Longitudinal analysis of the utility of liver biochemistry in hospitalised COVID-19 patients as prognostic markers

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Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine transaminase; APTT, Activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, Body mass index; BR, bilirubin; CHD, Coronary heart disease; CIs, confidential intervals; CKD, Chronic kidney disease; CRP, C-reactive protein; DM, Diabetes mellitus; GGT, Gamma-glutamyl transferase; HIC, Health Informatics Collaborative; HRs, hazard ratios; HTN, Hypertension; ICU, intensive care unit; INR, international normalised ratio; IQR, Interquartile range; K-M, Kaplan-Meier; LLN, Lower limit of normal; NIHR, National Institute for Health Research; NHS, National Health Service; OUH, Oxford University Hospitals; PT, prothrombin time; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SD, standard deviation; ULN, Upper limit of normal.

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Abstract

Background & Aims: The association of liver biochemistry with clinical outcomes of SARS-CoV-2 infection is currently unclear and the utility of longitudinally-measured liver biochemistry as prognostic markers for mortality is unknown. We aimed to determine whether abnormal liver biochemistry, assessed at baseline and at repeat measures over time, was associated with death in hospitalised patients with COVID-19 compared to those without COVID-19, in a UK population.

Approach & Results: We extracted routinely collected clinical data from a large teaching hospital in the UK, matching 585 hospitalised SARS-CoV-2 RT-PCR-positive patients to 1165 hospitalised RT-PCR-negative patients for age, gender, ethnicity and pre-existing comorbidities. 26.8% (157/585) of COVID-19 patients died, compared to 11.9% (139/1165) in the non-COVID-19 group (p<0.001). At presentation, a significantly higher proportion of the COVID-19 group had elevated alanine aminotransferase (20.7% vs. 14.6%, p=0.004) and hypoalbuminaemia (58.7% vs. 35.0%, p<0.001), compared to the non-COVID-19 group. Within the COVID-19 group, those with hypoalbuminaemia at presentation had 1.83-fold increased hazards of death compared to those with normal albumin (adjusted HR 1.83, 95% CI 1.25-2.67), whilst the hazard of death was ~4-fold higher in those aged ≥75 years (adjusted HR 3.96, 95% CI 2.59-6.04) and ~3-fold higher in those with pre-existing liver disease (adjusted HR 3.37, 95% CI 1.58-7.16). In the COVID-19 group, alkaline phosphatase (ALP) increased (R=0.192, p<0.0001) and albumin declined (R=-0.123, p=0.0004) over time in patients who died.

Conclusion: In this UK population, liver biochemistry is commonly deranged in patients with COVID-19. Baseline hypoalbuminaemia and rising ALP over time could be prognostic markers for death but investigation of larger cohorts is required to develop a better understanding of the relationship between liver biochemistry and disease outcome.
Within a year of the first case being reported there had been over 100 million confirmed cases of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and ~2.5 million deaths have been reported globally by the end of February 2020; the UK is one of the worst affected countries with over 4 million confirmed cases and over 121,000 deaths reported in this time period (1). The clinical syndrome caused by SARS-CoV-2, COVID-19, primarily affects the respiratory system but other organs, including the heart, gastrointestinal tract and liver may be affected, and a systemic sepsis syndrome may develop (2).

Data on liver biochemistry in COVID-19 patients have been reported from China, the USA and Italy. These studies report that 37-69% of patients with COVID-19 had at least one abnormal liver biochemistry on hospital admission (3-9) while 93% had at least one abnormal liver biochemistry over the course of disease (6). Specially, the prevalence estimates of elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin (BR) in hospitalised COVID-19 patients are 9%-28%, 14%-35% and 6%-23%, respectively (3-6, 10-12). Studies reported that liver biochemistry abnormalities are associated with longer hospital stay (4), or clinical severity (3, 12, 13), whereas other studies have not found a relationship between liver biochemistry and severity (10). The set of liver biochemistry tests reported for COVID-19 patients varies: ALT, AST, and total BR are typically included, with alkaline phosphatase (ALP) and Gamma-glutamyl transferase (GGT) less frequently reported.

Albumin is a non-specific marker of liver function, and has been less consistently assessed; it is typically reported in baseline patient characteristics, with limited investigation of its utility as a prognostic marker. However, a recent meta-analysis of 20 retrospective cohort studies from China reported lower baseline albumin levels in patients with severe COVID-19 compared to mild cases, but with significant heterogeneity between studies (14). Another meta-analysis demonstrated that hypoalbuminaemia could be included in prognostic machine learning models to predict severe COVID-19 or mortality (15).

Several studies have investigated potential associations between liver biochemistry and death in COVID-19 patients (6, 8-11, 16, 17), or included liver biochemistry in the development of predictive models (18-21). A report from Italy showed that ALP >150 U/L at hospital admission (without adjusting for relevant confounders) was associated with clinical deterioration in 292 COVID-19 patients (10), and another study from the USA reported that peak ALT >5 times upper limit of normal (ULN) during admission was associated with death in a cohort of 2,273 COVID-19 patients (11). However, another USA study reported that elevations in ALT and AST elevation on
admission were associated with length of stay, intensive care unit (ICU) admission and intubation but not death (6). Studies from Wuhan, China did not find associations of ALT (18, 19) or AST (19) elevation on admission with death in COVID-19 patients, whilst several studies have reported associations of elevated total bilirubin (16, 19) and low albumin on admission (19-21) with risk of death. Other studies considered abnormality of liver tests as one composite variable (7) or a composite endpoint (ICU admission or death) as the primary outcome (8, 9, 17), without specifying which individual liver test abnormalities are associated with the death endpoint. Given these variable associations between liver biochemistry and COVID-19 outcomes, the prognostic value of liver biochemistry derangement in COVID-19 needs further evaluation.

Having established a clinical data pipeline through the National Institute for Health Research (NIHR) Health Informatics Collaborative (HIC) (22, 23), our tertiary referral hospital in the UK is strongly placed to undertake analyses using electronic health data from hospitalised patients. Using this resource, we aimed to determine the prevalence of deranged liver biochemistry at baseline and over the disease course in COVID-19 patients, with comparison to a matched group of non-COVID-19 patients admitted during the same period. We also aimed to determine whether baseline liver biochemistry derangement was associated with risk of death in COVID-19 patients, and to compare longitudinal changes in liver biochemistry between COVID-19 patients who died and who survived.

Methods

Data collection

We used routinely collected clinical data from Oxford University Hospitals (OUH) National Health Service (NHS) Foundation Trust, a large teaching hospital trust in the South East of the UK, with ~1000 in-patient beds. The data are collected by the local NIHR HIC team in Oxford, being drawn automatically from operational systems into a data warehouse and linked to produce a comprehensive record for each patient with a data validation process, as previously described in our HIC methods paper (23). The management of the dataset is governed by the NIHR HIC Data Sharing Framework. All of the data used for this study were provided in anonymised form by OUH NHS Foundation Trust, with the prior approval of the Trust Information Governance Team, following the satisfactory completion of a Data Protection Impact Assessment.
The data extracted for this study included detailed information on demographics, body mass index (BMI), emergency admissions, blood test results, diagnostic codes, procedures, ICU admission, prescriptions, medicines administration, and discharge destination/outcome for all patients admitted to OUH between 1st January 2020 and 21st August 2020.

Inclusion and exclusion criteria
To select eligible data for adults with/without COVID-19, the inclusion criteria were: (a) at least one RT-PCR nose/throat swab having been undertaken, which is the validated clinical tests being deployed through our hospital diagnostic microbiology laboratory (COVID-19 patients were defined by at least one positive result; COVID-19-negative patients were defined by the absence of a positive result); (b) age ≥ 18 years when tested; (c) hospitalised patients; and (d) at least one episode of liver biochemistry recorded at the time or after the RT-PCR test (the set of liver biochemistry routinely tested by OUH clinical biochemistry laboratory comprises ALT, ALP, albumin, and BR; AST and GGT are not routinely tested). Exclusion criteria were: (a) SARS-CoV-2 RT-PCR test results reported as invalid; (b) missing age; and (c) pregnancy (antenatal/delivery/post-partum). Patients were followed until death, or until the last available clinical record.

