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Markers of liver function as potential prognostic indicators of SARS-CoV-2 infection: A retrospective analysis during the first and second waves of COVID-19 pandemic

Ludovica Esposito[1], Matteo Guarino[1], Benedetta Perna[1], Andrea Ciccarone[1], Alice Eleonora Cesaro[1], Francesca Manza[1], Adriana Pretula[1], Anna Costanzini[1], Martina Maritati[2], Elena Forini[3], Michele Domenico Spampinato[1], Roberto De Giorgio[1], Carlo Contini[2]

[1]Department of Translational Medicine, St. Anna University Hospital of Ferrara, University of Ferrara, Italy; [2]Infectious and Dermatology Diseases, St. Anna University Hospital of Ferrara, University of Ferrara, Italy; [3]Unit of Statistics, St. Anna University Hospital of Ferrara, University of Ferrara, Italy

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SUMMARY

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is known to cause a predominant respiratory disease, although extrapulmonary manifestations can also occur. One of the targets of Coronavirus disease 2019 (COVID-19) is the hepatobiliary system. The present study aims to describe the correlation between the increase of liver damage markers (*i.e.* alanine aminotransferase [ALT], aspartate aminotransferase [AST], total bilirubin [TB]) and COVID-19 outcomes (*i.e.*, in-hospital mortality [IHM] and intensive care unit [ICU] transfer).
Methods: All patients with confirmed SARS-CoV-2 infection admitted to the Infectious Diseases Unit of the St. Anna University-Hospital of Ferrara from March 2020 to October 2021 were retrospectively included in this single-centre study. ALT, AST and TB levels were tested in all patients and IHM or ICU transfer were considered as main outcomes. Co-morbidities were assessed using Charlson Comorbidity Index.
Results: A total of 106 patients were retrieved. No hepatic marker was able to predict IHM, whereas all of them negatively predicted ICU transfer (ALT: OR 1.005, 95%CI 1.001-1.009, p= 0.011; AST: OR 1.018, 95%CI 1.006-1.030, p= 0.003; TB: OR 1.329, 95%CI 1.025-1.724, p= 0.032). Age was the only parameter significantly related to mortality.
Conclusions: The present study, by correlating liver damage markers with COVID-19 outcome, showed that an increase of ALT, AST and TB predicted patients' severity, although not mortality.

Keywords: COVID-19, ICU, in-hospital mortality, liver disease; SARS-CoV-2 infection.

INTRODUCTION

Since 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic caused millions of deaths worldwide [1, 2]. From a pathogenetic standpoint SARS-CoV-2 enters the infected cells via different molecular mechanisms involving angiotensin converting enzyme-2 (ACE-2). Since ACE-2 expression has been identified in the liver and epithelial cells of the biliary tract, SARS-CoV-2 can target these tissues causing a possible liver/biliary injury [3-6]. Any hepatic damage occurring during Coronavirus disease 2019 (COVID-19) should be considered as a manifestation of SARS-CoV-2 infection, regardless any

Corresponding author
Carlo Contini
E-mail: cnc@unife.it

pre-existent liver disease [7, 8]. A wide range of pathogenetic mechanisms can contribute to liver abnormalities, specifically:

1) immune-mediated damage as a result of the massive inflammatory response;
2) direct cytotoxicity due to the viral replication in the hepatocytes;
3) anoxia due to COVID-19 related respiratory failure;
4) pharmacological liver damage mainly due to hepatotoxic antivirals;
5) reactivation of a pre-existing liver disease;
6) vascular injury resulting from hypercoagulative state [8-10].

Recently, a real-time polymerase-chain-reaction (RT-PCR) detected SARS-CoV-2 RNA in hepatocytes [11]. This study was aimed at describing the correlation between increased liver damage markers (*i.e.* alanine aminotransferase [ALT], aspartate aminotransferase [AST] and total bilirubin [TB]) and the outcome of COVID-19 patients. Two main end-points were considered: in-hospital mortality (IHM) and Intensive Care Unit (ICU) transfer from the initial recovery ward.

PATIENTS AND METHODS

All patients with confirmed SARS-CoV-2 infection (by RT-PCR on nose-/oro-pharyngeal swab test) admitted to the Infectious Diseases Unit of the St. Anna University-Hospital, Ferrara, Italy, from March 2020 to October 2021 were retrospectively retrieved in this single-centre study. Among them, patients with ALT, AST and TB levels tested on admission to the recovery ward were included in the analysis. Exclusion criteria were:

1) subjects with a false-positive COVID-19 test (with a positive antigenic swab taken at the time of admission to the Emergency Department, not subsequently confirmed by a molecular swab, were considered false positives) or incomplete report of liver indexes;
2) the presence of any anamnestic pre-existent liver disease;
3) serological tests positive for major hepatotropic viruses [12].

