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ORIGINAL ARTICLE

Age-adjusted mortality and predictive value of liver chemistries in a Viennese cohort of COVID-19 patients

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Abstract

Background and Aims: The coronavirus disease of 2019 (COVID-19) causes considerable mortality worldwide. We aimed to investigate the frequency and predictive role of abnormal liver chemistries in different age groups.

Methods: Patients with positive severe acute respiratory distress syndrome-coronavirus-2 (SARS-CoV-2) polymerase chain reaction (PCR) test between 03/2020-07/2021 at the Vienna General Hospital were included. Patients were stratified for age: 18–39 vs. 40–69 vs. ≥70 years (y). Aspartate aminotransferase (AST), alanine-aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and total bilirubin (BIL) were recorded.

Results: 900 patients (18–39 years: 32.2%, 40–69 years: 39.7%, ≥70 years: 28.1%) were included. Number of comorbidities, median D-dimer and C-reactive protein increased with age. During COVID-19, AST/ALT and ALP/GGT levels significantly increased. Elevated hepatocellular transaminases (AST/ALT) and cholestasis parameters (ALP/GGT/BIL) were observed in 40.3% ($n = 262/650$) and 45.0% ($n = 287/638$) of patients respectively. Liver-related mortality was highest among patients with pre-existing decompensated liver disease (28.6%, $p < .001$). 1.7% of patients without pre-existing liver disease died of liver-related causes, that is consequences of hepatic dysfunction or acute liver failure. Importantly, COVID-19-associated liver injury (16.0%, $p < .001$), abnormal liver chemistries and liver-related mortality (6.5%, $p < .001$) were most frequent among 40–69 years old patients. Elevated AST and BIL after the first positive SARS-CoV-2 PCR independently predicted mortality in the overall cohort and in 40–69 years old patients.

Abbreviations: 95% CI, 95% confidence interval; aHR, adjusted hazard ratio; ALP, alkaline phosphatase; ALT, alanine-aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; BIL, total bilirubin; BMI, body mass index; cACLD, compensated advanced chronic liver disease; COVID-19, coronavirus disease of 2019; CRP, C-reactive protein; dACLD, decompensated advanced chronic liver disease; EC, Ethics committee; GGT, gamma-glutamyl transferase; HR, hazard ratio; ICU, intensive care unit; INR, international normalized ratio; IQR, interquartile range; n, number; non-ACLD, non-advanced chronic liver disease; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory distress syndrome-coronavirus-2; ULN, upper limit of normal; WBC, white blood cell count.

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Conclusions: Almost half of the COVID-19 patients exhibit abnormal hepatocellular and cholestasis-related liver chemistries with 40–69 years old patients being at particularly high risk for COVID-19-related liver injury and liver-related mortality. Elevated AST and BIL after SARS-CoV-2 infection are independent predictors of mortality, especially in patients aged 40–69 years.

KEYWORDS
acute respiratory distress syndrome, COVID-19, liver chemistries, liver injury, SARS-CoV-2

1 | INTRODUCTION

The coronavirus disease of 2019 (COVID-19) pandemic, caused by severe acute respiratory distress syndrome-coronavirus-2 (SARS-CoV-2), is associated with substantial morbidity and mortality worldwide.[1] COVID-19 affects lungs, liver, intestinal and neuronal systems, causing acute respiratory distress syndrome (ARDS) and multiorgan failure.[2,3] Risk factors for mortality due to COVID-19 include old age, obesity, male sex and pre-existing comorbidities including liver disease.[4–6]

Previous studies have demonstrated liver enzyme abnormalities in a substantial number of patients with COVID-19.[7–11] Meta-analyses found liver transaminases to be elevated in approximately 20% of patients, and also reported increased parameters of cholestatic liver injury, that is alkaline phosphatase (ALP) in 6.1% and gamma-glutamyl transferase (GGT) in 21.1% of COVID-19 patients, respectively.[12,13]

Elevations of liver enzymes were more frequently observed in patients with severe courses of COVID-19 and in critically ill patients.[14–17] In a large retrospective Chinese study, elevated levels of aspartate aminotransferase (AST) and direct bilirubin at hospital admission were identified as independent predictors of COVID-19-associated mortality.[18] However, this study has some limitations, including the lack of detailed data on pre-existing liver disease and the severity of systemic inflammation.[19] Another study reported progressively increasing levels of hepatic transaminases in COVID-19 patients with severe courses of the disease.[20]

Examining post-mortem liver biopsies of patients with SARS-CoV-2 infection, viral particles were detected in the cytoplasm of hepatocytes, directly linking hepatocellular infection with COVID-19-associated liver injury.[21] Next to direct SARS-CoV-2-mediated cytotoxicity, other pathomechanisms such as an excessive proinflammatory state, hypoxemia, drug-induced liver injury, coagulopathy-associated vascular dysfunction, cardiac congestion and sepsis are likely all contributing to liver injury in COVID-19.[3,16]

The aim of this study was to investigate (i) the rate of abnormal liver chemistries at the first blood withdrawal after the first positive SARS-CoV-2 polymerase chain reaction (PCR) test and (ii) the trajectory of hepatic transaminases in a large Austrian cohort of patients with COVID-19. Moreover, we set out to determine (iii) the impact of liver abnormalities on clinical outcome of patients with COVID-19 in different age strata.

Lay summary

Investigating liver chemistries in a large cohort of SARS-CoV-2 infected patients, we observed abnormal hepatocellular and cholestasis-related liver chemistries in 40.3% and 45.0% of patients with COVID-19. Patients aged 40-69 years are at particularly high risk for COVID-19-related liver injury and liver-related mortality. Elevated AST and BIL after the first positive SARS-CoV-2 PCR test are independent predictors for mortality, especially in 40-69 years old patients.

