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ORIGINAL RESEARCH

Diabetes Mellitus May Exacerbate Liver Injury in Patients with COVID-19: A Single-Center, Observational, Retrospective Study

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ABSTRACT

Introduction: The spread of coronavirus disease 2019 (COVID-19) is having a profound effect on global health. In this study, we investigated early predictors of severe prognosis from the perspective of liver injury and risk factors for severe liver injury in patients with COVID-19.
Methods: We examined prognostic markers and risk factors for severe liver injury by analyzing clinical data measured throughout the course of the illness and the disease severity of 273 patients hospitalized for COVID-19. We assessed liver injury on the basis of aminotransferase concentrations and fibrosis-4 (FIB-4) index on admission, peak aminotransferase concentration during hospitalization, aminotransferase peak-to-average ratio, and albumin and total bilirubin concentrations. Furthermore, we analyzed age, aspartate aminotransferase (AST) concentrations, FIB-4 index on admission, hypertension, diabetes mellitus (DM), dyslipidemia, cerebral infarction, myocardial infarction, and body mass index as mortality risk factors.
Results: We identified advanced age as a risk factor. Among biochemical variables, AST concentration and FIB-4 index on admission were associated with high mortality. AST on admission and peak AST during hospitalization were significantly higher in the non-surviving (n = 45) than the discharged group (n = 228). Multivariable Cox hazards analyses for mortality showed significant hazard ratios for age, peak AST, and FIB-4 index on admission (p = 0.0001 and 0.0108, respectively), but not in a model including AST and FIB-4 index on admission. Furthermore, the AST peak was significantly higher among non-surviving patients with DM than in those without DM.
Conclusions: We found that advanced age, high AST, and FIB-4 index on admission and a higher peak AST during hospitalization are risk factors for poor COVID-19 prognosis. Furthermore, DM was a risk factor for exacerbation of liver injury among non-surviving patients. The AST concentration and FIB-4 index should be assessed periodically throughout hospitalization, especially in

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patients with high AST values on admission and those with DM.

Keywords: Aminotransferase; COVID-19; Diabetes mellitus; Fibrosis-4; Prognostic marker; SARS-CoV-2

Key Summary Points

Liver injury is associated with disease severity and mortality in patients with COVID-19; therefore, investigating early predictors of severe prognosis and risk factors for severe liver injury are essential.

Higher aspartate aminotransferase (AST) and FIB-4 index on admission and higher peak AST during hospitalization were found to be risk factors for poor COVID-19 prognosis.

Diabetes mellitus, one of the most common comorbidities in patients hospitalized for COVID-19, was significantly associated with severe liver injury in COVID-19 patients with a poor prognosis.

AST concentrations should be assessed periodically throughout hospitalization, especially in patients with diabetes mellitus hospitalized for COVID-19.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19) pandemic has created an ongoing global health crisis. There is published evidence that systemic inflammation and aberrant cytokine regulation are hallmarks of severe COVID-19 [1], and that SARS-CoV-2 infection damages both the hepatobiliary and respiratory systems in 15–65% of patients with COVID-19 [2, 3].

Several meta-analyses and studies have found that liver injury is associated with disease severity and mortality [4–9]. Furthermore, several studies have suggested that diabetes mellitus (DM) is linked to worse outcomes in patients with COVID-19 [10, 11]. However, few studies have investigated using biochemical prognostic markers of liver injury [12] to study the association between the severity of liver injury and DM in such patients. Here, we aimed to investigate early predictors of poor prognosis associated with liver injury and risk factors for severe liver injury in patients with COVID-19 by analyzing such patients' clinical data, particularly trends in liver biochemical and inflammatory-related markers, and their association with disease severity during hospitalization.

METHODS

Study Design and Cohort

This single-center, observational, retrospective study was conducted in Nozaki Tokushukai Hospital. The inclusion criteria were positivity for SARS-CoV-2 on a quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay of a nasopharyngeal swab sample in Nozaki Tokushukai Hospital between March 23, 2020 and December 31, 2020. We excluded individuals with asymptomatic or minor illness not requiring inpatient treatment (n = 443) or pre-existing liver diseases such as alcoholic hepatitis or cirrhosis, hepatitis C or B virus infection, or hepatic tumors (n = 12), to clarify the associations between liver injury and poor COVID-19 prognosis. We defined alcoholic liver disease as a habitual drinker for more than 5 years and daily alcohol consumption of more than 60 g/day in men and 40 g/day in women. Finally, 273 patients were included in this study (Supplementary Fig. 1).

Data Collection

The Research Ethics Committee of the Tokushukai Medical Group approved this study (Approval no. TGE01425-002). Written informed consent for RT-qPCR testing for SARS-CoV-2 was obtained from all included patients. The research was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments.

We collected the following basic patient data: age, sex, height, weight, and body mass

index (BMI). Liver injury was defined as high serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT), decreased serum albumin and total bilirubin, and fibrosis-4 (FIB-4) index. We also collected white blood cell counts, C-reactive protein, D-dimer, ferritin, and sialylated carbohydrate antigen data and information on the comorbidities of hypertension (HT), diabetes mellitus (DM), dyslipidemia (DL), cerebral infarction (CI), and myocardial infarction (MI).

