Longitudinal progression of clinical variables associated with graded liver injury in COVID-19 patients

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Abstract

Background Hospital-acquired liver injury is associated with worse outcomes in COVID-19. This study investigated the temporal progression of clinical variables of in-hospital liver injury in COVID-19 patients.

Methods COVID-19 patients (n = 1361) were divided into no, mild and severe liver injury (nLI, mLI and sLI) groups. Time courses of laboratory variables were time-locked to liver-injury onset defined by alanine aminotransferase level. Predictors of liver injury were identified using logistic regression.

Results The prevalence of mLI was 39.4% and sLI was 9.2%. Patients with escalated care had higher prevalence of sLI (23.2% vs. 5.0%, p < 0.05). sLI developed 9.4 days after hospitalization. sLI group used more invasive ventilation, anticoagulants, steroids, and dialysis (p < 0.05). sLI, but not mLI, had higher adjusted mortality odds ratio (= 1.37 [95% CI 1.10, 1.70], p = 0.005). Time courses of the clinical variables of the sLI group differed from those of the nLI and mLI group. In the sLI group, alanine aminotransferase, procalcitonin, ferritin, and lactate dehydrogenase showed similar temporal profiles, whereas white-blood-cell count, D-dimer, C-reactive protein, respiration and heart rate were elevated early on, and lymphocyte and SpO2 were lower early on. The top predictors of sLI were alanine aminotransferase, lactate dehydrogenase, respiration rate, ferritin, and lymphocyte, yielding an AUC of 0.98, 0.92, 0.88 and 0.84 at 0, −1, −2 and −3 days prior to onset, respectively.

Conclusions This study identified key clinical variables predictive of liver injury in COVID-19, which may prove useful for management of liver injury. Late onset of sLI and more aggressive care are suggestive of treatment-related hepatotoxicity.

Keywords SARS-CoV-2 · Liver failure · Liver dysfunction · Acute kidney injury · Aminotransferases · Aspartate aminotransferase · Multi-organ failure · Cytokine storm

Introduction

Coronavirus disease 2019 (COVID-19) [1, 2] caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved into a global pandemic with devastating morbidity and mortality. The widespread outbreaks and the likelihood of resurgences have strained and will likely continue to strain healthcare resources for extended period. SARS-CoV-2 infection affects many vital organs and, in severe cases, could result in multiple organ failure. Among many organs affected, in-hospital acquired liver injury has been reported to be associated with worse COVID-19 outcomes [3–10]. The effects of COVID-19 on the liver remain incompletely understood [3–10]. The liver could be damaged by direct viral infection, indirect host-immune responses (i.e., thrombosis, cytotropic and cytokine-mediated immune responses, amongst others) and/or hepatotoxicity from COVID-19 therapies that include antiviral, antibacterial, and immuno-modulatory drugs, amongst others [11–14].

The temporal progression of in-hospital liver injury and its relations with clinical variables and clinical outcomes are unknown. To our knowledge, there has been no systematic evaluation of the temporal profiles of clinical variables leading up liver injury in COVID-19, and how these temporal
profiles can predict liver injury and thus inform clinical decision making. Improved understanding of in-hospital liver injury associated with COVID-19 could lead to better patient management that include more frequent monitoring of liver function tests, particularly in patients treated with Remdesivir® and patients with advanced liver disease [14].

The goal of this study was to investigate the temporal progression of clinical variables associated with in-hospital liver injury in COVID-19 patients. We analyzed the temporal profiles of different clinical variables with time-lock to liver injury onset defined by alanine aminotransferase level. We grouped patients into graded (no, mild and severe) liver injury. The effects of treatments (such as invasive mechanical ventilation, anticoagulants, and steroids) were also analyzed with respect to graded liver injury.

**Methods**

**Study population and data collection**

This retrospective single-center study was approved by the Stony Brook Institutional Research Board with an exemption for informed consent and a HIPAA waiver. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines for cohort studies [15], except sample size and sensitivity analyses were not calculated because no a priori data exist for longitudinal liver injury in COVID-19.

