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Liver stiffness is associated with disease severity and worse clinical scenarios in coronavirus disease 2019: A prospective transient elastography study

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Abstract

Background: Pre-existing chronic liver disease is currently considered a poor prognostic factor for coronavirus disease 2019 (COVID-19). The present study aimed to investigate the association of liver stiffness measurement (LSM) with disease severity and clinical course of COVID-19.

Methods: We prospectively recruited consecutive hospitalised adult patients with COVID-19 in a 3-month period. Demographic, laboratory, clinical and vibration-controlled transient elastography (VCTE) features were recorded at entry, and all patients were prospectively followed-up. Severe liver fibrosis was defined as an LSM value higher than 9.6 kPA. Multivariate logistic regression analysis was performed to reveal factors associated with disease severity and outcomes.

Results: Out of 98 eligible patients with COVID-19, 12 (12.2%) had severe liver fibrosis. Patients with severe liver fibrosis had higher baseline disease severity ($P = .022$), more commonly required oxygen treatment at entry ($P = .010$), and had intensive-care unit (ICU) requirements during the 6 (1-39)-day median follow-up time ($P = .017$). The presence of severe liver fibrosis was independently associated with disease severity (odds ratio (OR): 7.685, 95% confidence interval (CI): 1.435-41.162, $P = .017$) and ICU requirement (OR: 46.656, 95% CI: 2.144-1015.090, $P = .014$). LSM was correlated with alanine aminotransferase levels ($P = .005$, r: 0.283), but not with other markers of acute hepatic injury or inflammation.

Conclusion: Initial VCTE application might help physicians identify patients who are more likely to have severe illness or worse clinical outcomes, in addition to other well-established clinical and laboratory factors. Further multicentre prospective studies are warranted to validate our results.

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection, has been responsible for a large number of hospital admissions and mortality since the beginning of the pandemic in December 2019.[1] The understanding of the disease has improved over time, and it has become apparent that it not only causes pneumonia but also may involve the gastrointestinal system, heart, kidney, and liver.[2-5] Several epidemiological studies have demonstrated the conditions associated with a more severe disease course, including the presence of type 2 diabetes mellitus, hypertension, cardiovascular disease,

chronic lung disease (chronic obstructive pulmonary disease and asthma), male sex, and older age.[6] Several laboratory features have also been associated with worse clinical outcomes in COVID-19, most remarkably the presence of lymphopenia and increased C-reactive protein.[7,8]

Despite these consistent factors associated with severe COVID-19, the impact of chronic liver disease on disease severity and progression has remained dubious. The observed increases in transaminase levels have been interpreted as hepatic injury of severe COVID-19 in several reports.[9] However, the aetiology of assumed liver damage remains unclear and is reported to be clinical of little concern.[10-12] Nevertheless, there are a few studies in the literature demonstrating a strong association of liver injury and/or pre-existing liver disease with COVID-19 outcomes,[13-16] but a recent pooled analysis of the accumulated literature has shown the opposite findings,[17] which was supported by the ensuing studies as well.[18] In general, having chronic liver disease is associated with increased severity and mortality for all-type pneumonias.[19,20] Nevertheless, the role of liver disease or injury in COVID-19 disease is still not clear, mainly owing to the solitary usage of laboratory results in the interpretation of liver damage, without the application of more advanced diagnostic tools such as transient elastography or liver biopsy.

Previous studies have demonstrated that non-invasive liver fibrosis indicators, including FIB-4 index and neutrophil to lymphocyte ratio, are associated with poor clinical outcomes in COVID-19.[21-26] Based on the evidence from these recent studies, it is rational to anticipate that patients with fibrosing NAFLD could be more prone to more severe COVID-19. In this regard, we aimed to confirm this hypothesis by investigating the associations of liver stiffness measurement (LSM) determined via vibration-controlled transient elastography (VCTE) (FibroScan; Echosens, Paris, France); which is a simple, reliable, and non-invasive ultrasound-based procedure to assess the severity of liver fibrosis,[27] with disease severity and clinical outcomes.