Definitions
We defined baseline as the date of the first positive SARS-CoV-2 RT-PCR test for a COVID-19 patient, and the date of the first negative test for a patient without COVID-19.

The normal ranges set by the hospital biochemistry lab are: ALT: 10-45 IU/L; ALP: 30-130 IU/L; BR: 0-21 umol/L; Albumin: 32-50 g/L. The reference ranges for other blood tests are provided in Supporting Table S1. We defined baseline liver biochemistry (ALT, ALP, BR, or albumin) as the liver biochemistry measured within 7 days of SARS-CoV-2 RT-PCR test and baseline derangement as at least one abnormal result at this time point. We defined peak/nadir liver biochemistry derangement as abnormality of the highest/lowest value recorded respectively at any point during follow-up. We defined liver biochemistry recovery as normalisation following derangement. The primary outcome was death during follow-up, and secondary outcomes included ICU admission and invasive ventilation.

Pre-existing comorbidities were defined by a historical diagnosis of a disease before baseline, and diagnosis codes retrieved were provided in Supporting Table S2. A full list of prescribed drugs searched for data extraction is provided in Supporting Table S3.
**Statistical analysis**

Statistical analysis was performed using R version 4.0.2. All significance tests performed were two-sided. \( p \) values <0.05 were deemed statistically significant.

**Propensity Score Matching**

We identified eligible adults, and conducted propensity score matching process to ensure the COVID-19 group was comparable to the non-COVID-19 group in terms of demographics and pre-existing conditions. We used the following variables to calculate propensity scores: age, gender, ethnicity, and pre-existing comorbidities (liver disease, diabetes mellitus (DM), hypertension (HTN), coronary heart disease (CHD), chronic kidney disease (CKD), and cancer). We performed propensity score matching using the package *MatchIt*, with the nearest-neighbour method applied, the matching ratio and calliper size set as 1:2 and 0.1 respectively, and without replacement.

**Comparison of COVID-19 and matched non-COVID-19 patients**

For continuous variables, we calculated median and interquartile range (IQR), or mean and standard deviation (SD), and used Wilcoxon test or t-test for comparison. For categorical variables, we computed number and percentage, and used chi-square or Fisher’s exact test for comparison. We used the Shapiro-Wilk test and graphical methods for normality check. We further stratified COVID-19 patients by the severity of disease into mild/moderate and severe/critical subgroups (using respiratory rate and oxygen saturation thresholds of \( \leq 30 \) breaths/min and \( \geq 90\% \), respectively, for mild/moderate illness; and >30 breaths/min and <90\%, respectively, for severe illness, as established by the WHO) (24) and then compared liver biochemistries and outcomes of each COVID-19 severity subgroup to those of non-COVID-19 group.

**Investigation on whether liver biochemistry predict outcomes in COVID-19 patients**

We compared the presence of clinical outcomes in the COVID-19 and non-COVID-19 groups. We also performed Kaplan-Meier (K-M) analysis to compare the survival probability over time between the two groups. Within the COVID-19 and non-COVID-19 groups, we compared demographics, BMI, comorbidities, baseline and peak/nadir liver biochemistry between those who died vs. survived. We then performed K-M analysis to compare the survival probabilities over time between subgroups with and without deranged baseline liver biochemistry. We used
univariate and multivariate Cox proportional-hazards models to investigate whether liver biochemistry predicted death, reporting hazard ratios (HRs) and 95% confidential intervals (CIs). Variables with p<0.1 in univariate analysis and/or clinically important parameters (demographics, comorbidities and drug use) were included for the final multivariate model. To investigate associations of additional patient characteristics with risk of death, and the robustness of HRs to adjustment for additional confounders, we performed sensitivity/subset analysis whereby associations were investigated in a subset of patients who were not missing data for confounders. In addition, we performed receiver operating characteristic (ROC) analysis on baseline liver biochemistry to examine predictive ability for COVID-19 death, reporting the area under an ROC curve (AUC), sensitivity, and specificity.

Longitudinal analysis of liver biochemistry in COVID-19 patients

We compared liver biochemistry between the COVID-19 group and non-COVID-19 group at each time point (to examine difference between groups), as well as investigating liver biochemistry changes over time by comparing liver biochemistry at subsequent time points to their baseline within each group. Within the COVID-19 group, we firstly examined the changing pattern over time of liver biochemistry in subgroups of patients who died and survived by fitting linear regression lines with 95% CIs, and reporting Pearson’s correlation coefficients and linear regression significance, as applied by other longitudinal analyses (25). We then used mixed effects models (lme4, sjPlot packages) (26, 27) to test if the change of liver biochemistry is significant, by considering fixed effects (the follow-up time, subgroup, and subgroup-by-time interaction) and random effect intercept for individuals.

Results

Identification, demographics, and outcomes of COVID-19 compared to non-COVID-19 groups

We identified 6311 eligible patients (585 adults with SARS-CoV-2 infection and 5726 without) according to the inclusion/exclusion criteria (Supporting Figure S1). Based on our 585 COVID-19-positive patients, we matched a cohort of 1165 COVID-19-negative patients. After matching, there were no significant differences in demographics and pre-existing comorbidities between the two groups (Table 1; Supporting Table S4). Median duration of follow-up was 58 [IQR: 14-104] days in the COVID-19 group and 50 [IQR: 20-78] days in the matched non-COVID-19 group, with no significant difference in the monitoring duration of liver biochemistry (interval between first and
last-available liver biochemistry test dates) between the groups (Table 1). Within the COVID-19 group, median follow-up duration was 10 days [IQR: 5-21] for those who died, and 79 days [IQR: 36-115] for those who survived. Admitting specialties are summarised in Supporting Table S5.

In patients with COVID-19, 26.8% (157/585) died compared to 11.9% (139/1165) in the non-COVID-19 group (p<0.001). The COVID-19 group had a higher rate of ICU admission (12.1% vs. 4.3%, p<0.001), and a higher rate of invasive ventilation use (8% vs. 2.5%, p<0.001) (Table 1). The K-M estimated probability of surviving >30 days after SARS-CoV-2 RT-PCR test was 77% for COVID-19 vs. 92% for non-COVID-19 patients (Figure 1).

Assessment of liver biochemistry in the COVID-19 compared to non-COVID-19 group

Baseline liver biochemistry was available in 492 COVID-19 and 974 non-COVID-19 patients. The median time interval between consecutive liver biochemistry measurements was 1 [IQR: 1-4] day and 2 [IQR: 1-7] days (p<0.001), for COVID-19 and non-COVID-19 groups respectively.

Overall, the COVID-19 group had a significantly higher proportion of patients with ≥1 deranged liver biochemistry test at baseline compared to the non-COVID-19 group (72.6% vs. 55.5%, p<0.001) (Supporting Table S6). At baseline, the COVID-19 group had a significantly higher median ALT value (25 IU/L vs. 19 IU/L, p<0.001) and a higher proportion of patients with ALT >ULN (20.7% vs.14.6%, p=0.004) than the non-COVID-19 group (Table 1). The COVID-19 group also had a lower median albumin (30 g/L vs. 34 g/L, p<0.001), lower platelets (216 x10^9/L vs. 245 x10^9/L, p<0.001), lower lymphocytes (0.9 x10^9/L vs. 1.2 x10^9/L, p<0.001) and a significantly higher CRP (78 mg/L vs. 20 mg/L, p<0.001), as compared to the non-COVID-19 group. Baseline vital signs were also more deranged in the COVID-19 group, whilst renal function was preserved (Table 1).

