Inactivated vaccines reduce the risk of liver function abnormality in NAFLD patients with COVID-19: a multi-center retrospective study

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Summary

Background Abnormal liver function was frequently observed in nonalcoholic fatty liver disease (NAFLD) patients infected with SARS-CoV-2. Our aim was to explore the effect of SARS-CoV-2 inactivated vaccines on liver function abnormality among NAFLD patients with COVID-19.

Methods The multi-center retrospective cohort included 517 NAFLD patients with COVID-19 from 1 April to 30 June 2022. Participants who received 2 doses of the vaccine (n = 274) were propensity score matched (PSM) with 243 unvaccinated controls. The primary outcome was liver function abnormality and the secondary outcome was viral shedding duration. Logistic and Cox regression models were used to calculate the odds ratio (OR) and hazard ratio (HR) for the outcomes. Sensitivity analysis was conducted to assess robustness.

Findings PSM identified 171 pairs of vaccinated and unvaccinated patients. Liver function abnormality was less frequent in the vaccinated group (adjusted OR, 0.556 [95% CI (confidence interval), 0.356–0.869], $p = 0.010$). Additionally, the vaccinated group demonstrated a lower incidence of abnormal bilirubin levels (total bilirubin: adjusted OR, 0.223 [95% CI, 0.072–0.690], $p = 0.009$; direct bilirubin: adjusted OR, 0.175 [95% CI, 0.080–0.384],

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$p < 0.001$) and shorter viral shedding duration (adjusted HR, 0.798 [95% CI, 0.641–0.994], $p = 0.044$) than the unvaccinated group. Further subgroup analysis revealed similar results, while the sensitivity analyses indicated consistent findings.

Interpretation SARS-CoV-2 vaccination in patients with NAFLD may reduce the risk of liver dysfunction during COVID-19. Furthermore, vaccination demonstrated beneficial effects on viral shedding in the NAFLD population.

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Keywords: COVID-19; Nonalcoholic fatty liver disease; Inactivated SARS-CoV-2 vaccine; Liver function abnormality; Direct bilirubin

Research in context

Evidence before this study

Prior to conducting this study, there was limited published evidence available regarding the impact of inactivated SARS-CoV-2 vaccination on liver function abnormalities in COVID-19 patients with nonalcoholic fatty liver disease (NAFLD). To assess the existing literature, a PubMed search was conducted on June 7th, 2023, using the following search terms: (COVID-19 OR severe acute respiratory syndrome coronavirus 2 OR SARS CoV-2) AND (vaccination OR vaccine) AND (liver function) AND (nonalcoholic fatty liver disease OR NAFLD). Only one relevant study was identified, which reported the safety and immunogenicity of COVID-19 vaccination in the NAFLD population. A similar search on ClinicalTrials.gov also yielded only one ongoing study focusing on vaccine immunogenicity that had not yet enrolled participants. Notably, liver function abnormalities were frequently observed in the NAFLD population and were associated with the progression of NAFLD and increased COVID-19-related mortality. However, it remains unclear whether the vaccines have hepatoprotective effect on NAFLD patients during SARS-CoV-2 infection. To address this knowledge gap, we conducted a multi-center retrospective study utilizing Logistic and Cox regression models to calculate odds ratios and hazard ratios for the outcomes. Sensitivity analysis was also conducted to ensure the robustness of the findings.

Added value of this study

This multi-center retrospective study provided insights into the effect of inactivated vaccination on liver function abnormality in NAFLD patients with COVID-19 and revealed that such vaccines could reduce the risk of liver function abnormalities in the individuals with NAFLD in face of COVID-19. Furthermore, our findings indicated the beneficial effects of inactivated vaccines on viral shedding in the NAFLD population. These results highlight the need to increase the vaccination rate within this population by providing evidence of the protective effects of vaccination on liver function in patients with NAFLD and COVID-19.

Implications of all the available evidence

Collectively, our work underscores the necessity of enhancing vaccination rates within the NAFLD population by substantiating the hepatoprotective effects of vaccination in individuals with NAFLD and COVID-19.

Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) has been growing, with an incidence of about 25% in the global population.[1,2] Compared with the general population, individuals with NAFLD have an increased susceptibility risk of COVID-19.[3,4] Furthermore, patients having COVID-19 with NAFLD are more likely to experience disease progression,[5–7] extended viral shedding,[5] a higher incidence of pulmonary thromboembolism,[8] prolonged hospitalization,[5] and increased use of hospital resources.[9] Patients with NAFLD and COVID-19 also exhibit an increased prevalence of liver function abnormalities (50%–59% vs. 14%–53% in the general population).[5,10] These hepatic abnormalities and dysfunction may persist up to 2 months after a negative SARS-CoV-2 test result[11] and more importantly, can potentially exacerbate NAFLD progression, thereby increasing the risk of developing nonalcoholic steatohepatitis (NASH) and hepatic fibrosis.[6,12,13] Therefore, implementing appropriate hepatoprotective measures for individuals with NAFLD in the face of SARS-CoV-2 infection is critical.

SARS-CoV-2 vaccination is important for preventing COVID-19 and reducing its severity.[14,15] However, the potential benefits of vaccination in the NAFLD population with immune imbalance[16] remains uncertain. After receiving a complete inactivated vaccination, patients with NAFLD have been shown to exhibit a high rate of neutralizing antibody seropositivity (95.5%),[17] similar to that observed in the general population.[18] However, it

should be noted that high antibody titers do not necessarily indicate vaccine efficacy.[19,20] Although recent vaccination efficacy studies have suggested that mRNA vaccination is associated with a decreased mortality risk in hospitalized patients with cirrhosis,[21] the primary cause of death in COVID-19 cases is predominantly attributed to respiratory complications, with a low incidence of liver failure-related mortality. Thus, previous studies may not fully reflect the effect of vaccination on liver health. Whereas, limited attention has been paid to whether vaccination can reduce liver damage in individuals with NAFLD who contract COVID-19. In patients with NAFLD, liver injury is critical in the pathological progression of the condition. Hence, investigating the potential effect of COVID-19 vaccination on liver damage in this immune imbalanced population is essential.

