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Original Article

Dynamic Humoral Immune Response to Primary and Booster Inactivated SARS-CoV-2 Vaccination in Patients with Cirrhosis

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Abstract

Background and Aims: Our aim was to determine the immune efficacy of a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) booster vaccination in cirrhotic patients who had received the primary series. ***Methods:*** We performed a longitudinal assessment in 48 patients with cirrhosis, 57 patients with chronic hepatitis B (CHB) and 68 healthy controls (HCs) to continuously track the dynamics of SARS-CoV-2 specific antibodies and memory B cells after receiving the primary series and booster dose at different times. A pseudovirus neutralization assay was used to determine neutralization against Omicron subvariants BA.2.12.1, BA.4 and BA.5 from serum samples collected from three cohorts. ***Results:*** Serum anti-receptor-binding domain (RBD) immunoglobulin (Ig)G and neutralizing antibody (NAb) levels in cirrhotic patients were elevated within 15–45 days after completing the primary series before rapidly declining and reaching a valley at around 165–195 days. After receiving the booster dose, both antibody levels were significantly increased to levels comparable to patients with CHB and HCs. Subgroup analysis showed that booster vaccination induced weaker antibody responses in patients with decompensated cirrhosis than in those with compensated cirrhosis. The SARS-CoV-2 memory B-cell response in cirrhotic patients was durable during follow-up regardless of the hepatic fibrocirrhosis grade. However, compared with the primary series, the booster dose did not result in an evident improvement of neutralization activity against the Omicron subvariants BA.2.12.1 and BA.4, and was followed by a significant decrease in the titer against BA.5. ***Conclusions:*** A booster dose elicited a robust and durable humoral response to the wild-type strain in cirrhotic patients but not the Omicron subvariants. Repeated vaccination of inactivated SARS-CoV-2 vaccine may not benefit cirrhotic patients in neutralization against newly circulating strains.

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Introduction

A primary two dose series of inactivated coronavirus disease 2019 (COVID-19) vaccinations induced a robust immune response against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and provided a protective effect in the general population.[1,2] However, for populations with severe comorbid conditions, the immune response after vaccination is of great concern.[3–8] Our previous studies have shown that humoral and cellular responses reduced by the primary series are influenced by the immune status in patients with chronic hepatitis B (CHB) and with severe liver disease.[9,10] Omicron subvariants have recently become the predominant SARS-CoV-2 globally, and they escape from the neutralizing antibody (NAb) responses elicited by the primary vaccination series to result in breakthrough infections.[11,12] These observations imply that patients with comorbid conditions such as liver cirrhosis should be considered a population remaining at high risk for SARS-CoV-2 infection despite having completed the primary vaccination series.

Cirrhosis is a common chronic disease and is accompanied by impaired immune function in some patients.[13–15] It is of great clinical value to assess the efficacy of booster doses and their neutralizing activity against the BA.4/BA.5 Omicron subvariants in this population. Currently, there are only a few reports describing the immune response to the booster

Keywords: SARS-CoV-2; Omicron subvariants; Cirrhosis; Booster dose; Humoral response.

Abbreviations: actMBCs, activated memory B cells; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; atyMBCs, atypical memory B cells; BMI, body mass index; BV, booster vaccination; CHB, chronic hepatitis B; CI, confidence interval; DB, direct bilirubin; ETV, entecavir; GGT, gamma-glutamyl transferase; GMT, geometric mean titer; HB, hemoglobin; HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HC, healthy control; Ig, immunoglobulin; MBCs, memory B cells; NAb, neutralizing antibody; PLT, platelet; PV, primary vaccination; RBC, red blood cell; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TB, total bilirubin; TDF, tenofovir disoproxil fumarate; WBC, white blood cell; WT, wild-type.

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dose,[14,16,17] and no data have been published on its protective effect against the Omicron subvariants in cirrhotic patients. Our aim was to determine whether the booster dose elicits a robust humoral immune response in cirrhotic patients who had received the primary series, to provide evidence to improve the vaccination strategies in this vulnerable population.

Methods

Study population and design

This prospective cohort study was conducted between June 2021 to July 2022 at the Department of Infectious Diseases of the Second Affiliated Hospital of Chongqing Medical University. A total of 48 patients with hepatitis B virus-related liver cirrhosis (HBV-LC) were enrolled. Clinical hepatologists made the diagnoses and assessments. A group of 57 patients with CHB and 68 healthy controls (HCs) were enrolled from our previous registered clinical study (NCT05007665). The baseline characteristics of the three cohorts are shown in Table 1. All participants had completed a two dose primary series of inactivated SARS-CoV-2 vaccine (BBIBP-CorV/CoronaVac) prior to enrollment. Peripheral blood samples were collected from cirrhotic patients at four times after the primary series and booster dose (T1-T4). The dynamics of serum SARS-CoV-2 antibody levels and vaccine-induced B-cell response and the neutralizing activity against the Omicron subvariants were predefined primary study endpoints. The study population and design are shown in Figure 1.

Adverse events (AEs)

Participant demographic data and AEs after the primary series and booster dose were recorded on questionnaires. AEs were verified by investigators and categorized following the scale of the National Medical Products Administration of China (version 2019).

Assay of anti-receptor-binding domain (RBD) immunoglobulin (Ig)G and NAbs

The anti-RBD IgG and NAbs in serum samples were evaluated by capture chemiluminescence immunoassays (MAGLUMI X8; Snibe, Shenzhen, China) following the manufacturer's instructions. The sensitivity, specificity, and cutoff values of the kits for anti-RBD IgG and NAbs were calculated. The assay is described in the Supplementary File 1.