Over follow-up, the COVID-19 group had more deranged liver biochemistry, with a higher median peak ALT (34 IU/L vs. 26 IU/L, p<0.001), a higher proportion with peak ALT >ULN (37.9% vs. 27.7%, p<0.001), a lower median nadir albumin (26 g/L vs. 29 g/L, p<0.001), and a higher prevalence of hypoalbuminaemia (79.0% vs. 59.5%, p<0.001) compared to the non-COVID-19 group (Supporting Table S6). COVID-19 patients also had significantly higher median ALT and lower median albumin values at time points throughout follow-up (7, 14, 21, and 28 days), compared to the non-COVID-19 group (all p<0.05) (Figure 2A-B). In the COVID-19 group, median ALT increased at 7 and 14 days, compared to baseline (both p<0.05) (Figure 2C), and median albumin decreased at 7 days (p<0.0001) and remained at low levels at subsequent time...
points (Figure 2D). We did not identify differences in ALP and bilirubin over time between these groups, other than at baseline or at 7 days (Supporting Figure S2).

Baseline vital signs were recorded for 423 patients with COVID-19, and used to stratify disease severity, with 65 severe/critical COVID-19 cases and 358 mild/moderate cases. As expected, a higher rate of death was observed in severe/critical cases compared to mild/moderate cases (43.1% vs. 27.1%, p=0.01). A higher proportion (31% vs. 19%, p=0.04) in the severe/critical group had elevated ALT at baseline. However, there were no other significant differences in liver biochemistry between mild/moderate and severe/critical cases at baseline or during follow up (Table 2).

Compared to the non-COVID-19 group, both the mild/moderate COVID-19 subgroup and severe/critical COVID-19 subgroup had a significantly higher prevalence of abnormal liver biochemistry both at baseline and during follow-up, with significantly higher rates of mortality, ICU admission rate, and the proportion requiring invasive ventilation (Supporting Table S7, S8). These results were consistent with those from the comparison between the whole COVID-19 group and the non-COVID-19 group.

**Demographics, comorbidities and liver biochemical characteristics associated with mortality in COVID-19 patients**

In the COVID-19 group, patients who died were significantly older than those who survived (median 82 years vs. 66 years respectively), significantly more likely to have pre-existing comorbidities including liver disease, DM, CHD and cancer, significantly more likely to have baseline hypoalbuminaemia (72.6% vs. 52.9%, p<0.001) and nadir albumin below the lower limit of the normal range during follow-up (96.2% vs. 72.7%, p<0.001) (Table 3). In patients who died, baseline ALP was higher (p=0.007) and peak BR was more likely to be abnormal compared to those who survived (p=0.016, Supporting Table S9). Equivalent data for the non-COVID-19 group are provided in Supporting Table S10.

Survival curves and logrank tests (unadjusted for relevant confounders) within the COVID-19 group showed that hypoalbuminaemia compared to a normal albumin at baseline was associated with an increase in mortality (Figure 3A). Surprisingly an elevated baseline ALT, compared to a normal ALT at baseline was associated with an increase in survival (Figure 3B). An elevated ALP or bilirubin at baseline were not significantly associated with a lower survival probability over time (Figure 3C-D).
242 (PT) measured, elevation of this parameter (and thus of INR) at baseline was significantly associated with a lower survival probability (Supporting Figure S3).

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245 In multivariate analysis (fully adjusted for demographics, comorbidities, and prescribed drug use before baseline) for the COVID-19 group, those with hypoalbuminaemia (i.e., <32 g/L) at baseline had a 1.83-fold-increased hazards of death compared to those with normal baseline albumin (adjusted HR 1.83, 95% CI 1.25-2.67). Those aged ≥75 years had a ~4-fold-increased hazards of death compared to those aged <75 years (adjusted HR 3.96, 95% CI 2.59-6.04), and those with pre-existing liver disease had a ~3-fold-increased hazards of death than those without pre-existing liver disease (adjust HR 3.37, 95% CI 1.58-7.16) (Table 4). However, we found no significant association between baseline ALT and hazards of death (adjusted HR 0.86, 95% CI 0.53-1.38, p=0.53) in fully adjusted analysis (Table 4). A one unit (1 g/L) decrease in albumin at baseline was associated with a 5% increase in hazards of death (adjusted HR 1.05, 95%CI 1.02-1.09), while age increase by 10 years was associated with 82% increase in hazards of death (adjusted HR 1.82, 95% CI 1.56-2.13) (Supporting Table S11). Similarly, a one unit decrease in nadir albumin during follow-up was associated with a 7% increase in hazards of death (adjusted HR 1.07, 95% CI 1.04-1.10) (Supporting Table S12). In the COVID-19 group, baseline albumin was significantly negatively correlated with age in those that survived (R=-0.264, p<0.0001) but not in those who died (R=-0.027, p=0.75) (Supporting Figure S4).

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247 The ROC analysis further demonstrated the prognostic value of baseline albumin for COVID-19 death, which has higher overall performance (AUC=0.642) compared to that of baseline ALP, BR or ALT (Figure 4A). The combination of baseline liver biochemistry parameters does not significantly improve the performance (AUC=0.659, p=0.08) (Figure 4A, Supporting Tables S13). Considering the other predictors (age and liver disease) identified from the multivariate model, we found adding albumin can further improve prediction of death (AUC significantly improved from 0.711 to 0.752, p=0.002, Figure 4B, Supporting Tables S14). Adding other baseline liver biochemistry parameters did not make further significant improvement (Figure 4B).

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249 For the subset of COVID-19 patients who had BMI data (survived vs. died, 246 vs. 96), HRs did not change materially after additional adjustment for BMI in the multivariate analysis (Supporting Table S15). Baseline albumin was weakly correlated with BMI both in those who died and survived (R=0.08, p=0.42 vs. R=0.099, p=0.12, respectively) (Supporting Figure S5).
Longitudinal assessment of liver biochemistry patterns in patients who died with COVID-19, compared to those who survived.

Within the COVID-19 group, patients who died during follow-up had significantly lower median albumin at baseline, 7 and 14 days after a positive SARS-CoV-2 RT-PCR, compared to the patients who survived (all p<0.001) (Figure 5A). There was no significant difference in ALT at any time point other than 7 days between those who died and survived (Figure 5B). For patients who died, ALP was higher at baseline and 28 days and BR was higher at 7 days compared to those who survived (all p<0.05) (Figure 5C-D).

In the COVID-19 group, ALT increased during the first two weeks (p<0.05) and remained elevated in patients who died (R=-0.020, p=0.6), whilst in patients who survived ALT decreased from 7 days onward with a significantly decreasing trend to normalisation (R=-0.101, p<0.0001) (Figure 6A-B). ALP significantly increased over time in those who died (R=0.192, p=0.0001), compared to those who survived (R=0.032, p=0.068) (Figure 6C-D). Albumin decreased significantly in both groups during the first 7 days and continued to decline in patients who died (R=-0.123, p=0.0004), but in patients who survived had an upward trend especially for follow up >1 month (R=0.311, p<0.0001) (Figure 6E-F). BR decreased throughout follow-up in both subgroups but the decline was not significant (R=-0.052, p=0.146; R=-0.013, p=0.483) (Supporting Figure S6A-B).

Consistently, the mixed effects model also showed that within the subgroup who died, albumin significantly decreased over time ($\beta=-0.12$, 95%CI: (-0.14, -0.10), p<0.001) while ALP significantly increased over time ($\beta=0.50$, 95%CI: (0.29, 0.70), p<0.001), but the change in ALT or BR was nonsignificant (p=0.817, p=0.489, respectively) (Supporting Table S16). Considering the interaction between subgroup and time, the mixed effects model further revealed that: (a) changes in albumin were significantly different over time between subgroups who died vs. survived (p<0.001), with decrease in the subgroup who died ($\beta_{time} + 1*\beta_{interaction}=-0.11$) and increase in the subgroup who survived ($\beta_{time} + 0*\beta_{interaction}=0.08$) based on the coefficients of time and interaction; (b) changes in ALP were also significantly different over time for the subgroups (p=0.005), increasing in the subgroup who died ($\beta_{time}+1*\beta_{interaction}=0.50$) whilst decreasing in the subgroup who survived ($\beta_{time}+0*\beta_{interaction}=-0.07$); (c) changes in ALT (p=0.126) or BR (p=0.356) were insignificantly different over time for the subgroups who died and survived (Table 5).
Among COVID-19 patients who had ≥2 longitudinal data points for ALT, ALP, BR and albumin, 468 had at least one liver biochemistry derangement during follow-up. Among them 26.9% (126/468) had normalised by the end of follow-up, while the remaining 73.1% still had at ≥1 abnormal liver biochemistry at the end of follow-up (Supporting Table S17).

