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Multiparametric Ultrasoud Findings Post Sars-Cov 2 Infection: Increased Risk of Liver Fibrosis?

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Abstract

Purpose: Increased transaminase levels are frequently detected in patients with Coronavirus disease 19 (COVID 19) and are associated with worse outcome. The association with abdominal radiological signs and severity grading, however, remains to be elucidated.

Our aim was to evaluate the long term effects of COVID 19 infection on liver ultrasound features and in particular on liver steatosis and liver stiffness (LS).

Material and methods: All consecutive patients referred to the outpatient post-COVID 19 Unit of the Fondazione Policlinico Gemelli 6 months after infection underwent B-mode ultrasound (US), sound speed estimation (SSE) and 2D-Shear-Wave Elastography (2D-SWE). Liver steatosis and fibrosis were defined as a SSE value ≤ 1.537 m/s and ≥ 7.1 kPa.

Results: Among 146 patients enrolled, 55 patients (37.7%) had liver steatosis, 11 patients (7.5%) had significative liver fibrosis. LS was positively correlated with liver steatosis at the follow up post- COVID-19 infection compared with those without liver steatosis. Furthermore, significative fibrosis was associated with obesity (p=0.02), diabetes (p<0.01), aminontransferases elevation during infection (p<0.01) and anti-IL6 therapy during hospitalization (p=0.02).

Conclusion: The new ultrasonographic techniques are an useful and non-invasive tool for the follow up of patients after COVID 19 infection to evaluate chronic liver injury.

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Introduction

The Coronavirus Disease 2019 (COVID-19), determined by the novel Coronavirus 2 (SARS-Cov-2), was first detected in November 2019 in Wuhan (Hubei Chinese province) and soon became a pandemic emergency. At present it is a worldwide-spread airborne infectious disease [1] with extremely heterogeneous manifestations, mostly related to the respiratory tract, ranging from asymptomatic disease to an uncomplicated mild flu-like illness or to severe acute respiratory distress syndrome, but it could also involve other organs and systems, determining musculoskeletal pain, vomiting, diarrhoea, liver disorders [2]. Liver damage has been detected in 14-53% of patients with COVID-19, especially in severely ill patients [3]. Some authors observed that at least half of the patients with severe COVID-19 infection had transaminases elevation [4,5] and patients admitted to intensive care units presented a higher risk of liver injury, with transient increase in aminotransferases even 20 times superior to the upper normal limit [6,7]. Nevertheless clinically significant acute liver damage seems to remain rare [8].

The exact mechanism of liver damage in COVID-19 patients is unclear. Moderate microvascular steatosis and mild lobular and portal activation were detected in a biopsy specimen of a deceased patient [9]. Possible mechanisms of liver injury are: immune mediated damage related to severe inflammatory response, direct viral cytotoxicity effect, drug induced liver injury, or reactivation of pre-existing liver disease [10]. The so called "cytokine storm" characterized by elevated levels of pro-inflammatory cytokines such as interleukin (IL) 6 and granulocyte colony stimulating factor has been described in patients with severe disease [11].

Pathological examination of COVID-19 patient showed hepatocyte degeneration accompanied by focal necrosis, moderate steatosis, infiltration of leukocytes into lobular and portal area, and sinusoidal congestion [9]. Thus, all these indicated liver involvement in patients infected by COVID-19.

Several recent studies have been demonstrating that COVID-19 infection also has been associated to higher frequency of liver steatosis on radiological imaging [12]. Moreover, it has been shown that transient elastography is a useful and non-invasive tool to assess onset and severity of acute liver injury in COVID-19 patients. Increased liver stiffness (LS) seems to be directly associated with transaminases elevation and with the possible development of complicated disease, necessity of intensive care hospitalization and mortality [13].

Despite the high prevalence of acute liver damage during COVID-19 infection, long term effects of the infection on the liver have not been explored so far. In particular, while the association has been assessed for steatosis and increased transaminases levels, the same does not apply for liver fibrosis, currently one of the most important conditions triggered by liver damage.