What's known

- Several comorbidities and laboratory features have been associated with worse clinical outcomes in coronavirus disease 2019 (COVID-19), most remarkably the presence of diabetes mellitus, hypertension, cardiovascular disease, chronic lung disease, cardiovascular disease, lymphopenia, and increased C-reactive protein.
- The role of liver disease or injury in COVID-19 is still not clear, mainly owing to the solitary usage of laboratory results in the interpretation of liver damage, without the application of more advanced diagnostic tools such as transient elastography or liver biopsy.

What's new

- In a prospectively followed cohort with all hospitalised patients for COVID-19, initial liver stiffness measurement determined by vibration-controlled transient elastography (VCTE) shows a significant association with disease severity and clinical outcomes.
- The positive association of severe liver fibrosis (>9.6 kPA) with higher rates of more severe COVID-19 disease, requirement of supplemental oxygen therapy, and intensive care unit points out the potential role of VCTE in COVID-19.

2 | METHODS

2.1 | Study population and sample selection

We prospectively recruited 98 consecutive adult inpatients with SARS-CoV-2 infection from Marmara University Hospital from June to September 2020. SARS-CoV-2 infection was documented by reverse-transcription polymerase chain reaction (PCR) assays of nasopharyngeal swab specimens in all patients. Patients were excluded if they met one of the following criteria: (a) younger than the age of 18, (b) pre-existing chronic liver disease (determined by the history and review of past laboratory results and/or abdominal ultrasound), (c) presence of ascites, (d) pregnancy, (e) history of liver transplantation, (f) significant alcohol consumption (≥20 g per week for women and ≥30 g per week for men) or (g) unable to give written informed consent. All demographic, laboratory, and clinical parameters were collected on the day of VCTE application. Eligible patients were confirmed to have negative hepatitis B surface antigen and anti-hepatitis C virus antibody results the day after VCTE application.

A total of 98 subjects without COVID-19 were separately recruited as a control cohort for the study (1:1) during their medical check-up programme in our hospital for the comparison of previously unknown non-alcoholic fatty liver disease (NAFLD) presence and LSM levels. The exclusion criteria used for COVID-19 patients were also implemented to control group candidates for eligibility in the study. The control group was constructed on two bases: (a) their comorbidities and habits were the representative of the general Turkish population; and (b) they were matched with the COVID-19 group in terms of age, sex, presence of diabetes mellitus, and hyperlipidaemia. The data collection protocol of controls was similar to that of COVID-19 patients on the day of VCTE application.

The study protocol was approved by the local research ethical review board of the Marmara University School of Medicine (Approval date: 01.06.2020, Approval No: 09.2020.574). The study was performed in accordance with the principles of the Helsinki Declaration. All participants provided written informed consent.

2.2 | Definitions

Elevated transaminase levels were defined as alanine aminotransferase (ALT) > 32.1 U/L for men and >23.15 U/L for women based on the most recent cut-off values determined for the Turkish

population,[28] which was also consistent with the previous cut-off values extracted from Asian populations.[29,30] Obesity was defined as body mass index (BMI) ≥ 30 kg/m^2. Diabetes mellitus was diagnosed based on history or haemoglobin A1c ≥ 6.5%. Hypertension was defined as blood pressure ≥ 130/85 mm Hg or specific drug use. Hyperlipidaemia was defined as total cholesterol higher than 200 ng/mL or low-density lipoprotein higher than 140 ng/mL. Other comorbidities were obtained from the patient's medical history. The presence of any coronary, organic heart disease or arrhythmia was defined as heart disease. The presence of chronic obstructive pulmonary disease (COPD) and/or asthma was defined as chronic lung disease. Immunosuppression was defined as a history of malignancy requiring chemotherapy in the past year or active usage of an immunosuppressive drug.

2.3 | Disease outcomes and follow-up

The hospitalisation time was calculated starting from the hospitalisation day to discharge or death. Chest involvement was categorised according to a visual quantitative evaluation of chest computed tomography (CT) imaging, as published.[31] All patients were classified for COVID-19 severity according to World Health Organization (WHO) clinical progression scale.[32] Severe disease was used to define patients with a WHO clinical progression scale score higher than 5, which corresponds to those requiring oxygen therapy.