Co-morbidities were assessed using Charlson Comorbidity Index (CCI). ICU transfer and IHM were considered the two main outcomes. The secondary aim of the study was to assess the effect of various treatments (*i.e.* azithromycin, low molecular weight heparin [LMWH], steroids, remdesevir, tocilizumab and oxygen treatment) on ICU admission and IHM. In this study, we set the limit for oxygen treatment at >9 L/min because it was the highest value acceptable for the admission to the Infectious Diseases Unit in our Hospital. Patients requiring >9 L/min of oxygen were moved to other wards (*i.e.* Internal Medicine, Pneumology or ICU). This is a retrospective observational study performed without direct involvement of patients. Thus, in agreement with our local ethics committee (Comitato Etico di Area Vasta Emilia Centro of Ferrara) a formal approval for this study was deemed unnecessary and, thereby, a project identification code was not assigned.

Statistical analysis

In this study categorical data were expressed as absolute values and percentages, while means and standard deviations (SD) were reported for continuous variables. Differences between patients deceased/discharged and transferred to ICU for COVID-19 severity were compared with the Pearson's X2, Fisher's and Mann-Whitney tests, as appropriate. The association between IHM/ICU admission and the investigated variables (*i.e.*, ALT, AST and TB) was studied with univariated and multivariated logistic regression analysis. All the liver damage markers were considered positive when above their cut-off (ALT >35 U/L in female and >50 U/L in male; AST >35 U/L in female and >50 U/L in male; TB >1,2 mg/dl). Odds ratios (ORs) and their 95% confidence intervals (CI) were reported. The MedCalc® Statistical Software version 19.8 (MedCalc Software Ltd, Ostend, Belgium) was used for statistical analyses and the significance level was set for $p<0.05$.

RESULTS

A total number of 723 patients were admitted to the Infectious Disease Unit from March 2020 to October 2021 for SARS-CoV-2 infection. A total number of 617 were excluded for incomplete details about liver function tests (n=495), anamnestic pre-existent liver disease (n=109) or serological tests positivity for major hepatotropic viruses (n=13); thus, 106 patients were eligible for the study. Among them, 50 subjects were female (47.2%) and 56 males (52.8%) with a mean age of 64.0±17.5 years. A total number of 89 patients

Table 1 - Demographic and mean laboratory features of the enrolled patients.

Features	*Values*
Age, mean (SD)	64.0 (17.5)
Female, n. (%)	50 (47.2)
Deceased, n. (%)	17 (16)
LOS, mean (SD)	21.0 (20.9)
CCI, mean (SD)	1.7 (2.5)
ALT U/L, mean (SD)	174 (539)
AST U/L, mean (SD)	69 (76)
TB mg/dL, mean (SD)	1.45 (3.89)

Note: Values are expressed as percentage or mean (SD). ALT: alanine aminotransferase; AST: aspartate aminotransferase; CCI: Charlson Comorbidity Index; LOS: Length of stay; SD: Standard deviation; TB: Total bilirubin.

(84.0%) were discharged, whereas 17 (16.0%) died because of COVID-19. Thirteen patients (12.3%) were transferred to ICU and among them 3 subjects died (23.1%). The mean length of stay [LOS] was 21±20.9 days ranging from 1 to 116 days. The mean CCI was 1.7±2.5 (range 0-12). Demographic and mean laboratory features are summarized in Table 1.

High levels of ALT, AST and TB were detected in 69 (65.1%), 53 (50.0%) and 21 (19.9%) patients, respectively. The mean levels and significance of ALT, AST and TB in relation to the two considered outcomes are summarized in Table 2. In the logistic regression stratified for age, CCI and P/F, neither ALT, AST nor TB influenced IHM, while age was the only parameter able to predict death. In

Table 2 - Median levels, SD and significance of ALT, AST and TB in relation with the two main outcomes.

	IHM			*ICU*		
	Deceased	*Survived*	*p*	*Transferred*	*Not transferred*	*p*
	Mean (SD)	*Mean (SD)*		*Mean (SD)*	*Mean (SD)*	
ALT (U/l)	398 (1282)	128 (132)	0.060	621 (1423)	107 (130)	*0.001
AST (U/l)	71 (145)	68 (60)	0.901	(52)	54 (43)	*<0.001
TB (mg/dl)	0.86 (0.57)	1.51 (4.25)	0.570	2.09 (2.54)	1.28 (4.09)	*0.001

**Significant levels of p-value.*
Note: ALT: alanine aminotransferase; AST: aspartate aminotransferase; ICU: Intensive care unit; IHM: In-hospital mortality; SD: Standard deviation; TB: Total bilirubin.