2 | PATIENTS AND METHODS

2.1 | Study population

Adult patients with positive SARS-CoV-2 PCR test at the Vienna General Hospital between 03/2020 and 07/2021 were included in this retrospective study. Clinical and laboratory parameters, including age, body mass index (BMI), comorbidities (i.e. pre-existing arterial hypertension, diabetes mellitus, hyperlipidemia, chronic liver disease, cardiovascular, lung, chronic kidney and malignant disease), liver chemistries (alkaline phosphatase [ALP], gamma-glutamyl transferase (GGT), AST, alanine-aminotransferase [ALT] and total bilirubin [BIL]), haemoglobin, platelet and white blood cell count (WBC), international normalized ratio (INR), D-dimer, serum sodium, creatinine, albumin and C-reactive protein (CRP), hospital admission, intensive care unit (ICU) admission, intubation, death, liver-related death and COVID-19-related death were assessed via chart review. Patients were stratified for age (18–39 years, 40–69 years, and ≥70 years). Liver-related death was defined as death directly associated with liver-related complications. Pre-existing liver disease was subdivided into non-advanced chronic liver disease (non-ACLD), compensated ACLD (cACLD) and decompensated ACLD (dACLD). Notably, due to the retrospective design of the study, not all parameters were available for every patient.

2.2 | Laboratory parameters

All parameters were assessed by standard laboratory assays. For every parameter, the last available value prior to the positive

SARS-CoV-2 PCR test (t0), as well as the first three available values after the positive SARS-CoV-2 PCR test (t1, t2 and t3 respectively) and the last available value (last) were recorded.

For parameters of hepatocellular (AST, ALT) and cholestatic liver injury (ALP, GGT, BIL), standard laboratory thresholds for men and women were used as upper limit of normal (ULN). Liver injury was defined as increased AST or ALT >3xULN or increased AP or BIL >2xULN, analogous to previous studies[18,20] and to the American College of Gastroenterology Clinical Guideline definition.[22]

2.3 | Statistical analysis

Categorical variables were reported as number (n) and proportion (%) of patients showing the parameter of interest. Where appropriate, the total number of available values (n total) was added. Continuous data were depicted as median and interquartile range (IQR). D'Agostino & Pearson and Shapiro-Wilk normality tests were implemented to test for normal distribution. Mann-Whitney U test was used for comparing non-normally distributed continuous variables between two groups. For comparison of non-normally distributed continuous variables in three or more groups, the Kruskal–Wallis test was computed. Dunn's multiple comparisons test was implemented as post hoc test. For group comparisons of categorical variables, Pearson's chi-squared or Fisher's exact test was used. Kaplan-Meier curves depicted differences in survival between groups of elevated versus non-elevated levels of the parameters of interest. Differences in survival between these groups were assessed by a log-rank test. Cox proportional hazard models were used to determine the impact of the parameters of interest on mortality. Multivariate analysis considered age, sex, creatinine, albumin, obesity, liver disease, diabetes mellitus, cardiovascular disease, lung disease and malignancy. Patients entered these models at the time of the first positive SARS-CoV-2 PCR test. IBM SPSS 22.0 statistic software (IBM) and GraphPad Prism 8 (Graphpad Software) were used for statistical analysis. A two-sided *p*-value of <.05 was considered as statistically significant.

2.4 | Ethics

The study was approved by the ethics committee (EC) of the Medical University of Vienna (EK1461/2020). It was performed in accordance with the current version of the Helsinki Declaration. Due to the retrospective design of the study, the EC waived the need for informed consent.

3 | RESULTS

3.1 | Patient characteristics in different age strata

In total, 900 patients with positive SARS-CoV-2 PCR test were included in this study. 52.4% of patients were male. The median age was 52.9 years with 290 (32.2%) patients between 18 and 39 years, 357 (39.7%) patients between 40 and 69 years and 253 (28.1%) patients ≥70 years old. Overall, 60 patients had pre-existing liver disease (non-ACLD: 8.3% [*n* = 41], cACLD: 20.0% [*n* = 12], dACLD: 11.7% [*n* = 7]). The main aetiologies included non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH) in 58.3% (*n* = 35), alcohol-related liver disease (ALD) in 16.7% (*n* = 10) and viral hepatitis in 8.3% (*n* = 5) of patients with pre-existing liver disease. With progressive age, the prevalence of comorbidities, including pre-existing liver disease, arterial hypertension, diabetes mellitus, dyslipidemia, cardiovascular disease, chronic renal deficiency, lung disease and malignancy increased. The rate of obesity was highest in 40–69 years old patients (Table 1).

At the blood withdrawal after the first positive SARS-CoV-2 PCR test, 10.3% (*n* = 66/652) of patients showed liver injury by biochemical definition. Parameters of hepatocellular liver injury (AST/ALT) were elevated in 40.3% (*n* = 262/650) of patients and parameters of cholestatic liver injury (ALP/GGT) in 45.0% (*n* = 287/638) of patients. Platelet count and albumin levels decreased throughout the age strata. Creatinine levels were significantly increased in patients ≥70 years. D-dimer and CRP were particularly elevated in patients ≥40 years old.

3.2 | Trajectory of liver values after SARS-CoV-2 infection

Over the course of the SARS-CoV-2 infection, plasma levels of parameters of hepatocellular injury (AST: $p < .001$; ALT: $p = .002$) and parameters of cholestatic liver injury (ALP: $p = .012$; GGT: $p < .001$) progressively increased (at t1/t2/t3 vs. t0), while there were no significant changes in BIL ($p = .347$; Figure-1, Table-S1).

Median levels of AST were already increased at the first blood withdrawal after the first positive SARS-CoV-2 PCR test as compared to the last previous value (t1: 32.0 U/L vs. t0: 25.0 U/L, $p < .001$). For ALT (t0: 25.0 U/L vs. t1: 27.0 U/L, $p = .700$) and parameters of cholestatic liver injury (ALP: t0: 77.0 U/L vs. t1: 72.5 U/L, $p = .999$; GGT: t0: 37.0 U/L vs. t1: 42.0 U/L, $p = .952$), the median values at the first positive SARS-CoV-2-PCR (t1) were similar to baseline values (t0).