Our COVID-19 Persons Under Investigation Registry consisted of 6678 patients clinically suspected of COVID-19 infection from February 7, 2020 to June 30, 2020 [16–20], of which 2892 were tested positive using real-time polymerase chain reaction test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on a nasopharyngeal swab specimen. Exclusion criteria included patients who were not hospitalized, without alanine aminotransferase (ALT) and aspartate aminotransferase (AST) data, less than 18 years old, and still in the hospital. There were 1361 hospitalized COVID-19 patients meeting the inclusion/exclusion criteria.

Patients were then divided into three groups based on liver injury (LI) defined by ALT level [21]: (1) no liver injury (nLI) with ALT < 1 ULN [< 40 U/L] and AST < 1 ULN [< 35 U/L], (2) mild liver injury (mLI) with ALT = 1–5 ULN and AST = 1–3 ULN, and (3) severe liver injury (sLI) with ALT ≥ 5 ULN and AST ≥ 3 ULN. In the general floor cohort, there were 605 nLI, 389 mLI, and 52 sLI patients. In the ICU cohort, there were 95 nLI, 147 mLI, and 315 sLI patients.

Although AST, ALT, ALP and total bilirubin are commonly used tests to assess liver injury and are associated with increased mortality risk in COVID-19 [22], we chose to time lock to ALT because ALT is more liver specific than the other liver function biomarkers [23–25] (see Discussion).

Demographics and chronic comorbidities, longitudinal vital signs, laboratory blood tests, and blood gases were extracted from electronic medical records. Demographic data included age, gender, ethnicity, and race. Chronic comorbidities included smoking, diabetes, hypertension, asthma, chronic obstructive pulmonary disease (COPD), coronary artery disease, heart failure, cancer, immunosuppression, and chronic kidney disease. Longitudinal laboratory tests included creatinine (Cr), procalcitonin (PROCAL), aspartate transaminase (AST), alanine aminotransferase (ALT), ferritin (FERR), lactate dehydrogenase (LDH), white blood cell count (WBC), C-reactive protein (CRP), lymphocytes count (LYMPH), D-dimer (DDIM), brain natriuretic peptide (BNP), alkaline phosphatase (ALP), total bilirubin, prothrombin time, total protein, and albumin. Longitudinal vital signs and blood gas variables included heart rate (HR), respiratory rate (RR), pulse oxygen saturation (SpO2), temperature (TEMP), and pH. FiO2 was administered and well documented in ICU patients but not usually so in general floor patients, and thus FiO2 was not tabulated.

Laboratory test variables were plotted across time with time lock (t = 0) to LI onset (defined by elevated ALT), along with data 3 days before and 3 days after LI onset. For comparison, time series data for no LI patients were time locked (t = 0) to 3 days after ED admission, along with data 3 days before and 3 days after that time point. For ease of comparison, individual laboratory test values were normalized to the no LI group at t = 0 for individual patients.

**Predictive models**

Logistic regression models were used to rank the importance of clinical variables leading to liver injury at each day prior to onset. Ranking was performed using all clinical variables except BNP, ALP, total bilirubin, total protein, and prothrombin time due to significant missing data at multiple time points. Prediction performance was evaluated for individual top variables and the combined top variables in predicting both sLI and mLI as well as sLI only at different days prior to onset. For the combined prediction models, AUC calculation excluded AST due to its strong correlation with ALT. Prediction performance was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve.

**Statistical analysis**

All statistical analyses were performed using Python packages (Sklearn and Statsmodels) and Statistical Analysis System (SAS) software (Cary, NC, USA). Frequencies and percentages for categorical variables between nLI and mild
LI and sLI using χ² or Fisher’s exact tests. Age, expressed as median (IQR), was compared between groups using ANOVA and post hoc pairwise t-test. Mortality rates were compared between groups with logistic regression adjusted with covariates (age, gender, ethnicity, and significant comorbidities). Differences between nLI, mLI, and sLI, for clinical variables in time series graphs were analyzed via linear mixed models and least-squares means. p-values < 0.05 were considered statistically significant and corrected using the Bonferroni method for appropriate, multiple tests.