Inactivated SARS-CoV-2 vaccines have been widely used in China. Individuals with NAFLD who received inactivated vaccination have been reported to exhibit high immunogenicity comparable to that in the general population, along with a favorable safety profile with transient adverse reactions.[17] However, no research exists on the occurrence of liver function abnormalities in SARS-CoV-2-vaccinated individuals with NAFLD after they contract COVID-19. Therefore, we conducted a multi-center retrospective study using data collected from three centers in China to investigate the association between inactivated SARS-CoV-2 vaccination and liver function in patients with COVID-19 and preexisting NAFLD.

Methods

Study design

This study was an investigator-initiated, retrospective, and multi-center cohort research comprising three centers in Shanghai, China. All experiments involving human patients were performed in accordance with the Declaration of Helsinki and approved by the Ethics Committees of Zhongshan Hospital affiliated to Fudan University (B2022-536[2]), Shanghai Eighth People's Hospital Affiliated to Jiangsu University (2022-04-034), and Shanghai Pudong Hospital (WZ-05). Written informed consent was obtained from all patients before their inclusion in the study.

Study population

This study consecutively enrolled hospitalized patients with NAFLD who tested positive for SARS-CoV-2 from three designated COVID-19 hospitals in Shanghai, namely Zhongshan Hospital, Shanghai Eighth People's Hospital, and Shanghai Pudong Hospital, between April 1, 2022 and June 30, 2022. According to specific public health policy, all individuals who tested positive were systematically admitted to hospitals. The hospitals included in our study are representative designated facilities strategically located in different geographical regions within Shanghai, covering the eastern, central and western areas of the city. During this period, the entire included population was infected with Omicron BA.2 or BA.2.2 variants in Shanghai. Patients were eligible if they met the following inclusion criteria: 1) age >18 years, 2) first positive SARS-CoV-2 test result, and 3) clinical diagnosis of NAFLD within the past 6 months. Patients were excluded based on the following exclusion criteria[22]: 1) positive hepatitis B surface antigen or anti-hepatitis C virus antibody, 2) excessive alcohol consumption (>20 g/day in men and >10 g/day in women), 3) evidence of liver diseases other than NAFLD, 4) liver decompensation, or 5) immunosuppressive status (such as HIV infection, cancer, organ transplantation, bone marrow transplantation, or application of immunosuppressive agents within the last 3 months).

Participants diagnosed with NAFLD were identified based on the following conditions: 1) hepatic steatosis detected by abdominal ultrasound (US) within the last 6 months (ultrasonic diagnostic criteria of parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, and bright vessel walls)[23,24] and 2) the exclusion of secondary causes of hepatic steatosis and other liver diseases.[25] However, we could not subdivide the NAFLD cohort into nonalcoholic fatty liver and NASH groups due to the absence of liver biopsies.[22] Their baseline liver function was assessed within the three-month period preceding the onset of COVID-19 infection. These follow-up liver function data was retrieved from the Shanghai medical treatment combination system.

All patients in the vaccinated group had received two doses of inactivated whole-virion SARS-CoV-2 vaccines (CoronaVac, BBIBP-CorV, or WIBP-CorV). Additionally, their last vaccine dose was administered 3 months before their COVID-19 diagnosis. Furthermore, the time interval between the patients' first and second SARS-CoV-2 vaccine doses was 3–8 weeks, according to the guidance issued by the National Health Commission of the People's Republic of China.[26] "Unvaccinated" patients were defined as those who had never received any COVID-19 vaccine dose. Patients who received other inactivated vaccines, non-inactivated vaccines, or only one dose of inactivated SARS-CoV-2 vaccines were excluded from the final analysis.

SARS-CoV-2 infection was confirmed via real-time reverse-transcription polymerase chain reaction (PCR) using naso-oropharyngeal swabs.[27,28] Patients with NAFLD who had a previously positive SARS-CoV-2 PCR test result were excluded from the analysis. The classification of COVID-19 severity was based on the Diagnosis and Treatment Protocol for COVID-19 Patients (Tentative 9th Edition) by the National Centers for Disease Control and Prevention of China (China CDC). Participants underwent nasopharyngeal swab sampling for RT-PCR every day until two consecutive negative

SARS-CoV-2 nucleic acid tests were obtained, with a cycle threshold (Ct) value below 35 and a minimum sampling interval of at least 24 h.

Outcome

The primary composite outcome was liver function abnormality. Liver function abnormality was defined as an increase over the upper limit of the normal range[5,27,29,30] within the week of COVID-19 diagnosis in any of the following parameters: alanine aminotransferase (ALT, >50 U/L), aspartate aminotransferase (AST, >35 U/L), γ-glutamyl transpeptidase (GGT, >60 U/L), alkaline phosphatase (ALP, >135 U/L), total bilirubin (TBIL, >21 U/L), or direct bilirubin (DBIL, >5.1 U/L). The secondary outcome was viral shedding duration, which was defined as the number of days from the date of the first positive PCR test result to the date of the first negative PCR test result, followed by consecutive negative results.[29] Data including demographics, medical history, liver function, and viral shedding duration were extracted from the electronic medical records in the relevant registries.

Statistical analysis

Categorical variables were described as frequencies and percentages, and continuous variables were represented as means and standard deviations (SDs) or medians and interquartile ranges (IQRs). The means of the continuous variables were compared using independent group *t*-tests for normally distributed data, and Mann–Whitney U test was applied for non-normally distributed data. Categorical variables were compared by employing the chi-square or Fisher's exact tests. A p value of <0.05 was considered statistically significant. The regression analysis results were presented as odds ratios (ORs), hazard ratios (HRs), and 95% confidence intervals (CIs). Analyses were performed via SPSS Statistics (version 26.0, IBM, Armonk, NY, USA) and R studio (version 2022.12.0 + 353, Posit Software, PBC).