Table 3 - Multivariate analysis stratified for age, CCI and PO_2 / FiO_2 ratio.

	IHM			*ICU*		
	OR	*95% C.I.*	*p*	*OR*	*95% C.I.*	*p*
ALT	1.001	0.999-1.002	0.135	1.005	1.001-1.009	0.011*
CCI	1.221	0.974-1-530	0.083	0.576	0.293-1.132	0.109
Age	1.086	1.032-1.142	0.001*	1.008	0.964-1.055	0.718
P/F ratio	0.471	0.046-4.796	0.525	1.408	0.228-8.696	0.713
AST	1.005	0.997-1.013	0.242	1.018	1.006-1.030	0.003*
CCI	1.243	0.986-1.566	0.065	0.590	0.330-1.055	0.075
Age	1.076	1.014-1.141	0.016*	1.027	0.975-1.082	0.315
P/F ratio	0.491	0.043-5.628	0.568	0.462	0.051-4.199	0.493
TB	0.862	0.548-1.354	0.518	1.329	1.025-1.724	0.032*
CCI	1.237	0.964-1.589	0.095	0.386	0.173-0.857	0.019*
Age	1.062	1.014-1.112	0.011*	1.024	0.978-1.072	0.309
P/F ratio	0.472	0.046-4.847	0.527	0.721	0.116-4.496	0.726

**Significant levels of OR.*
Note: ALT: alanine aminotransferase; AST: aspartate aminotransferase; CCI: Charlson comorbidity index; CI: confidence intervals; ICU: Intensive care unit; IHM: In-hospital mortality; OR: Odds ratio; P/F: PaO_2/FiO_2 ratio; SD: Standard deviation; TB: Total bilirubin.

the ICU-transferred subgroup each of the lab test (*i.e.* ALT, AST and TB) predicted ICU admission. The CCI resulted protective from ICU admission (OR 0.386, 95% CI 0.173-0.857, p=0.019). The levels of OR, 95% CI and significance of each involved parameter in relation with the two considered outcomes were reported in Table 3. The Hosmer-Lemeshow test showed a good calibration of the analysis (p= 0.746).

There was no correlation between different treatments (*i.e.* azithromycin, low-weight molecular heparin [LMWH], steroid, remdesevir, tocilizumab and oxygen >9 L/min) and the considered outcomes, except for oxygen treatment >9 L/min which was significantly related to ICU transfer.

A sensitivity analysis has been performed to stratify the cohort in two subgroups, *i.e.* patients with age <65 (group A) vs ≥80 years (group B). In group A, there were 2 cases (3.5%) of IHM and 9 (16%) patients were transferred to ICU. Although not statistically significant, levels of hepatic markers resulted to be higher in patients with eventful outcome or transferred to ICU. In group B, 11 (42.3%) patients died and 2 (7.6%) were transferred to ICU. In both groups, patients who died or were transferred to ICU showed higher values of ALT, AST and TB.

DISCUSSION

SARS-CoV-2 primarily affects the lungs causing a well-known interstitial pneumonitis; however, several extrapulmonary manifestations can occur [13]. One of the main targets involved in COVID-19 is the hepatobiliary system due to the binding of the virus to ACE-2 receptors densely expressed in hepatocytes and biliary epithelial cells [3]. The resulting hepato-biliary injury can influence patients' severity and outcome [14]. Recent studies on COVID-19 patients have shown that the incidence of liver injury ranged from 14.8 to 53%, as emerged by abnormal ALT/AST levels and associated with slightly elevated bilirubin values [7, 15, 16]. Increasing evidence has highlighted the close relationship of abnormal liver parameters with COVID-19 severity [8]. Furthermore, in addition to the previously mentioned pathogenetic mechanisms [8-10], co-infections with intracellular microorganisms (*e.g. Chlamydia pneumoniae* or *Mycoplasma pneumoniae*) might worsen liver damage and, consequently, patient's clinical conditions [11, 17-19]. Our evaluation, which expands previously published studies, provides further support to the role of SARS-CoV-2 in eliciting hepatocyte damage and the possible correlation between liver markers increase and the involved outcomes (*i.e.* IHM and ICU transfer) [14]. Since cytonecrosis indexes predict the progression to severe COVID-19 better than cholestatic ones, we assessed ALT, AST and TB to measure the direct effect of SARS-CoV-2 infection on the liver [20-23]. In our analysis the examined cohort had a low mean age and number of comorbidities, thus we assumed that a greater severity was ascribable to COVID-19 and not to an unknown pre-existing liver damage. In the univariate analysis, liver markers did not significantly affect IHM, although AST and ALT predicted ICU transfer. However, the examined parameters are indicators of multi-organ failure, which predicts higher severity in every clinical condition, and therefore they are not significant indicators of poor prognosis in COVID-19. Even though not statistically significant, ALT was the test which better expressed the risk of fatal outcome, conversely to Lei et al. who reported a better sensitivity of AST vs ALT in predicting COVID-19 severity and outcome [24]. This latter finding is consistent with the evidence that ALT is mainly expressed in the liver, whereas AST can be detected also in other tissues [25].