During the COVID-19 course, parameters of hepatocellular liver injury (AST: t1: 32.0 U/L vs. t3: 39.0 U/L, $p < .001$; ALT: t1: 27.0 U/L vs. t3: 30.0 U/L, $p = .183$) and parameters of cholestatic liver injury (ALP: t1: 72.5 U/L vs. t3: 78.0 U/L, $p = .184$; GGT: t1: 42.0 U/L vs. t3: 84 U/L, $p < .001$) increased, while median BIL levels remained unchanged (BIL: t1: 0.5 mg/dl vs. t3: 0.5 mg/dl, $p = .611$).

Finally, for the last available laboratory values, median ALP (t3: 78.0 U/L vs. last: 77.0 U/L, $p = .999$) and ALT levels (t3: 30.0 U/L vs. last: 28.0 U/L, $p = .999$) did not decrease, while levels of AST (t3: 39.0 U/L vs. last: 28.0 U/L, $p < .001$) and GGT (t3: 84.0 U/L vs. last: 44.0 U/L, $p < .001$) declined significantly.

3.3 | COVID-19-related liver injury in different age strata after the first positive SARS-CoV-2 PCRtest

Interestingly, the proportion of patients with COVID-19-related liver injury at the first blood withdrawal after the first positive

TABLE 1 Patient characteristics and comparison between patients stratified for age (18–39 years, 40–69 years, ≥70 years)

Patient characteristics	All patients($n = 900$)	Age			*p*-value
		18–39 years ($n = 290$)	40–69 years ($n = 357$)	≥70 years ($n = 253$)	
Sex, male/female (% male)	472/428 (52.4%)	151/139 (52.1%)	204/153 (57.1%)	117/136 (46.2%)	**.029**
Age, years (IQR)	52.9 (37.3)	29.6 (9.4)	55.4 (13.3)	79.8 (9.7)	**<.001**
Obesity, n/*n* total (%)	143/390 (36.7%)	24/69 (34.8%)	110/198 (44.4%)	31/123 (25.2%)	**.002**
Liver disease, n/*n* total (%)	60/741 (8.1%)	2/198 (1.0%)	27/298 (9.1%)	31/245 (12.7%)	**<.001**
Arterial Hypertension, n/*n* total (%)	323/738 (44.4%)	9/198 (13.3%)	129/295 (43.7%)	185/245 (75.5%)	**<.001**
Diabetes mellitus, n/*n* total (%)	141/740 (19.1%)	5/198 (2.5%)	68/296 (23.0%)	68/246 (27.6%)	**<.001**
Dyslipidemia, n/*n* total (%)	158/741 (21.3%)	7/198 (3.5%)	61/297 (20.5%)	90/246 (36.6%)	**<.001**
Cardiovascular disease, n/*n* total (%)	215/737 (29.2%)	6/198 (3.0%)	57/293 (19.5%)	152/246 (61.8%)	**<0.001**
Chronic renal insufficiency, n/*n* total (%)	91/751 (12.1%)	1/201 (0.5%)	23/303 (7.6%)	67/247 (27.1%)	**<.001**
Lung disease, n/*n* total (%)	126/743 (17.0%)	11/198 (5.6%)	53/298 (17.8%)	62/247 (25.1%)	**<.001**
Malignancy, n/*n* total (%)	111/741 (15.0%)	4/200 (2.0%)	48/296 (16.2%)	59/245 (24.1%)	**<.001**
Liver injury, n/*n* total (%)[a]	66/652 (10.3%)	11/130 (8.5%)	45/282 (16.0%)	11/240 (4.6%)	**<.001**
Alkaline phosphatase, $U \times L^{-1}$ (IQR)[a]	72.5 (46.0)	65.0 (34.0)	73.0 (51.0)	75.0 (53.0)	.080
Alkaline phosphatase > 2xULN, n/*n* total (%)	27/558 (4.8%)	2/117 (1.7%)	21/261 (8.0%)	4/180 (2.2%)	**.004**
Aspartate transaminase, $U \times L^{-1}$ (IQR)[a]	32.0 (30.0)	27.0 (20.0)	34.0 (32.0)	33.0 (29.0)	**.044**
Aspartate transaminase > 3xULN, n/*n* total (%)[a]	23/618 (3.7%)	5/121 (4.1%)	13/275 (4.7%)	5/222 (2.3%)	.338
Alanine aminotransferase, $U \times L^{-1}$ (IQR)[a]	27.0 (25.0)	28.0 (28.0)	30.0 (32.8)	23.0 (18.0)	**.001**
Alanine aminotransferase > 3xULN, n/*n* total (%)[a]	28/648 (4.3%)	8/130 (6.2%)	15/280 (5.4%)	5/238 (2.1%)	.099
Gamma-glutamyl transferase, $U \times L^{-1}$ (IQR)[a]	42.0 (84.5)	28.0 (57.5)	53.0 (143.0)	42.0 (62.0)	**<.001**
Gamma-glutamyl transferase > 2xULN, n/Total *n* (%)[a]	145/565 (25.7%)	18/117 (15.4%)	89/261 (34.1%)	38/187 (20.3%)	**<.001**
Bilirubin, $mg \times dl^{-1}$ (IQR)[a]	0.5 (0.4)	0.4 (0.3)	0.5 (0.5)	0.5 (0.3)	.137
Bilirubin > 2xULN, n/*n* total (%)[a]	23/638 (3.6%)	3/124 (2.4%)	17/275 (6.2%)	3/236 (1.3%)	**.009**
Thrombocytes, $G \times L^{-1}$ (IQR)[a]	213.5 (107.0)	229.5 (79.8)	218.0 (114.0)	203.0 (112.0)	**.005**
D-dimer, $mg \times dl^{-1}$ (IQR)[a]	1.5 (2.6)	0.5 (0.8)	1.3 (2.8)	1.3 (2.5)	**<.001**
Albumin, $g \times L^{-1}$ (IQR)[a]	33.7 (11.6)	42.7 (13.6)	32.7 (12.0)	32.6 (7.9)	**<.001**
Creatinine, $mg \times dl^{-1}$ (IQR)[a]	0.9 (0.5)	0.8 (0.3)	0.8 (0.4)	1.0 (0.7)	**<.001**
C-reactive pr/otein, $mg \times dl^{-1}$ (IQR)[a]	2.6 (8.1)	1.0 (3.3)	3.3 (9.6)	3.2 (7.3)	**<.001**
Follow-up and clinical outcomes	**All patients ($n = 697$)**	**Age**			***p*-value**
		18–39 years ($n = 175$)	**40–69 years ($n = 293$)**	**≥70 years ($n = 229$)**	
Median follow-up, days (IQR)	63.0 (139.0)				
Hospital admission, *n* (%)	458 (65.7%)	52 (29.7%)	200 (68.3%)	206 (90.0%)	**<.001**
Median hospital stay, days (IQR)	22.0 (33.0)	12.0 (25.0)	25.5 (39.0)	21.0 (28.0)	**.011**
ICU admission, *n* (%)	200 (28.7%)	23 (13.1%)	126 (43.0%)	51 (22.3%)	**<.001**
Median ICU stay, days (IQR)	22.0 (30.0)	18.0 (20.0)	29.0 (32.0)	11.0 (19.0)	**.002**
Intubation, *n* (%)	164 (23.5%)	17 (9.7%)	108 (36.9%)	39 (17.0%)	**<.001**