We further applied propensity score matching (PSM)[31] to address the possible imbalance between the vaccinated and unvaccinated groups of patients with COVID-19 and NAFLD. The PS model was adjusted for all potential confounders, including age, sex, body mass index (BMI), drinking habit, smoking habit, hypertension, diabetes, cardiovascular disease (CVD), hyperlipidemia, medications, baseline liver function, and COVID-19 severity. We then adopted the greedy nearest-neighbor matching algorithm with a 1:1 matching ratio and a caliper width of 0.2 SDs of the logit of the propensity score calculated by the multivariable logistic regression model.[32,33] Finally, the covariate balance between the two groups was evaluated using the absolute standardized mean difference (SMD), wherein a covariate with SMD <10% was considered well-balanced (Supplementary Figure S1).[34]

Additionally, we used multivariable binary logistic regression models to estimate the association between inactivated SARS-CoV-2 vaccination and the primary composite outcome. These models included a crude model and a multivariable model adjusted for potential confounders and effect modifiers, which were identified from previous literature[35–40] and incorporated into a directed acyclic graph (DAG, Supplementary Figure S2), using the online DAGitty tool (http://www.dagitty.net). The final minimally sufficient adjustment set comprised age, sex, BMI, hypertension, diabetes, CVD, and medications (hypotensive, hypoglycemic, and lipid-lowering drugs).

The Kaplan–Meier method was employed for the secondary outcome, and we utilized the log rank test to compare the unvaccinated and vaccinated groups. Next, we developed Cox proportional hazards models to calculate the association between inactivated SARS-CoV-2 vaccination and the secondary outcome. The status was defined as two continuous negative PCR test results. The "time" was established as the virus shedding duration, and the vaccinated group was designated as the "reference". The multivariate model included the adjusted covariates of age, sex, BMI, CVD, diabetes, hypertension, and medications selected from the DAG (Supplementary Figure S2). The proportional hazards assumption was confirmed to be met through log-log survival function plots (Supplementary Figure S3). Censors were defined as cases of death. Among the 511 individuals with complete data, all of them exhibited two continuous negative PCR test results, and no cases of censoring were observed.

We further conducted seven subgroup analyses using univariate Logistic and Cox proportional hazards regression models, where an interaction term in the matched models was applied to test the variation in the risk of liver function abnormality and virus elimination time across the baseline characteristics. The predefined subgroups were age (>65 years), sex, BMI (>28 kg/m^2), and the comorbidity subgroups of hypertension, diabetes, CVD, and hyperlipidemia (Supplementary Figure S4).

Finally, we conducted a sensitivity analysis to minimize the possibility of incurring bias and assess the robustness of the results. First, primary outcome analysis was restricted to the patients whose liver function parameters were within normal clinical ranges within 3 months before the index date to rule out previous liver function abnormality (Supplementary Table S1). Second, we excluded the patients with a medication history before the index date based on all the available data to avoid the potential effect of medications on hepatobiliary status (Supplementary Table S2). Third, we examined a potentially more specific definition of the secondary outcome, i.e., virus elimination time beyond 20 days (defined as prolonged viral elimination in existing studies)[41,42] (Supplementary Table S3). Fourth, an alternative statistical analysis was performed based on traditional methods introducing potential confounders using prior

knowledge and existing studies.[43,44] Consequently, three models were assessed via sequential adjustment for (a) smoking and drinking status and hyperlipidemia (model 1), (b) plus COVID-19 severity (model 2), and (c) plus COVID-19 severity, smoking and drinking status, and hyperlipidemia (model 3) (Supplementary Table S4). Fifth, missing values exclusively pertained to the primary composite outcome. In our main Logistic model, the primary composite outcome was deemed present if any of the remaining liver function parameters, excluding the missing parameter, displayed abnormalities. To further address missing data, three missing data handling models were established, employing the following methods: (a) complete case analysis (model 1), (b) worst-case imputation (model 2), and (c) worst and best imputation (model 3) (Supplementary Table S5).

Role of the funding source

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The authors have not been paid to write this article by a pharmaceutical company or other agency. The authors were not precluded from accessing data in the study, and they accept responsibility to submit for publication.

Results

Baseline characteristics

In this study, 4877 patients with a positive SARS-CoV-2 PCR test result were screened between April 1, 2022 and June 30, 2022 from Shanghai, among which 610 patients with NAFLD were identified. After excluding patients with only one dose of inactivated vaccine, last inactivated vaccination over 3 months before the diagnosis of SARS-CoV-2 infection, no liver function tests during hospitalization, or refusal to consent, 517 patients with NAFLD were stratified by SARS-CoV-2 vaccination status and included in the final analysis (Fig. 1). The baseline characteristics of the patients are presented in Table 1. Before PSM, the vaccinated group (n = 274) was observed to be comparatively younger (median [IQR] age, 66.50 [15.00] years vs. 71.50 [21.00] years, $p < 0.001$) than the unvaccinated group (n = 243). Additionally, the vaccinated group exhibited a higher proportion of male patients (63.5% vs. 54.3%, $p = 0.034$) and lower proportions of those with smoking habits (9.9% vs. 16.0%, $p = 0.185$), hypertension (32.5% vs. 53.1%, $p < 0.001$), and CVD (12.4% vs. 21.4%, $p = 0.006$) than the unvaccinated group. Furthermore, compared with the unvaccinated group, the vaccinated group had more patients with mild COVID-19 (81.4% vs. 68.7%, $p < 0.001$). Moreover, the prevalence of liver function abnormality at baseline (3 months before infection) was 32.8% in the vaccinated group and 31.7% in the unvaccinated group ($p = 0.778$).

After 1:1 matching based on PSM, the final study cohort included 171 pairs of vaccinated and unvaccinated patients, as shown in Table 1. Subsequently, the baseline characteristics were well balanced between the two groups (Supplementary Figure S1). After PSM, the prevalence of liver function abnormality at baseline in the vaccinated and unvaccinated groups was 31.0% and 32.7%, respectively ($p = 0.728$).

