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MAJOR ARTICLE

Association of Nonalcoholic Fatty Liver Disease With COVID-19 Severity and Pulmonary Thrombosis: CovidFAT, a Prospective, Observational Cohort Study

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Background. Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease associated with systemic changes in immune response, which might be associated with coronavirus disease 2019 (COVID-19) severity. The aim of this study was to investigate the impact of NAFLD on COVID-19 severity and outcomes.

Methods. A prospective observational study included consecutively hospitalized adult patients, hospitalized between March and June 2021, with severe COVID-19. Patients were screened for fatty liver by ultrasound and subsequently diagnosed with NAFLD. Patients were daily followed until discharge, and demographic, clinical, and laboratory data were collected and correlated to clinical outcomes.

Results. Of the 216 patients included, 120 (55.5%) had NAFLD. The NAFLD group had higher C-reactive protein (interquartile range [IQR]) (84.7 [38.6–129.8] mg/L vs 66.9 [32.2–97.3] mg/L; $P = .0340$), interleukin-6 (49.19 [22.66–92.04] ng/L vs 13.22 [5.29–39.75] ng/L; $P < .0001$), aspartate aminotransferase (58 [40–81] IU/L vs 46 [29–82] IU/L; $P = .0123$), alanine aminotransferase (51 [32–73] IU/L vs 40 [23–69] IU/L; $P = .0345$), and lactate dehydrogenase (391 [285–483] IU/L vs 324 [247–411] IU/L; $P = .0027$). The patients with NAFLD had higher disease severity assessed by 7-category ordinal scale, more frequently required high-flow nasal cannula or noninvasive ventilation (26, 21.66%, vs 10, 10.42%; $P = .0289$), had longer duration of hospitalization (IQR) (10 [8–15] days vs 9 [6–12] days; $P = .0018$), and more frequently had pulmonary thromboembolism (26.66% vs 13.54%; $P = .0191$). On multivariable analyses, NAFLD was negatively associated with time to recovery (hazard ratio, 0.64; 95% CI, 0.48 to 0.86) and was identified as a risk factor for pulmonary thrombosis (odds ratio, 2.15; 95% CI, 1.04 to 4.46).

Conclusions. NAFLD is associated with higher COVID-19 severity, more adverse outcomes, and more frequent pulmonary thrombosis.

Keywords. COVID-19; SARS-CoV2; non-alcoholic fatty liver disease; NAFLD; pulmonary thrombosis.

The clinical spectrum of coronavirus disease 2019 (COVID-19) disease ranges in severity from asymptomatic to critical illness with acute respiratory distress syndrome, multiple organ failure, and high mortality rates in specific patient populations [1]. There is growing evidence that components of metabolic syndrome, such as diabetes, obesity, or hyperlipidemia, all related to nonalcoholic fatty liver disease (NAFLD), increase susceptibility to infection and adverse outcomes [2]. NAFLD is the most common chronic liver disease, affecting about 25% of the Western population, and is linked to chronic low-grade inflammation, impaired immune response, and microvascular endothelial dysfunction, which might have a profound impact on COVID-19 outcomes [2–4].

Surprisingly, the publications on the impact of NAFLD on infections in the pre-COVID era are scarce, and COVID-19 unveiled the magnitude of the growing NAFLD pandemic, indicating a potential significant role in infectious diseases. Still, the impact of NAFLD on COVID-19 remains unclear. According to limited data, it seems that patients with NAFLD have higher risk for infection and symptomatic disease [5–7]. In COVID-19, NAFLD was associated with liver injury of uncertain clinical significance [8]. However, the data on NAFLD as a predictor of disease severity and mortality in patients with COVID-19 are conflicting; while some studies show no difference in clinical presentations, other show increased mortality [8–12]. Most of the published data originate from retrospective studies from the beginning of pandemic, and there are no prospective studies from the later period.

Here we have designed a prospective cohort study with the aim to investigate a possible association between NAFLD and COVID-19 severity, complications, and outcomes.