TABLE 1 (Continued)

Follow-up and clinical outcomes	All patients ($n = 697$)	Age			p-value
		18–39 years ($n = 175$)	40–69 years ($n = 293$)	≥70 years ($n = 229$)	
Median duration of intubation, days (IQR)	21.5 (29.0)	19.0 (14.0)	26.5 (31.0)	13.0 (19.0)	**.001**
Death, *n* (%)	154 (22.1%)	0 (0.0%)	61 (20.8%)	93 (40.6%)	**<.001**
COVID-19-related death, *n* (%)	128 (18.4%)	0 (0.0%)	45 (15.4%)	83 (36.2%)	**<.001**
Liver-related death, *n* (%)	24 (2.7%)	0 (0.0%)	19 (6.5%)	5 (2.2%)	**<.001**

[a]At the first blood withdrawal after the first positive SARS-CoV-2 PCR test.

p-values depicting statistically significant differences are presented as bold values.

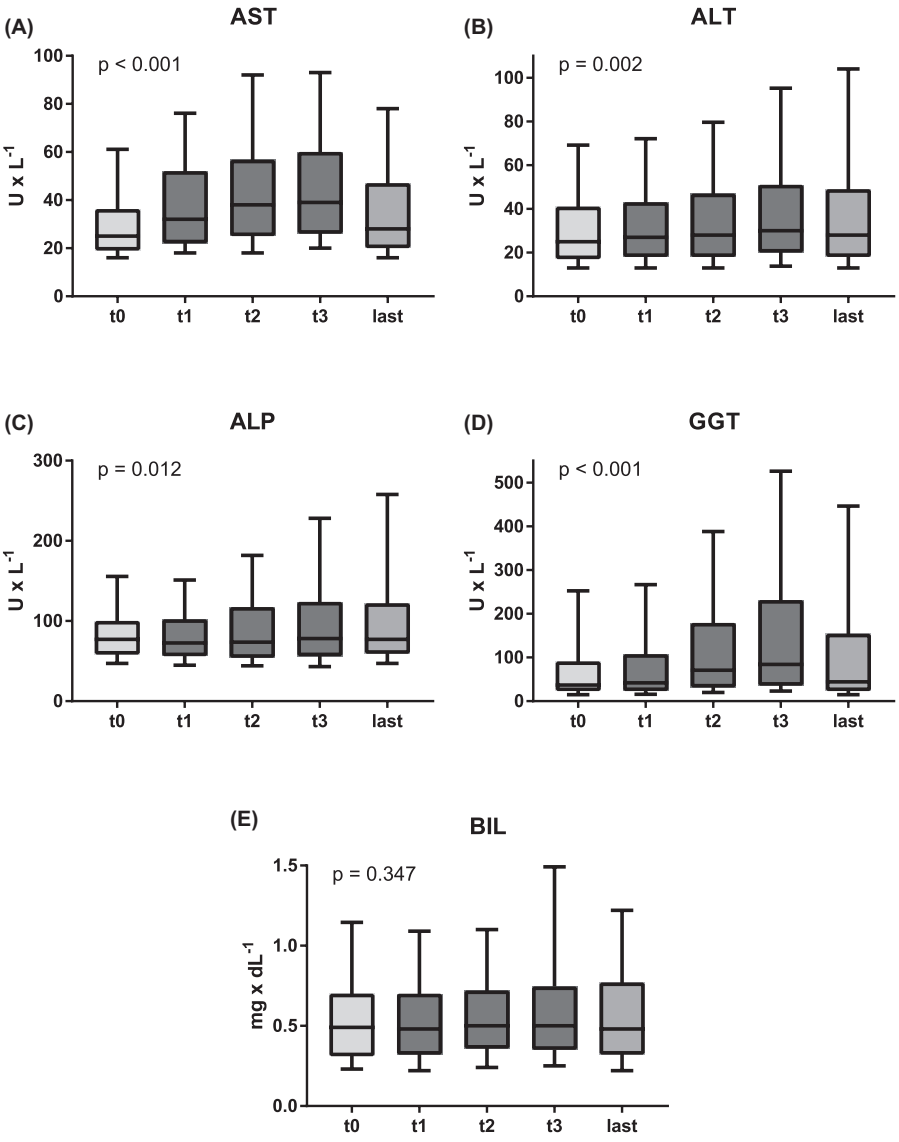


FIGURE 1 Trajectory of blood levels of (A) aspartate transaminase (AST), (B) alanine aminotransferase (ALT), (C) alkaline phosphatase (ALP), (D) gamma-glutamyl transferase (GGT) and (E) bilirubin. The borders of the whiskers are the 10th and the 90th percentile. t0 = last available value before SARS-CoV-2 infection; t1/t2/t3 = first/second/third available value after SARS-CoV-2 infection; last = last available value