Outcome

The vaccinated group was found to have a lower incidence of liver function abnormalities after COVID-19 than the unvaccinated group. The primary composite endpoint occurred in 217 (42.0%) patients, of which 118 (48.6%) were in the unvaccinated group and 99 (36.1%) in the vaccinated group (Supplementary Table S6). The crude OR for the composite outcome associated with vaccination was 0.599 (95% CI, 0.421–0.852; $p = 0.004$), while the adjusted OR was 0.559 (95% CI, 0.382–0.819; $p = 0.003$). In terms of the liver function parameters, the occurrence of TBIL levels above the upper limit of the normal range was lower in the vaccinated group than in the unvaccinated group (2.9% vs. 8.2%), with a crude OR of 0.335 (95% CI, 0.145–0.776; $p = 0.011$) and adjusted OR of 0.279 (95% CI, 0.114–0.680; $p = 0.005$). Similarly, the incidence of DBIL levels above the upper limit of the normal range was lower in the vaccinated group than in the unvaccinated group (6.7% vs. 26.2%), wherein the crude OR was 0.202 (95% CI, 0.114–0.360; $p < 0.001$) and adjusted OR was 0.175 (95% CI, 0.093–0.326; $p < 0.001$). In the PSM model, the primary composite outcome was observed in 140 (40.9%) patients, with 82 (48.0%) in the unvaccinated group and 58 (33.9%) in the vaccinated group, with associated crude and adjusted ORs of 0.557 (95% CI, 0.360–0.862; $p = 0.009$) and 0.556 (95% CI, 0.356–0.869; $p = 0.010$), respectively. Furthermore, the occurrence of TBIL levels above the upper limit of the normal range was 9.4% in the unvaccinated group and 2.3% in the vaccinated group (crude OR, 0.232 [95% CI, 0.076–0.709], $p = 0.010$; adjusted OR, 0.223 [95% CI, 0.072–0.690], $p = 0.009$). Additionally, the occurrence of DBIL levels above the upper limit of the normal range was 24.0% in the unvaccinated group and 5.6% in the vaccinated group (crude OR, 0.187 [95% CI, 0.087–0.403], $p < 0.001$; adjusted OR, 0.175 [95% CI, 0.080–0.384], $p < 0.001$) (Table 2).

Regarding the secondary outcome, 511 individuals had complete data on viral clearance duration, after excluding the 6 cases with missing data. A complete case analysis was conducted, revealing that the vaccinated group exhibited a shorter duration of viral clearance during COVID-19 than the unvaccinated group. Moreover, the median virus elimination time was 15 days (95% CI, 14.278–15.722) in all patients (Supplementary Table S7), whereas the vaccinated group exhibited a shortened virus elimination time (13

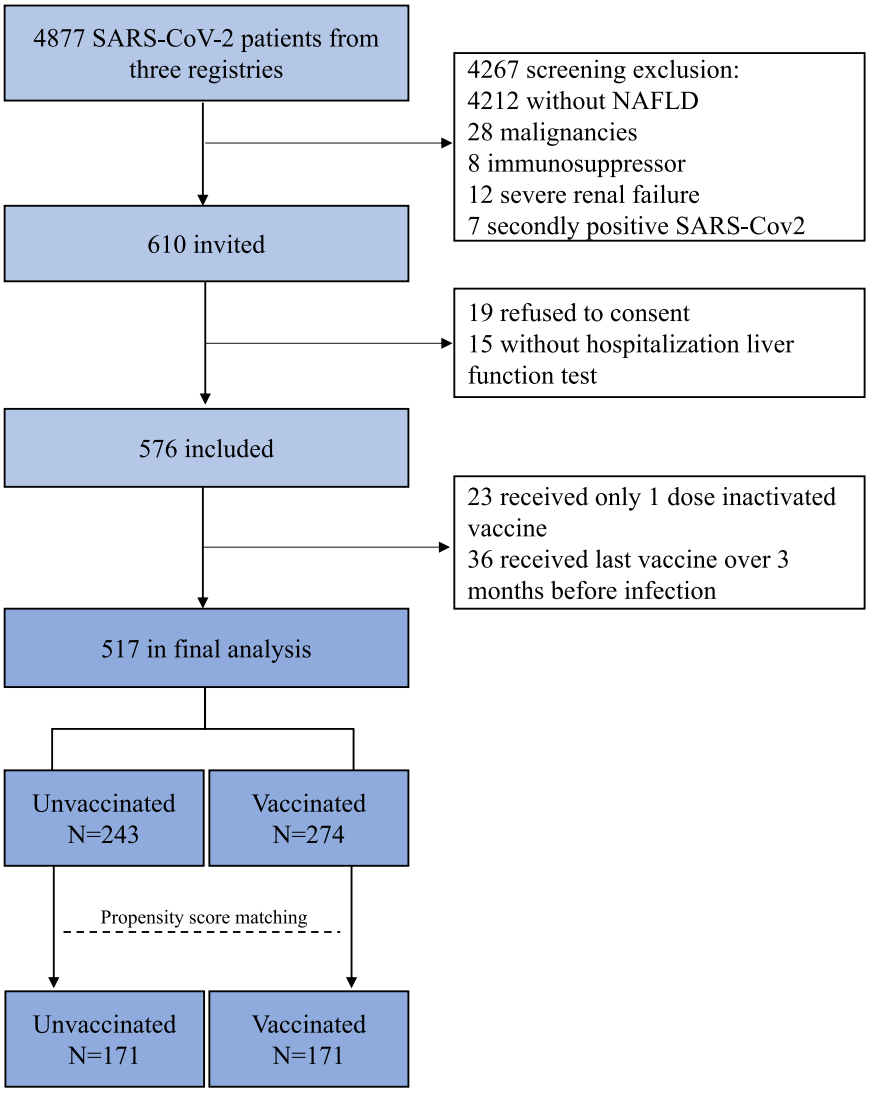

Fig. 1: **Flowchart of the current study.**

days, 95% CI, 11.653–14.347 vs. 16 days, 95% CI, 14.993–17.007) compared with the unvaccinated group. Furthermore, the crude HR for viral clearance was 0.727 (95% CI, 0.610–0.867; $p < 0.001$), and the adjusted HR was 0.791 (95% CI, 0.655–0.955; $p = 0.015$). After PSM, the difference in the median virus elimination time was reduced between the vaccinated and unvaccinated groups (14 days vs. 15 days, respectively). Additionally, the crude and adjusted HRs remained significant after PSM (crude OR, 0.781 [95% CI, 0.630–0.970], $p = 0.025$; adjusted HR, 0.798 [95% CI, 0.641–0.994], $p = 0.044$) (Fig. 2).