Discussion

Novelty and key findings

We used an automatic approach via an established NIHR HIC clinical data collating bioinformatic pipeline (22, 23) to capture a complete record of relevant clinical and laboratory parameters from hospitalised patients within the time period assessed. This resource allows us for the first time to fully analyse liver biochemistry abnormalities and outcome on a large cohort of patients with COVID-19 and propensity scoring matched non-COVID-19 controls. To our knowledge this is the first study to comprehensively: i) conduct longitudinal analyses of liver biochemistry patterns over time in COVID-19 patients compared to a matched cohort of non-COVID-19 patients attending hospital in the UK, ii) investigate whether albumin, in addition to other liver biochemistries, at baseline and during follow-up is associated with death in COVID-19 patients, and iii) analyse longitudinal liver biochemistry patterns in COVID-19 patients who subsequently die or survive.

The COVID-19 group exhibited a ~2-fold higher death rate, with a significantly lower survival probability, compared to the non-COVID-19 group. Among the COVID-19 group, a higher proportion of patients had at least one abnormal liver biochemistry, compared to the matched non-COVID-19 group. Patients with COVID-19 who died showed a decline in albumin and a greater increase in ALP over time compared to those who survived, and baseline hypoalbuminaemia was a significant predictor of death in COVID-19 patients on multivariate analysis with adjusting for relevant confounders.

Comparison to previous studies

In our study, rates of baseline and peak ALT derangement between COVID-19 and non-COVID-19 groups were significantly different, consistent with findings from a large USA cohort (11). The increase in ALT between baseline and at 14 days follow-up in the COVID-19 group is also consistent with previous reports (6).
Patients with pre-existing liver disease had an increased risk of mortality in COVID-19 which is consistent with the findings of previous studies (28, 29), although numbers with pre-existing liver disease in our study were small. Because some COVID-19 patients in our cohort were admitted to hospital for non-COVID-19 illnesses we analysed baseline liver biochemistry at the time of the SARS-CoV-2 RT-PCR rather than date of admission. This may partially explain differences between our study and previous studies which analysed liver biochemistry measured on hospital admission (3-6). Variable patterns of treatment between cohorts may also account for differences.

Although baseline hypoalbuminaemia was significantly associated with hazards of death in COVID-19, ALP and BR were not. Recent findings from two multi-centre studies support the prognostic association of albumin with death in COVID-19 (21, 30) and a prospective study also shows albumin is associated with a composite endpoint (ICU admission or death) (17). However, other studies of liver biochemistry in COVID-19 did not investigate albumin (6, 10, 11). Interestingly, a previous study found that hypoalbuminemia is a strong predictor of 30-day all-cause mortality in acutely admitted medical patients (31). The mechanism of the effect of hypoalbuminaemia is not certain; it may reflect the broad association between low albumin and critical illness, or be a marker for a characteristics of host (e.g. nutritional status, co-morbidity), or disease phenotype (e.g. immune activation) (32). As albumin is a cheap and widely available test, it can be usefully employed as a prognostic biomarker.

Due to variable population settings in previous studies (e.g., demographics, comorbidities), it is important to understand the populations in which prognostic models are developed (15) to ensure such models are externally valid and to also adjust for relevant confounders. In our cohort, a univariate Cox proportional-hazards model and K-M curve analysis suggests that an elevation in baseline ALT is weakly associated with lower hazards of death from COVID-19. However, the univariate analysis is unadjusted and may be confounded by additional factors. Interestingly, in a US cohort of 60 hospitalised COVID-19 patients, ALT at admission was also higher in those who survived compared to those who died (6). In our multivariate analysis, the association of baseline ALT and hazards of death was attenuated towards the null and became nonsignificant, after full adjustment for demographics, comorbidities, and prescribed drug use before baseline. Therefore, larger cohorts are warranted to investigate this unexpected association with more confidence. In the sensitivity analysis of COVID-19 patients stratified by disease severity, severe/critical cases were more likely to have elevated ALT at baseline compared to mild/moderate cases, and were more likely to die compared to mild/moderate cases, in line with previous studies (3, 12, 13).
The observed association of raised ALT with lower hazards of death is intriguing since recent data have suggested that genetic predisposition to fatty liver disease, conferred via possession of the PNPLA3 I148M variant (which is highly prevalent in the UK population) (33) is associated with both elevated ALT and a concomitant reduction in systemic inflammation (as measured by CRP), as well as preservation of albumin levels during COVID-19 (34). As such, elevated ALT at baseline may be a surrogate for non-alcoholic fatty liver disease (NAFLD) in a subset of patients; those with NAFLD may have a lower risk of severe COVID-19 outcomes, but additional studies are required to confirm this association and the underlying mechanism is not understood.

A study from the US (11) reported a positive association of ALT >5 times ULN with death, however we were unable to replicate this investigation as only a small number of participants had ALT elevated to this level; furthermore, the prevalence of comorbid disease and BMI >35kg/m² in the US study population was much higher compared to our cohort. Similarly, a large primary care cohort study (35) reported that BMI >40kg/m² was associated with an increased risk of COVID-19-related death, but we were underpowered to replicate this analysis.

**COVID-19 and liver biochemistry derangement**

Although derangements in liver biochemistry are common in COVID-19 patients, the reasons for the liver injury remain unclear, but may include direct viral damage, drug-induced liver injury, hypoxia, immune-mediated injury, sepsis, or cytokine release (36, 37). Angiotensin-converting enzyme 2 (ACE2), a functional receptor for SARS-CoV-2 (38) is found abundantly in the gastrointestinal tract and liver, in addition to presenting in alveolar type 2 cells (the major SARS-CoV-2 targeting cell type in lung). A recent study observed a higher expression of ACE2 in cholangiocytes (~60% of cells) compared to hepatocytes (<3%) (39). Given the hepatic distribution of the ACE2 receptor, SARS-CoV-2 may well cause damage of both bile ducts and liver (40, 41). Our stratification analysis by disease severity revealed that compared to non-COVID-19 patients, COVID-19 patients were more likely to have abnormal liver biochemistry and severe outcomes. Alternatively, the liver may be a bystander, with deranged liver biochemistry reflecting systemic disease (42). It is interesting that liver biochemistry parameters are not currently included in the existing risk stratification tools such as ISARIC scores for predicting clinical deterioration (43) or mortality risk (44) for COVID-19. These parameters (or albumin in particular) may be considered for inclusion in future scoring models or used for early ICU review.

**Caveats and limitations**

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Routinely collected liver biochemistry is not consistent between settings, and therefore the definitions of liver biochemistry derangement may vary across studies. Although AST and GGT have been investigated in previous studies, these parameters are not available for our population. It would be interesting to include GGT in future analysis, and correlation of LFTs with blood gases would be an approach to determine the extent to which deranged liver biochemistry may be associated with liver hypoxia. Other information like respiratory parameters could be included in the propensity score matching and multivariate analysis if available for future analyses. We recognise that analysis can be influenced by missing data, but we have reported missing values and investigated peak/nadir values in addition to baseline. We also undertook sensitivity analysis in a subset of patients with complete BMI measurements to investigate its association with death. Treatment outcomes are important, however, as changing approaches have been made to treatment for COVID-19 and the drugs used as part of clinical trials were not recorded in the current dataset, we have not undertaken an analysis of responses to treatment in this study. Our cohort in the South East of the UK may not be representative of populations elsewhere, especially in terms of ethnic diversity, so caution should be applied in extrapolation of results.