SARS-CoV-2 PCR test was particularly high in 40–69 years old patients (8.5% vs. 16.0% vs. 4.6%; $p < .001$). Consistently, median levels of parameters of hepatocellular injury (AST: 18–39 years: 27.0 U/L vs. 40–69 years: 34.0 U/L vs. ≥70 years: 33.0 U/L, $p = .044$; ALT: 18–39 years: 28.0 U/L vs. 40–69 years: 30.0 U/L vs. ≥70 years: 23.0 U/L, $p = .001$) were highest among patients between 40 and 69 years old. Parameters of cholestatic liver injury (ALP: 18–39 years: 65.0 U/L vs. 40–69 years: 73.0 U/L vs. ≥70 years: 75.0 U/L, $p = .004$; GGT: 18–39 years: 28.0 U/L vs. 40–69 years: 53.0 U/L vs. ≥70 years: 42.0 U/L, $p < .001$) showed similar results with

increased values especially in patients ≥40 years old. Finally, BIL levels >2xULN were observed primarily in patients of 40–69 years of age (6.2% vs. 18–39 years: 2.4% vs. ≥70 years: 1.3%, $p = .009$; Table 1, Figure 2).

3.4 | Clinical outcomes of patients in regard to the presence or absence of elevated liver chemistries

Follow-up data were available for 697 patients with a median follow-up duration of 63.0 [IQR 139.0] days. In total, 164 patients (23.5%) were intubated and 154 patients (22.1%) died, with 128 deaths (18.4%) being COVID-19-related and 24 deaths (3.4%) being liver-related. While the rate of hospital admissions, death and COVID-19 related death increased throughout the age strata, the rate of ICU admissions, intubations and median hospital stay was highest in 40–69 years old patients. There were no deaths among patients aged 18–39 years (Tables 1, 2, Figure 2).

Of all patients with liver-related death, 14 patients had no pre-existing liver disease (1.7% of patients without preexisting liver disease), 6 had non-ACLD (14.6% of non-ACLD patients), 1 had cACLD (8.3% of cACLD patients) and 2 had dACLD (28.6% of dACLD patients, $p < .001$). Liver disease aetiology did not impact on liver-related death (17.1% of NAFLD/NASH patients vs. 20.0% of ALD

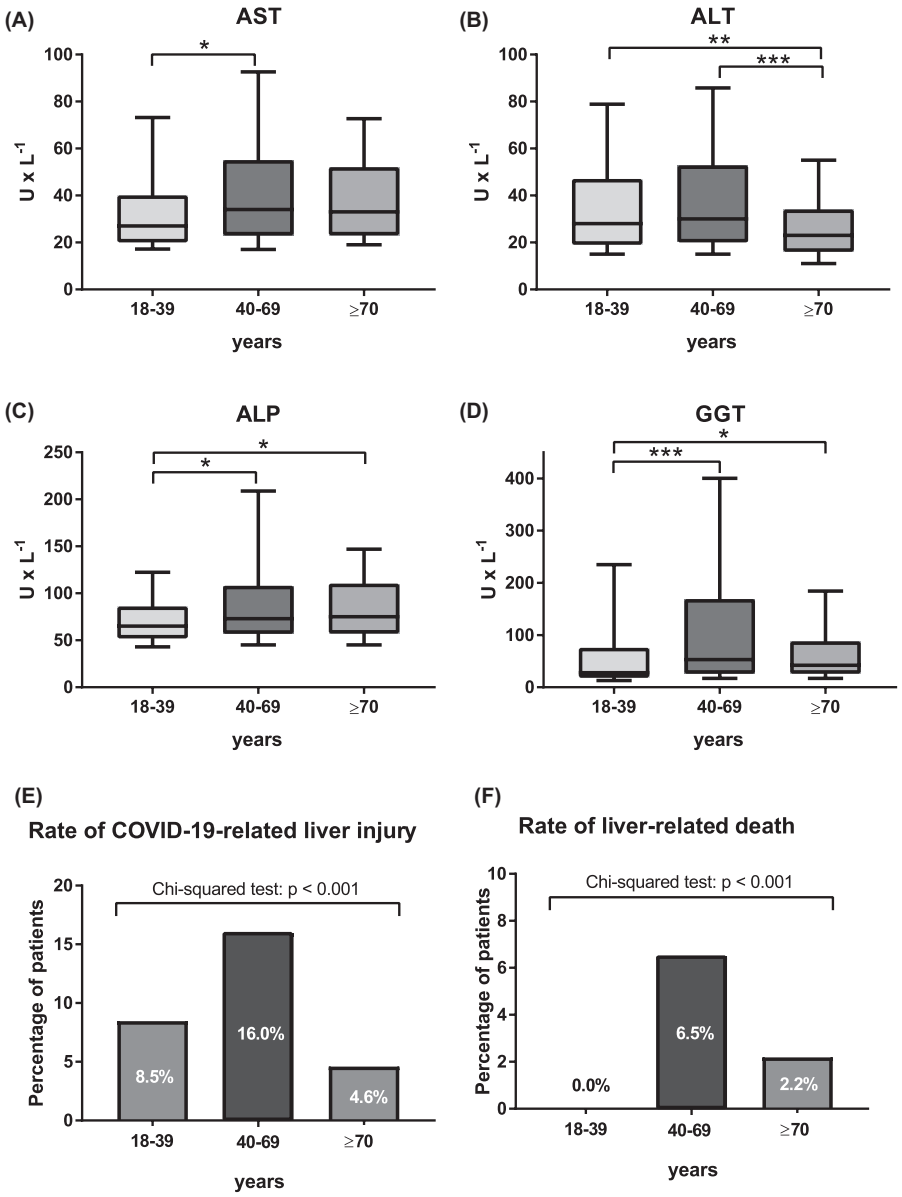


FIGURE 2 Comparison of plasma levels of (A) aspartate transaminase (AST), (B) alanine aminotransferase (ALT), (C) alkaline phosphatase (ALP) and (D) gamma-glutamyl transferase (GGT) between different age strata (i.e. patients aged 18–39 years, 40–69 years and ≥70 years) at blood withdrawal after the first positive SARS-CoV-2 PCR test. The borders of the whiskers are the 10th and the 90th percentile. Comparison of (E) proportion of patients with COVID-19-related liver injury between different age strata and (F) proportion of liver-related death among 40–69 years old and ≥70 years old patients

patients vs. 20.0% of viral hepatitis patients; $p = .701$). Importantly, liver-related death occurred significantly more often in 40–69 years old patients (18–39 years: 0.0% vs. 40–69 years: 6.5% vs. ≥70 years: 2.2%; $p < .001$).