Pseudovirus neutralization assay

Pseudotyped HIV-1 viruses expressing the spike of SARS-CoV-2 prototype (Wuhan-1) and its subvariants (Omicron BA.2.12.1, BA.4 and BA.5) were prepared by the Sino Biological Corporation (Beijing, China). The 50% serum pseudovirus neutralization titers (PVNT50) were calculated using the Reed–Muench method. The assay is described in the Supplementary File 1.

Flow cytometry for memory B cells (MBCs)

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood and used for detecting SARS-CoV-2-specific B cells. Biotinylated SARS-CoV-2 spike RBD protein was mixed with streptavidin-BV421 at a 4:1 molar ratio to obtain the antigen probe. PBMC were stained with the antigen probe and conjugated antibodies to detect SARS-CoV-2-specific B cells. $CD3^-CD19^+CD27^+$B cells were defined as $CD27^+$MBCs. $CD3^-CD19^+CD27^+CD21^-$B cells were defined as activated memory B cells (actMBCs). $CD3^-CD19^+CD27^+CD21^+$B cells were defined as resting memory B cells (rMBCs). $CD3^-CD19^+CD27^-CD21^-$B cells were defined as atypical memory B cells (atyMBCs). $CD3^-CD19^+CD27^-CD21^+$B cells were defined as intermediate memory B cells (intMBCs). Approximately 1×10^5 events were collected within a lymphocyte gate on the flow cytometer (CytoFLEX; Beckman Coulter, CA, USA). FlowJo (10.0.7r2; Treestar, OR, USA) was used for the analysis of the B-cell populations. The steps and gating strategies are shown in the Supplementary File 1 and Supplementary Figure 1.

Ethical considerations

The study was approved by the ethics committee of The Second Affiliated Hospital of Chongqing Medical University and conformed to the ethical guidelines of the Declaration of Helsinki. It was registered at www.chictr.org.cn (ChiCTR2100047936) and ClinicalTrials.gov (NCT05007665). Written informed consent was obtained from all participants before recruitment.

Statistical analysis

For categorical variables, chi-square and Fisher's exact tests were used for statistical comparison. For continuous variables, the Wilcoxon signed-rank test was used to compare paired-groups and the Mann-Whitney *U* test was used to compare unpaired groups. Propensity score matching was performed to balance the baseline characteristics of cirrhotic patients and controls. A two-sides p-value<0.05 was considered of statistical significance. Data were analyzed by SPSS (version 22.0.0; IBM Corp, Armonk, NY, USA) and GraphPad Prism (version 9.2.0; GraphPad Software Inc, La Jolla, CA, USA).

Results

Safety of the booster SARS-CoV-2 vaccine in patients with cirrhosis

AEs that occurred in cirrhotic patients within 7 and 30 days after the primary series and booster dose are shown in Table 2. The overall rate of AEs within 7 and 30 days after primary series was 12.5% (6/48) and 8.0% (2/25) after the booster dose. The most common local and systemic AEs in cirrhotic patients were pain and fatigue/diarrhea. No severe AEs (grade 3 or 4) occurred after the full vaccination course. We also compared the hepatic fibro-cirrhosis changes and activity of HBV infection in patients with cirrhosis before and after booster vaccination to observe the safety of booster dose. The results showed that liver function-associated indicators were stable and no significant clinical flare-ups had occurred (Supplementary Table 1).

SARS-CoV-2 antibody response and serum neutralization against Omicron subvariants elicited by a booster dose in patients with cirrhosis

We observed the dynamic changes in serum SARS-CoV-2 antibody responses in patients with cirrhosis after primary series and booster dose. The seropositivity rate of anti-RBD IgG was 89.6% (43/48) within 15–45 days after the second dose, decreased with time to 41.7% (10/24) at 166–195 days, and rose significantly to 96.2% (25/26) after the booster dose (Fig. 2A right panel). Anti-RBD IgG levels also decreased in the time after the second dose and increased significantly after the booster dose (0.8 AU/mL [interquartile range (IQR) 0.5–2.4] vs.14.9 AU/mL [IQR 3.3–42.3], p=0.001; Fig. 2A right panel). The seropositivity rate and NAb level showed

Table 1. Baseline characteristics of patients with cirrhosis, patients with CHB and HCs