Future studies
Further longitudinal studies of COVID-19 outcomes in diverse patient groups, including those with pre-existing liver disease are needed. The NIHR HIC program will continue to benefit the field of COVID-19 research, as data accumulated for further large teaching hospitals can be used to expand this analysis, resulting in a more generalizable study population and increased statistical power.

Conclusion
Liver biochemistry derangement is common in COVID-19 patients at the time of a SARS-CoV-2 RT-PCR test and during the clinical course of disease. Baseline hypoalbuminaemia and rising ALP over time are prognostic markers for death in COVID-19 patients, but investigation of larger cohorts is required to develop a better understanding of the relationship between liver biochemistry and disease outcome.
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Figure legends

Figure 1. K-M curves for the comparison of time to death in COVID-19 group vs. non-COVID-19 group since SARS-CoV-2 RT-PCR test. One in COVID-19 group has missing death date. p-value was based on the logrank test.

Figure 2. Comparison of ALT and albumin between hospitalised adults with COVID-19 and non-COVID-19 patients matched for age, gender, ethnicity and pre-existing comorbidities: (A) ALT comparison at baseline, and 7, 14, 21, 28 days; (B) Albumin comparison at baseline, and 7, 14, 21, 28 days; (C) ALT changes over time in COVID-19 and non-COVID-19 groups; (D) Albumin changes over time in COVID-19 and non-COVID-19 groups. ALT, Alanine transaminase. Green dash-dotted lines indicate the lower limits of normal and the upper limits of normal. * p-value <0.05, ** p-value <0.01, *** p-value <0.001, **** p-value <0.0001.

Figure 3. Survival K-M curves stratified by baseline liver biochemistry at the time of testing positive for SARS-CoV-2: (A) normal and low baseline albumin; (B) normal and elevated baseline ALT; (C) normal and elevated baseline ALP; (D) normal and elevated baseline bilirubin. K-M, Kaplan-Meier. p-values were based on the logrank test. One patient has missing death date.

Figure 4. ROC curves of predicting death with (A) baseline liver biochemistry (B) baseline liver biochemistry and other identified predictors of mortality. ALP, Alkaline phosphatase; ALT, Alanine transaminase; BR, bilirubin; ROC, receiver operating characteristic; AUC, the area under an ROC curve. Age (≥75 years or <75 years) and liver disease (yes/no) are used as binary variables, which were identified from multivariate Cox proportional-hazards models (Table 4). Baseline albumin + ALP + BR + ALT in the figure indicates including all baseline liver biochemistry parameters in the ROC analysis.

Figure 5. Comparison of liver biochemistry between patients with COVID-19 who died during follow-up and who survived to the end of follow-up: (A) Albumin comparison at baseline at baseline, 7, 14, 21, 28 days; (B) ALT comparison at baseline, 7, 14, 21, 28 days; (C) ALP comparison at baseline at baseline, 7, 14, 21, 28 days; (D) Bilirubin comparison at baseline at baseline, 7, 14, 21, 28 days. ALT, Alanine transaminase; ALP, Alkaline phosphatase. Green dash-dotted lines indicate the lower limits of normal and the upper limits of normal. * p-value <0.05, ** p-value <0.01, *** p-value <0.001, **** p-value <0.0001.

Figure 6. Longitudinal changes of ALT, ALP, albumin over time of COVID-19 patients stratified by death during follow-up. (A) ALT at baseline, 7, 14, 21, 28 days; (B) Changing trend over time (112 vs. 155 days) of ALT by linear regression line fitting with 95% CI; (C) ALP at baseline at baseline, 7, 14, 21, 28 days; (D) Changing trend over time (112 vs. 155 days) of ALP by linear regression line fitting with 95% CI;
(E) Albumin at baseline at baseline, 7, 14, 21, 28 days; (F) Changing trend over time (112 vs. 155 days) of albumin by linear regression line fitting with 95% CI. ALT, Alanine transaminase; ALP, Alkaline phosphatase; CI, Confidence interval. Green dash-dotted lines indicate the lower limits of normal and the upper limits of normal. * p-value <0.05, ** p-value <0.01, *** p-value <0.001, **** p-value <0.0001. R represents Pearson’s correlation coefficient and p indicates linear regression significance.

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Table 1. Baseline characteristics and outcomes of patients with and without COVID-19

|                                | COVID-19 group (n=585) | non-COVID-19 group (n=1165) | p-value |
|--------------------------------|------------------------|-----------------------------|---------|
| Overall follow-up duration     | 58 [14,104]            | 50 [20,78]                  | p=0.002 |
| (median [IQR], days)           |                        |                             |         |
| Duration of liver biochemistry | 38 [7, 79]             | 37 [10, 67]                 | p=0.51  |
| monitoring (median [IQR], days)|                        |                             |         |
| Gender (male), n(%)            | 312 (53.3)             | 629 (54.0)                  | 0.83    |
| Age at test (median [IQR])     | 73 [57, 84]            | 73 [58, 83]                 | 0.76    |
| Ethnicity category, n(%)       |                        |                             | 0.92    |
| Asian                          | 31 (5.3)               | 62 (5.3)                    |         |
| Black                          | 20 (3.4)               | 35 (3.0)                    |         |
| Mixed                          | 10 (1.7)               | 22 (1.9)                    |         |
| White                          | 415 (70.9)             | 804 (69.0)                  |         |
| Other                          | 9 (1.5)                | 23 (2.0)                    |         |
| Not stated                     | 100 (17.1)             | 219 (18.8)                  |         |
| BMI(median [IQR], kg/m²)       | 26 [23.2, 31.0]        | 26 [22.7, 30.5]             | 0.47    |
| BMI category*, n(%)            |                        |                             | 0.31    |
| <18.5                          | 17 (4.3)               | 40 (4.9)                    |         |
| ≥18.5 - <25                    | 140 (35.8)             | 311 (37.9)                  |         |
| ≥25 - <30                      | 115 (29.4)             | 238 (29.0)                  |         |
| ≥30 - <35                      | 77 (19.7)              | 122 (14.9)                  |         |
| ≥35 - <40                      | 26 (6.6)               | 73 (8.9)                    |         |
| ≥40                            | 16 (4.1)               | 37 (4.5)                    |         |
| Pre-existing comorbidities, n(%) |                        |                             |         |
| Liver disease (any)            | 17 (2.9)               | 33 (2.8)                    | 1       |
| Chronic viral hepatitis        | 5 (0.9)                | 5 (0.4)                     | 0.317   |
| Alcoholic liver disease        | 5 (0.9)                | 5 (0.4)                     | 0.317   |
| Hepatic failure, not elsewhere classified | 3 (0.5) | 1 (0.1) | 0.112 |
| Fibrosis and cirrhosis of liver | 7 (1.2) | 10 (0.9) | 0.606 |
| Other inflammatory liver diseases | 3 (0.5) | 5 (0.4) | 1 |
| Other diseases of liver        | 4 (0.7)                | 24 (2.1)                    | 0.041   |
| Liver disorders in diseases classified elsewhere | 0 (0.0) | 1 (0.1) | 1 |
| DM                             | 88 (15.0)              | 175 (15.0)                  | 1       |

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| Condition       | Baseline, n (%) | 30-day, n (%) | p-value |
|-----------------|----------------|--------------|---------|
| HTN             | 179 (30.6)     | 349 (30.0)   | 0.83    |
| CHD             | 59 (10.1)      | 113 (9.7)    | 0.86    |
| CKD             | 56 (9.6)       | 107 (9.2)    | 0.86    |
| Cancer          | 41 (7.0)       | 88 (7.6)     | 0.75    |

**Outcomes**

| Outcome                        | Baseline, n (%) | 30-day, n (%) | p-value |
|--------------------------------|-----------------|--------------|---------|
| Death                          | 157 (26.8)      | 139 (11.9)   | <0.001  |
| ICU admission                  | 71 (12.1)       | 50 (4.3)     | <0.001  |
| Used invasive ventilation in ICU | 47 (8.0)       | 29 (2.5)     | <0.001  |