Results

Figure 1 shows the patient selection flowchart. Of the 1361 hospitalized COVID-19 patients who met the inclusion and exclusion criteria, (1) 1046 patients were admitted to general floor and never transferred to ICU, (2) 214 patients upgraded to ICU from general floor, and (3) 101 patients directly admitted to ICU from emergency room. In the never-upgrade general floor group (n = 1046), 57.8% had nLI, 37.2% had mLI, and 5.0% had sLI. In the direct ICU plus, the upgraded ICU group (n = 315), 30.1% had nLI, 46.7% had mLI, and 23.2% had sLI.

Table 1A summarizes patient demographics, comorbidities and select treatments categorized by nLI, mLI, and sLI. Of all hospitalized COVID-19 patients, 51.43% of patients had nLI, 39.38% had mLI, and 9.18% had sLI. nLI patients were older than mLI patients (p < 0.05), followed by sLI patients (p < 0.05). Sex (p < 0.05) and ethnicity (p < 0.05) were significantly different amongst groups. Race was significantly different between nLI and mLI (p < 0.05) and between nLI and sLI (p < 0.05). Smoking, diabetes, hypertension, COPD, coronary artery disease, cancer, chronic kidney disease (p < 0.05), but not asthma and immunosuppression (p > 0.05), were significantly different between nLI and mLI. Smoking, coronary artery disease, heart failure and cancer (p < 0.05), but not diabetes, hypertension, asthma, COPD immunosuppression and chronic kidney disease (p > 0.05), were significantly different between nLI and sLI. There was no significant difference in comorbidities between mLI and sLI patients. Overall, nLI patients generally had more comorbidities than mLI and sLI patients. By comparison, in the ICU group, 30.1% of patients had nLI, 46.7% had mLI, and 23.2% had sLI.

Of all hospitalized COVID-19 patients, the unadjusted mortality rates for nLI, mLI, and sLI were, respectively, 14.3%, 12.3% and 36.0%. The adjusted mortality OR for mLI was 1.16 [95% CI 0.81, 1.68] (p = 0.415), not significantly different from nLI. The adjusted mortality OR for sLI was 1.37 [95% CI 1.10, 1.70] (p = 0.005), significantly different from nLI. Adjustment was made for age, gender, hypertension, and diabetes.

sLI patients received markedly more invasive and non-invasive mechanical ventilation, prophylactic and therapeutic anticoagulants, steroids, and dialysis than both mLI and nLI patients (Table 1B, p < 0.05). mLI patients received significantly more invasive mechanical ventilation, prophylactic anticoagulants, and steroids than nLI patients (p < 0.05).

The histograms of the days of hospitalization before patients develop liver injury are plotted in Fig. 2. mLI developed an average of 2.6 days after hospital admission and 43.9% of the mLI patients had liver injury on the day of hospital admission. sLI developed an average of 9.4 days after hospital admission, and 16.0% of patients had sLI on the day of admission.

Figure 3 depicts the time series of laboratory tests relative to liver injury onset for nLI, mLI, and sLI cohorts. For ease of comparison, these laboratory values were normalized to the nLI group at t = 0 by individual patients. The ALT of nLI group remained low and time invariant, whereas ALT of the mLI group was 3 × and the ALT of sLI group was 15 × that of nLI at t = 0. An apparent observation is that the time courses of sLI were markedly different from those of mLI and nLI, whereas those of mLI and nLI were similar.

For sLI, AST, ALP, and total bilirubin (liver enzymes) showed similar time courses. Procalcitonin, ferritin, LDH also showed similar time courses as ALT. Bilirubin, procalcitonin, and ferritin remained elevated or further increased days after sLI onset.