Variable	Patients with cirrhosis, *n*=48	Patients with CHB, *n*=57	HCs, *n*=68
Age in years	52.0 (46.3–57.0)	40.0 (34.0–48.0)	30.0 (25.0–44.5)
Sex			
Male	70.8 (34/48)	61.4 (35/57)	48.5 (33/68)
Female	29.2 (14/48)	38.6 (22/57)	51.5 (35/68)
BMI in kg/m^2	24.5 (22.4–24.5)	23.0 (21.0–25.0)	22.0 (20.1–24.4)
Vaccines			
BBIBP-CorV	31.3 (15/48)	36.8 (21/57)	72.1 (49/68)
CoronaVac	56.2 (27/48)	56.1 (32/57)	25.0 (17/68)
BBIBP-CorV + CoronaVac	12.5 (6/48)	7.0 (4/57)	2.9 (2/68)
Blood sampling after vaccination			
T1	100.0 (48/48)	/	/
T2	60.4 (29/48)	/	/
T3	50.0 (24/48)	100.0 (57/57)	100.0 (68/68)
T4	54.2 (26/48)	100.0 (57/57)	100.0 (68/68)
Interval days			
Between PV and T1	30.5 (25.5–39.5)	/	/
Between PV and T2	101.0 (93.0–104.0)	/	/
Between PV and T3	188.5 (172.5–192.5)	174.0 (172.0–182.0)	246.5 (191.5–274.5)
Between BV and T4	84.0 (39.0–112.0)	69.0 (44.0–85.0)	29.0 (28.0–38.5)
Compensation status			
Decompensated cirrhosis	27.1 (13/48)	/	/
Compensated cirrhosis	72.9 (35/48)	/	/
Child–Pugh score			
Class A, score 5–6	54.5 (6/11)	/	/
Class B, score 7–9	45.5 (5/11)	/	/
Class C, score 10–15	0 (0/11)	/	/
HBeAg			
Positive	26.8 (11/41)	17.5 (10/57)	/
Negative	73.2 (30/41)	82.5 (47/57)	/
HBV DNA in IU/mL	10.0 (10.0–50.0)	50.0 (10.0–500.0)	/
<100 IU/mL	95.0 (38/40)	71.9 (41/57)	/
>100 IU/mL	5.0 (2/40)	28.1 (16/57)	/
Antiviral treatment			
ETV	66.7 (32/48)	22.8 (13/57)	/
TDF	14.6 (7/48)	19.3 (11/57)	/
Others	14.6 (7/48)	17.5 (10/57)	/
Non-antiviral therapy	4.2 (2/48)	40.3 (23/57)	/
Comorbidity			
Hypertension	4.2 (2/48)	0 (0/57)	0 (0/68)
Type 2 diabetes	2.1 (1/48)	0 (0/57)	0 (0/68)
Chronic lung diseases	4.2 (2/48)	0 (0/57)	0 (0/68)
Osteoporosis	2.1 (1/48)	0 (0/57)	0 (0/68)

(continued)

Table 1. *(continued)*

Variable	Patients with cirrhosis, *n*=48	Patients with CHB, *n*=57	HCs, *n*=68
Laboratory tests			
RBC as 10^{12}/L	4.8 (4.4–5.2)	4.8 (4.5–5.3)	4.8 (4.5–5.4)
HB in g/L	150.0 (139.5–158.5)	148.0 (136.0–159.0)	143.5 (135.0–158.5)
WBC as 10^9/L	5.6 (4.7–6.4)	5.5 (4.9–6.4)	6.2 (5.3–7.1)
Neutrophil as 10^9/L	2.8 (2.1–3.5)	/	/
Lymphocyte as 10^9/L	1.7 (1.5–2.3)	1.8 (1.5–2.0)	1.9 (1.7–2.4)
PLT as 10^9/L	142.0 (107.0–179.5)	193.0 (167.0–226.0)	244.5 (211.5–289.0)
ALB in g/L	46.1 (43.2–48.2)	48.4 (46.7–49.6)	48.6 (46.6–50.3)
ALT in U/L	23.0 (18.5–33.5)	20.0 (16.0–34.0)	15.5 (12.0–22.5)
AST in U/L	26.0 (23.5–33.5)	22.0 (18.0–32.0)	19.0 (17.0–21.5)
ALP in U/L	95.0 (76.0–107.0)	67.0 (57.0–80.0)	/
GGT in U/L	29.5 (19.5–44.5)	18.0 (12.0–32.0)	19.5 (14.0–31.5)
TB in μmol/L	13.2 (8.1–18.5)	13.0 (10.7–16.1)	9.0 (7.6–11.9)
DB in μmol/L	4.2 (2.7–5.9)	4.2 (3.3–5.2)	3.3 (2.8–3.8)

Categorical variables are presented as frequencies [% (*n*/*n*)], continuous variables are presented as median (interquartile range). When the HBV DNA level was lower than the detection limit (20 IU/mL), 10 IU/mL was assigned for calculation. When the HBV DNA level was lower than 100 IU/mL, 50 IU/mL was assigned for calculation. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BV, booster vaccination; CHB, chronic hepatitis B; DB, direct bilirubin; ETV, entecavir; GGT, gamma-glutamyl transferase; HB, hemoglobin; HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HC, healthy control; PLT, platelet; PV, primary vaccination; RBC, red blood cell; TB, total bilirubin; TDF, tenofovir disoproxil fumarate; WBC, white blood cell.

similar trends. The seropositivity rate decreased from 79.2% (38/48) to 50.0% (12/24) and then increased to 92.3% (24/26) after the booster dose (Fig. 2B right panel). NAb levels also rose significantly after the booster dose compared with 166–195 days after the second dose (0.1 μg/mL [IQR 0.1–0.2] vs. 0.8 μg/mL [IQR 0.3–4.6], p=0.001; Fig. 2B right panel). Correlation analysis showed a strong positive correlation between anti-RBD IgG and NAb level after the booster dose (R=0.95, p<0.001; Supplementary Fig. 2).

Subgroup analysis showed that booster vaccination induced a weakened anti-RBD IgG response in patients with decompensated cirrhosis (0.7 AU/mL [IQR 0.2–1.6] vs. 11.4 AU/mL [IQR 2.9 21.2], p=0.068), compared with compensated cirrhosis (0.8 AU/mL [IQR 0.5–2.8] vs. 21.5 AU/mL [IQR 3.5–79.1], p=0.008; Fig. 2C). Similar results were observed in the NAb response (Fig. 2D). To determine the effect of hepatic fibro-cirrhotic changes on the antibody response induced by the booster dose, we used the Child–Pugh score to assess hepatic function. In this study, 11 patients had known Child–Pugh scores. Six were Child–Pugh class A and 5 were Child–Pugh class B (Table 1). The antibody responses to the booster vaccination were poor in Child–Pugh class B patients (p=0.127 for anti-RBD-IgG, p=0.05 for NAbs; Supplementary Fig. 3A, B) despite the small sample size. In addition, HBV e antigen activity did not affect the antibody response in cirrhotic patients (Supplementary Fig. 3C, D).