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METHODS

Study Design and Patients

A prospective, noninterventional monocentric observational study was conducted at the University Hospital for Infectious Diseases Zagreb (UHID), Croatia (COVID-FAT, ClinicalTrials.gov Identifier: NCT04982328). Included were consecutively hospitalized adult patients with confirmed COVID-19 between March and June 2021. Inclusion criteria were severe disease, defined as bilateral pneumonic infiltrates on chest imaging, Sp02 ≤94% on room air and/or dyspnea or respiratory frequency ≥24 breaths/min. Exclusion criteria were history of chronic liver disease, significant alcohol consumption, active cancer, pregnancy, immunocompromised patients, and palliative management. Patients who required oxygen supplementation for <24 hours, who were admitted to the intensive care unit (ICU) within the first 24 hours, and those who died in the first 48 hours of hospital admission were excluded from the study. A flowchart of the study's design is presented in Supplementary Figure 1. All participants gave written informed consent. The study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by Ethics Committee of the UHID Zagreb (code 01-673-4-2021).

Data Collection and Definitions

At the time of presentation, the following data were collected: demographic, clinical, comorbidities, use of chronic medications, body mass index, waist/hip ratio, baseline clinical status, and selected laboratory data. Clinical evolution, including oxygen requirements, invasive and noninvasive ventilation, and complication rates (including pulmonary thrombosis [PT]), was assessed daily.

Patients were treated according to standard of care with remdesivir (up to 5 days), corticosteroids, low–molecular weight heparin (LMWH), and tocilizumab at the discretion of the managing physician. Multislice computed tomography (MSCT) pulmonary angiography was performed according to hospital protocol in all patients who required high-flow nasal cannula (HFNC) oxygen therapy or noninvasive ventilation (NIV), in those who required oxygen supplementation >15 L/02, or if there was clinical suspicion of PT.

Upon admission, patients were screened for fatty liver by ultrasound and/or by measurement of the difference between liver and spleen computed tomography (CT) attenuation in patients who underwent CT angiography. Patients were subsequently diagnosed with NAFLD according to current guidelines that require: (1) evidence of liver steatosis, (2) no significant alcohol consumption, (3) no competing causes of liver steatosis, and (4) no coexisting causes of chronic liver disease [4, 13].

Outcomes

Patients' clinical status was assessed daily using a 7-category ordinal scale from day 0 to day 28, hospital discharge, or death. The categories were defined as follows: (1) discharged, (2) hospitalized but ready to be discharged, (3) requiring low-flow supplemental oxygen, (4) requiring HFNC or NIV, (5) requiring invasive mechanical ventilation, (6) requiring extracorporeal membrane oxygenation, and (7) death. Overall survival and the proportion of patients with clinical improvement were assessed. Other outcomes measured were duration of hospitalization, time to recovery (measured as discharge or readiness for discharge), presence of complications, and pulmonary thrombosis.

Statistical Analysis

Clinical characteristics and laboratory and demographic data were evaluated and descriptively presented. The Fisher exact test and Mann-Whitney *U* test were used to compare the 2 groups. All tests were 2-tailed; a *P* value <.05 was considered statistically significant. Time to hospital discharge or readiness for discharge was evaluated using the Kaplan-Meier method. Risk factors associated with negative outcomes were investigated using a univariate model and, subsequently, a multivariable Cox regression model by estimating the hazard ratio (HR) and its 95% confidence intervals. Multivariable Cox proportional hazards models were developed using backward elimination with $P < .1$ to retain variables in the model. Binary logistic regression analysis was used to assess the independent predictors of PT. Variables were entered in a backward stepwise logistic regression model. Statistically nonsignificant predictors were progressively excluded based on a likelihood ratio test. The strength of an association was expressed as an odds ratio (OR) and its corresponding 95% CI. Statistical analyses were performed using GraphPad Prism, version 9.1.1 (San Diego, CA, USA), and MedCalc, version 20.008 (MedCalc Software, Ostend, Belgium).