For the sLI, WBC, D-dimer, CRP, HR, RR were elevated throughout, whereas lymphocyte, SpO2 and prothrombin time were lower throughout. Cr, total protein and albumin appeared to peak earlier relative ALT. BNP, temperature and pH were mostly similar amongst groups, although there were some differences at some time points. A drop in pH was seen on the day of the sLI onset.
**Table 1** (A) Demographic characteristics, comorbidities and (B) treatments, ICU admission, length of stay (LOS), and unadjusted mortality rate of no liver injury (nLI), mild liver injury (mLI) and severe liver injury (sLI) patients

| | nLI (n = 700) | mLI (n = 536) | sLI (n = 125) | p value |
|---|---|---|---|---|
| (A) Demographics | | | | |
| Age, mean (sd) | 66.9 (17.2)<sup>a</sup> | 58.4 (16.0)<sup>b</sup> | 56.7 (17.5)<sup>b</sup> | <0.001 |
| Sex | | | | |
| Male | 332 (47.4%)<sup>a</sup> | 356 (66.4%)<sup>b</sup> | 96 (76.8%)<sup>b</sup> | <0.001 |
| Female | 368 (52.6%) | 180 (33.6%) | 29 (23.2%) | |
| Ethnicity | | | | |
| Hispanic/Latino | 21.0%<sup>a</sup> | 32.6%<sup>b</sup> | 21.6%<sup>b</sup> | <0.001 |
| Non-Hispanic/Latino | 69.0% | 51.9% | 56.8% | |
| Unknown | 10.0% | 15.5% | 21.6% | |
| Race | <0.001 | | |
| Caucasian | 62.2% | 45.2% | 46.4% | |
| African American | 8.9% | 5.8% | 4.0% | |
| Asian | 2.1% | 5.0% | 5.6% | |
| Unknown | 26.1% | 42.7% | 43.2% | |
| Comorbidities | | | | |
| Smoking history | 0.171 | | | |
| Current smoker | 5.0% | 3.9% | 4.8% | |
| Former smoker | 24.6% | 18.7% | 19.2% | |
| Never smoked | 63.3% | 72.2% | 71.2% | |
| Unknown | 7.1% | 5.2% | 4.8% | |
| Diabetes | 31.0%<sup>a</sup> | 23.0%<sup>b</sup> | 24.0%<sup>b</sup> | 0.005 |
| Hypertension | 54.3%<sup>a</sup> | 45.2%<sup>b</sup> | 49.6%<sup>b</sup> | 0.005 |
| Asthma | 5.3% | 7.3% | 7.2% | 0.376 |
| COPD | 11.1%<sup>a</sup> | 6.4%<sup>b</sup> | 8.0%<sup>ab</sup> | 0.009 |
| CAD | 18.0%<sup>a</sup> | 13.6%<sup>a</sup> | 9.6%<sup>a</sup> | 0.015 |
| Heart failure | 12.6%<sup>a</sup> | 5.1%<sup>b</sup> | 4.0%<sup>b</sup> | <0.001 |
| Cancer | 13.3%<sup>a</sup> | 6.7%<sup>b</sup> | 5.6%<sup>ab</sup> | <0.001 |
| Immunosuppression | 10.1%<sup>a</sup> | 5.8%<sup>b</sup> | 5.6%<sup>ab</sup> | 0.005 |
| CKD | 13.7%<sup>a</sup> | 6.4%<sup>b</sup> | 10.4%<sup>ab</sup> | <0.001 |
| # of comorbidities per patient | | | | |
| 0 | 26.3% | 38.3% | 37.6% | 0.155 |
| 1 | 24.3% | 25.9% | 29.6% | |
| 2 | 22.6% | 21.3% | 17.6% | |
| 3 | 14.1% | 10.0% | 7.2% | |
| 4 | 7.6% | 3.7% | 4.0% | |
| 5 | 3.7% | 0.4% | 3.2% | |
| 6 | 1.3% | 0.4% | 0.8% | |
| 7 | 0.1% | 0 | 0 | |
| (B) Treatments | | | | |
| IMV | 8.1%<sup>a</sup> | 22.3%<sup>b</sup> | 52.0%<sup>c</sup> | <0.001 |
| Noninvasive MV | 6.9%<sup>a</sup> | 9.9%<sup>b</sup> | 22.4%<sup>c</sup> | <0.001 |
| Anticoagulants | | | | |
| Prophylactic | 35.6%<sup>a</sup> | 44.0%<sup>b</sup> | 58.4%<sup>c</sup> | <0.001 |
| Therapeutic | 16.3%<sup>a</sup> | 15.3%<sup>a</sup> | 33.6%<sup>b</sup> | <0.001 |
| Steroids | 13.3%<sup>a</sup> | 26.1%<sup>b</sup> | 52.0%<sup>c</sup> | <0.001 |
| Dialysis | 5.4%<sup>a</sup> | 4.7%<sup>b</sup> | 16.0%<sup>c</sup> | <0.001 |
| % in ICU admission | 13.57%<sup>a</sup> | 27.43%<sup>b</sup> | 58.40%<sup>c</sup> | <0.001 |
| Length of stay | 7.9 (7.8)<sup>a</sup> | 12.8 (13.6)<sup>b</sup> | 20.2 (16.6)<sup>f</sup> | <0.001 |
| Mortality (unadjusted) | 14.3%<sup>a</sup> | 12.3%<sup>c</sup> | 36.0%<sup>c</sup> | <0.001 |