We further compared antibody responses in cirrhotic patients to CHB patients and HCs. Propensity score analysis was used to identify two cohorts of CHB patients and HCs who were statistically matched on a 1:1 basis to cirrhotic patients. We matched for crucial variables that are known to impact the immune response to COVID-19 vaccine, including age, sex, body mass index, vaccine type, and interval days. The comparable variables are shown in Supplementary Tables 2 and 3. The anti-RBD IgG and NAb levels in cirrhotic patients and CHB patients 165–195 days after the primary series were comparable. After receiving the booster dose, the anti-RBD IgG and NAbs levels remarkably increased in both groups, but there was no significant difference between cirrhotic patients and CHB patients. Additionally, the com-

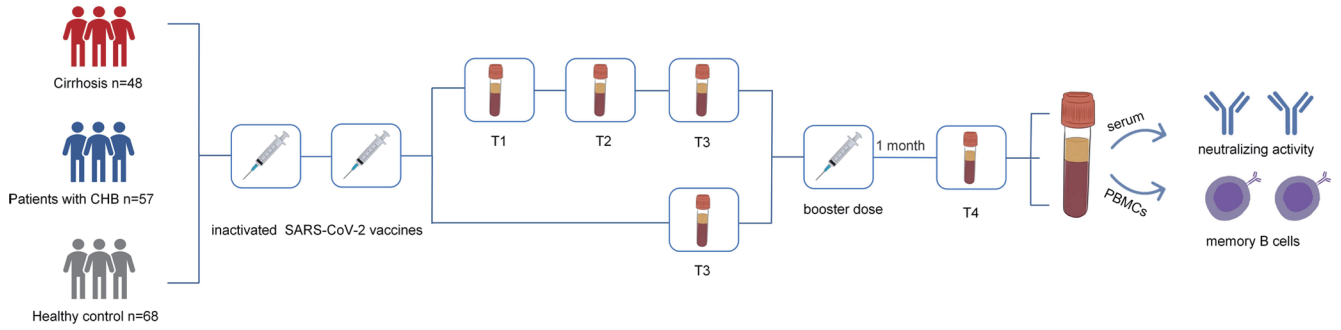

Fig. 1. Study design and flowchart of the three cohorts vaccinated with primary series and booster dose.

Table 2. Adverse events after the primary series and booster dose in patients with cirrhosis

	Primary series (*n*=48)	Booster dose (*n*=25)
Overall adverse events within 7 days	6 (12.5%)	2 (8.0%)
Overall adverse events within 30 days	6 (12.5%)	2 (8.0%)
Local adverse events		
Pain	3 (6.3%)	2 (8.0%)
Swelling	/	/
Redness	/	/
Itch	1 (2.1%)	/
Systemic adverse events		
Fatigue	1 (2.1%)	/
Headache	/	/
Nausea	/	/
Limb numbness	/	/
Lower extremity edema	/	/
Fever	/	/
Diarrhea	1 (2.1%)	/
Abdominal pain	/	/
Grade 3 and 4 adverse events	/	/

parison of cirrhotic patients and HCs yielded similar results (Fig. 2E, F).

Given concern of the antibody escape of emerging Omicron subvariants, we performed a pseudovirus neutralization assay using sera from five cirrhotic patients to evaluate the neutralization of Omicron subvariants BA.2.12.1, BA.4 and BA.5. One month after the booster dose, the serum geometric mean titers (GMTs) against Omicron subvariants BA.2.12.1, BA.4, and BA.5 were 1.4-fold, 3.6-fold and 4.3-fold lower, respectively than the titers against wild-type (WT) strain (Fig. 3A). The patients showed only a mild improvement of serum neutralization activity against WT, BA.2.12.1 and BA.4 strains after the booster dose (WT, 2.7-fold; BA.2.12.1, 1.9-fold; BA.4, 2.4-fold). There was no enhancement effect against BA.5 (Fig. 3B). When compared with CHB patients and HCs, the cirrhotic patients had lower GMTs against the WT strain and BA.4 and BA.5 Omicron subvariants (Fig. 3C).

The results revealed that the booster vaccination significantly restored the SARS-CoV-2 specific WT antibody levels but failed to elicit a robust neutralization response against the BA.2.12.1, BA.4 and BA.5 Omicron subvariants in patients with cirrhosis. Hepatic fibro-cirrhosis grades may be related to the weakened WT antibody response.

SARS-CoV-2 memory B-cell response to a booster dose in patients with cirrhosis

To further evaluate the durable humoral immune response of cirrhotic patients after the booster dose, we observed the SARS-CoV-2-specific B-cell response in circulation. The frequency of RBD^+CD27^+MBCs at T4 after receiving the booster dose remained stable (p=0.057; Fig. 4A left panel). The frequency of $RBD^+CD27^+CD38^+$MBCs within the RBD^+CD27^+MBC population increased after the primary series, peaking at T3 (p=0.003) and remained stable at T4 after the booster dose (p=0.638; Fig. 4B left panel).

To have more insight into the functional phenotypes, we gated on RBD^+B cells and compared the frequencies of actMBCs ($CD27^+CD21^-$), rMBCs ($CD27^+CD21^+$), atyMBCs ($CD27^-CD21^-$), and intMBCs ($CD27^-CD21^+$). Figure 4C shows the percentages of the four subpopulations of RBD^+MBCs at each time. Importantly, the frequencies of RBD^+ actMBCs were increased significantly between T1 and T3 after the primary series (p=0.001), and remained stable after the booster dose at T4 (Fig. 4D upper panel). RBD^+ rMBCs had an opposite trend, decreasing significantly after the primary series (p=0.017) and then remained stable after booster dose at T4 (Fig. 4D lower panel).