RESULTS

Baseline Patient Characteristics

Overall, 216 Caucasian patients were included in the study (137; 63.43% males; median age [IQR], 61 [50–67] years). One hundred twenty patients (55.5%) were diagnosed with NAFLD, and 46 (38.33%) patients had "lean-weight NAFLD." Patients with NAFLD were younger and had a higher median body mass index (BMI) and waist-hip ratio, as presented in Table 1. There were no differences in other comorbidities or in the use of chronic medications.

Time since symptom onset to hospital admission (IQR) was similar between groups (10 [7–12] days vs 10 [8–13] days; $P = .2297$). On admission, required oxygen supplementation to maintain SpO2 ≥90% was similar between groups (median [IQR], 7L [3–25] O2/min).

Patients with NAFLD had significantly higher inflammatory markers, including C-reactive protein (CRP), procalcitonin, and interleukin-6, and higher aspartate aminotransferase,

Table 1. Baseline Patients' Characteristics

	NAFLD (n = 120)	Non-NAFLD (n = 96)	Difference (95% CI)[a]	*P* Value[b]
Age, median (IQR), y	59 (49.25–64.75)	63 (55–71)	4.00 (1.00 to 8.00)	.0096
Male, No. (%)	78 (65)	59 (61.46)	0.006 (–0.12 to 0.14)	.6700
Overweight (BMI 25–30 kg/m^2), No. (%)	22 (18.33)	22 (22.92)	0.04 (–0.07 to 0.16)	.4968
Obesity (BMI >30 kg/m^2), No. (%)	74 (61.67)	26 (27.08)	0.34 (0.21 to 0.46)	.0001
BMI, median (IQR), kg/m^2	31.25 (28.67–34.66)	27.17 (24.22–30.67)	–4.08 (–6.45 to –2.81)	<.0001
Waist-hip ratio, median (IQR)	1.04 (0.97–1.10)	0.90 (0.84–1.05)	–0.13 (–0.17 to –0.06)	<.0001
Comorbidities, No. (%)				
Diabetes mellitus	20 (16.67)	10 (10.42)	0.06 (–0.04 to 0.15)	.2358
Arterial hypertension	47 (39.17)	45 (46.88)	0.07 (–0.06 to 0.21)	.2707
Gastritis/GERD	5 (4.17)	5 (5.21)	0.01 (–0.06 to 0.07)	.7539
Dyslipidemia	16 (13.33)	15 (15.62)	0.02 (–0.08 to 0.12)	.6979
Cardiovascular disease	7 (5.83)	10 (10.42)	0.04 (–0.04 to 0.12)	.3092
Use of chronic medications, No. (%)				
ACE inhibitors	33 (27.50)	28 (29.17)	0.02 (–0.11 to 0.14)	.8793
Beta blockers	22 (18.33)	22 (22.29)	0.04 (–0.07 to 0.16)	.4968
Acetylsalicylic acid	12 (10.00)	15 (15.63)	0.05 (–0.05 to 0.15)	.2226
Proton pump inhibitors	14 (11.67)	7 (7.29)	0.04 (–0.05 to 0.13)	.3576
Statins	11 (9.17)	13 (13.54)	0.04 (–0.05 to 0.13)	.3847
Metformin	11 (9.17)	6 (6.25)	0.03 (–0.05 to 0.11)	.4598
Other perioral antidiabetic	10 (8.33)	3 (3.13)	0.05 (–0.02 to 0.12)	.1515
Duration of illness on admission, median (IQR), d	10 (7–12)	10 (8–13)	0.00 (0.00 to 2.00)	.2297
Laboratory findings on admission, median (IQR)				
C-reactive protein, mg/L	84.7 (38.6–129.8)	66.9 (32.2–97.3)	–17.7 (–30.0 to –1.1)	.0340
Procalcitonin, µg/L	0.13 (0.09–0.25)	0.09 (0.07–0.17)	–0.04 (–0.05 to –0.01)	.0035
Interleukin-6, ng/L	49.19 (22.66–92.04)	13.22 (5.29–39.75)	–35.9 (–51.4 to –13.5)	<.0001
Ferritin, µg/L	899 (501–1378)	623 (437–1417)	–275.5 (–431.0 to 128.0)	.2581
White blood cell count, ×109/L	6.5 (4.9–9.3)	7.2 (5.5–9.4)	0.70 (–0.40 to 1.20)	.3270
Lymphocyte's count, 10^9/L	0.76 (0.57–1.08)	0.75 (0.51–1.04)	–0.0004 (–0.17 to 0.14)	.4431
Neutrophil/lymphocyte ratio	6.96 (3.94–10.49)	8.09 (4.94–10.79)	1.13 (–0.51 to 1.97)	.2327
Hemoglobin, g/L	140 (132–147)	138 (129–146)	–2.00 (–5.00 to 2.00)	.2893
Platelets, ×10^9/L	183 (138–253)	187 (151–251)	4.00 (–10.00 to 33.00)	.3182
Bilirubin, µmol/L	11 (9–14)	11 (9–15)	0.00 (–1.00 to 1.00)	.6197
Aspartate aminotransferase, IU/L	58 (40–81)	46 (29–82)	–12.00 (–17.00 to –2.00)	.0123