There is now increased availability of noninvasive tests for the assessment of liver injury, steatosis and fibrosis stage that have been extensively used in the last years in the follow up of patients with chronic liver disease

In particular one of the major advance in liver fibrosis staging has been the introduction of liver stiffness measurement using ultrasound (US) two dimensional (2D) shear-wave elastography (SWE). This is a widely used non-invasive tool to measure LS already adopeted into clinical practice as a point of care test for the evaluation of liver fibrosis of different aethiologies [14].

Several quantitative or semi-quantitative US-methods have been tested over the past few years to diagnose and evaluate hepatic steatosis [15]. Preliminary results have demonstrated the ability and reproducibiliry of a new quantitative US method based on sound speed estimation (SSE) in detecting and grading hepatic steatosis using liver histology and mangetic resonance as reference standard [16,17]. The analysis of the radiofrequency echoes detected by the system may allow to calculate sound speed and indirectly estimate liver steatosis: an increase of fat in the liver causes a decrease in the speed of the sound.

By knowing the growing association between acute liver damage and COVID-19 established by recent studies we wanted to evaluate the relationship between COVID-19 infection and chronic liver damage. In particular, this study aims to clarify the role of US-based techniques in the follow up of patients with COVID-19 infection.

Methods

Patients

Between September 2020 and April 2021 all consecutive patients referred to the outpatient post-COVID-19 Unit of the Fondazione Policlinico Gemelli in Rome for multidisciplinar re-evaluation 6 months after COVID-19 infection were enrolled in this prospective study [18]. Inclusion criteria were age > 18 years and resolution of COVID-19 acute infection that required in-hospital treatment. Subjects without previous hospital admission for COVID-19 infection or with not appropriate US visualization of the liver were excluded. Other exclusion criteria was pre-existing chronic liver disease of various aethiology and liver cancer.

The diagnosis and clinical management of COVID-19 patients were in accord to the practice guidelines [19].

COVID-19 infection was diagnosed through real time- polymerase chain reaction (RT-PCR) assay performed on biological fluids from respiratory tract (throat swabs, nasopharingeal swabs or lower respiratory tract lavage) [20]. Patients received treatment with lopinavir/ritonavir, idrossiclorochine, anti-IL6 according to patients' clinical characteristics and physicians' decisions. For those with severe symptoms, methylprednisolone (1–2 mg per kg weight per day) and empirical antibiotics (moxifloxacin or piperacillin/tazobactam or meropenem) would be administered [20].

Resolution of COVID-19 acute infection was defined as at least two consecutive RT-PCR negative results 24 hours apart.

Study design

During multidisciplinary re-evaluation all patients underwent B-mode US, SSE and 2D-SWE. At the time of US examination, height and weight were recorded form each patient in order to calculate Body Mass Index (BMI). Patients with a Body Mass Index (BMI) ≥30 kg/m2 were defined as obese. The following variables were retrospectively collected from medical records: comorbidities (diabetes mellitus, arterial hypertension, dyslipidemia), medical treatment, history of chronic liver disease (Non-Alcoholic Fatty Liver Disease [NAFLD], viral, autoimmune and others) and laboratory tests during hospital admission (complete blood count and liver biochemistries including Alanine Aminotransferase [ALT], Aspartate Transaminase [AST], total bilirubin, Gamma-Glutamyl Transferase [GGT], alkaline

phosphatase). We retrospectively collected also patient's medical treatment during hospitalization and in particular antiviral therapy, idrossicloroquine, anti-interleukin (IL) 6, antibiotics, subcutaneous heparin, corticosteroids.

The presence of liver steatosis and fibrosis were related to different clinical and laboratoristic parameters recorded.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee and received institutional review board approval from the 'Fondazione Policlinico Universitario A. Gemelli IRCCS' Ethical Committee. All patients were informed about the objective of the study and signed an informed consent.

Ultrasound techniques

Liver US, SSE and LS studies were performed with Aixploree-Mach30 ultrasound system (SuperSonic Imagine, SSI, France) equipped with a wideband C1-6 MHz curvilinear probe by two trained operators (M.A.Z and M.E.A. respectively with 15 and 10-years of experience in liver US) not aware of the clinical data of the patients. Each examination was performed in duplicate and the mean value was used for the analysis.

