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Original Article

Safety and Immunogenicity After Primary and Booster Inactivated SARS-Cov-2 Vaccination in Patients with Autoimmune Liver Diseases

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Abstract

Background and Aims: SARS-CoV-2 vaccines-associated autoimmune liver diseases have been reported in several case reports. However, the safety and immunogenicity after primary and booster inactivated SARS-CoV-2 vaccination in patients with autoimmune liver diseases (AILD) is still unknown. ***Methods:*** Eighty-four patients with AILD were prospectively followed up after the second dose (primary) of inactivated SARS-CoV-2 vaccine. Some of them received the third dose (booster) of inactivated vaccine. Adverse events (AEs), autoimmune activation, and liver inflammation exacerbation after primary and booster vaccination were recorded. Meanwhile, dynamics of antireceptor-binding-domain IgG (anti-RBD-IgG), neutralizing antibodies (NAbs) and RBD-specific B cells responses were evaluated. ***Results:*** The overall AEs in AILD patients after primary and booster vaccination were 26.2% and 13.3%, respectively. The decrease of C3 level and increase of immunoglobulin light chain κ and λ levels were observed in AILD patients after primary vaccination, however, liver inflammation was not exacerbated, even after booster vaccination. Both the seroprevalence and titers of anti-RBD-IgG and NAbs were decreased over time in AILD patients after primary vaccination. Notably, the antibody titers were significantly elevated after booster vaccination (10-fold in anti-RBD-IgG and 7.4-fold in NAbs, respectively), which was as high as in healthy controls. Unfortunately, the inferior antibody response was not enhanced after booster vaccination in patients with immunosuppressants. Changes of atypical memory B cells were inversely related to antibody levels, which indicate that the impaired immune memory was partially restored partly by the booster vaccination. ***Conclusions:*** The well tolerability and enhanced humoral immune response of inactivated vaccine supports an additional booster vaccination in AILD patients without immunosuppressants.

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Introduction

More than 3 years after the first reported case of coronavirus disease 2019 (COVID-19), the pandemic is still overwhelming around the world.[1] As of December 1, 2022, COVID-19 has led to over 630 million infections worldwide and claimed over 6.6 million lives (World Health Organization COVID-19 Dashboard). Vaccination is still an effective measure for the prevention of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, severe disease, and mortality.[2]

Recent case reports found that autoimmune hepatitis (AIH) developing after SARS-CoV-2 vaccine,[3–8] and vaccine could also act as a trigger in the disease course of AIH.[9] In addition, it has been reported that SARS-CoV-2 vaccination can be associated with liver injury, and some patients showed features of immune-mediated hepatitis,[10] which lead to concerns of the safety of vaccines. Autoimmune liver diseases (AILD), as a special type of chronic liver disease, including AIH, primary biliary cholangitis (PBC), and AIH + PBC (overlap syndrome). The liver damage of AILD is mainly caused by immune disorders and can be induced or aggravated by drug exposure, viral infection, and even vaccina-

Keywords: SARS-CoV-2; Autoimmune liver disease; Inactivated SARS-CoV-2 vaccine; Safety; Antibody responses; Memory B cells.

Abbreviations: AEs, adverse events; AIH, autoimmune hepatitis; AILD, autoimmune liver disease; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibody; AMA-M2, antimitochondrial antibody-M2; ANA, antinuclear antibody; AST, aspartate aminotransferase; BMI, body mass index; CI, confidential interval; C3, complement 3; C4, complement 4; DB, direct bilirubin; GGT, gamma-glutamyl transferase; HB, hemoglobin; HCs, health care workers; Ig, immunoglobulin; MBCs, memory B cells; NAb, neutralizing antibody; PBC, primary biliary cirrhosis; PBMC, peripheral blood mononuclear cell; PLT, platelet; RBC, red blood cell; RBD, receptor binding domain; SAE, serious adverse event; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TB, total bilirubin; WBC, white blood cell.

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tion.[5,9–12] Thus, patients with AILD may be more vulnerable to SARS-CoV-2 vaccines in theory, which has discouraged most AILD patients from accepting vaccination. However, relevant safety data are limited.