Table 1. Continued

	NAFLD (n = 120)	Non-NAFLD (n = 96)	Difference (95% CI)[a]	*P* Value[b]
Alanine aminotransferase, IU/L	51 (32–73)	40 (23–69)	−11.00 (−15.00 to −1.00)	.0345
Gamma-glutamyl transferase, IU/L	60 (34–116)	44 (31–77)	−16.00 (−22.00 to −1.00)	.0335
Lactate dehydrogenase, IU/L	391 (285–483)	324 (247–411)	−67.00 (−96.00 to −20.00)	.0027
Creatinine kinase, IU/L	146 (85–420)	98 (59–351)	−48.00 (−69.00 to −2.00)	.0357
Serum albumins, g/L	39.6 (36.9–41.3)	39.3 (36.4–41.5)	−0.300 (−1.40 to 0.80)	.7412
Fibrinogen, g/L	5.8 (5.2–6.4)	5.9 (5.3–6.4)	0.10 (−0.20 to 0.40)	.5319
D-dimers, mg/L	0.76 (0.48–1.30)	0.86 (0.53–1.53)	0.09 (−0.05 to 0.24)	.2327

Abbreviations: BMI, body mass index; GERD, gastroesophageal reflux disease; IQR, interquartile range; NAFLD, nonalcoholic fatty liver disease.

[a]Presented are standardized differences between medians or proportions with corresponding 95% CIs.

[b]Fisher exact or Mann-Whitney *U* test, as appropriate.

alanine aminotransferase, and gamma-glutamyl transferase (Table 1). There were no differences in laboratory findings in patients with NAFLD and obesity compared with patients with NAFLD without obesity (Supplementary Table 1).

Patients were treated according to current standard of care: remdesivir (138, 63.88%), corticosteroids (dexamethasone [183, 84.72%] and/or methylprednisolone [55, 25.46%]), LMWH (214, 99.07%), and tocilizumab (21, 9.72%). Details of treatment regimens used are provided in Supplementary Table 1. Except for remdesivir, which was more frequently prescribed in patients with NAFLD (84, 70%, vs 54, 56.2%; $P = .0459$), there were no other differences in the choice of treatment between groups.

Clinical Course and Outcomes

At admission, patients with NAFLD had higher disease severity, assessed by a 7-category ordinal scale, as presented in Figure 1. Fourteen (11.7%) patients with NAFLD and 2 (2.1%) without NAFLD required HFNC within 24 hours after hospital admission ($P = .0080$).

The percentage of patients with clinical improvement by day 7 and day 14 was significantly higher in patients without NAFLD. By day 7, 27 (22.5%) patients with NAFLD compared with 39 (40.6%) without NAFLD were ready to discharge ($P = .0048$). By day 14, 86 (71.7%) patients with NAFLD and 85 (88.5%) without NAFLD were discharged from the hospital ($P = .0024$).