We defined disease severity on admission and during hospitalization according to the following criteria of the Ministry of Health, Labour and Welfare: mild, no respiratory symptoms and no requirement for supplemental oxygen $SpO_2 \geq 96\%$ (stage 1); moderate, dyspnea or respiratory symptoms without respiratory insufficiency $93\% < SpO_2 < 96\%$ or $SpO_2 \leq 93\%$ or requirement for supplemental oxygen (stage 2); and severe, requirement for intensive care unit (ICU) entry and invasive mechanical ventilation or death (stage 3). We classified the patients into two groups according to outcomes, a discharged group ($n = 228$) and a non-surviving group ($n = 45$), and analyzed their clinical data, liver biochemistry markers, inflammatory markers, and comorbidities. We also assembled the following four groups according to AST peaks and AST peak/average ratio (0–3) and analyzed the association between AST findings and prognosis based on AST peaks and AST peak/average ratios: Group 0, ≤ 21 IU/l, < 1.05, respectively; Group 1, > 21 IU/l ≤ 30 IU/l, $1.05 \leq$ and < 1.24, respectively; Group 2, > 30 IU/l ≤ 54 IU/l, $1.24 \leq$ and < 1.53, respectively; and Group 3, > 54 IU/l, $1.53 \leq$, respectively.

Treatment During Hospitalization

All patients received supportive care in accordance with their clinical symptoms. Blood pressure, pulse rate, and oxygen saturation were monitored more than three times per day and supplemental oxygen given to hypoxemic patients. We started treatment as soon as possible after admission, selecting medication according to the status of each patient from among the following: dexamethasone, methylprednisolone pulse, aspirin, favipiravir, remdesivir, ribavirin, tocilizumab, nafamostat mesylate, ciclesonide, and high-dose ascorbic acid. Antibiotics were administered if considered needed. To determine positivity or negativity for SARS-CoV-2, RT-qPCR assays were performed on nasopharyngeal swab samples several times during hospitalization, beginning at least 5 days after admission.

Statistical Analysis

Statistical analysis was performed with JMP Pro 14 (SAS Institute, Cary, NC, USA). Differences in means and proportions between the discharged and non-surviving groups were examined using Student's *t* test and Fisher's exact test, respectively. Kaplan–Meier curves and log–rank tests were used to compare times to specified events according to AST peak concentrations. A Cox proportional hazards regression model analysis was used to evaluate hazard ratios for differences in mortality. As potential risk factors, age, sex, BMI, hypertension, diabetes, dyslipidemia, history of cerebral infarction, and history of myocardial infarction were included. In model 1, AST at admission was included as a risk factor. In model 2, peak value of AST was included. In model 3, peak value of AST and FIB-4 index at admission were included. A p value less than 0.05 was considered to denote statistical significance.

RESULTS

Patient Baseline Characteristics and Laboratory Results (Table 1)

In the non-surviving group, 31.3% ($n = 14$) of patients were categorized as having mild disease, 42.4% ($n = 19$) moderate, and 26.7% ($n = 12$) severe on admission. As shown in Table 1, the time from symptom onset or PCR confirmation to discharge or death and the duration of hospitalization were significantly longer in the non-surviving group ($p = 0.001$, $p = 0.031$, respectively). The average age was

Table 1 Clinical data in the discharge and non-surviving groups

Outcomes	Discharge		Non-surviving		*p*
	Mean or *N* (%)	(SD)	Mean or *N* (%)	(SD)	
n	228		45		
Severity score at admission (Stage 1/2/3 [%])	121/105/2 (53.1/46/0.9)		14/19/12 (31.1/42.2/26.7)		< 0.0001
Times from onset or PCR-confirmation to discharge or death period (days)	18.1	(9.6)	23.7		0.001
Hospitalization period (days)	14.9	(9.1)	18.3		0.031
Time to admission (days)	4.7	(4.9)	5.4		0.470
Age (years)	56.9	(22.5)	81.6		< 0.0001
Sex (female/male [%])	99/129 (43.4/56.6)		20/25 (44.4/55.6)		1
Height (cm)	162.9	(10.0)	156		0.0002
Weight (kg)	62.4	(15.9)	54.8		0.0103
BMI	23.3	(4.4)	22.2	(3.6)	0.200
AST on admission (IU/l)	33.7	(26.2)	44.0	(30.0)	0.021
ALT on admission (IU/l)	32.1	(32.4)	31.4	(29.0)	0.890
GGT on admission (IU/l)	57.1	(72.3)	45.4	(39.6)	0.310
ALB on admission (mg/dl)	3.6	(0.4)	3.2	(0.7)	0.130
T-Bil on admission (mg/dl)	0.7	(0.3)	0.7	(0.3)	0.950
Fibrosis-4 index on admission	2.0	(1.8)	4.4	(2.6)	< 0.0001
WBC on admission (/ul)	6137	(1649)	6830	(3285)	0.610
CRP on admission (mg/dl)	9.7	(8.4)	7.8	(8.1)	0.610
D-dimer on admission (mg/ml)	0.9	(0.4)	3.2	(3.4)	0.140
Ferritin on admission (ng/ml)	733	(505)	460	(451)	0.220
KL-6 on admission (U/ml)	355	(137)	488	(421)	0.280
Hypertension (no/yes [%])	189/39 (82.9/17.1)		31/14 (68.9/31.1)		0.039
Diabetes mellitus (no/yes [%])	183/45 (80.3/19.7)		33/12 (73.3/26.7)		0.320
Dyslipidemia (no/yes [%])	211/17 (92.5/7.5)		43/2 (95.6/4.4)		0.750
Cerebral infarction (no/yes [%])	217/11 (95.2/4.8)		37/8 (82.2/17.8)		0.005
Myocardial infarction (no/yes [%])	226/2 (99.1/0.9)		43/2 (95.6/4.4)		0.130
Date of AST peak (days)	3.9	(5.4)	11.2	(10.3)	< 0.0001
AST peak (IU/l)	40.8	(34.5)	133.1	(264.7)	< 0.0001
AST average (IU/l)	28.1	(13.7)	55.8	(59.0)	< 0.0001