Patients with elevated AST at the first blood withdrawal after the first positive SARS-CoV-2 PCR test were more frequently admitted to the hospital (73.9% vs. 90.7%; $p < .001$) and to the ICU (27.8% vs. 51.0%; $p < .001$), they were intubated more often (21.8% vs. 43.3%; $p < .001$) and showed higher mortality (21.2% vs. 32.0%; $p = .006$), more COVID-19-related deaths (16.3% vs. 28.9%; $p = .001$) and more liver-related deaths (2.3% vs. 8.2%; $p = .001$).

Elevated AST was associated with more frequent hospital admission, ICU admission and intubation in all age strata. Moreover, in patients aged 18–39 years, elevated AST ($n = 27/93$; 29.0%) was linked to longer median hospital stay (9.5 days vs. 16.5 days; $p = .046$, Table-S2). COVID-19-related death occurred more often in 40–69 years ($n = 93/247$; 37.7%, Table-S3) and ≥70 years old patients with elevated AST ($n = 74/203$; 36.5%, Table-S4). However, overall death (19.5% vs. 31.2%; $p = .037$) and liver-related death (3.2% vs. 15.1%; $p = .027$) was associated with elevated AST levels only in patients aged 40–69 years.

3.5 | Association of survival and elevated liver enzymes after the first positive SARS-CoV-2 PCRtest

Assessed by log-rank test, elevated levels of AST ($n = 194/543$; $p < .001$), ALP ($n = 108/489$; $p = .004$) and GGT ($n = 244/493$; $p = .005$) were associated with shorter survival, while ALT ($n = 156/565$; $p = .253$) and BIL ($n = 44/557$; $p = .217$) were not. In univariate Cox regression analysis, elevated AST (HR: 2.10; 95% CI: 1.25–3.51; $p = .005$) and ALP (HR: 1.70; 95% CI: 1.17–2.46; $p = .005$) were associated with increased mortality (Table-S6). After adjustment for potentially confounding factors (pre-existing liver disease, age, albumin, diabetes mellitus, cardiovascular disease, lung disease and malignancy), AST (aHR: 1.47; 95% CI: 1.01–2.14; $p = .043$) and BIL (aHR: 2.20; 95% CI: 1.22–3.98; $p = .009$) independently predicted survival after SARS-CoV-2 infection. In contrast, ALT (aHR: 0.95; 95% CI: 0.60–1.50; $p = .842$), ALP (aHR: 0.93; 95% CI: 0.60–1.44: $p = .651$) and GGT (aHR: 0.95; 95% CI: 0.61–1.50; $p = .834$) were not independently linked to mortality in multivariate analysis (Table-3, Figure-3).

Shorter survival in 40–69 years old patients was linked to elevated AST ($n = 93/247$; $p = .004$), GGT ($n = 128/235$; $p = .003$) and BIL ($n = 31/246$; $p = .005$; Table-S5). Importantly, elevated AST (aHR: 1.78; 95% CI: 1.04–3.06; $p = .037$) and BIL (aHR: 2.18; 95% CI: 1.15–4.13; $p = .017$) independently predicted mortality in 40–69 years old patients, while elevated GGT did not (aHR: 0.88; 95% CI: 0.45–1.74; $p = .719$).

In ≥70 years old patients, only increased AST levels were linked to a shorter time of survival ($n = 74/203$; $p = .034$). Assessed by univariate Cox regression analysis, AST >ULN was associated with increased mortality (HR: 1.62; 95% CI: 1.03–2.54; $p = .037$, Table-S7). However, after adjustment for age, albumin and lung disease, elevated AST did not predict mortality in this cohort (aHR: 1.36; 95% CI: 0.80–2.32; $p = .259$).

4 | DISCUSSION

In this study, we thoroughly characterized the patterns and trajectories of liver abnormalities in a large Austrian cohort of patients with COVID-19. Importantly, we identified the patient cohort of 40–69 years of age as a particularly vulnerable group for COVID-19-associated liver injury and liver-related death due to COVID-19. Moreover, in accordance with previous studies,[18] we identified increased levels of AST and BIL around the time of the first positive SARS-CoV-2 PCR test as an independent risk factor for mortality.

Both parameters of hepatocellular and of cholestatic liver injury progressively increased following SARS-CoV-2 infection, which is in line with previous studies.[12,18] Subsequently, the liver chemistries, including AST and GGT regressed to pre-COVID infection levels. Interestingly, ALP levels often remained elevated, suggesting prolonged/persistent cholestatic injury in a considerable number of patients with COVID-19.

TABLE 2 Clinical outcomes of COVID-19 patients with and without elevated AST at blood withdrawal after the first positive SARS-CoV-2 PCR

Follow-up and clinical outcomes	AST ≤ULN($n = 349$)	AST > ULN($n = 194$)	p-value
Hospital admission, *n* (%)	258 (73.9%)	176 (90.7%)	**<.001**
Median hospital stay, days (IQR)	22.0 (41.0)	32.0 (36.0)	.261
ICU admission, *n* (%)	97 (27.8%)	99 (51.0%)	**<.001**
Median ICU stay, days (IQR)	24.5 (38.0)	32.0 (29.0)	.775
Intubation, *n* (%)	76 (21.8%)	84 (43.3%)	**<.001**
Median duration of intubation, days (IQR)	26.0 (34.0)	27.0 (28.0)	.981
Death, *n* (%)	74 (21.2%)	62 (32.0%)	**.006**
COVID-19-related death, *n* (%)	57 (16.3%)	56 (28.9%)	**.001**
Liver-related death, *n* (%)	8 (2.3%)	16 (8.2%)	**.001**

p-values depicting statistically significant differences are presented as bold values.