Disease progression was defined as at least one numerical increase in WHO clinical progression scale during the median follow-up period of 6 (1-39) days. The disease outcomes were determined as follows: (a) disease progression, (b) intensive care unit requirement at the follow-up, and (c) mortality. Any drug use for the treatment of COVID-19 at any time of hospitalisation was recorded, and all patients were followed-up for 6 (1-39) days prospectively for the development of outcomes.

2.4 | Performance of vibration-controlled transient elastography

LSM and controlled-attenuation parameter (CAP) were both measured using a FibroScan® 530 compact device equipped with both M and XL probes by a physician trained and certified by the manufacturer at the beginning of hospitalisation.[33] All patients were asked to fast for at least 3 hours before the examination as per the manufacturer's instructions. Probe selection was performed using the automatic probe selection tool embedded in the device software. For the examination, the patients were placed in the dorsal decubitus position and the transducer probe was positioned in the intercostal space of the right lobe of the liver. Reliable VCTE measurements were defined as at least 10 valid measurements and having an interquartile-range-to-median ratio of ≤0.3.[34] CAP is an average estimate of ultrasound attenuation at 3.5 MHz and is expressed in dB/m. LSM is an average estimate of stiffness (Young's modulus) at a shear wave frequency of 50 Hz and is expressed as kPa.[35] Severe liver fibrosis (fibrosis ≥ 3) was defined as LSM higher than 9.6 kPA, as published several times.[27,36,37] Patients with hepatic steatosis were defined by a CAP value of ≥274 dB/m, and they were diagnosed with NAFLD owing to the exclusion of both secondary causes and significant daily alcohol consumption.[38,39]

2.5 | Statistical analysis

Descriptive statistics were used to describe the general characteristics of the study population. Continuous data are displayed as the mean ± standard deviation (SD) or median (minimum-maximum) when appropriate, and the distribution normality of the variables was assessed with the Kolmogorov-Smirnov or Shapiro-Wilk test. Categorical data are expressed as counts and proportions. For the comparison of continuous variables between two groups, Student's t-test was used when the data conformed to a normal distribution; otherwise, the Mann-Whitney U test was used. The chi-square test was used to compare categorical parameters. To reveal the association of LSM with disease outcomes, we performed multivariate logistic regression analysis with forward stepwise selection using the significant parameters ($P < .05$) from the univariate analysis and the well-acknowledged prognostic parameters for COVID-19 described in the literature. All variables were checked for multicollinearity by correlation coefficients to determine eligibility for multivariate analysis. When multicollinearity was detected between two parameters, only the parameter with the highest odds ratio in the univariate logistic regression analysis was included in the multivariate model to prevent estimation bias. Spearman's rho test was performed to investigate the possible correlations between LSM and markers of acute hepatic injury (AST and ALT) and inflammation (ferritin and C-reactive protein). Statistical significance was defined as $P < .05$. All statistical analyses were conducted using SPSS software version 20.0 (IBM, Armonk, NY, USA).

3 | RESULTS

3.1 | Baseline demographics and laboratory features

Baseline demographics and VCTE characteristics are displayed in Table 1. The mean age of the whole cohort was 55.2 ± 13.9 years. Out of 98 patients with SARS-CoV-2 infection, 12 (12.2%) had severe liver fibrosis (LSM ≥ 9.6 kPA). Patients with severe liver fibrosis had a higher BMI ($P = .028$) and more commonly had diabetes mellitus ($P = .015$). There were no other differences between severe and non-severe liver fibrosis patients regarding other types and numbers of comorbidities. The median LSM of the whole cohort was 5.8 (2.6-16.1) kPA, and the mean CAP value was 261.2 ± 57.6 dB/m.