Table 1 continued

Outcomes	Discharge		Non-surviving		*p*
	Mean or *N* (%)	(SD)	Mean or *N* (%)	(SD)	
AST peak average ratio	1.3	(0.5)	1.7	(1.0)	< 0.0001

For continuous variables, mean and standard deviation are described and for categorical variables, number and percentage of each strata is described

Differences in means and proportions between the discharge and death groups were examined using Student's *t* test and Fisher's exact test, respectively

significantly higher in the non-surviving (81.6 years) than in the discharged group (56.9 years) ($p < 0.0001$). There was no difference in prognosis between men and women (Table 1). Heights and weights were both significantly lower in the non-surviving group; however, there was no significant difference between the groups in BMI.

Among the data on admission, only the AST concentration and FIB-4 index were significantly higher in the non-surviving group (Table 1, $p = 0.021$ and $p < 0.0001$, respectively). Besides, FIB-4 index on admission was higher in patients with DM than in those without DM (3.1 ± 2.2, 2.2 ± 2.1, $p = 0.0076$). However, there were no significant differences in the other assessed liver biochemical and inflammation-related variables between the two outcome groups (Table 1). Regarding prognosis, the morbidity of patients with HT or CI was significantly higher in the non-surviving than the discharged group ($p = 0.039$ and $p = 0.005$, respectively), but not in those with DM (Table 1). Because ALT and GGT are related to AST, they were not examined to minimize the number of tests. To further analyze the association between COVID-19 prognosis and AST concentration over the course of the illness, we analyzed the timing and size of AST peaks, average AST, and AST peak/average ratios. The AST peaks occurred significantly later, and AST peaks, average AST concentrations, and AST peak/average ratios were significantly higher in the non-surviving than in the discharged group (Table 1). We also examined whether AST was a more sensitive and reliable marker than the other assessed liver biochemical variables and inflammation markers throughout the course of illness (Supplementary Table 1). The other variables were checked less frequently than was AST, especially in the discharged group. In both groups, the peaks of all other variables occurred later than those of AST. These results suggest that higher peak and longer duration of high AST concentrations are risk factors for COVID-19 mortality.

AST Concentrations and Mortality in the Discharged and Non-Surviving Groups (Fig. 1)

We analyzed AST concentrations and prognoses in the discharged and non-surviving groups throughout, and for up to 2 months after, hospitalization to clarify the association between mortality and AST concentration (Fig. 1). In the non-surviving group, AST concentrations were continuously higher than those in the discharged group until 21 days after admission. Additionally, most deaths (30/45) occurred within 22 days after admission. Over the subsequent 14 days, we found similar AST concentrations in the two outcome groups. However, around 1 month after hospitalization, an additional three patients died shortly after steep increases in their AST concentrations (Fig. 1).

Variation in AST Over Time During Hospitalization (Fig. 2)

Timing of AST Peaks During the Course of Illness (Fig. 2A)

Given the above results, we examined the relationship between AST peak concentrations and