Additionally, there were no reported deaths among the 517 participants. And two patients (0.4%) were admitted to the ICU because of critical pneumonia, all of whom were from the unvaccinated group (0.8%).

Subgroup analysis

Subgroup analyses were performed to analyze the associations of the primary composite and secondary outcomes with inactivated vaccination based on the stratification by individual characteristics including demographic and behavioral features. The subgroup analysis results after matching are presented in Fig. 3. The association between vaccination and the risk of liver function abnormality showed differences across the sex and BMI groups ($p = 0.008$ and $p = 0.030$, respectively, for interaction). The OR for male patients was 0.332 (95% CI, 0.185–0.597; $p < 0.001$), whereas that for female patients was 1.108 (95% CI, 0.567–2.164; $p = 0.765$). Furthermore, the OR for low BMI was 0.385 (95% CI, 0.220–0.673; $p < 0.001$), while that for high BMI was 1.058 (95% CI, 0.516–2.170; $p = 0.879$). In

Characteristics	Unmatched				Matched			
	Unvaccinated N = 243	Vaccinated N = 274	Standard mean difference	p value	Unvaccinated N = 171	Vaccinated N = 171	Standard mean d ifference	p value
Demographics and history								
Age, median (IQR)	71.50 (21.00)	66.50 (15.00)	0.488	**<0.001**	67.00 (17.00)	68.00 (10.00)	0.049	0.731
Male, n (%)	132 (54.3%)	174 (63.5%)	0.187	**0.034**	97 (56.7%)	99 (57.9%)	0.024	0.827
BMI (kg/m^2), mean (SD)	27.02 (3.23)	27.36 (4.40)	0.038	0.319	26.59 (3.56)	27.43 (4.20)	0.043	0.335
Habitual smoker, n (%)	39 (16.0%)	27 (9.9%)	0.185	**0.035**	21 (12.3%)	20 (11.7%)	0.018	0.868
Habitual drinker, n (%)	13 (5.3%)	17 (6.2%)	0.037	0.678	11 (6.4%)	10 (5.8%)	0.024	0.822
Hypertension, n (%)	129 (53.1%)	89 (32.5%)	0.426	**<0.001**	74 (43.3%)	76 (44.4%)	0.024	0.827
Diabetes, n (%)	127 (52.3%)	128 (46.7%)	0.111	0.208	85 (49.7%)	88 (51.5%)	0.035	0.746
Hyperlipidemia, n (%)	69 (28.4%)	76 (27.7%)	0.015	0.868	49 (28.7%)	50 (29.2%)	0.013	0.905
Coronary heart disease, n (%)	52 (21.4%)	34 (12.4%)	0.242	**0.006**	30 (17.5%)	31 (18.1%)	0.015	0.888
Medications, n (%)	185 (76.1%)	194 (70.8%)	0.121	0.172	126 (73.7%)	127 (74.3%)	0.013	0.902
Hypotensive drugs, n (%)	129 (53.1%)	89 (32.5%)	0.426	**<0.001**	74 (43.4%)	76 (44.4%)	0.024	0.827
Hypoglycemic drugs, n (%)	121 (49.8%)	123 (44.9%)	0.098	0.265	80 (46.8%)	85 (49.7%)	0.059	0.588
Lipid-lowering drugs, n (%)	52 (21.4%)	44 (16.1%)	0.137	0.119	37 (21.6%)	30 (17.5%)	0.103	0.340
Baseline liver function								
Liver abnormality, n (%)	77 (31.7%)	90 (32.8%)	0.025	0.778	56 (32.7%)	53 (31.0%)	0.038	0.728
TBIL (U/L), median (IQR)	10.75 (6.30)	11.15 (6.50)	0.068	0.221	10.60 (7.20)	10.90 (7.20)	0.008	0.586
Normal, n (%)	172 (96.1%)	192 (96.5%)	0.162	0.856	115 (94.3%)	119 (95.2%)	0.217	0.766
1–2 ULN, n (%)	7 (3.9%)	5 (2.5%)			7 (5.7%)	4 (3.2%)		
2–3 ULN, n (%)	0 (0.0%)	2 (1.0%)			0 (0.0%)	2 (1.6%)		
>3 ULN, n (%)	0 (0.0%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
DBIL (U/L), median (IQR)	2.65 (1.80)	2.90 (1.90)	0.060	0.658	2.85 (2.00)	2.80 (2.20)	0.136	0.455
Normal, n (%)	162 (92.0%)	192 (93.2%)	0.169	0.679	117 (91.4%)	113 (90.4%)	0.193	0.764
1–2 ULN, n (%)	12 (6.8%)	11 (5.3%)			9 (7.0%)	9 (7.2%)		
2–3 ULN, n (%)	2 (1.1%)	1 (0.5%)			2 (1.6%)	1 (0.8%)		
>3 ULN, n (%)	0 (0.0%)	2 (1.0%)			0 (0.0%)	2 (1.6%)		
ALT (U/L), median (IQR)	21.50 (20.80)	24.00 (23.80)	0.051	0.901	21.50 (28.50)	23.50 (22.80)	0.023	0.699
Normal, n (%)	168 (87.5%)	194 (87.0%)	0.121	0.841	117 (86.0%)	124 (89.2%)	0.221	0.454
1–2 ULN, n (%)	23 (12.0%)	25 (11.2%)			19 (14.0%)	13 (9.4%)		
2–3 ULN, n (%)	1 (0.5%)	4 (1.8%)			0 (0.0%)	2 (1.4%)		
>3 ULN, n (%)	0 (0.0%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
AST (U/L), median (IQR)	24.50 (13.80)	24.00 (10.00)	0.035	0.309	24.50 (14.50)	23.00 (9.80)	0.062	0.116
Normal, n (%)	163 (81.9%)	187 (84.6%)	0.162	0.501	118 (81.9%)	116 (86.6%)	0.245	0.325
1–2 ULN, n (%)	34 (17.1%)	29 (13.1%)			25 (17.4%)	15 (11.2%)		
2–3 ULN, n (%)	1 (0.5%)	4 (1.8%)			0 (0.0%)	2 (1.5%)		
>3 ULN, n (%)	1 (0.5%)	1 (0.5%)			1 (0.7%)	1 (0.7%)		
ALP (U/L), median (IQR)	71.50 (26.00)	76.00 (33.00)	0.073	0.559	70.00 (23.00)	76.00 (31.00)	0.092	0.819
Normal, n (%)	175 (98.3%)	201 (97.6%)	0.215	0.625	119 (99.2%)	126 (97.7%)	0.120	0.350
1–2 ULN, n (%)	1 (0.6%)	5 (2.4%)			1 (0.8%)	3 (2.3%)		
2–3 ULN, n (%)	2 (1.1%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
>3 ULN, n (%)	0 (0.0%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
GGT (U/L), median (IQR)	34.50 (27.00)	32.00 (29.00)	0.187	**0.002**	36.00 (33.00)	32.00 (36.00)	0.199	**0.011**
Normal, n (%)	158 (86.8%)	190 (87.2%)	0.174	0.962	116 (87.2%)	118 (87.4%)	0.224	0.981
1–2 ULN, n (%)	24 (13.2%)	25 (11.5%)			17 (12.8%)	14 (10.4%)		
2–3 ULN, n (%)	0 (0.0%)	3 (1.4%)			0 (0.0%)	3 (2.2%)		
>3 ULN, n (%)	0 (0.0%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
SARS-CoV-2 severity								
Asymptomatic, n (%)	25 (10.3%)	24 (8.8%)	0.353	**<0.001**	18 (10.5%)	16 (9.4%)	0.041	0.742
Mild, n (%)	167 (68.7%)	223 (81.4%)			130 (76.0%)	131 (76.6%)		