Patients with severe liver fibrosis had higher leukocyte counts, lactate dehydrogenase, fibrinogen, and C-reactive protein levels and

TABLE 1 Baseline demographics and transient elastography characteristics

	COVID-19 (n = 98)	LSM < 9.6 (n = 86)	LSM ≥ 9.6 (n = 12)	*P*
Age, years	55.2 ± 13.9	55.1 ± 14.4	58.1 ± 10.9	.492
Male sex	58 (59.2)	52 (60.5)	6 (50.0)	.490
Body-mass index	29.7 (20.1-48.7)	30.2 ± 5.1	33.7 ± 7.5	.028*
Obesity	47 (48.5)	38 (44.7)	9 (75.0)	.066
Smoking	9 (9.2)	8 (9.3)	1 (8.3)	.913
Diabetes mellitus	22 (22.4)	16 (18.6)	6 (50.0)	.015*
Hypertension	38 (38.8)	31 (36.0)	7 (58.3)	.138
Hyperlipidaemia	5 (5.1)	5 (5.8)	–	.391
Heart disease	13 (13.3)	10 (11.6)	3 (25.0)	.201
Chronic lung disease	14 (14.3)	14 (16.3)	–	.131
Chronic renal failure	1 (1.2)	1 (1.2)	–	.707
Immunosuppression/ Malignancy	6 (6.2)	6 (7.0)	–	.366
Number of comorbidities				
0	37 (37.8)	35 (40.7)	2 (16.7)	
1	35 (35.7)	30 (34.9)	5 (41.7)	.231
>1	26 (26.5)	21 (24.4)	5 (41.7)	
NAFLD (CAP > 274 dB/m)	41(41.8)	32 (37.2)	9 (75.0)	.013*
LSM, kPA (VCTE)	5.8 (2.6-16.1)	5.5 ± 1.6	11.6 ± 2.2	<.001*
CAP, dB/m (VCTE)	261.2 ± 57.6	255.2 ± 57.1	303.9 ± 42.3	.002*
IQR (VCTE)	31.5 ± 14.5	31.9 ± 14.3	28.2 ± 13.7	.426
IQR/median (VCTE)	17 (3-29)	16.7 ± 7.5	18.1 ± 6.8	.476

Abbreviations: CAP, controlled attenuation parameter; IQR, interquartile range; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; VCTE, vibration-controlled transient elastography.

*P value < .05.

maximum ALT levels (all P values < .05). Forty-nine (50%) patients had high transaminase-elevated liver enzymes at entry. The laboratory characteristics are detailed in Table 2.

3.2 | COVID-19-related clinical features and outcomes

COVID-19-related clinical characteristics and outcomes are shown in Table 3. The median onset of COVID-19 symptoms before admission was 2 days (0-14) in the whole cohort. The median day of hospitalisation was 6 (1-39) days. The initial median WHO disease severity index score was 4 (2-6) in the whole cohort. Sixty-one patients (62.2%) required supplemental oxygen at entry. The baseline disease severity score (P = .022) and initial requirement of oxygen treatment (P = .010) were higher in patients with severe liver fibrosis than in those without severe liver fibrosis. The severe and non-severe liver fibrosis groups did not differ from each other with regard to hospitalisation time and severity of chest CT infiltration.

The most commonly used drugs for the treatment of COVID-19 were favipiravir in 78 (79.6%) patients and hydroxychloroquine in 70 (71.4%) patients, followed by corticosteroids in 24 (24.5%) patients and tocilizumab in 17 (17.3%) patients. Convalescent plasma was given to 9 (8.2%) patients.

Disease progression was observed in 37 (37.8%) patients. During the follow-up, 12 patients (12.2%) required a transfer to the intensive care unit (ICU), 5 of them (5.1%) underwent mechanical ventilation (MV), and 2 (2.0%) patients died. ICU requirement (P = .017) was more common in patients with severe liver fibrosis than in those without severe fibrosis. We were not able to perform mortality analyses because of the low number of deaths.