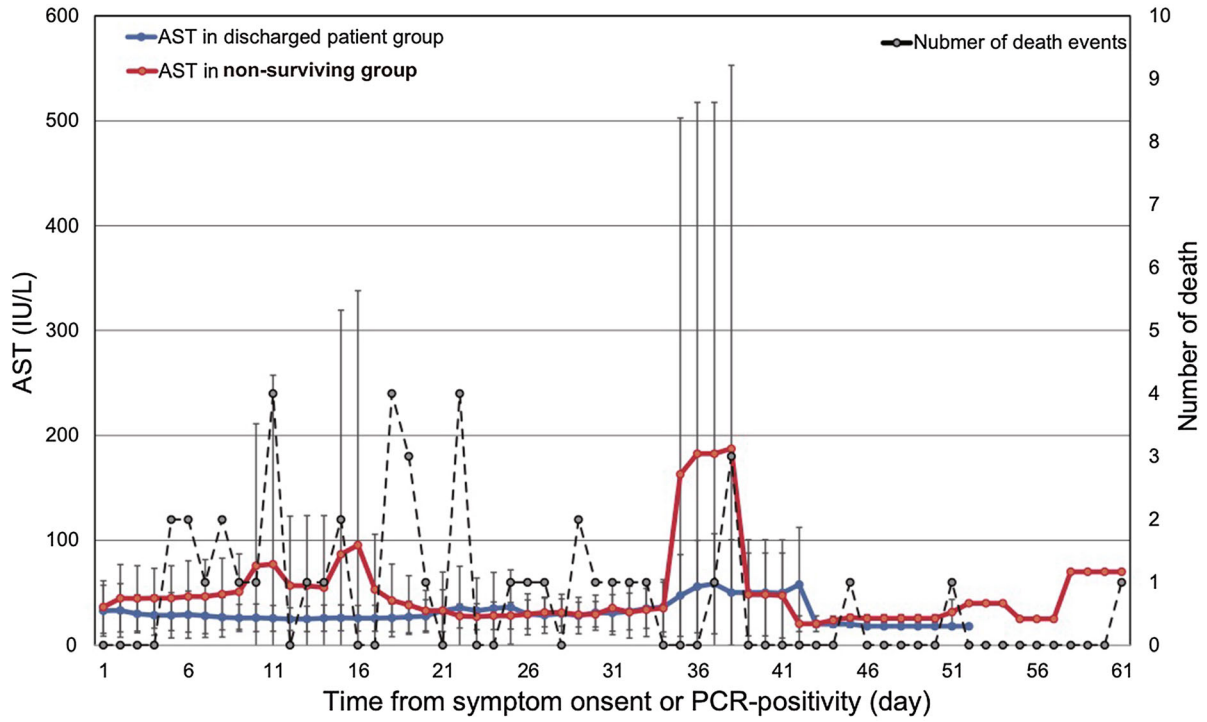


Fig. 1 Changes in AST concentrations according to outcome group and mortality during hospitalization. Points (*blue*: discharged group, *red*: non-surviving group) and *bars* indicate the means and standard deviations, respectively, of AST concentrations in the discharged and non-surviving groups. *Black points* indicate the number of deaths each day

duration of COVID-19. We first examined the timing of AST peaks during the course of illness (Fig. 2A). AST concentrations peaked within 10 days in most patients in the discharged group, and most were discharged within 1 month. In contrast, among the patients whose AST peaks occurred more than 10 days after admission, the duration of COVID-19 tended to be long, regardless of disease severity on admission. We also noted this trend in the non-surviving group, in whom the duration of COVID-19 was longer in patients whose AST concentrations peaked more than 10 days after admission. These results suggest that there is a relationship between the duration of illness and the timing of AST peaks, regardless of disease severity (Fig. 2A).

Association Between AST Peak Concentrations and Timing of AST Peaks (Fig. 2B)

To further investigate the relationship between liver injury and the course of illness, we analyzed AST peak concentrations, timing of AST peaks, and disease severity on admission in the discharged and non-surviving groups (Fig. 2B). Compared with those in the non-surviving group, in the discharged group, a smaller proportion of patients had AST peaks > 50 IU/l and the AST peaks occurred earlier. Disease severity was primarily moderate or severe on admission in the discharged group (Fig. 2B).

Association Between AST Peak/Average Ratios and Timing of AST Peaks (Fig. 2C)

To better understand the degree and course of liver injury, we analyzed the AST peak/average ratios and the timing of AST peaks. In both outcome groups, most patients with high AST peaks had either moderate or severe disease. A greater proportion of patients had AST peak/average ratios larger than 1.4 in the non-surviving than in the discharged group (Fig. 2C).

Associations Between AST Peaks and Interval Between Death and Disease Onset or Positive Diagnosis of COVID-19 in the Non-Surviving Group (Fig. 2D)

We also assessed the peak of liver injury during the course of COVID-19 in the non-surviving group. AST peak concentrations of less than 200 IU/l were observed in 42/45 patients; the sizes of the AST peaks were not related to the interval between admission and death (Fig. 2D).

In summary, AST peaks tended to be higher and occur later in the non-surviving than the discharged group. Furthermore, AST peaks tended to be higher in patients with moderate or severe disease on admission, regardless of prognosis.

Relationships Between Overall Survival and AST Peak and AST Peak/Average Ratio Quartiles (Fig. 3)

Next, we analyzed the relationship between prognosis and AST peak concentrations in the non-surviving group. To identify whether the greatest severity of liver injury or progression of liver injury over the clinical course was more strongly related to mortality, we compared the overall survival rates of patients classified on the basis of AST peaks (Fig. 3A) and AST peak/average ratios (Fig. 3B) in the non-surviving group. Compared with the other groups, the patients in group 3 had significantly poorer prognoses. In addition, AST peaks were more strongly associated with prognosis than were AST peak/average ratios (log–rank test $p < 0.0001$ (Fig. 3A), $p = 0.004$ (Fig. 3B), respectively) (Fig. 3).