(Table 1 continues on next page)

Characteristics	Unmatched				Matched			
	Unvaccinated N = 243	Vaccinated N = 274	Standard mean difference	p value	Unvaccinated N = 171	Vaccinated N = 171	Standard mean d ifference	p value
(Continued from previous page)								
Moderate, n (%)	43 (17.7%)	26 (9.5%)			22 (12.9%)	23 (13.5%)		
Severe, n (%)	6 (2.5%)	1 (0.4%)			1 (0.6%)	1 (0.6%)		
Critical, n (%)	2 (0.8%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		

BMI, body mass index; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; ULN, upper limit of normal. Bold *p* values denoted statistical significance at the $p < 0.05$ level.

Table 1: **Characteristics of COVID-19 patients with NAFLD at baseline before and after propensity-score matching.**

contrast, no difference was observed in the association between inactivated vaccination and the risk of liver function abnormality between the subgroups of age, hypertension, diabetes, CVD, and hyperlipidemia. In the case of the secondary outcome, the findings were consistent with the endpoint results (Supplementary Figure S4).

Sensitivity analysis

In all sensitivity analyses (Supplementary Tables S1–S5), vaccination was consistently associated with a decreased risk of liver function abnormality after COVID-19 infection. Compared with our main model analysis, the alternative analysis based on the traditional methods of identifying and adjusting for confounders revealed that the OR estimates of the primary composite outcome were minimally altered, with the ORs changing from 0.559 (95% CI, 0.382–0.819; $p = 0.003$) to 0.586 (95% CI, 0.398–0.864; $p = 0.007$) (Supplementary Table S4).

Discussion

Our study was a multi-center retrospective cohort investigation of the association between inactivated SARS-CoV-2 vaccination and liver function in patients having NAFLD with COVID-19 and revealed the following findings: (1) among all patients with NAFLD, 42.0% (217/517) exhibited liver function abnormalities upon admission, (2) inactivated SARS-CoV-2 vaccination was associated with a reduced incidence of liver function abnormality (36.1% [vaccinated group] vs. 48.6% [unvaccinated group], $p = 0.004$), and (3) inactivated SARS-CoV-2 vaccination was linked to a shorter virus shedding duration in vaccinated patients with NAFLD compared with those who were unvaccinated (median virus elimination time, 13 days vs. 16 days, $p < 0.001$). Therefore, our study results prove the potential protection of inactivated SARS-CoV-2 vaccination against liver function abnormalities among individuals with NAFLD who contract COVID-19.

Liver function abnormalities have been observed in the general population after COVID-19 infection, with a reported incidence of 14%–53%.[45] In the case of patients with NAFLD and COVID-19, their characteristic immune imbalance[16] and increased COVID-19 susceptibility[3] lead to a higher prevalence of liver function abnormalities (50%–59%).[46] Recent evidence from clinical autopsy samples and molecular studies has supported the hepatic tropism of SARS-CoV-2 in individuals with NAFLD.[47] The inflamed hepatocytes in patients with NAFLD exhibit mitochondrial

Outcome	Matched						
	All (n = 342)	Unvaccinated (n = 171)	Vaccinated (n = 171)	Crude		Adjusted[a]	
				OR (95% CI)	p[b] value	OR (95% CI)	p[b] value
Liver abnormality, n (%)	140 (40.9%)	82 (48.0%)	58 (33.9%)	0.557 (0.360–0.862)	**0.009**	0.556 (0.356–0.869)	**0.010**
TBIL >1 ULN, n (%)	20 (5.8%)	16 (9.4%)	4 (2.3%)	0.232 (0.076–0.709)	**0.010**	0.223 (0.072–0.690)	**0.009**
DBIL >1 ULN, n (%)	46 (14.6%)	37 (24.0%)	9 (5.6%)	0.187 (0.087–0.403)	**<0.001**	0.175 (0.080–0.384)	**<0.001**
ALT >1 ULN, n (%)	57 (16.7%)	30 (17.5%)	27 (15.8%)	0.881 (0.499–1.557)	0.663	0.887 (0.494–1.594)	0.689
AST >1 ULN, n (%)	70 (20.5%)	42 (24.6%)	28 (16.4%)	0.601 (0.353–1.026)	0.062	0.607 (0.354–1.043)	0.071
ALP >1 ULN, n (%)	13 (3.8%)	7 (4.1%)	6 (3.5%)	0.847 (0.279–2.574)	0.769	0.826 (0.265–2.571)	0.741
GGT >1 ULN, n (%)	51 (15.2%)	26 (15.6%)	25 (14.8%)	0.942 (0.519–1.709)	0.843	0.950 (0.518–1.743)	0.868

OR, odds ratio; CI, confidence interval; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; ULN, upper limit of normal. Bold *p* values denoted statistical significance at the $p < 0.05$ level. [a]In Logistic regression model, adjusted variables for comparing unvaccinated and vaccinated cohorts included age, gender, BMI, diabetes, coronary heart disease, hypertension and medications. [b]Odds ratios and *p* values were calculated based on Logistic regression model.