3.3 | Factors associated with disease severity and ICU requirement

There was bivariate collinearity between LSM, BMI, and ALT levels (all P-values < .05). Therefore, only LSM was included in the multivariate logistic regression analysis to prevent biased estimation and

TABLE 2 Initial laboratory characteristics

	COVID-19 (n = 98)	LSM < 9.6 (n = 86)	LSM ≥ 9.6 (n = 12)	*P*
Leukocyte count, g/L	5000 (2600-12 300)	4.900 (2.600-11.700)	6.450 (2.700-12.300)	.042*
Hemoglobin, g/L	13.5 ± 1.5	13.6 ± 1.5	13.3 ± 1.4	.550
Lymphocyte count, g/L	1.150 (200-2.500)	1124.7 ± 445.1	1316.6 ± 552.4	.269
Lymphocyte ratio, %	22.6 ± 8.6	22.7 ± 8.4	22.3 ± 9.8	.911
AST, U/L	35 (14-124)	34 (14-105)	38 (16-124)	.757
Maximum AST, U/L	57 (14-239)	52 (14-239)	66 (31-183)	.104
ALT, U/L	29 (9-180)	29 (9-180)	28.5 (16-57)	.811
Maximum ALT, U/L	54 (9-276)	53 (9-266)	77.5 (31-276)	.052
Elevated transaminase levels	44 (44.9)	39 (45.3)	5 (41.7)	.810
ALP, U/L	68 (30-165)	68 (30-165)	59.5 (32-115)	.168
GGT, U/L	33 (11-239)	35 (11-239)	33.5 (18-141)	.942
LDH, U/L	292 (133-979)	259 (133-979)	436 (232-679)	.001*
Albumin, mg/dL	3.9 (2.5-5.1)	3.8 (2.5-4.5)	3.7 (3.1-4.0)	.130
Fibrinogen, mg/L	453 (248-1122)	439 (248-1122)	597.5 (439-690)	.006*
C-reactive protein, mg/L	33.8 (1.8-289.5)	36.1 (2.4-289.5)	92.2 (14.5-199.6)	.040*
Ferritin, μg/L	166 (10-1825)	168.0 (9.5-1825.0)	152.3 (72.3-1067.0)	.433

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase.

*P value < .05.

to give the highest odds ratio amongst the three dependent variables in the univariate logistic regression analysis conducted for the association with disease severity and ICU requirement. As a result, LSM > 9.6 kPA was independently associated with disease severity (OR: 7.685, 95 CI%: 1.435-41.162, $P = .017$) in addition to increasing age (OR: 1.062, 95% CI: 1.016-1.110, $P = .008$), presence of diabetes mellitus (OR: 5.428, 95% CI: 1.113-26.473, $P = .036$), presence of chronic lung disease (OR: 3.908, 95% CI: 0.976-15.648, $P = .05$), and higher CRP levels (OR: 1.018, 95% CI: 1.006-1.030, $P = .004$) (Table 4). LSM > 9.6 kPA was also independently associated with ICU requirement (OR: 46.656, 95% CI: 2.144-1015.090, $P = .014$), in addition to the presence of immunosuppression (OR: 44.405, 5% CI: 2.052-960.803, $P = .016$) and decreasing lymphocyte counts (OR: 0.991, 95% CI: 0.994-0.999, $P = .008$) (Table 5).

3.4 | Association of LSM with inflammatory and acute hepatic damage markers

Disease severity and outcomes were not different between COVID-19 patients with normal and elevated transaminases (all *P*-values > .1). Thorax CT severity was more frequent in higher grades in patients with elevated transaminase ($P = .003$). LSM and CAP values were significantly higher, as well as BMI values, in patients with elevated transaminases (all *P*-values < .05) (Supplementary Table 1). LSM was only correlated with ALT levels ($P = .005$, r: 0.283), and no correlation was observed with AST ($P = .097$, r: 0.168) and inflammatory markers, such as CRP ($P = .075$, r: 0.182) and ferritin ($P = .160$, r: 0.144).

3.5 | Unknown NAFLD presence and LSM levels in subjects with and without COVID-19

The demographic and VCTE-related characteristics of the controls and COVID-19 patients are displayed in Supplementary Table 2. We compared the VCTE results of our COVID-19 patients with a separate 1:1 matched control group without COVID-19 (n = 98) in terms of age, sex, and the presence of several comorbidities, including diabetes and hyperlipidaemia (Supplementary Table 2). COVID-19 patients were more frequently smokers, and they had higher rates of hypertension and chronic lung disease. BMI levels were higher and obesity was more common in the COVID-19 group (all *P* values < .05). NAFLD presence was not different between the two groups (control: 35 [35.7%] vs COVID-19:41 [41.8%], $P = .379$). The median LSM value was higher in patients with COVID-19 than in those without COVID-19 (Control: 5.1 [2.9-10.3] vs COVID-19:5.8 [2.6-16.1], $P = .013$).