Risk Factors for COVID-19 Mortality (Table 2)

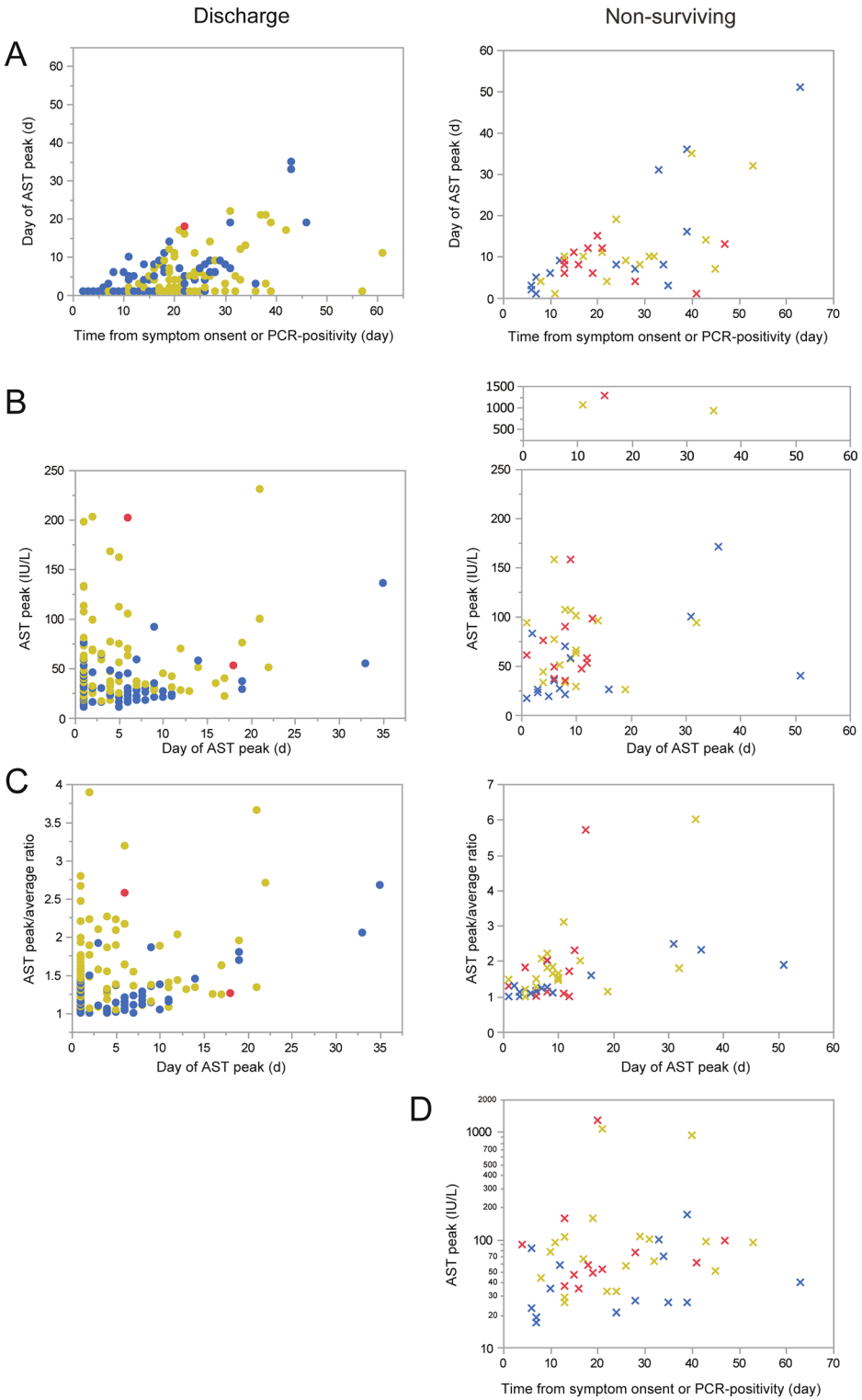
Finally, we evaluated the possible mortality risk factors of age, sex, AST concentrations, BMI, and FIB-4 index on admission and selected comorbidities (HT, DM, DL, CI, MI), as shown in Table 2. Because ALT and GGT are related to AST, we did not examine them to minimize the number of tests. As well as age, AST concentration on admission, and peak AST concentration were associated with mortality (Model 1, $p = 0.0053$; Model 2: $p = 0.0001$, respectively). Compared with AST on admission, peak AST concentrations and FIB-4 index on admission independently showed stronger associations with mortality (Model 3, $p = 0.0001$; $p = 0.0108$, respectively, Table 2). However, none of the other assessed laboratory variables or comorbidities related to vasculitis showed significant associations with mortality.

Association Between DM and Liver Injury (Fig. 4)

To clarify the risk factors for severe liver injury, we investigated the relationship between liver injury and several comorbidities, including HT, DM, and a history of MI or CI in the discharged and non-surviving groups (Fig. 4). In the non-surviving group, AST peaks and AST peak/average ratios were moderately but significantly higher in patients with DM than in those without DM ($p = 0.12$, 0.05 and 0.0076, respectively); however, there were no significant differences in the discharged group ($p > 0.05$).