Considering that antibody responses decay over time, booster dose programs have been recommended by World Health Organization.[13] Our previous study has revealed that the antibody responses were compromised in AILD patients after primary inactivated vaccination.[14] In addition, another previous study also showed an inferior antibody response to mRNA or Johnson & Johnson vaccine in patients with AILD and with immunosuppressants.[15] However, it is currently not known whether booster dose of inactivated vaccine is likely to improve antibody responses in AILD patients.

In this prospective study, we aimed to investigate the safety, humoral responses to primary and booster inactivated vaccines in patients with AILD. This study therefore had three main objectives. First, to evaluate whether the primary and booster vaccination would activate autoimmune response or aggravate liver inflammatory. Second, to investigate whether the booster vaccination could elevate the antibody responses in AILD patients. Third, to depict the dynamic changes of immune memory responses after primary and booster vaccination.

Methods

Study design and participants

In this prospective observational study, patients with AILD after the second dose (primary) of inactivated vaccine (BBIBP-CorV from Beijing Institute of Biological Products/Corona-Vac from the Chinese company Sinovac Biotech) were consecutively recruited from the Second Affiliated Hospital of Chongqing Medical University since August 1, 2021. And the third dose (booster) of inactivated vaccine was recommended to population at least 6 months interval after primary vaccination in China. Participants were followed up at 1 month (T1), 3 months (T2) and 6 months (T3) after primary vaccination, and some of them completed the booster inactivated vaccines and continued to follow-up at 1 month (T4) after booster vaccination. The inclusion criteria for patients with AILD were: (1) 18 years of age or older; 2) diagnosed with an AILD by relevant guidelines, including clinical manifestation, autoantibodies, liver function or liver biopsy.[16–18] The inclusion criteria for healthy controls (HCs) were: (1) 18 years of age or older; (2) without chronic liver disease, hypertension, diabetes, and other basic diseases. The exclusion criteria were: (1) history of SARS-CoV-2 infection; (2) Hepatitis B/C virus or human immunodeficiency virus infection; (3) other major diseases, such as tumors, renal failure; and (4) pregnancy. In addition, we included, as an HC group, 68 healthcare workers at our hospital over 6 months after primary inactivated vaccination, without personal history of COVID-19 or major comorbidities. All the 68 HCs blood sampling before and after booster vaccination.

Data and sample collection

For all participants recruited in this study, adverse events (AEs) within 7 days were recorded by questionnaire.[19] All AEs were recorded and graded according to the scale issued by National Medical Products Administration of China (version 2019). AEs related to vaccination were judged by investigators. Demographic characteristics and clinical data (including autoimmune test and liver function) were obtained by questionnaire or electronic medical record. At each visit, serum was used to test the antibody responses and PBMCs was used to examined the B-cell responses.

Assay of liver function and autoimmune indexes

The liver function indices in serum samples were detected by biochemical detection instrument, including albumin (ALB), aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin (TB), etc. Autoimmune indexes were detected by western blotting, including antinuclear antibody (ANA), antimitochondrial antibody (AMA), antimitochondrial M2 (AMA-M2), IgA, IgG, IgM, complement 3 (C3), C4, Ig light chains kappa (κ) and lambda (λ), and others.

Assay of anti-RBD-IgG and NAbs

The antireceptor-binding-domain IgG (anti-RBD-IgG) and neutralizing antibodies (NAbs) in serum samples were evaluated using capture chemiluminescence immunoassays by MAGLUMI X8 (Snibe, Shenzhen, China), as previously described.[20] The sensitivity and specificity of the kits (Snibe) for anti-RBD-IgG are 100% and 99.6%, respectively, and those for NAbs are 100% and 100%, respectively. The cut-off value was 1.00 AU/mL for anti-RBD-IgG and 0.15 μg/mL for NAbs.