Patients with NAFLD more frequently required HFNC or NIV (26, 21.66%, vs 10, 10.42%; $P = .0289$) and had longer duration of hospitalization (IQR) (10 [8–15] vs 9 [6–12] days; $P = .0018$). Six (5%) patients with NAFLD and 3 (3.12%) controls required invasive mechanical ventilation. However, there were no differences in in-hospital mortality between groups (8, 6.67%, vs 3, 3.12%; $P = .3529$).

There were no differences in outcomes between obese and "lean-weight" NAFLD patients: requirement of HFNC/NIV (18, 24.32%, vs 8, 17.39%; $P = .4950$), PE (22, 29.73%, vs 10, 21.74%; $P = .3992$), mortality (5, 6.76%, vs 2, 4.35%; $P = .7064$), and duration of hospitalization (IQR) (10 [8–16] days vs 10 [7–15] days; $P = .4873$) (Supplementary Table 1).

Next, we examined the impact of NAFLD on time to recovery, as defined by time to hospital discharge or readiness for discharge. Multivariable Cox regression analysis identified age >60 years, pulmonary thrombosis, HFNC/NIV (Supplementary Figure 2), aspartate aminotransferase >60, and NAFLD (Figure 2) as being negatively associated with time to recovery (Table 2). Other comorbidities, use of chronic medications, inflammatory markers, and choice of treatment (remdesivir vs no remdesivir, tocilizumab vs no tocilizumab, dexamethasone in low [<8 mg] or high dose [>8 mg]) were not associated with readiness for discharge in our model.

Association of NAFLD With Pulmonary Thrombosis

Overall, 45 (20.83%) patients were diagnosed with pulmonary thrombosis during hospitalization: 32 (26.66%) with NAFLD and 13 (13.54%) without NAFLD ($P = .0191$). PT was described as massive in 6 patients with NAFLD, segmental in 14 patients with NAFLD and 5 non-NAFLD, and subsegmental in 12 patients with NAFLD and 8 non-NAFLD.

In order to identify factors associated with PT, we performed multivariable logistic regression analysis, which identified baseline CRP >100 mg/dL, lactate-dehydrogenase (LDH) >390 IU/L, lymphocyte count $<1200 \times 10^9$/L, D-dimers >1.1 mg/L, and NAFLD as being associated with PT (Table 3). Age, BMI, obesity, and other comorbidities were not associated with PT in our model.