Subgroup analysis showed that booster vaccination induced similar RBD^+CD27^+MBC and $RBD^+CD27^+CD38^+$MBC responses in patients with decompensated and compensated cirrhosis (Fig. 4A right panel and 4B right panel). The sample sizes of patients with Child–Pugh class B (n=1 at T1, n=2 at T3) were insufficient for analysis. In addition, HBV e antigen activity did not have an impact on the memory B-cell responses in cirrhotic patients (Supplementary Fig. 3E, F). Changes of RBD^+MBCs like RBD^+actMBCs and RBD^+rMBCs after the booster dose in decompensated and compensated cirrhotic patients were similar (Supplementary Fig. 3G, H).

We next compared the B-cell responses in cirrhotic patients with those in CHB patients and HCs before and after the booster vaccination. The frequencies of RBD^+CD27^+MBCs decreased after the booster dose in CHB patients and HCs (Fig. 4E), but differences in the frequencies of $RBD^+CD27^+CD38^+$MBCs in the three cohorts were not significant (Fig. 4F). The results demonstrated that the SARS-CoV-2 memory B-cell response in cirrhotic patients was durable after receiving the booster dose regardless of the hepatic fibro-cirrhosis grade.

Discussion

This study focused primarily on the dynamic changes in serum anti-RBD IgG, NAbs, and neutralizing activity against Omicron subvariants (BA.2.12.1, BA.4, and BA.5) in cirrhotic patients after receiving the primary vaccination series and a