**Baseline liver biochemistry**

| Test                          | Baseline, n (%) | 30-day, n (%) | p-value |
|-------------------------------|-----------------|--------------|---------|
| ALT, >ULN, n (%)              | 102 (20.7)      | 142 (14.6)   | 0.004   |
| ALT (median [IQR], IU/L)      | 25 [16, 40]     | 19 [13, 32]  | <0.001  |
| ALP, >ULN, n (%)              | 98 (19.8)       | 213 (21.6)   | 0.47    |
| ALP (median [IQR], IU/L)      | 85 [66, 114]    | 90 [71, 124] | 0.005   |
| Bilirubin, >ULN, n (%)        | 29 (5.9)        | 127 (13.0)   | <0.001  |
| Bilirubin (median [IQR], umol/L) | 9 [6, 13]    | 10 [7, 16]   | <0.001  |
| Albumin, <LLN, n (%)          | 291 (58.7)      | 346 (35.0)   | <0.001  |
| Albumin (median [IQR], g/L)   | 30 [27, 34]     | 34 [29, 37]  | <0.001  |

**Baseline blood clotting tests**, median[IQR]

| Test                        | Baseline, median [IQR] | 30-day, median [IQR] | p-value |
|-----------------------------|-------------------------|----------------------|---------|
| Prothrombin time, seconds   | 10.9 [10.4, 11.4]       | 10.9 [10.4, 11.8]    | 0.093   |
| APTT, seconds               | 24.8 [23.0, 27.7]       | 24.4 [22.6, 27.1]    | 0.014   |
| INR                         | 1.0 [1.0, 1.1]          | 1.0 [1.0, 1.1]       | 0.073   |

**Baseline renal function tests**

| Test                        | Baseline, median [IQR], umol/L | 30-day, median [IQR], umol/L | p-value |
|-----------------------------|--------------------------------|----------------------------|---------|
| Creatinine (median [IQR])   | 82 [65, 111]                   | 81 [65, 117]                | 0.71    |
| Elevated creatinine, n(%)   | 151 (29.9)                     | 321 (31.3)                  | 0.63    |
| Urea (median [IQR]), mmol/L  | 6.3 [4.3, 9.8]                 | 6.2 [4.5, 9.9]              | 0.64    |
| eGFR (median [IQR]), ml/min/1.73m² | 76 [49, >90]  | 76 [47, >90]               | 0.65    |
| eGFR <90 ml/min/1.73m², n(%) | 369 (73.2)                     | 746 (72.6)                  | 0.86    |

**Other tests at baseline**, (median[IQR])

| Test                        | Baseline, median [IQR] | 30-day, median [IQR] | p-value |
|-----------------------------|------------------------|----------------------|---------|
| CRP, mg/L                   | 78 [27, 148]           | 20 [4, 93]           | <0.001  |
| Platelets, x10^9/L          | 216 [160, 281]         | 245 [195, 311]       | <0.001  |
| Lymphocytes, x10^9/L        | 0.9 [0.6, 1.3]         | 1.2 [0.8, 1.8]       | <0.001  |

**Baseline vital signs**

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|                                | Median [IQR]             |             |     |
|--------------------------------|-------------------------|-------------|-----|
| Temperature tympanic, °C       | 37 [36.5, 38]           | 36.5 [36, 37] | <0.001 |
| Heart rate, bpm                | 86 [76, 97]             | 81 [72, 95]  | <0.001 |
| Oxygen saturation, %           | 95 [94, 97]             | 96 [95, 98]  | <0.001 |
| Respiratory rate               | 20 [18, 22]             | 18 [17, 19]  | <0.001 |
| Diastolic blood pressure, mmHg | 70 [64, 78]             | 72 [64, 81]  | 0.076 |
| Systolic blood pressure, mmHg  | 129 [118, 143]          | 133 [121, 149]| <0.001 |

Data are the median [IQR] or number (%) unless otherwise indicated. For categorical variables, Fisher exact test was performed for comparison on cells with small counts (<5), otherwise Chi-square test was used. For continuous variables, Wilcoxon test was used for comparison due to non-normality. † 194 vs. 344 missing on BMI data; BMI categories were reported based on WHO classification. ‡ 93 vs. 191 patients with vs. without COVID-19 did not have baseline data available on all the four liver biochemistries. In detail, 93 vs. 191 missing on ALT; 91 vs. 180 missing on ALP; 93 vs. 191 missed data in bilirubin; 89 vs. 177 missing in albumin. ‡ 158 vs. 303 patients with vs. without COVID-19 did not have baseline data available on blood clotting tests. § 162 vs. 324 patients with vs. without COVID-19 did not have baseline data available on vital signs. ALT, Alanine transaminase; ALP, Alkaline phosphatase; APTT, Activated partial thromboplastin time; BMI, Body mass index; CHD, Coronary heart disease; CKD, Chronic kidney disease; CRP, C-reactive protein; DM, Diabetes mellitus; HTN, Hypertension; INR, International normalised ratio; IQR, Interquartile range; LLN, Lower limit of normal; NA, not applicable; ULN, Upper limit of normal.
Table 2. Comparison of demographics, comorbidities, outcomes, baseline and peak/nadir liver biochemistries between COVID-19 subgroups stratified by disease severity.

|                          | Mild/Moderate COVID-19 cases (n=358)† | Severe/Critical COVID-19 cases (n=65)‡ | p-value |
|--------------------------|---------------------------------------|----------------------------------------|---------|
| Gender (male), n(%)      | 198 (55.3)                            | 31 (47.7)                              | 0.32    |
| Age at test (median [IQR]) | 74 [59, 84]                          | 71 [56, 84]                            | 0.54    |
| Ethnicity category, n(%) |                                       |                                        | 0.44    |
| Asian                    | 19 (5.3)                              | 1 (1.5)                                |         |
| Black                    | 11 (3.1)                              | 2 (3.1)                                |         |
| Mixed                    | 5 (1.4)                               | 3 (4.6)                                |         |
| White                    | 260 (72.6)                            | 48 (73.8)                              |         |
| Other                    | 4 (1.1)                               | 1 (1.5)                                |         |
| Not stated               | 59 (16.5)                             | 10 (15.4)                              |         |
| BMI (median [IQR]), kg/m²| 26.9 [23.6, 30.9]                     | 25.7 [21.6, 32.0]                      | 0.58    |
| BMI category, n(%)       |                                       |                                        | 0.47    |
| <18.5                    | 9 (3.4)                               | 2 (5.4)                                |         |
| ≥18.5 - <25              | 90 (34.0)                             | 15 (40.5)                              |         |
| ≥25 - <30                | 85 (32.1)                             | 6 (16.2)                               |         |
| ≥30 - <35                | 52 (19.6)                             | 10 (27.0)                              |         |
| ≥35 - <40                | 18 (6.8)                              | 3 (8.1)                                |         |
| ≥40                      | 11 (4.2)                              | 1 (2.7)                                |         |
| Pre-existing comorbidities, n(%) |                |                                        |         |
| Liver disease            | 10 (2.8)                              | 2 (3.1)                                | 1       |
| DM                       | 52 (14.5)                             | 7 (10.8)                               | 0.54    |
| HTN                      | 109 (30.4)                            | 13 (20.0)                              | 0.12    |
| CHD                      | 36 (10.1)                             | 5 (7.7)                                | 0.72    |
| CKD                      | 33 (9.2)                              | 3 (4.6)                                | 0.33    |
| Cancer                   | 27 (7.5)                              | 3 (4.6)                                | 0.56    |
| Outcomes                 |                                       |                                        |         |
| Death, n(%)              | 97 (27.1)                             | 28 (43.1)                              | 0.012   |
| ICU admission, n(%)      | 37 (10.3)                             | 18 (27.7)                              | <0.001  |
| Used invasive ventilation in ICU, n(%) | 23 (6.4)                              | 14 (21.5)                              | <0.001  |
| Baseline liver biochemistry |                                   |                                        |         |
| ALT, >ULN, n(%)          | 66 (19.0)                             | 20 (31.2)                              | 0.041   |
| ALT (median [IQR]), IU/L | 24.0 [17.0, 39.0]                     | 29.5 [18.8, 50.2]                      | 0.063   |
| ALP, >ULN, n(%)          | 71 (20.4)                             | 10 (15.6)                              | 0.48    |