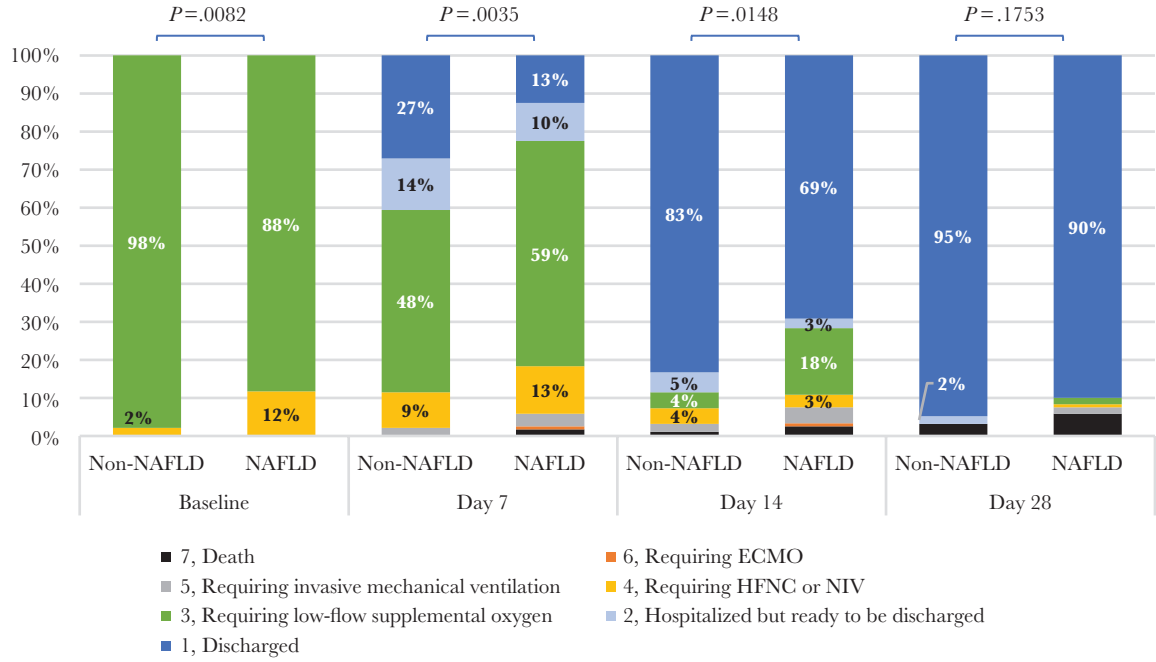

Figure 1. Seven-category ordinal scale at baseline and days 7, 14, and 28, stratified by the presence of NAFLD. Figure shows the patients' clinical status as assessed on the 7-category ordinal scale on admission and at days 7, 14, and 28, according to the presence of NAFLD. Categories on the ordinal scale were as follows: (1) discharged or ready for discharge; (2) hospitalization in a non-ICU without supplemental oxygen; (3) non-ICU hospitalization with supplemental oxygen; (4) ICU or non-ICU hospitalization with noninvasive ventilation or high-flow oxygen; (5) ICU hospitalization with mechanical ventilation; (6) ICU hospitalization with ECMO or mechanical ventilation and additional organ support; and (7) death. The Wilcoxon rank-sum test was used to calculate differences between groups. Abbreviations: ECMO, extracorporeal membrane oxygenation; HFNC, high-flow nasal cannula; ICU, intensive care unit; NAFLD, nonalcoholic fatty liver disease; NIV, noninvasive ventilation.

DISCUSSION

In this prospective cohort study, we found a significant association between NAFLD and COVID-19 severity, clinical course, and outcomes. In addition, this appears to be independent of other components of metabolic syndrome. This might have implications for clinical management and future studies for several reasons.

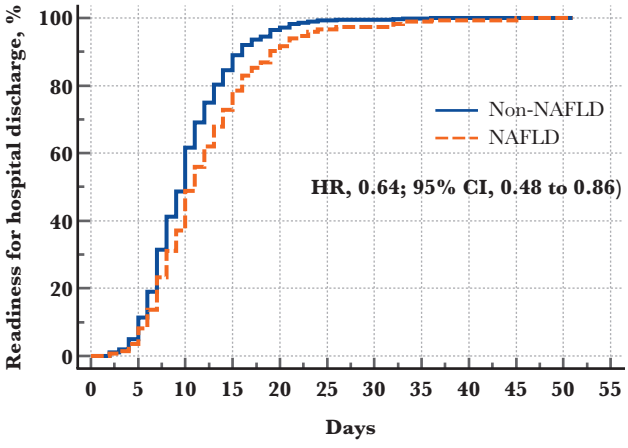

Figure 2. Kaplan-Meier curves and Cox proportional hazard ratios for time to discharge or readiness for discharge in patients with and without nonalcoholic fatty liver disease. Abbreviations: HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease.

First, the prevalence of NAFLD (55%) was high in our cohort. Notably, only a few patients were previously diagnosed with NAFLD, which is consistent with the finding that 95% of patients are unaware of having NAFLD [14]. Overrepresentation of NAFLD in hospitalized COVID-19 patients has been reported with varied prevalence: 52% in the United States, 42% in Mexico, and 30% in Israel, the UK, and China [9, 10, 12, 15, 16]. The underdiagnosing of NAFLD emphasizes the need for large-scale health care campaigns and screening strategies.