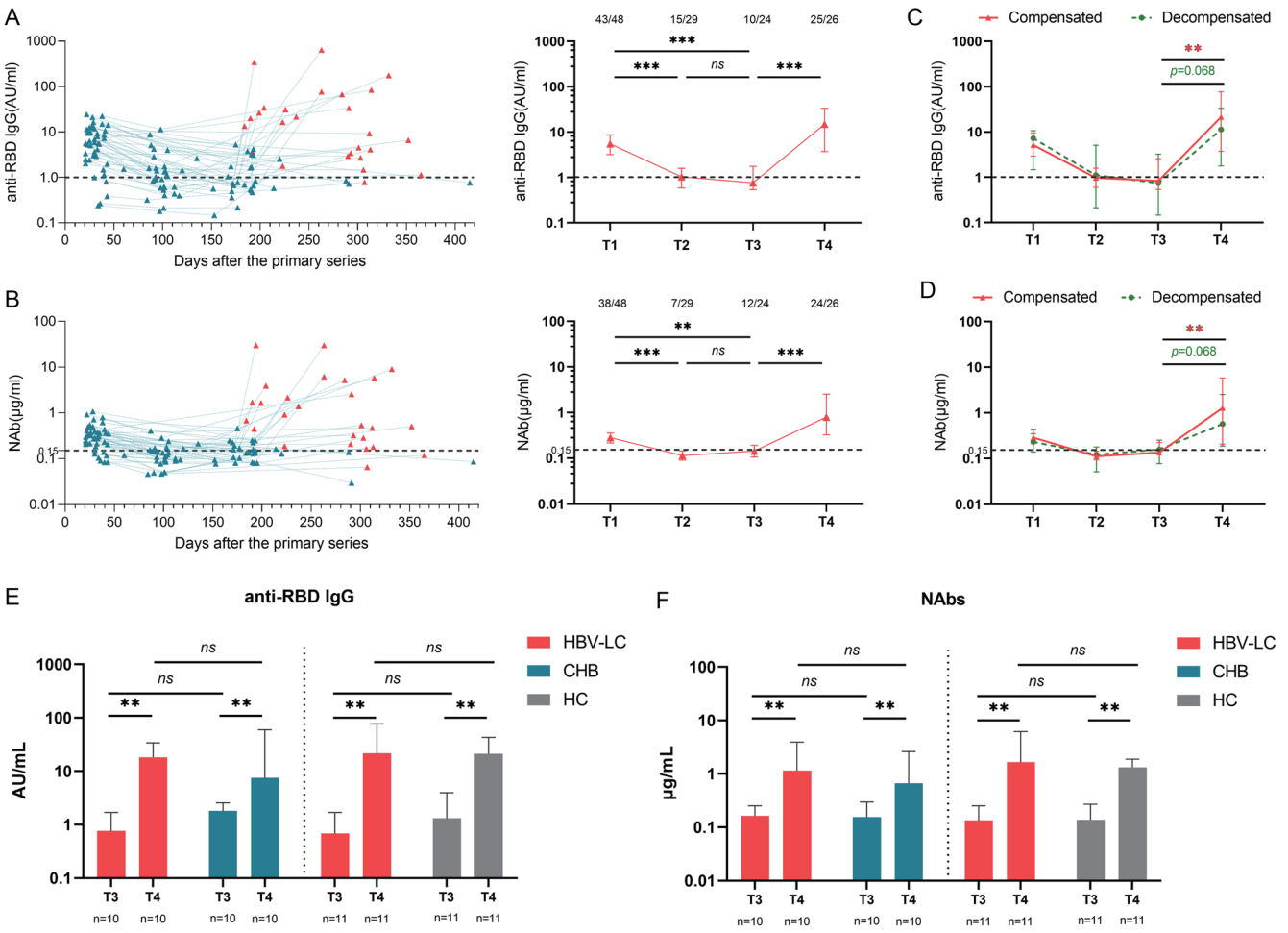

Fig. 2. Dynamics of SARS-CoV-2 antibody responses to the primary series and booster dose in patients with cirrhosis. (A) Longitudinal analysis of anti-RBD-IgG in patients with cirrhosis over time. (B) Longitudinal analysis of NAbs in patients with cirrhosis over time. (C) Longitudinal analysis of anti-RBD-IgG in patients with compensated and decompensated cirrhosis. (D) Longitudinal analysis of NAbs in patients with compensated and decompensated cirrhosis. (E) Comparison of anti-RBD-IgG titer in cirrhotic patients, CHB patients, and HCs after a booster dose. (F) Comparison of NAbs titer in cirrhotic patients, CHB patients, and HCs after a booster dose. The error bars represent median and 95% CI. The horizonal dotted lines indicate the lower limit of quantitation. Significance of differences of unpaired data determined by 2-tailed Mann-Whitney *U* tests and for paired data, by 2-tailed Wilcoxon signed-rank tests. *p<0.05, **p<0.01, ***p<0.001; ns, not significant. CHB, chronic hepatitis B; CI, confidence interval; HC, healthy control; Ig, immunoglobulin; NAb, neutralizing antibody; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

booster. The kinetics of the SARS-CoV-2-specific B-cell response was also examined.

No serious AEs were reported in patients with cirrhosis in the first 30 days after receiving the primary series and booster doses, including severe thromboembolism and myocarditis. No clinical flare-ups or hepatic parenchymal changes occurred after the booster dose. Overall, the primary series and booster dose were safe and well tolerated in patients with cirrhosis. Therefore, it is recommended that they receive a booster dose and that clinical flare-ups are closely monitored after vaccination.

Anti-RBD IgG and NAbs levels in cirrhotic patients were elevated between 15 and 45 days after the primary series and rapidly decline thereafter, reaching a trough between days 165 and 195. That matched the previously reported kinetics of specific antibodies after SARS-CoV-2 infection.[18–20] Around 30 days after receiving the booster dose, serum SARS-CoV-2 specific antibodies in patients with cirrhosis increased significantly again, reaching levels similar to those in patients with CHB and in HCs. Enhancement of the antibody response in cirrhosis patients by the booster dose was consistent with previous studies.[16,17] Indeed, our previous study showed that the antibody titer in patients with cirrhosis was lower than that in healthy people 1 month after primary series.[9] This emphasized the necessity of booster vaccination in cirrhotic patients because of the catch-up effect of antibody responses. Another clinical implication of our findings is the observation that the degree of antibody response impairment was proportional to the severity of liver dysfunction, suggesting patients with decompensated cirrhosis are a vulnerable population that needs to be prioritized for additional vaccination.

Interestingly, the results of pseudovirus neutralization assays show that the booster dose did not significantly enhance the serum neutralization activity against Omicron subvariants, and that repeated vaccination of inactivated SARS-CoV-2 vaccine might dampen neutralization activity against BA.5. This suggested that repeated vaccination with inactivated vaccine mainly recalled previous memory, and that such vaccination-induced immune imprinting might reflect "original