All patients fasted more than 8 h and were placed in the supine position with the right arm in maximal abduction. First B-mode scanning was performed in order to better visualize the right liver lobe and to choose the better position for SSE and 2D SWE according to a standardized protocol

The SSE was performed with the Sound Speed PLUS (SSp PLUS) technique. Measurements were obtained from the right hepatic lobe through intercostal spaces with the probe axis orthogonal to the liver capsule. The operator selected the most appropriate area in the right liver lobe (usually 5th or 6th segment) with the upper part of the biggest box on liver capsule and the smallest box free of large vessels. The median value of 3 successful measurements was obtained from each patient with suspended normal breathing.

The results were expressed in meter per second (m/sec) and a value a cut off ≤ 1.537 m/s identified the presence of steatosis [17].

The LS evaluation were performed with the 2D-SWE technique by selecting the most appropriate area of the right lobe at least 5 cm in diameter, free of large vessels, and 2 cm below the liver capsule. Measurements were obtained by positioning a 15-mm-diameter region of interest (ROI) in the center of a color map with complete and homogeneous filling, obtained during a breath hold. The median value of 3 successful LS measurements was obtained from each patient. Measurements were considered reliable if the stability index of the single analysis was at least 90% of median values [21]. The results were expressed in kilopascals (kPa) and a value >7.1 kPa was considered to be elevated and to reflect significant fibrosis [14].

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for continuous variables or as frequency and percentage for categorical variables.

To verify the relationship of different parameters with post COVID liver steatosis (SSE ≤ 1.537 m/s) or significant liver fibrosis (2D SWE ≥ 7,1 kPa) we applied the Pearson's Chi square test for categorical variables and Student's t test for continuous variables.

Parameters associated with significant liver fibrosis were tested in a logistic regression model. Odds ratio and corresponding 95% confidence intervals (CI) were derived from univariate logistic regression analysis. The variables that by the univariate analysis showed an association with liver fibrosis were included in multiple linear regressions models.

Statistical analysis was performed using the STATA® Software version 14.0 (Stata Corporation; College Station TX, USA).

Results

Clinical characteristics of enrolled patients

One hundred fifty-eight eligible patients were recruited during the study period. Ten patients were excluded for pre-existing chronic liver disease (8 NAFLD, 2 viral hepatitis). Two patients were not included in the final evaluation for inadequate visualization of right liver lobe. Overall, we included 146 patients (77 men, 69 women; mean age 54.8 years)

The mean BMI was 26.2 kg/m2; arterial hypertension and diabetes were present respectively in 33.5% and 10.9% of patients. Hepatic steatosis, defined as SSE ≤ 1.537 m/s, was present in 55 patients (37.6%). The mean value of 2D-SWE was 4.9 kPa; significant fibrosis, defined as 2D-SWE ≥7,1, was present in 11 patients (7.5%). The mean in-hospital days were 14.8 days and 57 patients (38%) had ALT elevation. Medical therapy during hospitalization includes idrossicloroquine (60.9% of patients), antibiotics (54.1%), antivirals (51.4%), enoxaparine (48.6%). Only 10.9% of patients received corticosteroid treatment.

Demographic and clinical data of the study population are provided in **Table 1**.

There was no difference regarding the comorbidities and the medical history between patients' groups with or without acute liver damage during COVID-19 infection, except for laboratory parameters (AST, GGT and ALP levels) and enoxaparine and corticosteroid administration **(Table 1)**. Patients with acute liver damage have higher LS and lower SSE values compared with patients without (5.2 *vs* 4.8 kPa, p = 0.04 and 1530 vs 1548 m/sec, p < 0.01 respectively) **(Figure 1)**.

Liver steatosis post COVID-19 infection

During the follow up after COVID-19 infection Doppler US examination was normal in all patients, and none of the subjects showed US signs suggestive for liver cirrhosis. The overall SSE value was 1541 m/s. A decrease in SSE suggestive of liver steatosis (<1537 m/s) was detected in 37.7% of the whole cohort. Patients with or without liver steatosis were compared with regard to demographic and clinical features including laboratory tests and medical therapy during hospital admission. Interestingly, subjects with SSE (<1537 m/s) had a significantly higher LS values (5.3 vs 4.7 kPa, p < 0.01) without significant differences in aminotransferases and GGT levels **(Table 2)**. No significative differences in age, sex and BMI, were found between the two groups. Liver steatosis was neither associated with comorbidities (obesity, diabetes, hypertension) nor with medical therapy during acute infection.