4 | DISCUSSION

In the present study, we reported an association of LSM determined by VCTE with COVID-19 severity and clinical outcomes in 98 prospectively followed patients with COVID-19. The main findings of the present study can be summarised as follows: (a) approximately 12% of the hospitalised COVID-19 patients had severe liver fibrosis, (b) LSM ≥ 9.6 kPA was associated with more severe COVID-19 disease, (c) LSM ≥ 9.6 kPA was associated with higher rates of supplemental oxygen therapy, and (d) LSM ≥ 9.6 kPA was associated with ICU requirement at the follow-up. We are aware of one small

TABLE 3 COVID-19-related clinical features and outcomes

	Whole cohort (n = 98)	LSM < 9.6 (n = 86)	LSM > 9.6 (n = 12)	*P* value
Hospitalisation time, days	6 (1-39)	6 (1-39)	9.5 (3-28)	.343
Time from symptom onset to FibroScan, days	2 (0-14)	2 (0-14)	3.5 (0-14)	.092
WHO disease severity scale	4 (2-6)	4 (2-6)	5 (3-6)	.022*
Chest CT severity				
0	13 (13.3)	12 (14.0)	1 (8.3)	
1 (<25%)	56 (57.1)	51 (59.3)	5 (41.7)	.130
2 (25%-75%)	21 (21.4)	18 (20.9)	3 (25.0)	
3 (>75%)	8 (8.2)	5 (5.8)	3 (25.0)	
Oxygen requirement at the entry	61 (62.2)	25 (29.1)	8 (66.7)	.010*
Disease progression	37 (37.8)	33 (38.4)	4 (33.3)	.736
ICU requirement at the follow-up	12 (12.2)	8 (9.3)	4 (33.3)	.017*
MV requirement at the follow-up	5 (5.1)	5 (5.8)	–	.391
Mortality	2 (2.0)	2 (2.3)	–	.594
Treatments used for COVID-19				
Hydroxychloroquine	70 (71.4)	63 (73.3)	7 (58.3)	.284
Favipiravir	78 (79.6)	67 (77.9)	11 (91.7)	.268
Tocilizumab	17 (17.3)	12 (14.0)	5 (41.7)	.018*
Corticosteroid	24(24.5)	20 (23.3)	4 (33.3)	.447
Convalescent plasma	9 (9.2)	7 (8.1)	2 (16.7)	.338
Low-molecular-weight heparin	82 (83.7)	70 (81.4)	12 (100.0)	.102

Abbreviations: CT, computer tomography; ICU, intensive care unit; MV, mechanical ventilation; WHO, World Health Organization.

*P value < .05.

previous study that investigated the association of LSM determined by VCTE with the COVID-19 disease course. The prior study consisted of only 32 patients, which is approximately one-third of our cohort, and therefore lacked an adequate number of patients to reach statistical power.[40] Nevertheless, that study found associations of LSM with the clinical course of COVID-19 patients in terms of the length of hospitalisation, the requirement of ICU treatment, and even mortality. However, these associations were only found in univariate analyses, and they were not able to show whether LSM is an independent factor associated with these outcomes. One other criticism for the prior study is the cut-off selection of LSM as 5.0 kPa, which is very low for reflecting significant (the generally accepted LSM cut-off is 7.2 to 7.5 kPa) or severe fibrosis (the generally accepted LSM cut-off is 9.6 kPa). In our study, we performed the same analysis with LSM cut-off values of 5 and 7.5 kPa and could not demonstrate any association with COVID-19 outcomes in multivariate analysis (data not presented). That is, we demonstrated that an LSM cut-off value of 9.6 kPa is the most appropriate threshold to predict worse COVID-19 outcomes.

