2 infection, leading to severe COVID-19 disease. Thus, abnormal LFTs at admission predict the outcome of COVID-19 patients.

| Table 4 | Factors associated with moderate severe COVID-19 after adjusting for confounding factors. |
|---------|----------------------------------------------------------------------------------------|
| OR      | 95%CI | p-value |
| MS (MRF | 2.45 | 1.07–5.61 | 0.033 |
| 1 + 2 + 3 + 4) | |
| NFS | 1.181 | 1.01–1.37 | 0.033 |
| FIB-4 (1.3–2.67) | 2.006 | 1.09–3.67 | 0.024 |
| FIB-4 > 2.67 | 4.756 | 2.68–8.43 | < 0.001 |
| AST ULN | 0.406 | 0.27–0.61 | < 0.001 |
| AST > 2 ULN | 3.187 | 1.99–5.08 | < 0.001 |
| ALT ULN | 0.716 | 0.48–1.05 | 0.09 |
| Hepatocellular type < ULN | 2.265 | 1.48–3.45 | < 0.001 |
| Hepatocellular type > 2 ULN | 3.404 | 2.12–5.47 | < 0.001 |
| GGT > 1 – 3 ULN | 1.676 | 0.97–2.88 | 0.062 |
| Albumin ULN | 0.422 | 0.21–0.82 | 0.01 |
| Albumin | 0.871 | 0.82–0.92 | < 0.001 |
| Leucocytes count | 1.104 | 1.05–1.16 | < 0.001 |
| Neutrophil count | 1.136 | 1.07–1.21 | < 0.001 |
| CRP > 200 mg/L | 2.785 | 1.66–4.66 | < 0.001 |
| CRP < 100 mg/L | 0.441 | 0.29–0.65 | < 0.001 |
| Fibrinogen | 1.317 | 1.05–1.64 | 0.015 |
| Lupus-anticoagulant | 4.453 | 1.49–13.23 | 0.007 |

OR: odd ratio; CI: confidence interval; MS: metabolic syndrome, MRF: metabolic risk factors: 1-obesity; 2-diabetes, 3-dyslipidemia, 4-hypertension.
Adjustment for confounding factors that could disturb LFTs, as follows: hypoxemia (SaO2 < 93% related to severe pneumonia) [16], and hepatotoxic drugs (acetaminophen, antibiotics) administrated before the hospitalization. Hypotension defined by systolic blood pressure < 90 mmHg was not considered because of the low number of cases < 10.

Authors’ contributions
ST conceived and designed the study, collected patient data, performed data analysis and interpretation, and drafted the manuscript. MD conceived and designed the study, analysis, and interpretation of the manuscript and contributed to the critical revision of the manuscript. FC, JM contributed to data collection. TFB, DM, FM contributed to the critical revision of the manuscript. TF performed statistical analysis. All the others contributed to COVID-19 recruitment. All authors contributed to the manuscript revision and approved the submitted final version.
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