DISCUSSION

Accumulating evidence has shown that liver injury is associated with COVID-19 disease severity and mortality. Recent studies have reported that patients with severe and/or critical COVID-19 have significantly more abnormal liver biochemistry on admission than do patients with less severe disease [6, 13] and that high aminotransferase concentrations on admission predict all-cause mortality, especially in patients with severe disease [14]. However, few studies have investigated the diagnostic and prognostic value of abnormal laboratory findings [15, 16]. Therefore, we examined the associations between liver injury and COVID-19 disease severity and comorbidity by analyzing laboratory test results throughout the duration of hospitalization. We found that advanced age, higher AST concentrations on admission, and higher FIB-4 index on admission can be used as early prognostic markers.


Discharge
Non-surviving
A
Day of AST peak (d)
Time from symptom onsent or PCR-positivity (day)
B
AST peak (IU/L)
Day of AST peak (d)
C
AST peak/average ratio
Day of AST peak (d)
D
AST peak (IU/L)
Time from symptom onsent or PCR-positivity (day)

◀**Fig. 2** Severity of COVID-19 on admission: *blue*, mild; *green*, moderate; *red*, severe (*left*: discharged group, *right*: non-surviving group). *Circles* indicate discharged patients (*left*), crosses indicate deceased patients (*right*). **A** Timing of AST peak. **B** Association between size and timing of AST peak. **C** Association between AST peak/average ratio and timing of AST peak. **D** Association between AST peak and interval between death and symptom onset or positive diagnosis of COVID-19 in the non-surviving group

These findings suggest that the potential for liver injury, particularly as indicated by AST concentration and FIB-4 index, should be investigated upon admission regardless of disease severity. According to Wu et al., patients with abnormal liver biochemistry are at increased risk of poor prognosis [17]. Therefore, liver biochemistry in COVID-19 patients should be screened on admission and intensely monitored and managed during hospitalization.

Another important question is whether liver injury only reflects prognosis or seriously influences the course of illness. Some studies have found evidence for liver tropism of SARS-CoV-2; however, multiple other factors may cause liver injury [2, 18, 19]. Crucial questions remain and need to be clarified by further research. Nevertheless, our results may shed some light on this issue. Notably, in the non-surviving group, patients with DM had significantly greater liver injury than did those without DM. In contrast, we found no differences between patients with versus without DM in the discharged group. Furthermore, we found no differences in liver injury between patients with versus without any of the other assessed comorbidities (data not shown). These results indicate that DM is not an independent prognostic factor for mortality, however, DM is likely one of the factors that exacerbate liver injury in COVID-19 patients. We speculated this mechanism in previous published studies. A previous meta-analysis found DM to be a significant risk factor for death of COVID-19 patients, probably because patients with DM have higher circulating angiotensin-converting enzyme-2 concentrations [20]. However, it is well known that a large proportion of patients with DM develop non-alcoholic fatty liver disease (NAFLD) and that its inflammatory complication, non-alcoholic steatohepatitis [21, 22], is associated with severity of fibrosis [23].

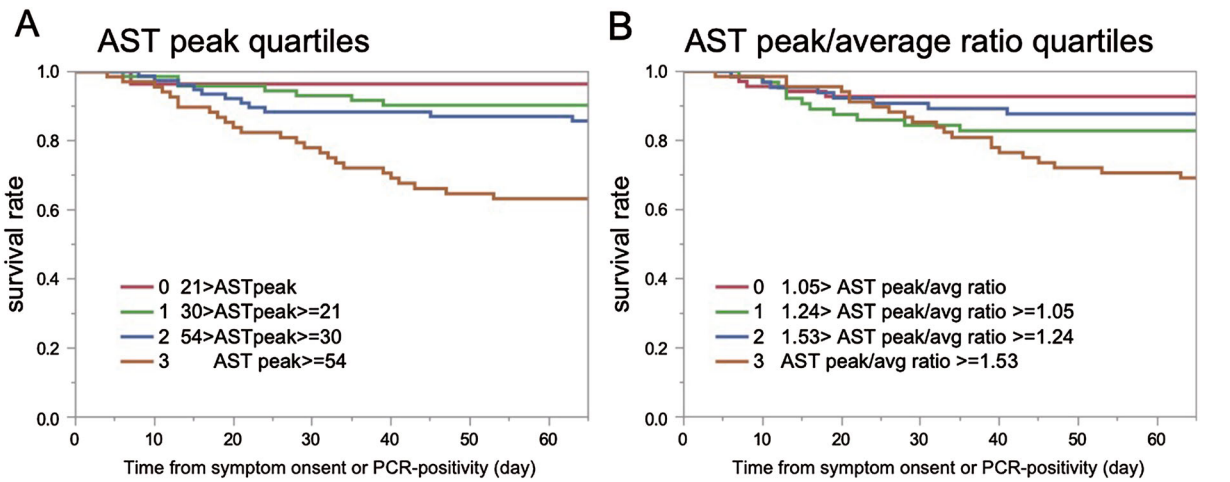


Fig. 3 Relationship between overall survival and AST peak and AST peak/average ratio quartiles. Kaplan–Meier curves of overall survival of hospitalized patients with COVID-19 according to AST values. **A** Patients were divided into AST peak quartiles (0, 21 IU/l > AST peak; 1, 30 IU/l > AST peak ≥ 21 IU/l; 2, 54 IU/l > AST peak ≥ 30 IU/l; 3, AST peak ≥ 54 IU/l). **B** Patients were divided into AST peak/average ratio quartiles (0, 1.05 > AST peak/avg ratio; 1, 1.24 > AST peak/avg ratio ≥ 1.05; 2, 1.53 > AST peak/avg ratio ≥ 1.24; 3, AST peak/avg ratio ≥ 1.53)