A few studies have reported that patients with severe COVID-19 were more likely to have NAFLD than those with non-severe COVID-19 disease.[41-43] However, the prognosis of NAFLD is determined by the severity of liver fibrosis.[44] In this regard, a recent study investigated the potential role of non-invasive fibrosis scores, including the fibrosis-4 (FIB-4) index and NAFLD fibrosis score (NFS), in COVID-19 patients and metabolic-associated fatty liver disease (MAFLD), a relatively new terminology used for patients with hepatic steatosis.[24] These studies have demonstrated that patients with MAFLD with increased FIB-4 or NFS have a higher likelihood of developing severe COVID-19 illness regardless of other prognostic comorbidities. This was a reasonable and rational approach, as these non-invasive fibrosis scores are valid and accurate in both predicting and excluding advanced fibrosis in various circumstances.[45,46] Our study carried this opinion one step further by suggesting the independent role of severe liver fibrosis determined by a more advanced tool, namely, FibroScan. As suggested by a previous study, our results also support the theory that severe liver fibrosis might exacerbate virus-induced hyperinflammation, possibly through the hepatic

TABLE 4 Factors associated with severe COVID-19

	Univariate analysis	Multivariate analysis		
	P value	Odds ratio	95% CI	*P* value
Age	.016*	1.062	1.016-1.110	.008*
Male sex	.838	0.927	0.304-2.829	.894
Diabetes mellitus	.471	5.428	1.113-26.473	.036*
Hypertension	.727	0.629	0.185-2.138	.458
Heart disease	.397	0.947	0.218-4.112	.942
Chronic lung disease	.045*	3.908	0.976-15.648	.05*
Immunosuppression	.985	0.285	0.016-5.031	.392
LSM ≥ 9.6	.019*	7.685	1.435-41.162	.017*
Lymphopenia	.937	1.000	0.999-1.001	.634
C-reactive protein	.03*	1.018	1.006-1.030	.004*

Abbreviations: CI, confidence interval; LSM, liver stiffness measurement.
*P value < .05.

TABLE 5 Factors associated with ICU requirement in COVID-19

	Univariate analysis	Multivariate analysis		
	P value	Odds ratio	95% CI	*P* value
Age	.321	1.044	0.983-1.109	.162
Male sex	.573	0.902	0.155-5.240	.908
Diabetes mellitus	.729	0.711	0.055-9.146	.794
Hypertension	.359	0.072	0.005-1.131	.061
Heart disease	.659	2.140	0.174-26.249	.552
Chronic lung disease	.529	0.345	0.014-8.759	.519
Immunosuppression	.002*	44.405	2.052-960.803	.016*
LSM ≥ 9.6	.017*	46.656	2.144-1015.090	.014*
Lymphocyte count	.005*	0.997	0.994-0.999	.008*
C-reactive protein	.02*	0.991	0.971-1.1011	.367

Abbreviations: CI, confidence interval; ICU, intensive care unit; LSM, liver stiffness measurement.
*P value < .05.

release of multiple proinflammatory cytokines, thereby contributing mechanistically to severe COVID-19.

Non-alcoholic steatohepatitis (NASH), which is severe form of NAFLD, was shown to have a strong association with the risk of COVID-19 in a population-based study from the United States.[47] The association of NASH presence and worse clinical outcomes in COVID-19 is rational, as circulating inflammatory markers are more pronounced in patients with NASH than in those with simple steatosis or without steatosis. However, we need more proof and explanation of whether the existence of NAFLD or NASH is associated with a higher incidence of COVID-19. As an additional analysis, we compared the VCTE results of our COVID-19 patients with those of a separate control group without COVID-19 (n = 98) and found slightly increased rates of previously unknown NAFLD presence in COVID-19 patients than in controls, although the difference was not significant. The median LSM value was higher in patients with COVID-19, which raised the question of whether this could be a reflection of COVID-19-related acute hepatic inflammation. In fact, this outcome was more likely the reflection of a higher obesity rate in the COVID-19 group. Studies investigating the association of biopsy-proven NASH with COVID-19 with larger and better-matched prospectively followed cohorts are needed to clarify this issue.