Second, the patients with NAFLD had a more severe form of disease at admission, more frequently required HFNC or NIV, and had less probability for early discharge. Multivariable analysis identified NAFLD as being negatively associated with

Table 2. Multivariable Cox Regression Analysis of Factors Associated With Time to Discharge or Readiness for Discharge

	Hazard Ratio (95% CI)	*P* Value
Age >60 y	0.64 (0.48 to 0.85)	.0025
Pulmonary thrombosis	0.66 (0.45 to 0.95)	.0278
HFNC/NIV	0.26 (0.16 to 0.42)	<.0001
AST >60 IU/L	0.99 (0.99 to 0.99)	.0057
NAFLD	0.64 (0.48 to 0.86)	.0005

The strength of association was expressed as HR and its corresponding 95% CI. The area under the ROC curve in the fully adjusted model was 0.86 (95% CI, 0.81 to 0.90).

Abbreviations: AST, aspartate aminotransferase; HFNC/NIV, high-flow nasal cannula/noninvasive ventilation; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; ROC, receiver operating characteristic.

Table 3. Multivariable Logistic Regression Analysis of Factors Associated With Pulmonary Thrombosis

	Odds Ratio (95% CI)	*P* Value
C-reactive protein >100 mg/dL	2.48 (1.17 to 5.24)	.0171
Lactate dehydrogenase >390 IU/L	2.36 (1.08 to 5.11)	.0299
Lymphocyte count <1200 × 10^9/L	4.74 (1.89 to 11.87)	.0011
D-dimers >1.1 mg/L	2.45 (1.19 to 5.58)	.0153
NAFLD	2.15 (1.04 to 4.46)	.0399

The strength of association was expressed as OR and its corresponding 95% CI. The area under the ROC curve in the fully adjusted model was 0.83 (95% CI, 0.77 to 0.88).

Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; ROC, receiver operating characteristic.

time to recovery. A significant association between NAFLD and COVID-19 severity has consistently been reported [2, 17]. A retrospective study from the United States showed that patients with NAFLD had significantly longer length of stay, ICU admission rate, and need for mechanical ventilation [18]. Similarly, Jin et al. in their retrospective study (n = 202, 76 patients with NAFLD, early 2020) showed that patients with NAFLD had a 2-fold higher risk of disease progression and longer viral shedding time [9].

Third, NAFLD was associated with COVID-19 disease severity regardless of BMI, and there was no significant difference between obese and "lean-weight NAFLD" in clinical outcomes. In contrast, a small retrospective study from China (n = 66) showed a 6-fold increased risk of severe COVID-19 in obese NAFLD patients [19]. While obesity was linked with COVID-19 severity in studies that did not include NAFLD as a variable, others showed no such association [20–22]. Similarly, a large UK study showed that obese patients without fatty liver are not at increased risk for symptomatic disease [7]. Our findings provide directions for future COVID-19 prognostic research, as NAFLD should be included as a variable, especially in patients with components of metabolic syndrome.

Next, we found that patients with NAFLD have higher inflammatory biomarkers, which were previously shown to be negative predictors of COVID-19 outcomes [23]. Several studies also showed higher CRP in patients with NAFLD and COVID-19 [12, 20]. As serum CRP and IL6 are natively elevated in NAFLD patients [24], this might be a possible link with worse outcomes. A recent comparative analysis of gene expression data sets offered additional explanations: Upregulation of ACE2 receptor and proprotein convertase FURIN might enable efficient viral replication, and increased expression of JAK1 and STAT1 corresponds with increased expression of cytokines and higher basal expression of CXCL10 and IL6 and proinflammatory signals [25]. Importantly, this was NAFLD-specific. Indeed, we have learned that not all patients respond to corticosteroids and IL-6R inhibitors. This might call for different anti-inflammatory therapeutic strategies in patients with NAFLD.