Liver stiffness post COVID-19

LS was determined in all patients with COVID-19 six months

Table 1: Demographic and clinical data of the study population. Laboratory reports and therapy are related to acute COVID 19 infection. Steatosis and liver stiffness values are related to 6 months follow up.

Characteristc	All patients (n = 146)	ALT < 45 UI/L (n = 89)	ALT > 45 UI/L (n = 57)	p value
Age, years Mean (SD)	54.8 (13.8)	55.4 (14.9)	53.8 (11.9)	0.5
Sex, n (%) Male/Female	77 (52.7)/69 (47.3)	45 (50.5)/44 (49.5)	32 (56.1)/25 (43.9)	0.51
BMI	26.2 (4.5)	25.9 (4.5)	26.6 (4.4)	0.29
Obesity (BMI>30), n (%)	24 (16.4)	15 (16.8)	9 (15.8)	0.32
Hypertension, n (%)	49 (33.5)	32 (35.9)	17 (29.8)	0.44
Diabetes, n (%)	16 (10.9)	10 (11.2)	6 (10.5)	0.89
Dyslipidemia, n (%)	54 (37)	35 (39.3)	19 (34.5)	0.46
Hospital length, days mean (SD)	14.8 (11.2)	14.6 (12.3)	15.1 (12.2)	0.8
Laboratory values mean (SD)				
AST (UI/L)	40.7 (57.4)	21.9 (9.2)	77.9 (87)	**<0.01**
total bilirubin (mg/dl)	0.7 (0.3)	0.7 (0.4)	0.7 (0.2)	0.56
GGT (UI/L)	58.6 (84.7)	39.3 (38.9)	97.2 (128.6)	**<0.01**
ALP (UI/L)	68.3 (45.5)	59 (17.8)	86.7 (71.8)	**<0.01**
D-dimer (ng/ml)	1588 (4092)	1882 (5067)	1088 (1225)	0.38
Lymphocytopenia n (%)	36 (24.6)	21 (23.6)	15 (26.3)	0.14
Therapy				
Antivirals	75 (51.4)	48 (43.9)	27 (47.3)	0.44
Hydroxychloroquine	89 (60.9)	53 (59.5)	36 (63.1)	0.66
Anti-IL 6	35 (23.9)	21 (23.6)	14 (24.5)	0.89
Antibiotics	79 (54.1)	46 (51.7)	33 (57.9)	0.46
Enoxaparin	71 (48.6)	36 (40.4)	35 (61.4)	**0.01**
Steroids	16 (10.9)	6 (6.7)	10 (17.5)	**0.04**
SSE (m/sec) mean (SD)	1541.0 (38.7)	1548.0 (35.0)	1530.0 (42.8)	**< 0.01**
2D-SWE, kPa, mean (SD)	4.9 (1.1)	4.8 (1.0)	5.2 (1.2)	**0.04**

Significant p values are in bold

N: Number of Patients; SD: Standard Deviation; BMI: Body Mass Index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT: Gamma Glutamyl Transpeptidase; ALP: Alkaline Phosphatase; IL6: Interleukin 6; SSE: Sound Speed Estimation; 2D-SWE: Two Dimensional Shear Wave Elastography

Table 2: Demographic and clinical data of the study population. Laboratory reports and therapy are related to acute COVID 19 infection. Steatosis and liver stiffness values are related to 6 months follow up.