TABLE 3 Independent risk factors for mortality in COVID-19 patients between 40 and 69 years old. Next to the univariate analysis (i), multivariate models including (ii) aspartate transaminase (AST), (iii) gamma-glutamyl-transferase (GGT) and (iv) bilirubin at blood withdrawal after the first positive SARS-CoV-2 PCR are shown

Parameter of interest	HR	95% CI	*p*-value
(i) univariate (unadjusted) analysis			
Alkaline phosphatase, >ULN	1.54	0.89–2.67	.121
Aspartate transaminase, >ULN	2.10	1.25–3.51	**.005**
Alanine aminotransferase, >ULN	0.89	0.51–1.55	.671
Gamma-glutamyl transferase, >ULN	2.31	1.31–4.07	**.004**
Bilirubin, >ULN	2.35	1.27–4.35	**.007**
Liver disease (present vs. absent)	2.56	1.41–4.65	**.002**
Age, 10 years	2.04	1.40–3.00	**<.001**
Sex (male)	1.94	1.12–3.36	**.019**
Creatinine, mg × dl^{-1}	1.16	1.01–1.34	**.040**
Albumin, g × L^{-1}	0.91	0.88–0.95	**<.001**
Obesity (yes)	1.17	0.68–2.01	.572
Diabetes mellitus (yes)	1.13	0.64–2.00	.682
Cardiovascular disease (yes)	1.18	0.65–2.14	.596
Lung disease (yes)	1.68	0.96–2.94	.070
Malignancy (yes)	1.56	0.89–2.73	.120
(ii) multivariate (adjusted) model including AST			
Aspartate transaminase, >ULN	1.78	1.04–3.06	**.037**
Liver disease (present vs. absent)	1.73	0.90–3.31	.100
Age, 10 years	1.37	0.93–2.01	.107
Sex (male)	1.52	0.83–2.79	.173
Creatinine, mg × dl^{-1}	1.06	0.91–1.25	.445
Albumin, g × L^{-1}	0.92	0.88–0.96	**<.001**
Lung disease (yes)	0.79	0.41–1.54	.484
(iii) multivariate (adjusted) model including GGT			
Gamma-glutamyl-transferase, >ULN	0.88	0.45–1.74	.719
Liver disease (present vs. absent)	1.93	1.03–3.63	**.040**
Age, 10 years	1.33	0.91–1.95	.139
Sex (male)	1.66	0.91–3.04	.099
Creatinine, mg × dl^{-1}	1.04	0.89–1.22	.617
Albumin, g × L^{-1}	0.93	0.89–0.97	**<.001**
Lung disease (yes)	0.85	0.44–1.66	.643
Age, 10 years	1.37	0.93–2.01	.107
(iv) multivariate (adjusted) model including bilirubin			
Bilirubin, >ULN	2.18	1.15–4.13	**.017**
Liver disease (present vs. absent)	1.65	0.86–3.14	.130
Age, 10 years	1.49	0.81–2.73	.186
Sex (male)	1.52	0.83–2.79	.199
Creatinine, mg × dl^{-1}	1.07	0.91–1.26	.431
Albumin, g × L^{-1}	0.93	0.89–0.96	**<.001**
Lung disease (yes)	0.81	0.42–1.55	.525

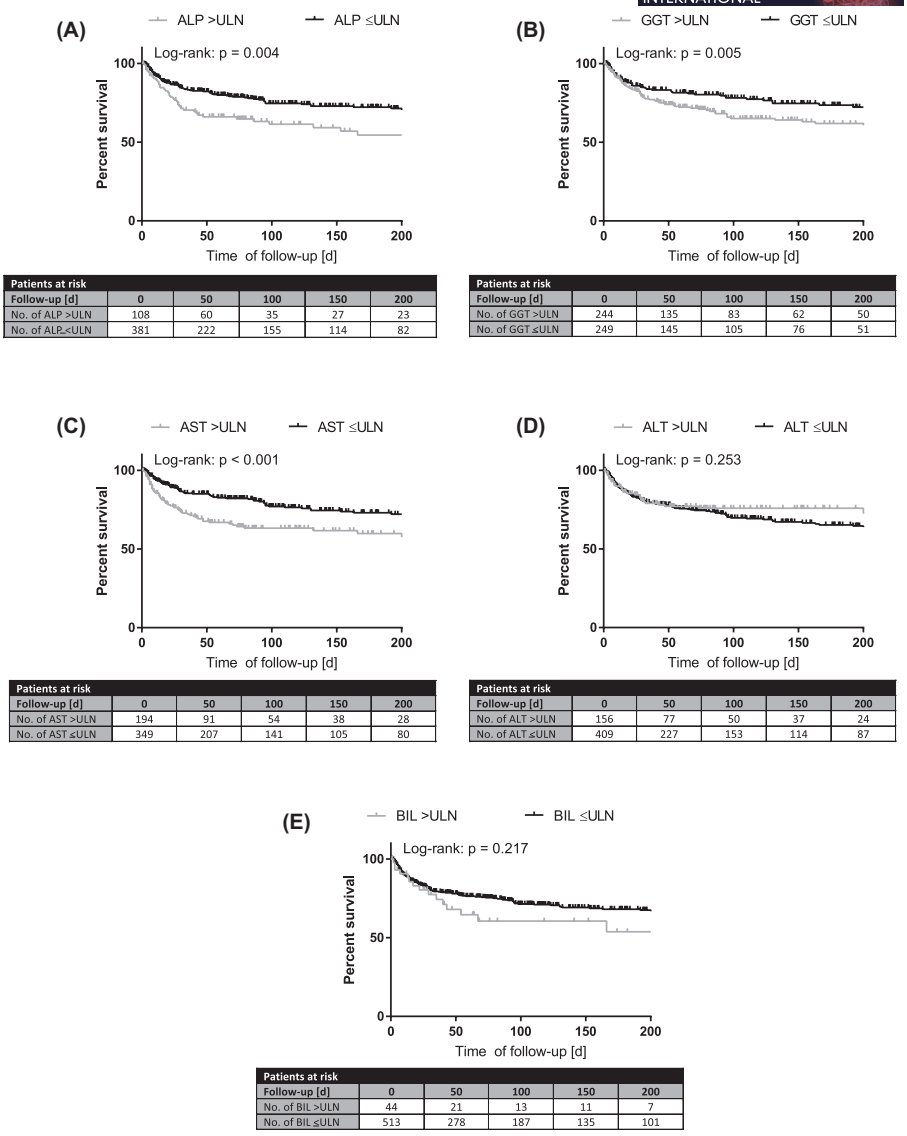
p-values depicting statistically significant differences are presented as bold values.