The severe liver fibrosis rate of 12% detected in our study is conspicuously higher than the prevalence estimates (2%) reported in a large Asian population-based study using the same LSM cut-off value.[48] This discrepancy initially raises the suspicion of a selection bias, however, this is most likely caused by the fact that our cohort consisted of hospitalised patients for COVID-19 with several comorbidities while that prevalence study was representative of the normal healthy population in Hong-Kong Chinese. It is also possible

that there may have been some false positive LSM findings owing to concurrent inflammatory liver changes in the setting of COVID-19 acute illness.[49] Liver injury because of SARS-CoV-2 infection is an unravelled issue. It is hypothesised that COVID-19-related liver injury is possible via the inflammatory response and virus-induced cytotoxic T cells, the direct cytopathic effect of the virus via the ACE2 receptor, hypoxia and circulatory changes, and/or the hepatotoxic effect of drugs used for treatment.[43,50] Several studies highlighted the liver tropic effect of SARS-CoV-2 and showed that liver injury defined by increased transaminases is more commonly observed in severe COVID-19 disease.[51-53] However, not all abnormal liver function tests mean that patients with COVID-19 have liver injury, and elevated aminotransferases might partially be caused by myocardial or muscle injury.[10,54] The studies showing the association of increased aminotransferase in the COVID-19 disease course only used laboratory tests to assign acute liver damage but did not use any other advanced tool to prove it. A recent study, which investigated LSM with VCTE, was the first to use a reliable tool to show liver injury.[40] They demonstrated that LSM was correlated with markers of acute liver damage (AST, ALT, and ferritin), which supported the suitability of VCTE to ensure liver involvement in COVID-19. The present study, with a larger cohort that excluded all patients with pre-existing liver disease, demonstrated the correlation of LSM with ALT only, but not with AST, ferritin or CRP, in COVID-19 disease. Our findings raise the suspicion of whether the liver involvement of COVID-19 is a real entity or whether is it the underlying liver disease that affects the prognosis of these patients, as LSM was not correlated with inflammatory markers but with liver-specific transaminase only.

Both NAFLD and COVID-19 are considered sexually dimorphic diseases. NAFLD affects men and women differently, as women are protected from NAFLD with an around 50% decreased risk with favourable prognosis and less progression to hepatocellular carcinoma compared with men.[55] Similarly, men are considered more prone to COVID-19 disease as well as poorer prognosis.[56] In our study, we found no gender disparity regarding the disease severity and clinical outcomes.

Our study has several limitations. First, the lack of a large sample size prevented us from performing further subgroup analysis. Nevertheless, this is the largest study to investigate the association of LSM determined by VCTE with COVID-19 disease severity and outcomes. Second, the exclusion of BMI as a covariable from multivariate analysis because of its multicollinearity with LSM might be considered a confounding condition. Obesity has been shown to be strongly associated with increased COVID-19 mortality and severity.[57] It provides a unique microenvironment for the pathogenesis of the disease and is characterised by a chronic, low-inflammatory state, which may lead to the production of exhausted immune cells, and the organism becomes more vulnerable to infections and less responsive to antimicrobial drugs. When obesity was added to other covariables for multivariate logistic regression analysis, we found that the significance of factors associated with disease severity and ICU requirement did not change, and obesity was amongst them; however, the odds ratios did change (data not presented). In this regard, we presented the multivariate analysis, as in the present results, to prevent estimation bias because of the coexistence of linear parameters and to give a more accurate risk increase of outcomes for each factor. Finally, although this was a prospective follow-up study, we could not perform a control VCTE after recovery, which could have provided information on whether the increased LSM was temporary because of acute COVID-19 infection or a permanent state. Nevertheless, the lack of a correlation between LSM and inflammatory markers prohibited us from interpreting such a potential association of LSM with acute liver injury in COVID-19.

In conclusion, LSM determined by VCTE is a useful parameter to reflect disease severity and is associated with ICU requirements for patients with COVID-19. Initial bedside LSM determined by VCTE might help physicians identify COVID-19 patients with higher susceptibility to worse clinical scenarios, in addition to other well-established clinical and laboratory factors. Further multicentre studies from different regions are warranted to validate our results.

DISCLOSURES

Yusuf Yilmaz has served as a speaker for Gilead, Echosens, Bilim Pharmaceuticals, and advisory board member for Novonordisk, and has received research funding from Biocodex, Gilead. Other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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