Another study reported that DM is an independent predictor of advanced liver disease in patients with NAFLD [24]. DM being one of the pathogenic mechanisms in NAFLD onset and progression points to a possible role in liver injury in patients with severe COVID-19 [25, 26]. Moreover, several studies have suggested that DM is a critical comorbidity in patients infected with SARS-CoV-2 because it is associated with dysregulation of the renin–angiotensin system [27, 28], and stimulated angiotensin II (Ang II) is a potential factor in the development of liver fibrosis [29]. These studies suggest that DM involves liver fibrosis and SARS-CoV-2 aggravates this condition. Several studies reporting associations between

Table 2 Risk factors for prognosis of death in hospitalized COVID-19 in different liver injury indicators in Cox regression analyses

Model 1: AST on admission				
Factors	**Risk ratio**	**Lower 95%**	**Upper 95%**	***p* value**
Age	1.07	1.03	1.10	< 0.0001
Sex (male)	0.90	0.40	2.05	0.8165
AST on admission	1.01	1.00	1.02	0.0118
BMI	0.96	0.86	1.07	0.5043
Hypertension	1.42	0.64	3.02	0.3703
Diabetes mellitus	0.93	0.39	2.02	0.8605
Dyslipidemia	0.69	0.10	2.42	0.6042
Cerebral infarction	1.86	0.65	4.60	0.2258
Myocardial infarction	5.32	0.27	30.87	0.2058
Model 2: Peak value of AST				
Factors	**Risk ratio**	**Lower 95%**	**Upper 95%**	***p* value**
Age	1.07	1.04	1.10	< 0.0001
Sex (male)	0.98	0.44	2.21	0.9784
Peak value of AST	1.002	1.00	1.00	0.0021
BMI	1.01	0.90	1.12	0.8454
Hypertension	1.04	0.48	2.15	0.9002
Diabetes mellitus	0.86	0.37	1.85	0.7183
Dyslipidemia	0.75	0.12	2.62	0.6989
Cerebral infarction	1.99	0.70	4.92	0.1782
Myocardial infarction	3.50	0.18	20.44	0.3217
Model 3: Peak value of AST and FIB-4 index				
Factors	**Risk ratio**	**Lower 95%**	**Upper 95%**	***p* value**
Age	1.05	1.02	1.08	0.0053
Sex (male)	0.82	0.36	1.87	0.6360
Peak value of AST	1.003	1.001	1.004	0.0001
FIB-4 index on admission	1.22	1.04	1.41	0.0108
BMI	0.98	0.87	1.09	0.7304
Hypertension	1.18	0.54	2.50	0.6628
Diabetes mellitus	0.83	0.35	1.83	0.6634
Dyslipidemia	0.69	0.11	2.41	0.6157
Cerebral infarction	1.83	0.64	4.57	0.2241
Myocardial infarction	3.33	0.17	19.79	0.2241