Group comparison of categorical variables in frequencies and percentages used χ² test or Fisher exact tests. Group comparison of age in mean and standard deviations used the ANOVA. p values are based on overall Chi-square tests. a b c denote significant groups based on pairwise tests with Bonferroni correction for p values.

COPD chronic obstructive pulmonary disease, CAD coronary artery disease, CKD chronic kidney disease
Table 2 shows the prediction performance (AUCs) of individual top variables and the combined top variables for both sLI and mLI as well as sLI only at different days prior to onset. AUCs were high on the day of onset and decreased −2 and −3 days before. For prediction of both sLI and mLI, the top predictors were ALT, AST, LDH, lymphocyte, and RR; the AUCs of the predictive model combining these predictors were 0.81, 0.79, 0.78 and 0.68 at 0, −1, −2 and −3 days prior, respectively. For prediction of sLI only, the top predictors were ALT, AST, LDH, RR, ferritin, and lymphocyte; the AUCs of the predictive model combining these predictors were 0.98, 0.92, 0.88 and 0.84 at 0, −1, −2 and −3 days prior, respectively. Prediction of sLI was overall better than that of sLI and mLI.

Discussion

This study investigated the temporal progression of clinical variables associated with graded hospital-acquired liver injury in COVID-19 patients. The major findings are: (1) sLI COVID-19 patients have higher prevalence of younger age, more male sex, invasion ventilation, anticoagulants, steroids and dialysis, (2) sLI, but not mLI, has significantly higher adjusted mortality than nLI, (3) COVID-19 patients develop sLI 9.4 days after hospitalization on an average, (4) the clinical variables time courses of the sLI group are markedly different from those of the nLI and mLI group, (5) for the sLI, ALT and a few liver panel laboratory variables, as well as procalcitonin, ferritin, and lactate dehydrogenase show similar temporal trajectories, whereas white-blood cell count, D-dimer, C-reactive protein, heart rate, respiration rate are elevated from the onset, and lymphocyte, SpO2 and prothrombin time are lower from the onset, and (6) the top predictors of sLI were ALT, AST, LDH, RR, ferritin and lymphocyte, and the AUCs of the predictive model combining these predictors were 0.98, 0.92, 0.88 and 0.84 at 0, −1, −2 and −3 days prior to liver injury onset, respectively.

Liver injury confers a high mortality rate

The sLI group received more invasive mechanical ventilation, anticoagulant, steroid, and dialysis, more likely to be admitted to ICU, stayed longer in the hospital, and had a higher adjusted mortality odds ratio, despite being younger and having fewer comorbidities, suggesting that sLI is an independent risk factor of worse COVID-19 outcomes. Mortality ORs varied and have been reported to be 1.48 [95% CI 1.12–1.96] for elevated ALT [4], 1.03 [95% CI 1.01–1.05] for elevated AST (another marker liver injury) [26], and 3.56 for ALT > 120 U/L [22]. These different findings are not unexpected and may depend on the timing of COVID-19 pandemic, patient cohorts, patient management by different hospitals, and definitions of liver injury, amongst others.