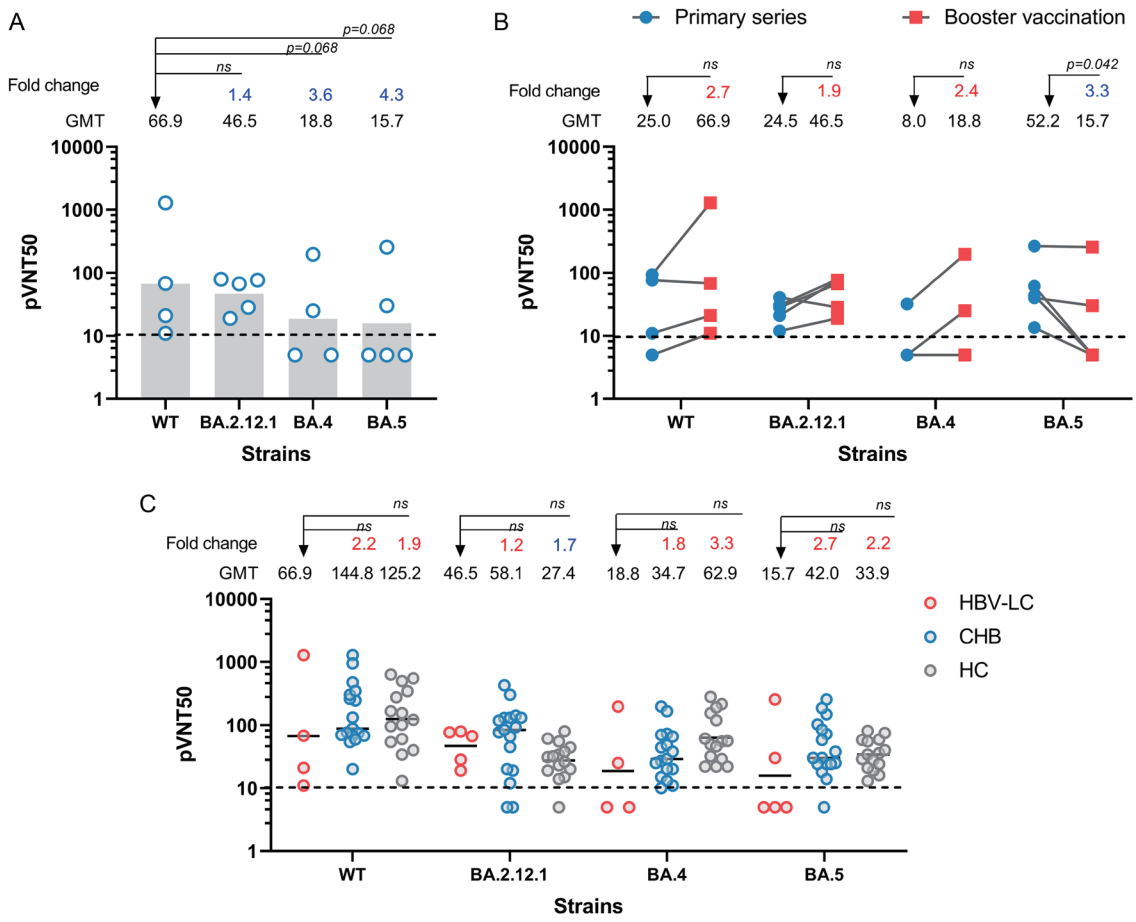

Fig. 3. Neutralization of the WT strain and Omicron subvariants by participant serum collected 1 month after the primary series and after the booster dose. (A) Neutralization of the WT strain and Omicron subvariants 1 month after booster dose in serum samples collected from cirrhotic patients (n=5). The GMT fold-change vs. the titers against the WT strain are shown, with red indicating fold-increase and blue indicating fold-decrease. (B) Neutralization of the WT strain and Omicron subvariants 1 month after the primary series and 1 month after the booster dose from serum samples collected from cirrhotic patients (n=5). The GMT fold-change after the booster dose vs. the titers after the primary series are shown, with red indicating fold-increase and blue indicating fold-decrease. (C) Neutralization of the WT strain and Omicron subvariants 1 month after the booster dose from serum samples collected from cirrhotic patients (n=5), CHB patients (n=17) and HCs (n=15). The GMT fold-change vs. the titers in cirrhotic patients are shown, with red indicating fold-increase and blue indicating fold-decrease. Horizonal dotted lines indicate the lower limit of quantitation. Significance of difference of unpaired data determined by 2-tailed Mann-Whitney U tests and, for paired data by 2-tailed Wilcoxon signed-rank tests. *p<0.05, **p<0.01, ***p<0.001; ns, not significant. CHB, chronic hepatitis B; GMT, geometric mean titer; HC, healthy control; WT, wild-type.

antigenic sin" as described following influenza vaccination. More evidence should be provided by real world study of BA.5 breakthrough infection and measurements of Omicron BA.5-specific humoral responses. In addition, Omicron subvariants partly escaped serum neutralization elicited by the booster dose, and the enhancement effect in cirrhosis patients was worse than that reported in healthy people.[12,21] The consideration is limited to patients with breakthrough infection, and a booster dose is highly recommended for most people to protect against severe COVID-19. Here we call for caution on immune imprinting especially in vulnerable populations when designing future vaccination and booster strategies.

As RBD-specific MBCs are thought to produce protective NAbs following repeated antigen exposure,[22] variations in their kinetics are crucial for predicting the durability of protection against reinfection. That is why RBD$^+$MBCs were the primary target of our attention. Different B-cell subpopulations with unique functional characteristics support both the acute and chronic phases of humoral immunity. CD27$^+$CD38$^+$MBCs, defined as antibody secreting cells (ASCs), rapidly enter the peripheral blood during acute viral infection, producing pathogen-specific antibodies. rMBCs, also called classical MBCs, persist for months to years and resist antigens by proliferating and differentiating into ASCs. actMBCs are cells that recently left germinal centers and are already primed to become antibody-producing ASCs.[22] This study observed a sustained increase in ASCs and actMBCs in the peripheral blood of cirrhosis patients within 6 months after the primary series. rMBCs continued to decline, suggesting that although circulating antibody levels decreased over time, MBCs were durable and had the ability to continuously differentiate into ASCs. At 1 month after a booster dose, the B-cell responses were stable regardless of the severity of liver dysfunction. Interestingly, RBD$^+$CD27$^+$MBCs were stable in patients with cirrhosis and decreased significantly in both CHB patients and HCs after a booster dose. That might be explained by the fact that MBCs were more able to continually differentiate into ASCs in HCs and CHB patients than in cirrhosis patients. The immunological mechanisms need to be further elucidated with larger sample sizes and longer follow-up.

This study has strengths and limitations. The strengths include tracking WT antibody and memory B cells at four