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| ALP (median [IQR]), IU/L | 84.0 [66.0, 116.2] | 80.0 [60.8, 104.0] | 0.19 |
|------------------------|-------------------|-------------------|-----|
| Bilirubin, >ULN, n(%)  | 21 (6.1)          | 2 (3.1)           | 0.52|
| Bilirubin (median [IQR]), umol/L | 9.0 [6.0, 14.0] | 10.0 [7.0, 13.2] | 0.44|
| Albumin, <LLN, n(%)   | 201 (57.8)        | 39 (60.9)         | 0.74|
| Albumin (median [IQR]), g/L | 30.5 [27.0, 34.0] | 30.5 [26.8, 33.2] | 0.52|

**Peak/Nadir liver biochemistry**

| ALT (median [IQR]), IU/L | 37.0 [23.0, 65.8] | 39.0 [26.0, 97.0] | 0.39|
|------------------------|-------------------|-------------------|-----|
| ALT, >ULN, n(%)        | 148 (41.3)        | 29 (44.6)         | 0.72|
| ALP (median [IQR]), IU/L | 108.0 [81.0, 158.0] | 95.0 [75.0, 152.0] | 0.16|
| ALP, >ULN, n(%)        | 133 (37.2)        | 18 (27.7)         | 0.19|
| Bilirubin (median [IQR]), umol/L | 12.0 [8.0, 17.0] | 13.0 [9.0, 16.0] | 0.67|
| Bilirubin, >ULN, n(%)  | 52 (14.5)         | 5 (7.7)           | 0.20|
| Albumin (median [IQR]), g/L | 25.0 [21.0, 29.0] | 25.0 [21.0, 28.0] | 0.55|
| Albumin, <LLN, n(%)    | 307 (85.8)        | 57 (87.7)         | 0.83|

Data are the median [IQR] or number (%) unless otherwise indicated. For categorical variables, Fisher exact test was performed for comparison on cells with small counts (<5), otherwise Chi-square test was used. For continuous variables, Wilcoxon test was used for comparison due to non-normality.

† Mild/Moderate: baseline respiratory rate ≤30 breaths/min and oxygen saturation (SpO2) ≥90%.
‡ Severe/Critical: respiratory rate >30 breaths/min, or oxygen saturation(SpO2) <90%. # 93 in mild/moderate cases vs. 28 in severe/critical cases missing on BMI data; BMI categories were reported based on WHO classification. ALT, Alanine transaminase; ALP, Alkaline phosphatase; APTT, Activated partial thromboplastin time; BMI, Body mass index; CHD, Coronary heart disease; CKD, Chronic kidney disease; CRP, C-reactive protein; DM, Diabetes mellitus; HTN, Hypertension; INR, International normalised ratio; IQR, Interquartile range; LLN, Lower limit of normal; NA, not applicable; ULN, Upper limit of normal.
Table 3. Demographics, pre-existing comorbidities, baseline and peak/nadir liver biochemistry of COVID-19 patients who survived and died

|                                    | Survived (n=428) | Died (n=157) | p-value |
|------------------------------------|------------------|--------------|---------|
| Gender = male, n(%)                | 221 (51.6)       | 91 (58.0)    | 0.21    |
| Age at test (median [IQR]), years  | 66 [54, 80]      | 82 [75, 89]  | <0.001  |
| Age ≥ 75 years, n(%)               | 158 (36.9)       | 120 (76.4)   | <0.001  |
| Ethnicity category, n(%)           |                  |              |         |
| Asian                              | 29 (6.8)         | 2 (1.3)      | 0.015   |
| Black                              | 18 (4.2)         | 2 (1.3)      | 0.14    |
| Mixed and other                    | 17 (4.0)         | 2 (1.3)      | 0.17    |
| White                              | 287 (67.1)       | 128 (81.5)   | 0.001   |
| Not stated                         | 77 (18.0)        | 23 (14.6)    | 0.41    |
| BMI (median [IQR]), kg/m²          | 27 [23.4, 31.3]  | 25 [22, 30.2] | 0.076 |
| BMI category*, n(%)                |                  |              |         |
| <18.5 (underweight)                | 10 (3.5)         | 7 (6.7)      | 0.28    |
| 18.5-24.9 (normal weight)          | 98 (34.3)        | 42 (40.0)    | 0.35    |
| 25.0-29.9 (pre-obesity/overweight) | 87 (30.4)        | 28 (26.7)    | 0.55    |
| 30.0-34.9 (obesity class I)        | 57 (19.9)        | 20 (19.0)    | 0.96    |
| 35.0-39.9 (obesity class II)       | 21 (7.3)         | 5 (4.8)      | 0.49    |
| ≥40.0 (obesity class III)          | 13 (4.5)         | 3 (2.9)      | 0.65    |
| Used invasive ventilation in ICU, n(%) | 41 (9.6)       | 6 (3.8)      | 0.036   |
| ICU admission, n(%)                | 59 (13.8)        | 12 (7.6)     | 0.061   |
| Pre-existing comorbidities, n(%)   |                  |              |         |
| Liver disease                      | 6 (1.4)          | 11 (7.0)     | 0.001   |
| DM                                 | 53 (12.4)        | 35 (22.3)    | 0.005   |
| HTN                                | 121 (28.3)       | 58 (36.9)    | 0.055   |
| CHD                                | 35 (8.2)         | 24 (15.3)    | 0.018   |
| CKD                                | 36 (8.4)         | 20 (12.7)    | 0.16    |
| Cancer                             | 24 (5.6)         | 17 (10.8)    | 0.045   |
| ≥1 liver biochemistry abnormal at baseline†, n(%) | 235 (67.9)    | 122 (83.6)   | 0.001   |
| ≥1 liver biochemistry abnormal at baseline (excl. albumin), n(%) | 123 (35.5) | 55 (37.7)    | 0.73    |
| Baseline ALT (median [IQR]), IU/L   | 25 [16, 43]      | 23 [16, 36]  | 0.11    |
| Baseline ALT categories*, n(%)     |                  |              |         |
| normal                             | 267 (77.2)       | 123 (84.2)   | 0.099   |
| >1-2ULN                            | 49 (14.2)        | 15 (10.3)    | 0.31    |
| >2-3ULN                            | 16 (4.6)         | 5 (3.4)      | 0.72    |
|                   | Baseline albumin (median [IQR], g/L) | Baseline albumin (<LLN), n(%) | ≥1 peak/nadir liver biochemistry abnormal, n(%) | ≥1 peak liver biochemistry abnormal (excl. albumin), n(%) | Peak ALT (median [IQR]), IU/L | Peak ALT categories, n(%) |
|-------------------|--------------------------------------|--------------------------------|-----------------------------------------------|--------------------------------------------------------|-----------------------------|---------------------------|
|                   | Baseline albumin (median [IQR], g/L) | Baseline albumin (<LLN), n(%) | ≥1 peak/nadir liver biochemistry abnormal, n(%) | ≥1 peak liver biochemistry abnormal (excl. albumin), n(%) | Peak ALT (median [IQR]), IU/L | Peak ALT categories, n(%) |
| Baseline albumin (median [IQR], g/L) | 31 [28, 34]                         | 28 [25, 32]                   | 352 (82.2)                                    | 238 (55.6)                                             | 37 [20, 71]                 | normal (58.9)              |
| Baseline albumin (<LLN), n(%) | 185 (52.9)                           | 106 (72.6)                     | 153 (97.5)                                    | 91 (58.0)                                              | 32 [19, 52]                 | >1-2ULN (21.7)              |
| ≥1 peak/nadir liver biochemistry abnormal, n(%) | 352 (82.2)                           | 153 (97.5)                     | 352 (82.2)                                    | 238 (55.6)                                             | 37 [20, 71]                 | normal (58.9)              |
| ≥1 peak liver biochemistry abnormal (excl. albumin), n(%) | 238 (55.6)                           | 91 (58.0)                      | 238 (55.6)                                    | 91 (58.0)                                              | 32 [19, 52]                 | >1-2ULN (21.7)              |
| Peak ALT (median [IQR]), IU/L | 37 [20, 71]                           | 32 [19, 52]                    | 37 [20, 71]                                    | 32 [19, 52]                                            | 37 [20, 71]                 | normal (58.9)              |
| Peak ALT categories, n(%) | normal (58.9)                         | 252 (58.9)                     | 11 (7.0)                                      | 13 (8.3)                                               | 13 (8.3)                    | normal (58.9)              |
| >1-2ULN           | 11 [14.0]                            | 28 (6.5)                       | 11 (7.0)                                      | 11 (7.0)                                               | 11 (7.0)                    | >1-2ULN (21.7)              |
| >2-3ULN           | 11 [14.0]                            | 28 (6.5)                       | 11 (7.0)                                      | 11 (7.0)                                               | 11 (7.0)                    | >1-2ULN (21.7)              |
| >3ULN             | 15 (8.3)                             | 55 (12.9)                      | 15 (8.3)                                      | 55 (12.9)                                              | 15 (8.3)                    | >3ULN (6.5)                 |
| Nadir albumin (median [IQR]), g/L | 27 [22, 32]                           | 23 [19, 27]                    | 27 [22, 32]                                    | 23 [19, 27]                                            | 27 [22, 32]                 | normal (58.9)              |
| Nadir albumin (<LLN)§, n(%) | 311 (72.7)                           | 151 (96.2)                     | 311 (72.7)                                    | 151 (96.2)                                             | 311 (72.7)                  | normal (58.9)              |