Characteristc	SSE > 1537 m/sec (n = 91)	SSE < 1537 m/sec (n = 55)	p value
Age, years Mean (SD)	53.8 (13.2)	56.5 (14.7)	0.26
Sex, n (%) Male/Female	46 (50.5)/45 (49.5)	31 (56.4)/24 (43.6)	0.46
BMI	25.8 (4.8)	26.6 (4.0)	0.29
Obesity (BMI>30), n (%)	15 (16.5)	9 (16.4)	0.98
Hypertension, n (%)	28 (30.7)	21 (38.2)	0.35
Diabetes, n (%)	8 (8.8)	8 (14.5)	0.28
Dyslipidemia, n (%)	35 (38.4)	19 (34.5)	0.63
Hospital length, days mean (SD)	13.7 (10.7)	16.4 (12.0)	0.21
Laboratory values mean (SD)			
AST (UI/L)	45.3 (9.2)	81.9 (38.5)	0.91
ALT (UI/L)	53.0 (86.3)	53.4 (55.0)	0.98
ALT> 45 UI/L	19 (20.9)	19 (34.5)	0.11
total bilirubin (mg/dl)	0.7 (0.4)	0.7 (0.3)	0.45

GGT (UI/L)	66.5 (102.2)	46.6 (45.4)	0.22
ALP (UI/L)	72.4 (53.6)	61.9 (28.5)	0.23
D-dimer (ng/ml)	1363 (2276)	1902 (5780)	0.54
Lymphocytopenia n (%)	19 (20.8)	17 (30.1)	0.21
Therapy			
Antivirals	45 (49.4)	30 (54.5)	0.55
Hydroxychloroquine	54 (59.3)	35 (63.6)	0.61
Anti-IL 6	21 (23.1)	14 (25.4)	0.74
Antibiotics	53 (58.2)	26 (47.3)	0.19
Enoxaparin	45 (49.4)	26 (47.3)	0.79
Steroids	13 (14.3)	3 (5.4)	0.09
2D-SWE, kPa, mean (SD)	4.7 (1.0)	5.3 (1.2)	**<0.01**

Significant p values are in bold

N: number of patients; SD: Standard Deviation; BMI: Body Mass Index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT: Gamma Glutamyl Transpeptidase; ALP: Alkaline Phosphatase; IL6: Interleukin 6; SSE: Sound Speed Estimation; 2D-SWE: Two Dimensional Shear Wave Elastography

Table 3: Patients' characteristic stratified according to the presence of significative fibrosis (2D-SWE ≥ 7.1 kPa) six months after infection.

Characteristc	2D-SWE< 7.1 kPa (n = 135)	2D-SWE ≥ 7.1 kPa (n = 11)	p value
Age, years Mean (SD)	54.5 (13.8)	58.3 (14.2)	0.38
Sex, n (%) Male/Female	70 (51.9)/65 (48.1)	7 (63.6)/4(36.4)	0.54
BMI	25.9 (4.4)	29.2 (4.7)	**0.01**
Obesity (BMI>30), n (%)	19 (14.0)	5 (45.4)	**0.02**
Hypertension, n (%)	43 (31.8)	6 (54.5)	0.18
Diabetes, n (%)	11 (8.1)	5 (45.4)	**< 0.01**
Dyslipidemia, n (%)	52 (38.5)	2 (18.2)	0.21
Hospital length, days mean (SD)	14.1 (10.6)	21.4 (15.2)	0.05
Laboratory values mean (SD)			
AST (UI/L)	39.2 (59.1)	55.3 (21.6)	0.37
ALT (UI/L)	51.8 (78.0)	65.1 (39.6)	0.58
ALT> 45 UI/L	30.0 (22.2)	8.0 (72.7)	**0.01**
total bilirubin (mg/dl)	0.67 (0.32)	0.89 (0.31)	**0.04**
GGT (UI/L)	55.9 (84.3)	85.8 (82.3)	0.29
ALP (UI/L)	60.0 (47.4)	60.5 (17.3)	0.57
D-dimer (ng/ml)	1635 (4283)	1108 (748)	0.73
Lymphocytopenia n (%)	33 (24.4)	3 (27.3)	0.39
Therapy			
Antivirals	69 (51.1)	6 (54.5)	0.82
Hydroxychloroquine	81 (60.0)	8 (72.7)	0.52
Anti-IL 6	29 (21.5)	6 (54.5)	**0.02**
Antibiotics	73 (54.1)	6 (54.5)	0.97
Enoxaparin	63 (46.7)	8 (72.7)	**0.12**
Steroids	15 (11.1)	1 (9.1)	**0.83**
SSE (m/sec), mean (SD)	1542 (37.7)	1522 (46.7)	0.09