In the logistic regression, age was the only parameter predicting fatal outcome; however, this variable did not influence the risk of ICU transfer. As demonstrated by Li et al., ALT, AST and TB were able to identify critically ill patients requiring an intensive care setting, thus supporting our hypothesis that liver damage worsens patients' clinical conditions [26]. In contrast, CCI was a protective factor for ICU transfer since this decision is strictly related to the number of comorbidities. Indeed, patients with a high CCI are usually at high risk of mortality (regardless the intensity of care) and, therefore, are not usually transferred/admitted to ICU.

Various pharmacological agents and therapeutical strategies have been investigated by numerous clinical trials and observational studies; these data were assessed to determine which treatment was actually effective in COVID-19. Considering the secondary outcome, none of the proposed treatments resulted effective in reducing mortality. Our

results are in agreement with the latest guidelines of the Italian Drug Agency, which advise against the use of antibiotics and LWMH and limit the use of corticosteroids, remdesevir and tocilizumab to selected patients [27]. In the absence of a causal treatment/adequate host response to infection, oxygen therapy can be only a supportive measure for vital functions without improving patient's survival [28]. Moreover, none of the analyzed therapies was able to predict ICU admission, except for oxygen treatment >9 L/min. This could be explained by considering oxygen supply as an indicator of severe respiratory failure [29], thus eventually requiring ventilatory support (and therefore a high level of intensity care). The sensitivity analysis highlighted that age is the only parameter able to negatively influence mortality, whereas liver markers indicated most severe patients requiring a higher intensity of care.

We would like to acknowledge different limitations of our study. First, it is a single-centre and retrospective analysis with a small sample size which considerably reduced the statistical power of our results. Secondly, in order to evaluate only patients with liver damage related to SARS-CoV-2, this analysis excluded subjects with any pre-existent hepatic disease. Therefore, the study design did not allow the assessment of COVID-19 effects on those patients previously affected by chronic liver injuries. Thirdly, only liver damage tests assessed on admission to the Infectious Diseases Unit were considered, thus preventing the analysis of the AST/ALT and TB trend during hospitalization which may affect patients' outcome. Finally, we examined only ALT, AST and TB and not other markers of liver function (*e.g.* coagulation or albumin).

In conclusion, although SARS-CoV-2 infection is mainly known to target the respiratory system, hepatic involvement in COVID-19 has been demonstrated in several studies. This single-centre, retrospective analysis supported and expanded the evidence that SARS-CoV-2 exerts a damaging effect to the liver and highlighted the correlation between increased hepatic markers, *i.e.* AST/ALT and TB and disease severity, as expressed by the ICU admission from a medical ward. Further prospective and multicentric studies on large cohorts are awaited to establish the impact of hepatic involvement in SARS-CoV-2 infection on patients' outcome.

Author contributions

Conceptualization, MG, CC, CC, MG, RDG; AC; methodology, MG, BP, ACo, AG, MDS and CC.; software EF, MM.; validation, MG, ACo, MM, RDG and CC; formal analysis, EF; investigation, AC; resources, AC and EF; data curation, LE, AC and EF.; writing-original draft preparation, LE, MG, AEC, FM, AP and CC; writing-review and editing, MG, BP, RDG and CC; visualization, LE, MG, BP, ACo, MM, MDS, RDG and CC ; supervision, AG, RDG; project administration, CC; funding acquisition, RDG and CC. All authors have read and agreed to the published version of the manuscript.

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Data availability sdtatement

The datasets generated and/or analyzed during the current study are not publicly available due to privacy policy but are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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