Similar to previous studies,[12,18,20] we observed COVID-19-related liver injury at blood withdrawal after the first positive SARS-CoV-2 PCR test in approximately 10% of patients. Interestingly, in our cohort pronounced elevation of liver chemistries (i.e. AST/ALT >3xULN and ALP/GGT/BIL >2xULN) were particularly frequent in 40–69 years old patients. Consequently, this was also the patient group with the highest rate of COVID-19-related liver injury (16.0%) – even if D-dimer and CRP levels as established parameters for COVID-19 severity[5,23,24] did not differ between 40–69 years old and ≥70 years old patients. However, the higher rate of obesity (44.4%) in the 40–69 years old patients suggests that there could be a considerable proportion of patients with hepatic steatosis, that is undiagnosed NAFLD, in this age group that could predispose to COVID-19-associated liver injury.

Interestingly, the rate of cholestatic liver injury (i.e. elevated ALP/GGT/BIL) was particularly high in our cohort (45.0% at the time of the first blood withdrawal after the positive PCR test), as compared to previous reports.[12,13,18] However, most data on cholestatic liver injury was derived from Asian patients, while large European and American studies often neither assessed ALP, nor GGT, nor BIL.[25,26] Thus, there may be differences in the proportion of elevation in these parameters of cholestasis between Asian and European patients. One may speculate that prolonged cholestatic injury is an "early" or "mild" version of the full spectrum of cholestatic disease described as COVID-19-associated sclerosing cholangitis after severe SARS-CoV-2 infection, that may even require liver transplantation.[3,27–29] Further studies should assess the best strategy to follow up patients with ALP elevation after COVID-19.

Mortality, as well as COVID-19-related mortality, was highest among the oldest patient cohort (≥70 years), which confirms older age as an important risk factor for mortality in COVID-19.[4,5,26,30,31] Correspondingly, the hospital admission rate was also highest in SARS-CoV-2-infected patients ≥70 years of age. In contrast, there was not a single death recorded for COVID-19 patients 18–39 years of age.

While overall mortality increased with age, liver-related mortality was highest in patients aged 40–69 years old. This fits the observation of more frequent liver injury in this patient cohort and



Patients at risk					
Follow-up [d]	0	50	100	150	200
No. of ALP >ULN	108	60	35	27	23
No. of ALP ≤ULN	381	222	155	114	82

Patients at risk					
Follow-up [d]	0	50	100	150	200
No. of GGT >ULN	244	135	83	62	50
No. of GGT ≤ULN	249	145	105	76	51

Patients at risk					
Follow-up [d]	0	50	100	150	200
No. of AST >ULN	194	91	54	38	28
No. of AST ≤ULN	349	207	141	105	80

Patients at risk					
Follow-up [d]	0	50	100	150	200
No. of ALT >ULN	156	77	50	37	24
No. of ALT ≤ULN	409	227	153	114	87

Patients at risk					
Follow-up [d]	0	50	100	150	200
No. of BIL >ULN	44	21	13	11	7
No. of BIL ≤ULN	513	278	187	135	101

FIGURE 3 Overall survival binary for elevated/non-elevated plasma levels of (A) alkaline phosphatase (ALP), (B) gamma-glutamyl transferase (GGT), (C) aspartate transaminase (AST), (D) alanine aminotransferase (ALT) and (E) bilirubin at blood withdrawal after the first positive SARS-CoV-2 PCR. Survival comparison by log-rank test

indicates that abnormalities of liver chemistries translate into worse liver-related outcomes. Thus, 40–69 years old patients with abnormal liver chemistries at COVID-19 diagnosis should be closely monitored, since they are at a particularly high risk for a severe course and worse outcomes.

Stratified for the severity of pre-existing liver disease, liver-related mortality was highest in dACLD patients followed by non-ACLD and cACLD patients. This is in line with previous studies, which indicated an increased risk of liver-related complications particularly in dACLD patients.[32,33] However, importantly, we also demonstrated that a small percentage (1.7%) of patients without pre-existing liver disease also died of liver-related causes, confirming that severe liver function impairment and acute liver failure is a rare but significant complication of COVID-19.[3]

Our observed association of elevated AST and BIL levels at COVID-19 diagnosis with mortality is in line with previous studies[18,34] reporting the prognostic value of AST and BIL in patients with COVID-19. Especially elevated AST at the first blood withdrawal after the first SARS-CoV-2 PCR test seems to predict a severe course of COVID-19,[35,36] since AST was associated with more frequent hospital admission, ICU admission and intubation in all age strata. In 40–69 years old patients, it was also linked to higher overall mortality, COVID-related death and liver-related death.

Our study also has limitations: firstly, due to the retrospective study design, selection bias cannot be ruled out. Second, not all parameters were available for all patients at all time points. However, the findings of this study are in line with the existing literature and the missing data is mostly attributable to patients without hospital admission. Thus, we are confident that our data is reflecting the clinical scenario of hospitalized patients with COVID-19. Thirdly, due to our monocentric study design, external validation of our results is required. Of note, this study mostly included unvaccinated patients

infected with early variants of SARS-CoV-2. In the light of recent developments, further studies are needed to re-evaluate the prevalence and prognostic impact of liver chemistry elevation in vaccinated patients or patients infected with the latest variants of the virus.

In conclusion, our large-scaled Austrian COVID-19 cohort study identified 40–69 years old patient as a particular risk group for liver injury of both hepatocellular and cholestatic patterns and for liver-related mortality. Elevated AST and BIL levels at COVID-19 diagnosis were an independent predictor of mortality, especially in patients aged 40–69 years.

DECLARATION

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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