Clinical variables time courses offer novel insight into COVID-19-related liver injury

In contrast to previous studies [3–6] which typically assessed clinical variables at admission, our study analyzed graded liver injury and their temporal dynamics with respect to other clinical variables. Another novelty of this study is that we time-locked these longitudinal clinical variables to liver injury onset. If only clinical variables at hospital admission were used across patients without time-lock, most of these clinically significant effects observed in our study would likely be averaged out. A key observation is that the sLI time courses of many clinical variables were markedly different from those of mLI and nLI patients, indicating that sLI is associated with more severe disease. For example, LDH, procalcitonin, and ferritin were significantly higher at
the time of sLI onset as compared to the onset of mLI and nLI, and these elevations suggest more severe systemic inflammation in sLI. Similarly, lymphocyte count was significantly lower in sLI compared to mLI and nLI, indicative of greater immunological stress in sLI [27]. It is not surprising that the time courses of AST, ALP, and total bilirubin were similar to that of ALT given that AST, ALP, and total bilirubin can reflect both hepatocellular and cholestatic injury [23, 24]. The indicators that change early on and gradually worsen can serve as early warning signs of liver injury because they allow physicians to intervene and, thus, should be closely monitored in COVID-19 patients.

Although AST, ALT, ALP, and total bilirubin are commonly measured to assess liver functions and their elevations have been associated with increased mortality risk in COVID-19 [22], we chose to time lock to ALT because ALT is more liver specific than AST [23–25]. AST may also be elevated by acute injury to the heart, kidney, and brain, which are also affected by COVID-19 [24, 25]. We did not time lock to bilirubin or ALP because elevations in bilirubin can also indicate extrahepatic disorders and ALP can also be elevated by kidney injury [24, 25].

### Potential mechanisms of liver injury

The mechanisms of liver injury associated with COVID-19 are incompletely understood. The liver could be damaged by direct viral infection, indirect host-immune responses, and treatment drug toxicity [11–13]. Direct SARS-CoV-2 infection is mediated by the angiotensin-converting enzyme 2 (ACE2) receptor [28]. The observed expression of ACE2 is far greater in cholangiocytes of the bile duct epithelium than in hepatocytes of the liver parenchyma, suggesting that direct cytopathic target for SARS-CoV-2 is cholangiocytes [29]. We found that ALP, a measure of bile duct injury, is not as severely elevated as AST and ALT during the time course of both mild and severe LI. Thus, we cannot yet conclude that COVID-19 liver injury is driven by direct tropism of cholangiocytes. Hypoxia, a relatively common complication of COVID-19, is also known to regulate hepatocyte ACE2 expression [12, 30]. Our results show that both mLI and sLI patients display elevation in respiratory rate as compared to nLI patients, suggesting that persistent hypoxic conditions might be upregulating ACE2 expression in hepatocytes to contribute to direct liver injury over the course of hospitalization. Secondary damage to the liver, such as by cytokine storm-driven inflammation and the resultant distributive shock, is another plausible mechanism of LI [11, 12]. Procalcitonin, ferritin, and D-dimer were elevated before the threshold for severe LI is met. Both procalcitonin and ferritin are markers of inflammation, LDH is a marker of cell death across different organs, and D-dimer is an indicator of blood clots associated with early mortality in COVID-19 [27]. The increasing elevation of these markers prior to the onset of sLI is suggestive of inflammation and vasculopathy at the systemic level which may cause organ injury [31]. Note that procalcitonin, ferritin, and LDH are not only associated with systemic inflammation, but are also associated with bacterial infections, and therefore their observed elevations may be the result of bacterial co-infection or secondary infection in COVID-19 [32]. In particular, procalcitonin is known to be highly correlated with bacterial infection [33]. However, concomitant bacterial infection has been shown to be relatively infrequent in COVID-19, occurring in an estimated 8.1% of critically ill patients [34]. In this study, we did not collect data on specific COVID-19-related infections and further studies
are needed to evaluate different COVID-19-related infections and treatments.