These data were risk ratio per unit

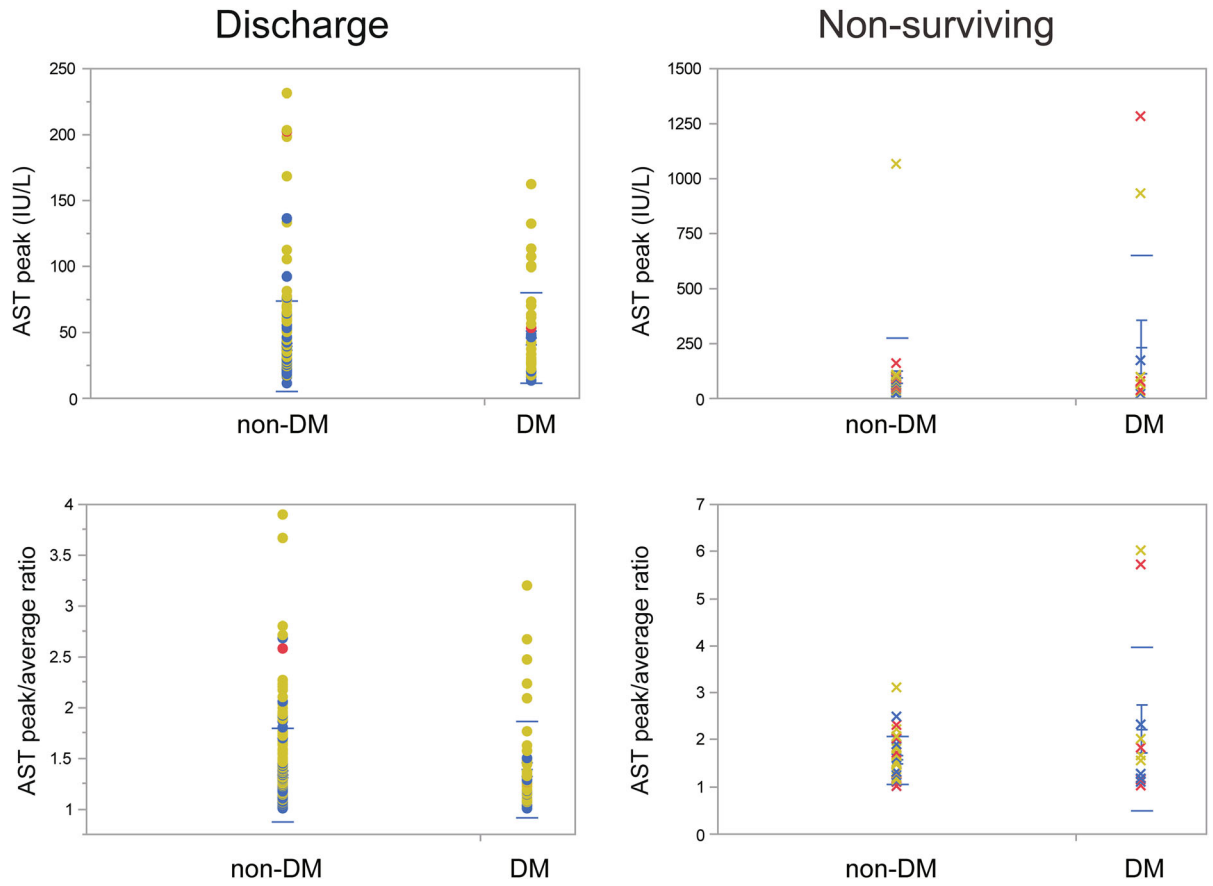


Fig. 4 Association between DM and liver injury (*left*: discharged group, *right*: non-surviving group)

liver fibrosis according to the FIB-4 index and risk of ICU admission and mortality [30, 31] offer support for this speculation. In our study, FIB-4 index on admission was significantly higher in patients with than without DM. We found that a patient with DM have higher aminotransferase concentration on admission, her FIB-4 indexes show rapid progression of liver fibrosis as detected by computed tomography, and she had high hepatocyte growth factor concentrations of approximately 2.0 ng/ml (normal concentrations < 0.39 ng/ml). Given these results, we speculate that high Ang II in patients with severe or longer duration SARS-CoV-2 infection facilitates development of NAFLD and/or liver fibrosis [32]; thus, liver injury and COVID-19 severity work synergistically. Recently, promising treatments for inhibiting liver fibrosis—Ang II inhibitors [33] and 6His-tagged recombinant human cytoglobin [34]—have been gaining attention. Inhibition of liver fibrosis may help to suppress COVID-19-induced liver injury. Further studies that evaluate the liver status of patients with COVID-19 are necessary for improving patient prognosis.

This study has four main limitations. First, it was performed in a single center and the proportion of critically ill patients was higher than in the overall COVID-19 cohort in Japan. Second, patients with critical COVID-19 are more likely to have repeated laboratory tests than those with non-critical disease and this may have shifted the peak of aminotransferase concentrations. We therefore analyzed the absolute number and frequency of blood tests and divided them by hospitalization days. The non-surviving group (6.90 ± 6.18) had more blood tests than did the discharged group (4.29 ± 4.30), however, frequency divided by

hospitalization days was similar in these two groups (0.5 ± 0.27, 0.46 ± 0.29, respectively). Third, considering the frequency of liver injury in patients with COVID-19, distinguishing between drugs and the underlying disease as causes of hepatotoxicity is challenging. However, one study has reported that certain drugs are a major cause of liver injury in such patients [35]. On the basis of these findings, we did not administer those drugs to patients with severe liver dysfunction, which may have reduced the potential bias from drug-induced hepatotoxicity. In addition, FIB-4 on admission is not affected by those drugs. Fourth, we were unable to evaluate and compare liver images between patients with or without DM. Regarding the last point, we consider that evaluation of liver images and NAFLD in patients with COVID-19 should be included in a future study.

CONCLUSIONS

This study provides insight into the potential use of the higher AST concentrations and FIB-4 index on admission and higher AST peaks during hospitalization as prognostic markers in patients with COVID-19. Additionally, AST peaks were significantly higher in non-surviving patients with DM than in those without DM. These findings suggest that it is critical to address any liver dysfunction while treating patients hospitalized for COVID-19, especially those with DM. Additional studies are necessary to clarify whether specific treatments for liver injury, such as inhibiting liver fibrosis and controlling DM, can mitigate COVID-19 disease severity.

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Compliance with Ethics Guidelines. This study was approved by the Ethics Committee of the Tokushukai Medical Group (TGE01425-002) and was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments. Written informed consent for RT-qPCR testing for SARS-CoV-2 was obtained from all included patients. The need for consent to participate in the study was waived because it was a retrospective study of anonymized data. All authors have consented to publication. This study did not include animal research, clinical trials, or the use of plants.

Data Availability. The data sets analyzed during the current study are available from the corresponding author on reasonable request.

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