Table 2: **Comparison of primary composite outcome between propensity-score matched groups.**

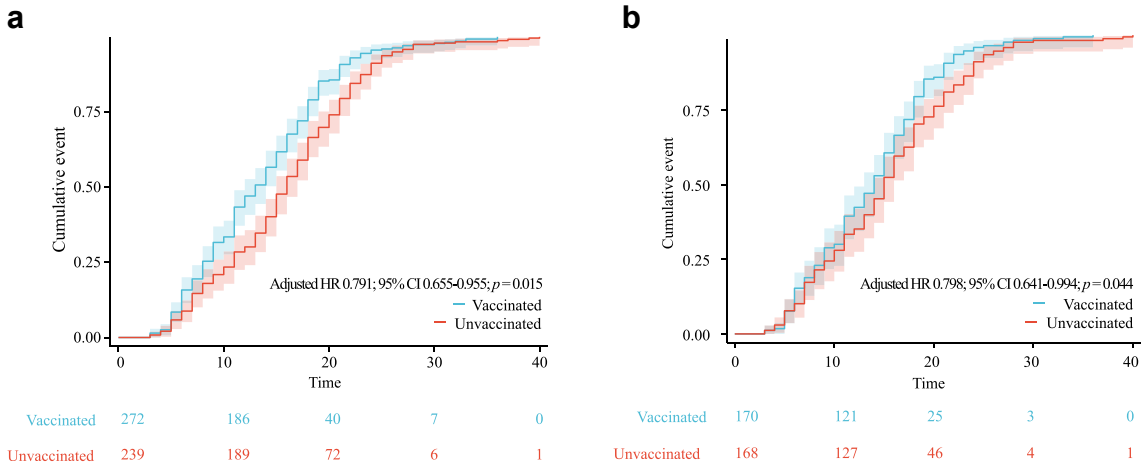

***Fig. 2:* Kaplan–Meier survival curves for secondary outcome of virus shedding duration.** The vaccination shortened the time to SARS-CoV-2 viral clearance in NAFLD population before propensity score matching (a) and after matching (b). Hazard ratios are adjusted by confounders based on directed acyclic graph using the Cox regression model. HR, hazard ratio.

dysfunction,[48] which may upregulate the expression of SARS-CoV-2 entry receptors in the liver[49] and facilitate viral infection. Moreover, the virus can directly target hepatocytes after it enters the liver, further exacerbating mitochondrial dysfunction.[50] Consequently, the interaction between NAFLD and COVID-19 could amplify the severity of both conditions. In our sensitivity analysis, we further selected 350 participants who had normal liver function within the past 3 months and found that 122 (34.9%) had shown abnormal liver function after hospital admission for COVID-19. This observed liver dysfunction in a significant proportion of the patients suggests a close association between COVID-19 infection and hepatobiliary damage, consistent with prior pathological evidence.[47] Furthermore, we found that full vaccination with inactivated vaccines could reduce the risk of liver function abnormalities in patients having COVID-19 with preexisting NAFLD, suggesting that COVID-19 vaccines might help maintain hepatic homeostasis during SARS-CoV-2 infection.

Characteristic	Unvaccinated	Vaccinated	OR (95% CI)	P value	Interaction P
All patients	171	171	0.557 (0.360–0.862)	**0.009**	
Age					
<=65 yrs	32 (46.4%)	18 (29.0%)	0.473 (0.229–0.976)	0.043	0.601
>65 yrs	50 (49.0%)	40 (36.7%)	0.603 (0.348–1.045)	0.071	
Gender					
Female	27 (36.5%)	28 (38.9%)	1.108 (0.567–2.164)	0.765	**0.008**
Male	55 (56.7%)	30 (30.3%)	0.332 (0.185–0.597)	**<0.001**	
BMI					
<=28 kg/m²	54 (49.5%)	31 (27.4%)	0.385 (0.220–0.673)	**0.001**	**0.030**
>28 kg/m²	28 (45.2%)	27 (46.6%)	1.058 (0.516–2.170)	0.879	
Hypertension					
NO	49 (50.5%)	35 (36.8%)	0.571 (0.321–1.017)	0.057	0.057
YES	33 (44.6%)	23 (30.3%)	0.539 (0.276–1.054)	0.071	
Diabetes					
NO	51 (59.3%)	30 (36.1%)	0.388 (0.209–0.723)	0.003	0.102
YES	31 (36.5%)	28 (31.8%)	0.813 (0.433–1.526)	0.519	
CVD					
NO	66 (46.8%)	47 (33.6%)	0.574 (0.355–0.930)	0.024	0.760
YES	16 (53.3%)	11 (35.5%)	0.481 (0.172–1.345)	0.163	
Hyperlipidemia					
NO	55 (45.1%)	37 (30.6%)	0.537 (0.317–0.908)	0.020	0.845
YES	27 (55.1%)	21 (42.0%)	0.590 (0.266–1.307)	0.193	

***Fig. 3:* Subgroup analyses.** Forest plot of subgroup analysis of association between inactivated vaccines and liver function abnormality in the NAFLD population by baseline demographic and disease characteristics. Odds ratios for patient subgroups are from univariate analyses using the Logistic regression model. OR, Odds ratio; CI, confidence interval; BMI, body mass index; CVD, coronary heart disease.