For categorical variables, Fisher exact test was performed for comparison on cells with small counts (<5), otherwise Chi-square test was used. For continuous variables, Wilcoxon test was used for comparison due to non-normality. § BMI categories were reported based on WHO classification. † 82 vs. 11 in alive subgroup vs. died subgroup had not all liver biochemistry baseline data available. ‡ 82 vs. 11 in alive subgroup vs. died subgroup missing baseline ALT. § 78 vs. 11 in alive subgroup vs. died subgroup missing baseline albumin. ALT, Alanine transaminase; ALP, Alkaline phosphatase; CHD, Coronary heart disease; CKD, Chronic kidney disease; DM, Diabetes mellitus; HTN, Hypertension; IQR, Interquartile range; LLN, Lower limit of normal; ULN, Upper limit of normal.
Table 4. Univariate and multivariate Cox proportional-hazards models investigating associations of baseline liver biochemistry derangement with death among adults with confirmed COVID-19

| Variables                        | Univariate analysis | Multivariate analysis |
|----------------------------------|---------------------|----------------------|
|                                  | Crude HR (95% CIs)  | p-value              |
|                                  | Adjusted HR (95% CIs) | p-value              |
| Age ≥75 years                    | 4.39 (3.03-6.36)    | <0.001               |
|                                  | 3.96 (2.59-6.04)    | <0.001               |
| Gender (male)                    | 1.24 (0.91-1.71)    | 0.18                 |
|                                  | 1.21 (0.86-1.71)    | 0.28                 |
| Ethnicity (white)                | 1.87 (1.25-2.8)     | 0.002                |
|                                  | 1.13 (0.71-1.8)     | 0.61                 |
| Baseline ALT (>ULN)              | 0.63 (0.4-0.98)     | 0.04                 |
|                                  | 0.86 (0.53-1.38)    | 0.53                 |
| Baseline ALP (>ULN)              | 1.21 (0.83-1.78)    | 0.33                 |
| Baseline bilirubin (>ULN)        | 1.5 (0.83-2.7)      | 0.18                 |
| Baseline albumin (<LLN)          | 2.01 (1.4-2.9)      | <0.001               |
|                                  | 1.83 (1.25-2.67)    | 0.002                |
| Pre-existing liver disease       | 2.66 (1.44-4.9)     | 0.002                |
|                                  | 3.37 (1.58-7.16)    | 0.002                |
| Pre-existing DM                  | 1.7 (1.16-2.48)     | 0.006                |
|                                  | 1.22 (0.77-1.93)    | 0.39                 |
| Pre-existing HTN                 | 1.33 (0.96-1.84)    | 0.085                |
|                                  | 0.7 (0.44-1.11)     | 0.13                 |
| Pre-existing CHD                 | 1.72 (1.11-2.65)    | 0.015                |
|                                  | 1.16 (0.67-2.02)    | 0.59                 |
| Pre-existing CKD                 | 1.34 (0.83-2.17)    | 0.23                 |
|                                  | 0.84 (0.46-1.51)    | 0.56                 |
| Pre-existing Cancer              | 1.71 (1.04-2.84)    | 0.036                |
|                                  | 1.06 (0.58-1.93)    | 0.85                 |

Analysis were performed on the 492 COVID-19 patients who had baseline liver biochemistry data (survived vs. died: 346 vs. 146); the cut off of age was based on the median age in the cohort; variables (except for demographics and comorbidities) with p<0.1 in univariate analysis were included for multivariate analysis; in multivariate analysis, HRs were fully adjusted for drugs use before baseline (including antiviral drugs, antibiotics, anticoagulants, acetaminophen, immunosuppressants, statins) to reduce confounding effects. ALT, Alanine transaminase; ALP, Alkaline phosphatase; CHD, Coronary heart disease; CKD, Chronic kidney disease; DM, Diabetes mellitus; HTN, Hypertension; HR, Hazards ratio; LLN, lower limit of normal; ULN, upper limit of normal.
Table 5. Changes of liver biochemistries over the time assessed by linear mixed effects model for patients with COVID-19 between groups (Died vs. Survived)

|                | Coefficient (β) | 95%CI                  | p-value |
|----------------|-----------------|------------------------|---------|
| Albumin        |                 |                        |         |
| (Intercept)    | 27.72           | (27.16, 28.29)         | <0.001  |
| Group          | -1.68           | (-2.76, -0.60)         | 0.002   |
| Time           | 0.08            | (0.07, 0.08)           | <0.001  |
| Interaction (Group x Time) | -0.19      | (-0.2, -0.17)         | <0.001  |
| ALP            |                 |                        |         |
| (Intercept)    | 110.49          | (101.83, 119.16)       | <0.001  |
| Group          | 5.34            | (-11.37, 22.04)        | 0.531   |
| Time           | -0.07           | (-0.17, 0.02)          | 0.133   |
| Interaction (Group x Time) | 0.57      | (0.17, 0.97)          | 0.005   |
| ALT            |                 |                        |         |
| (Intercept)    | 47.9            | (41.04, 54.76)         | <0.001  |
| Group          | -1.66           | (-14.95, 11.63)        | 0.807   |
| Time           | -0.19           | (-0.30, -0.08)         | 0.001   |
| Interaction (Group x Time) | 0.34      | (-0.09, 0.77)         | 0.126   |
| BR             |                 |                        |         |
| (Intercept)    | 10.56           | (9.60, 11.52)          | <0.001  |
| Group          | 2.17            | (0.32, 4.01)           | 0.021   |
| Time           | -0.02           | (-0.03, -0.01)         | <0.001  |
| Interaction (Group x Time) | 0.02      | (-0.02, 0.07)         | 0.356   |

Note: Group={0, 1}, where 0 indicates survived, 1 indicates died. CI, confidence interval; ALP, Alkaline phosphatase; ALT, Alanine transaminase; BR, Bilirubin.
p < 0.0001

|           | COVID-19 group | Non-COVID-19 group |
|-----------|----------------|--------------------|
| 0 days    | 584            | 1165               |
| 30 days   | 364            | 792                |
| 60 days   | 288            | 469                |
| 90 days   | 194            | 199                |
| 120 days  | 91             | 50                 |