Significant p values are in bold

N: number of patients; SD: Standard Deviation; BMI: Body Mass Index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; GGT: Gamma Glutamyl Transpeptidase; ALP: Alkaline Phosphatase; IL6: Interleukin 6; SSE: Sound Speed Estimation; 2D-SWE: Two Dimensional Shear Wave Elastography

Table 4: Independent predictors of significative liver fibrosis (2D-SWE ≥ 7.1 kPa) evaluated by logistic regression models.

	Univariate analysis		Multivariate analysis	
	OR (CI 95%)	p	OR (CI 95%)	P
Age	1.02 (0.97 – 1.07)	0.38		
Sex (female)	0.61 (0.17 – 2.20)	0.45		
Obesity	4.91 (1.36 –17.7)	0.01	6.28 (1.06 –37.20)	0.04
Hypertension	2.57 (0.74 – 8.87)	0.13		
Diabetes	9.39 (2.46 – 35,71)	<0.01	7.95 (1.43 – 44.25)	0.01
Dyslipidemia	0.35 (0.07 – 1.73)	0.19		
Hospital lenght	1.04 (0.99 – 1.09)	0.06		
Lymphocytopenia	0.81 (0.19 – 3.37)	0.78		
ALT > 45	6.41 (1.58 – 25.79)	0.01	7.07 (1.39 – 36.11)	0.02
AST	1.00 (0.99 – 1.01)	0.40		
Total bilirubin	3.92 (0.86 – 17.67)	0.07		
GGT	1.00 (0.99 – 1.01)	0.32		
ALP	0.99 (0.96 – 1.02)	0.58		
D-dimer	0.99 (0.99 – 1.01)	0.73		
Antivirals	1.15 (0.33 – 3.94)	0.83		
Hydroxychloroquine	1.78 (0.45 – 7.00)	0.41		
Anti- IL 6	4.39 (1.25 – 15.4)	0.02	2.19 (0.47 – 10.14)	0.31
Antibiotcs	1.02 (0.30 – 3.50)	0.97		
Enoxaparin	3.04 (0.77 – 11.98)	0.11		
Steroids	0.80 (0.09 – 6.69)	0.83		
Liver steatosis	4.99 (1.26 – 19.72)	0.02	6.37 (1.03 – 39.56)	0.05

OR: Odds Ratio; CI: Confidence Interval; AST: aspartate aminotransferase; ALT: Alanine Aminotransferase; GGT: Gamma Glutamyl Transpeptidase; ALP: alkaline phosphatase; IL6: interleukin 6.

after infection in order to detect the presence of significative fibrosis (2D SWE <7.1 kPa). Interestingly liver fibrosis was associated to obesity (p=0.02), diabetes (p<0.01) and aminontransferases elevation (ALT>45 UI/l) during hospitalization (p<0.01) **(Table 3, Figure 2)**. Furthermore, the length of hospitalization was higher in patients with significative fibrosis (21.4 vs 14.1 days, p=0.05). As concerning medical treatment during hospital admission, anti-IL6 drugs have been used more often in patients with significative fibrosis (54.6% vs 21.5%, p=0.02) (Table 3).

Correlation of comorbidities, aminotransferases elevation, drugs and LS

The logistic regression analysis showed that in COVID-19 patients a higher LS (>7.1 kPa) was significantly associated with obesity (OR 4.91, CI 1.36 – 17.7, p=0.01), diabetes (OR 9.39, CI 2.46 – 35.71, p<0.01) and liver steatosis (OR 4.99, CI 1.26 – 19.72, p=0.02). Furthermore, elevated aminotransferases levels and anti-IL6 therapy during hospitalization were associated with significative fibrosis at univariate logistic model (OR 6.41, CI 1.58 – 25.79, p=0.01 and OR 4.39, CI 1.25 – 15.40, p=0.02, respectively). (Table 4)

The same parameters except for anti-IL6 therapy resulted independently associated with significative fibrosis at multivariate logistic regression model: obesity (OR 6.28, CI 1.06 – 37.2, p=0.04), diabetes (OR 7.95 CI 1.43 – 44.25, p=0.01), liver steatosis (OR 6.37, CI 1.03 – 39.56, p=0.05) and elevation of ALT during hospitalization (OR 7.07, CI 1.39 – 36.11, p=0.02).