Finally, our study showed that patients with NAFLD have higher incidence of PT. Thromboembolic rates in COVID-19 are high and associated with higher mortality [26]. It seems that PT in COVID-19 patients is not associated with traditional risk factors such as age, history of malignancy, smoking, etc. A large multicenter cohort study showed that male sex, a longer delay from symptom onset to hospitalization, and systemic inflammation were independent predictors of PT [27]. The pathogenic mechanism includes COVID-19 endothelitis caused by direct viral infection and diffuse endothelial inflammation [28]. Several small postmortem analyses showed high steatosis prevalence in COVID-19 patients with thrombosis [29, 30]. To the best of our knowledge, this is the first prospective study suggesting an association between NAFLD and PT in COVID-19. The possible explanation includes higher levels of pro-inflammatory proteins and cytokines associated with systemic inflammation and higher plasma levels of von Willebrand factor and plasminogen activator inhibitor type 1 [31, 32]. There is an ongoing debate on dosing LMWH in patients with COVID-19 and the lack of protocols regarding who should be screened for PT [33]. Our findings might be important in identifying patients who might benefit from therapeutic doses of LMWH and who should be screened for PT. These should be confirmed in further studies.

In our study, NAFLD was associated with COVID-19 outcomes independently of other components of metabolic syndrome. This can be partially explained with low prevalence of class III obesity in our cohort. It seems that more than BMI, central fat distribution and metabolic consequences of obesity might be risk factors for COVID-19 severity, as recently described [34–36]. The specific role of NAFLD itself, not simply as a part of metabolic syndrome, has been already documented in noncommunicable diseases such as in heart failure, chronic kidney disease, and malignant diseases [37]. Similarly, NAFLD has been associated with severity of community-acquired pneumonia or *Clostridioides difficile* enterocolitis [38, 39]. It seems reasonable to speculate that altered immune responses and proinflammatory states in patients with NAFLD could predict COVID-19 severity, as described above.

Our study has a few associated caveats. The diagnosis of NAFLD was based on abdominal ultrasound, which is operator dependent; fibrosis stage was not evaluated as fibrosis scores may not be reliable in this setting, and elastography was not available at the COVID-19 department; therefore, the effect of advanced NAFLD on clinical outcomes was not analyzed; exclusion of patients who were admitted to the ICU in the first 24 hours or died during the first 48 hours could have led to selection bias, in which less severe patients were included, and this might be reflected in our mortality analysis.

Nevertheless, we report the first prospective data in a well-defined cohort study on the association of COVID-19 and NAFLD from the later phase of pandemic. The inconsistency in

previously published data highlights the strengths of our study, as most of the studies were retrospective and from the mere beginning of the pandemic when there was no explicit classification of the COVID-19 disease, "standard of treatment" included possible hepatotoxic drugs, and standard of care was not well defined; a significant proportion of data are from Asian countries, mainly China, so there are not enough data from other ethnic populations. Furthermore, in previous studies, the definition of NAFLD varied and mainly included patients previously diagnosed with NAFLD, or the diagnosis was based on steatosis scores (mainly hepatic steatosis index, fatty liver index, or NAFLD score) or CT scans, which might not reliably distinguish preexisting steatosis from COVID-19-associated liver injury. The possible impact of different NAFLD diagnostic scores on COVID-19 outcomes was recently suggested in a large-scale population-based cohort study from South Korea [40]. The authors showed a significant association between the presence of NAFLD and COVID-19 severity using several diagnostic criteria, and they justifiably argue that the impact might vary depending on the definition used [40]. There is also an ongoing debate on the new definition of MAFLD [41], which was used in some studies, but has not yet been included in official guidelines. Due to the study design, we were not able to collect the patients' prepandemic NAFLD scores; therefore, our results cannot directly be compared with previous studies.

In conclusion, we have shown a significant association between NAFLD, COVID-19 severity, and pulmonary thrombosis. The rapidly increasing prevalence of NAFLD requires novel therapeutic and prophylactic approaches based on better understanding of the immunopathogenesis of COVID-19 in these patients. Obviously, NAFLD should be included as a variable in future studies investigating COVID-19 outcomes and treatment strategies.

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