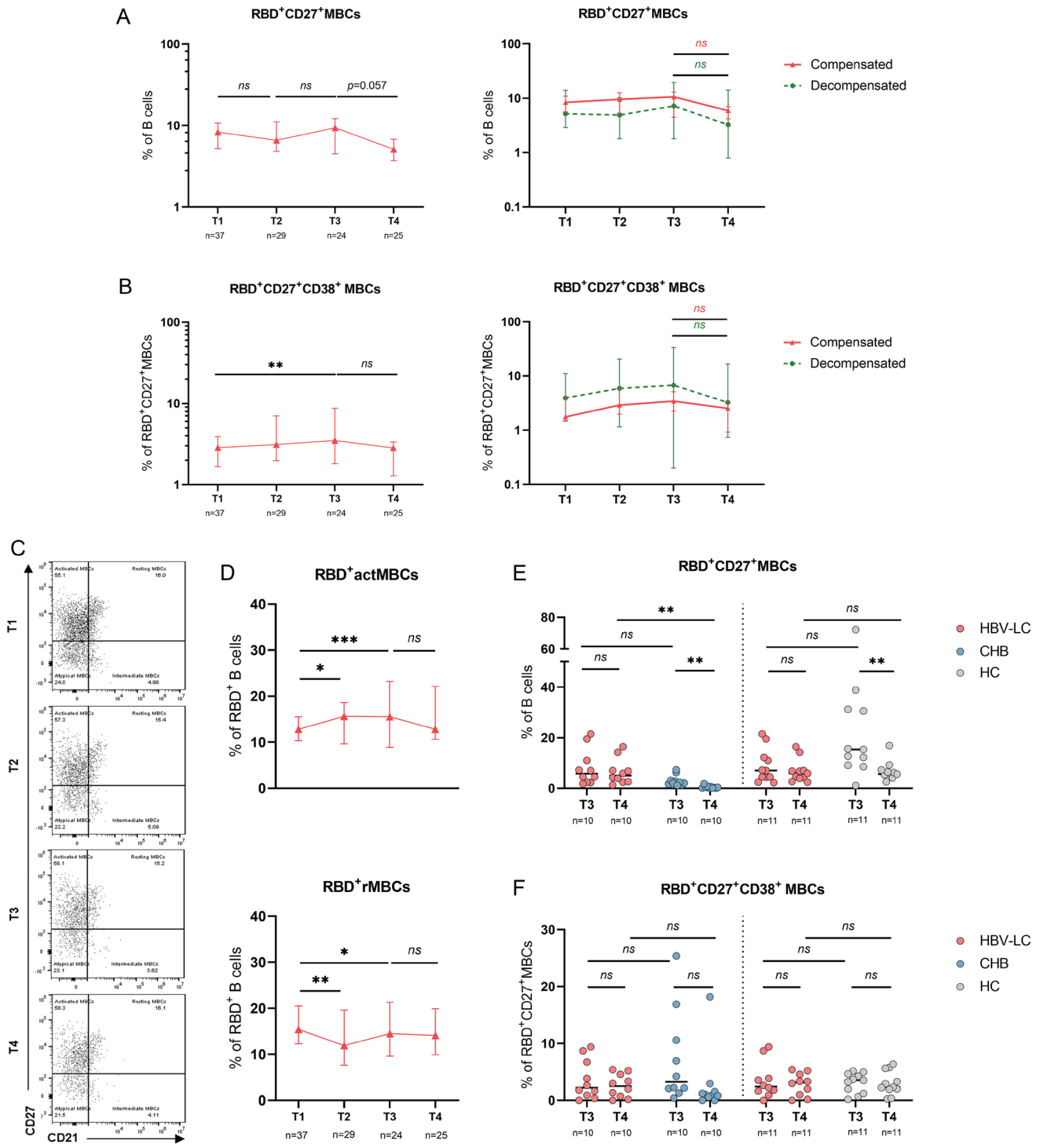

Fig. 4. Kinetics of SARS-CoV-2 memory B-cell responses to primary series and the booster dose in patients with cirrhosis. (A) Longitudinal analysis of the frequencies of RBD$^+$CD27$^+$MBCs in all cirrhotic patients (left panel) and in patients with compensated and decompensated cirrhosis (right panel) over time. (B) Longitudinal analysis of the frequencies of RBD$^+$CD27$^+$CD38$^+$MBCs in all cirrhotic patients (left panel) and in patients with compensated and decompensated cirrhosis (right panel) over time. (C) Representative gating of RBD-specific rMBCs, intMBCs, actMBCs, atyMBCs in total RBD$^+$B cells in patients with cirrhosis at four different times. (D) Longitudinal analysis of the frequencies of RBD$^+$actMBCs (upper panel) and RBD$^+$rMBCs (lower panel) in patients with cirrhosis. (E) Comparison of frequencies of RBD$^+$CD27$^+$MBCs in cirrhotic patients, CHB patients, and HCs after a booster dose. (F) Comparison of frequencies of RBD$^+$CD27$^+$CD38$^+$MBCs in cirrhotic patients, CHB patients, and HCs after a booster dose. The error bars represent medians with 95% CIs. Significance of differences of unpaired data determined by two-tailed Mann-Whitney *U* tests and, for paired data by two-tailed Wilcoxon signed-rank tests. *p<0.05, **p<0.01, ***p<0.001; ns, not significant. actMBCs, activated memory B cells; atyMBCs, atypical memory B cells; CHB, chronic hepatitis B; CI, confidence interval; HC, healthy control; intMBCs, intermediate memory B cells; MBCs, memory B cells; RBD, receptor-binding domain; rMBCs, resting memory B cells; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

different times and the assessment of serum neutralizing activity against Omicron subvariants. The limitations include a small sample size and lack of samples available from all three cohorts at each time of analysis.

In summary, the primary series and booster dose were safe and well tolerated in patients with cirrhosis. A booster dose was significantly restore WT antibody levels but did not improve serum neutralizing ability against Omicron subvariants in cirrhotic patients. Therefore, careful considerations of immune imprinting should be taken when designing future booster strategies.

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Conflict of interest

HR has been an editor-in-chief of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Concept and design (HR, XS, DC), funding acquisition (HR, XS, DC), participant recruitment (QZ, LW, XH, YZ, TH), experiment performance (QZ, LW, XH, YZ, TH, ZC, GZ, DX, MP, MC), acquisition, analysis or interpretation of data (QZ, LW, XH, HR, DC, XS), and drafting and critical revision of manuscript (QZ, HR). All authors were involved in writing the paper and gave final approval of the submitted and published versions.

Ethical statement

The study was approved by the ethics committee of The Second Affiliated Hospital of Chongqing Medical University and conformed to the ethical guidelines of the Declaration of Helsinki. It was registered at www.chictr.org.cn (ChiCTR2100047936) and ClinicalTrials.gov (NCT05007665). Written informed consent was obtained from all participants before recruitment.

Data sharing statement

No additional data are available.

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