In our study, the NAFLD population demonstrated a predominantly hepatocellular pattern of liver injury after COVID-19, rather than a cholestatic pattern. The proportions of patients exhibiting abnormal AST and ALT levels, which reflect hepatocellular damage, were 20.7% (107/517) and 16.2% (84/517), respectively. In contrast, the number of patients with abnormal ALP levels (indicative of biliary system injury) was relatively low at 3.5% (18/517). Moreover, elevated AST and DBIL levels on admission have been shown to be independently associated with an increased risk of COVID-19-related death.[51,52] Therefore, liver function abnormalities should be taken seriously, despite their mild nature (Supplementary Table S8).[5]

Our study suggests that inactivated SARS-CoV-2 vaccination might, to some extent, reduce the incidence of liver function abnormalities, particularly demonstrating a potential reduction in bilirubin levels. This finding was supported by our analysis after PSM that showed the vaccinated group had lower adjusted ORs for TBIL (adjusted OR, 0.223 [95% CI, 0.072–0.690], $p = 0.009$) and DBIL (adjusted OR, 0.175 [95% CI, 0.080–0.384], $p < 0.001$) than the unvaccinated group. Our observations also noted declining trends in indicators such as AST (16.4% vs. 24.6%, adjusted OR, 0.607 [95% CI, 0.354–1.043], $p = 0.071$) and ALT (15.8% vs. 17.5%, adjusted OR, 0.887 [95% CI, 0.494–1.594], $p = 0.689$). Bilirubin, as a comprehensive marker reflecting liver function, is influenced by a complex interplay of factors, including bile stasis and hepatocellular damage.[53,54] This complexity suggests that bilirubin may be more sensitive to vaccination-induced changes when compared to other markers, while trends in other markers of hepatocellular damage might require larger sample sizes to establish their significance. In summary, our study suggests that vaccines may offer a multi-dimensional protection of liver function in NAFLD individuals. The reduction in bilirubin levels hints at the possibility of comprehensive protection, although we acknowledge the need for further research to validate these preliminary findings.

Besides, our study also indicated that inactivated vaccination was associated with a shortened duration of viral clearance in individuals with NAFLD. This rapid decline in the viral load suggests the possibility of a relatively milder degree of liver injury within the vaccinated group. Based on all these results, vaccination may have a promising role in mitigating liver-related complications caused by COVID-19 in patients having NAFLD.

Considering that individuals with chronic liver disease (CLD), including NAFLD and cirrhosis, typically exhibit increased COVID-19 severity and mortality,[55] global guidelines have strongly recommended vaccination for these individuals with immune dysregulation and immunosuppression, aiming to reduce the likelihood of COVID-19-associated severe outcomes. However, clinical evidence supporting the COVID-19 vaccination recommendations in this patient population is limited, with only a few studies investigating the adverse reactions to vaccination and vaccine immunogenicity in individuals with CLDs.[17,26,27,30] Moreover, no researchers have specifically addressed the effect of vaccination on liver function in individuals with NAFLD after COVID-19 infection. Our study demonstrated the hepatoprotective effect of inactivated vaccines in individuals with NAFLD who contract COVID-19. Additionally, inactivated SARS-CoV-2 vaccines have been widely used in many developing countries, such as China, India,[56] Chile,[57] Brazil,[57] and various African nations, amounting to approximately 50 countries worldwide. Thus, our study not only provides supporting evidence for the global guidelines but also has significant guiding value for countries using inactivated vaccines. We also noted that the COVID-19 vaccination proportion was lower (52.9%) in the hospitalized patients with NAFLD included in our study than in the global population (70.1%).[58] The low vaccination rate among patients with NAFLD in our study may be attributed to concerns regarding the potential passive effect of the vaccines on the liver. Our research findings may help address these concerns and reduce vaccine hesitancy. Therefore, our study results highlight the need to increase the vaccination rate within this population by providing evidence of the protective effects of vaccination on liver function in patients with NAFLD and COVID-19.

Our study has several limitations that should be considered. First, we only included NAFLD inpatients with COVID-19 in three designed hospitals of the central, western and eastern regions in Shanghai, which may have led to some selection bias. Second, despite our rigorous efforts to address confounding variables through PSM and sensitivity analyses, unmeasured factors may have affected the accuracy of our findings. For example, personal living habits, such as diet and exercise, could have influenced the development of hepatobiliary abnormalities in our study population. Nevertheless, the anti-epidemic measures implemented in China during the study period led to the patients working remotely from home and receiving government-supplied food. Therefore, their living habits were similar, which might have minimized the influence of such external factors on the study outcomes. Finally, our sample size was small. Thus, large-scale prospective follow-up studies are required to further explore the causality of vaccination and hepatobiliary abnormalities in the NAFLD population.

In conclusion, our study suggests that complete inactivated vaccination may reduce the risk of liver function abnormalities and shorten the virus shedding time in individuals with NAFLD during COVID-19 infection. Therefore, we suggest that full vaccination should be undertaken in the NAFLD population.

Contributors

Ruyi Xue, Si Zhang, Wenqing Tang and Zhixue Chen designed study, collected and analyzed data, and wrote manuscript. Zhixue Chen, Wenqing Tang, Nana Feng, Fansheng Meng, Huibin Wu, Yitong Zhao, Yuxin Dai, Jindan Xue, Jingya Wang, Dejie Chu, Beilin Zhan, Yuqin Li and Anjun Xu collected and revised clinical, laboratory data. Zhixue Chen, Minzhi Lv and Huajie Xu performed statistical analysis, reviewed, interpreted, and checked clinical data. Ruyi Xue, Ling Dong, Si Zhang and Dejun Wu provided valuable suggestions for study design and data analysis. Ruyi Xue and Si Zhang contributed equally, designed the project, edited manuscript, and supervised the study. Ruyi Xue and Si Zhang accessed and verified the data. All authors reviewed and/or edited the final manuscript and were responsible for the decision to submit the manuscript.

Data sharing statement

The de-identified dataset for analysis will be made available to researchers on request after publication. Requests for data should be addressed to the corresponding authors.

Declaration of interests

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104912.

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