Taken together, given the relative late onset of sLI, high rate of IMV, anticoagulant and steroid use in sLI, there was likely hepatotoxicity from COVID-19 therapies that include antiviral, antibacterial, steroids, anticoagulants, and immuno-modulatory drugs, amongst others. In particular, the disproportional host-immune responses (including inflammation) are known to cause liver injury in COVID-19 patients [11–14, 35]. Alternative interpretations are possible and prospective studies are warranted.

**Predictive model using a few clinical variables accurately predicts liver injury a few days prior**

The top predictors of both sLI and mLl versus sLI only were essentially identical and they were ALT, AST, LDH, RR, ferritin and lymphocyte, which is encouraging. Prediction performance of sLI was overall better than that of both sLI and mLl, as expected, because the clinical variables of mLl were similar to those of nLI, but both were different from those of sLI. We were able to construct a prediction model using these longitudinal clinical variables to predict liver injury. The predictive model using these combined predictors yielded excellent prediction performance with a 0.84–0.98 AUC at 0–3 days prior to sLI. This is the first time that temporal progression of clinical variables is used to predict COVID-19-related liver injury at different days prior to onset. Additional testing of this predictive model using independent dataset from different hospitals is warranted.

**Comparison with AKI suggests that pathogenesis of liver injury and AKI is likely different**

It is informative to compare in-hospital liver injury with in-hospital AKI in the same cohorts of COVID-19 patients [18], as AKI is also known to be associated with higher mortality rate. Overall, sLI prevalence was 9.18% and mLl prevalence was 39.38%, compared to AKI prevalence of 29.9%. In patients with ICU care, sLI prevalence was 23.2% and mLl prevalence was 46.7% had mLl, compared to AKI prevalence of 37.3%. sLI had a higher IMV rate than AKI (52% vs 35.3%), higher steroid use (52.0% vs 36.2%), and higher dialysis rate (16%, vs 1.7–8.5%) but similar anticoagulant use (58.4% vs 53.9% for prophylactic, and 33.6% vs 28.7% for therapeutic AC). Adjusted odds ratio of sLI (OR = 1.37 [95% CI 1.10, 1.70] p = 0.005) was lower than that of AKI (OR = 4.67 [95% CI 3.1, 7.0], p < 0.001 vs non-AKI). sLI onset was 9.4 days whereas the AKI onset was 3.3 days. Of note, 69.6% of patients had both sLI and AKI, 49.8% of patients had both mLl and AKI, and 35.6% nLI patients had no AKI. These comparisons suggest that the pathogenesis of liver injury and AKI is likely different, although there could be some common elements.

**Limitations**

There are several limitations in this study. First, although the majority of patient records did not indicate pre COVID-19 liver disease, undiagnosed prior history of liver injury or disease might exist. COVID-19 could make pre-existing liver disease worse. Second, this is retrospective study from a single academic hospital. These findings need to be replicated in a large retrospective cohort from other institutions as well as in form of prospective studies. Third, our study was not adequately powered to address potential ethnicity or racial differences because the majority of our cohorts were Caucasian or unknown race. Finally, it is unclear whether such liver injury is transient or persistent, although it would not be surprising that some COVID-19 survivors will be more susceptible to COVID-19-related liver complications in the future.

**Conclusions**

This study characterized the temporal progression of readily available clinical and laboratory variables associated with in-hospital liver injury in COVID-19 patients, providing important insights in graded liver injury pathogenesis. Severe, but not mild, liver injury confers a higher risk of mortality and is an independent risk factor of worse COVID-19 outcomes. The relatively late onset of severe liver injury and more aggressive treatments suggest hepatotoxicity may in part be due to drugs used to treat COVID-19. The earliest clinical predictors of liver injury are identified, and they accurately predict severe liver injury a few days prior to onset. This approach may prove useful for better management of COVID-19 patients with liver injury.

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**Author contributions** JL: collected, curated, analyzed and edited paper; HA: curated, drafted and edited paper; SF: edited and revised paper; WH: analyzed and edited paper; TD: designed, supervised, wrote paper, and approved final version.

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** Authors declare no conflicts of interest.

**Ethical approval and consent to participate** This retrospective single-center study was approved by the Institutional Research Board of Stony brook University (IRB 2020-00207) with an exemption for informed consent and a HIPAA waiver.
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