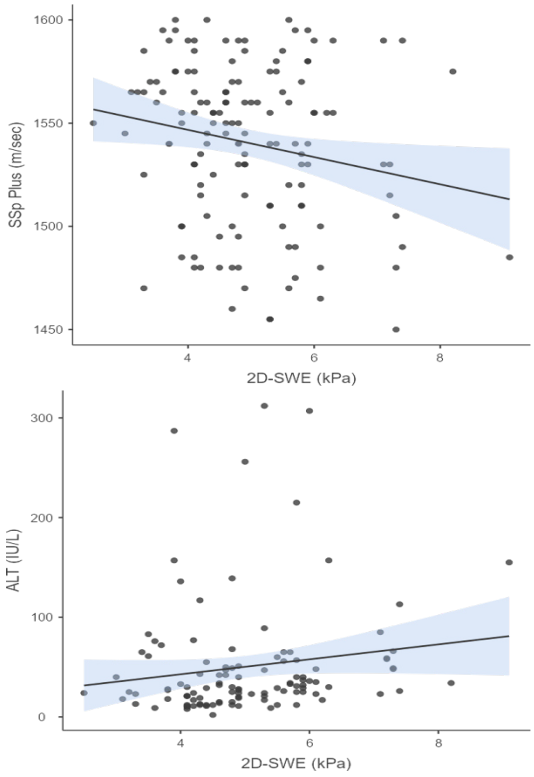

Figure 1: Liver Stiffness (A) and SSE (B) in patients with and without ALT elevation (> 45 IU/L).
SSE: Sound Speed Estimation; **ALT:** Alanine Aminotransferase; **kPa:** kilopascal.

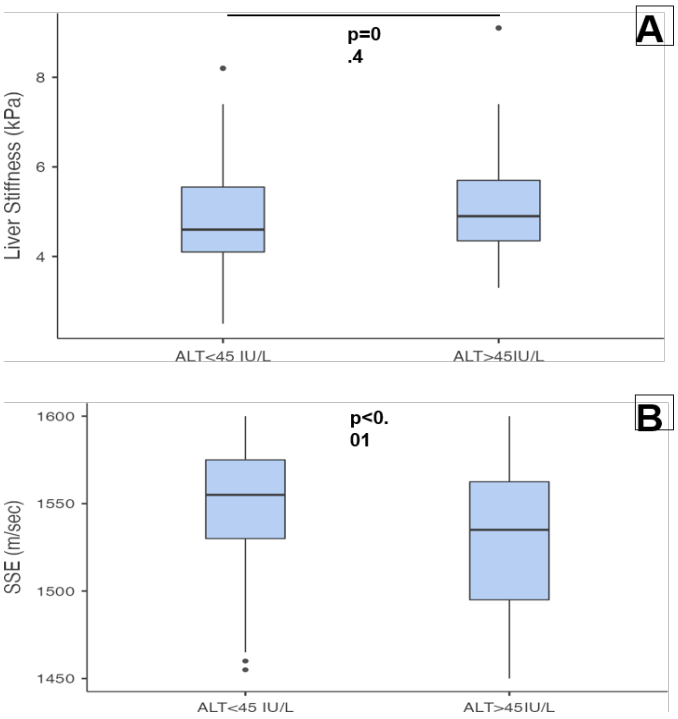

Figure 2: Scatter dot plot demonstrating the correlation between Liver Stiffness and SSE (A), BMI (B), ALT (C).
SSE: Sound Speed Estimation; BMI: Body Mass Index; ALT: Alanine Aminotransferase; SSp: Sound Speed PLUS; 2D-SWE: Two Dimensional Shear Wave Elastography.

Discussion

COVID-19 is a highly contagious disease with extensive influence on public health. Many studies emphasised that liver injury is a common complication of COVID-19 [3, 22-24]. In particular, elevated liver enzymes have been described in 17% up to 57% of affected individuals [25,26].

Despite the apparently low clinical severity of the liver damage associated to COVID-19, it is important to determine patient's outcome [8].

With respect to the ultrasound findings, in previous studies, most of the patients with only mildly elevated liver enzymes showed normal appearance of the liver whereas the majority of those with severely elevated liver enzymes had suspicious findings, specifically signs of acute hepatitis or vascular complications [27].

This is the first study performed to evaluate the long term effects of COVID-19 infection on liver involvement. We described abdominal US features of COVID-19 patients six months after infection and the correlation of these features with clinical profiles.

Particularly, in our study we analysed two groups of patients stratified according to the presence/absence of post- COVID-19 hepatic steatosis and fibrosis and we could identify different clinical and biochemical parameters associated to ultrasound findings, namely the presence of comorbidities such as diabetes and hypertension as well as acute liver damage and anti-IL6 therapy during COVID-19 infection.

Many studies conducted in recent years have reported the usefulness of elastographic methods in clinical diagnostic imaging [14, 28, 29].

In a recent paper transient elastography and the linear correlation between LS and direct markers of acute liver damage (AST, ALT and GGT) supports the suitability of LS measurements to ensure liver involvement in COVID-19 [13] and to predict outcome [30].

This is the first study to suggest, to our knowledge, a possible association between COVID-19 infection and chronic liver disease. In particular we found higher LS values six months after infection in patients with comorbidities such as obesity and diabetes.

It has already been demonstrated that patients with comorbidities (cardiovascular diseases, arterial hypertension, diabetes mellitus, renal disease, chronic obstructive pulmonary disease, tumours, HIV) are more likely to develop severe symptoms, potentially leading to cytokine storm and the risk of a fatal outcome during COVID-19 infection [31]. According to these reports we found a strong association between comorbidities and increased liver stiffness as sign of chronic liver damage. Even if the ethiology of chronic liver damage in COVID-19 remains unclear we could hypothesize that the increased burden of adipose tissue in obesity and the metabolic abnormalities associated to diabetes may amplify the proinflammatory response to viral infection owing to direct, indirect and epigenetic mechanisms, ranging from immune system activity attenuation to chronic inflammation.

Moreover, LS correlated well with acute injury, reflected by increased aminotransferase levels and longer hospitalization during the acute phase. Such results are in line with several previous studies demonstrating the critical relationship between viral infection and systemic inflammation [8,26,32].

On the other hand, drug-induced liver damage is also possible as demonstrated by the association of LS to anti-IL 6 therapy.

Thus, we speculate chronic liver injury in COVID-19 patients might result from viral infection of liver cells, inflammatory responses and drug reactions. The hypothesis is supported by indirect evidences and it should be confirmed by a large sample of pathological examinations in future. Even though it is difficult to make a clear statement in a small cohort, it will allow an earlier identification of high-risk patients for immediate effective intervention in future.

Obviously, our study had several limitations. Firstly, it was a single-center study with a limited number of cases that might have determined a bias of results. Secondly data about pre-existing chronic liver disease were only retrospectively collected and pre-infection occurrence of steatosis or fibrosis have not been ascertained by imaging data. Thirdly, histologic confirmation of US results has not been performed. However, even if the value of histologic assessment in liver disease remains unquestioned, it has been extensively demonstrated that US parameters are able to noninvasively provide equivalent information while avoiding the limitations of liver biopsy in the management of patients with chronic liver disease.

In conclusion, US parameters for the measurement of hepatic steatosis and fibrosis may prove to be useful for non-invasive and comprehensive estimation of the status of liver parenchyma after COVID-19 infection, especially in a subgroup of patients with coexisting comorbidities and acute injury. It remains however unclear, whether these changes are caused by COVID-19 itself, secondary hyperinflammation or drug reactions. Further studies with prospective, multicenter designs and larger numbers of participants are needed to clarify the role of

novel Coronavirus in chronic liver damage.

Conflict-of-interest statement

No potential conflicts of interest. No financial support.

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