Flow cytometry assay of memory B cells

Detection of SARS-CoV-2-specific memory B cells (MBCs) by flow cytometry was as previously described.[21] In brief, for SARS-CoV-2 specific MBCs responses, biotinylated SARS-CoV-2 Spike RBD protein (40592-V08H2-B; Sino Biological, Beijing, China) was mixed with Streptavidin BV421 (405225; Biolegend, San Diego, CA, USA) at 4:1 molar ratio for 1 h at 4°C to obtain the antigen probe. According to the manufacturer's instruction, peripheral blood mononuclear cells (PBMCs) were stained for 30 m at 4°C using antigen probe (1:33.3) and the following conjugated antibodies: antihuman CD3 (1:50) (300430; Biolegend), antihuman CD19 (1:50) (302212; Biolegend), antihuman CD21 (1:50) (354918; Biolegend), antihuman CD27 (1:50) (356406; Biolegend). After staining, cells were rewashed and resuspended in a 200ul FACS buffer. Samples were then evaluated by flow cytometry (CytoFLEX; Beckman Coulter, Brea, CA, USA) and analyzed using FlowJo (version 10.0.7r2; Treestar, Woodburn, OR, USA).

Statistical analysis

For categorical variables, chi-square and Fisher's exact tests were used determine significance. For continuous variables, Wilcoxon signed-rank tests were used to compare between-group differences, Mann-Whitney U tests were used for unpaired group comparisons, and Kruskal-Wallis H tests were used for multiple-group comparisons. Change of antibody titers and memory B cells with time were described by using geom_smooth [ggplot2 package] of a linear model. A two-sided p-value of <0.05 was considered significant. Data were analyzed with SPSS (version 24.0.0; IBM Corp., Armonk, NY, USA), and visualized with GraphPad Prism (version 9.2.0; GraphPad Software Inc, La Jolla, CA, USA) and R (version 3.5.3).

Results

Participant characteristics

Overall, 84 patients with AILD and 68 HCs were enrolled between August 1, 2021 and May 6, 2022. Some patients were difficult to complete the follow-up, and did not receive the

Table 1. Demographic characteristics of AILD patients

Variable	All patients, *n*=84	AIH, *n*=47	PBC, *n*=17	AIH + PBC, *n*=20
Age in years	54.9 (49.3–60.8)	54.5 (48.0–60.0)	54.5 (46.0–63.0)	56.0 (52.0–58.8)
Sex				
Male	13 (15.5)	7 (14.9)	4 (23.5)	2 (10.0)
Female	71 (84.5)	40 (85.1)	13 (76.5)	18 (90.0)
BMI in kg/m^2	22.4 (21.0–23.8)	22.7 (21.2–24.1)	22.0 (21.0–23.0)	21.9 (21.0–23.4)
Therapy				
Immunosuppressants	29 (34.5)	16 (34.0)	0	13 (65.0)
Prednisolone/Prednisone	27 (32.1)	15 (31.9)	0	12 (60.0)
Azathioprine	16 (19.0)	9 (19.1)	0	7 (35.0)
Mycophenolate mofetil	2 (2.4)	1 (2.1)	0	1 (5.0)
Other therapies[a]	52 (61.9)	28 (59.6)	17 (100.0)	7 (35.0)
Treatment naïve	3 (3.6)	3 (6.4)	0	0
Liver cirrhosis				
Yes	19 (22.6)	10 (21.3)	6 (35.3)	3 (15.0)
No	5 (77.4)	37 (78.7)	11 (64.7)	17 (85.0)
Vaccine				
BBIBP-CorV	23 (27.4)	14 (29.8)	3 (17.6)	6 (30.0)
Corona-Vac	54 (64.3)	27 (57.4)	13 (76.5)	14 (70.0)
mixed	7 (8.3)	6 (12.8)	1 (5.9)	0
Days after primary vaccination	81.1 (35.8–99.0)	81.3 (34.5–98.5)	78.0 (30.0–97.0)	83.6 (41.5–105.0)
Days after booster vaccination	32.3 (21.0–40.0)	32.6 (23.0–41.0)	31.0 (21.0–35.0)	31.7 (12.0–21.0)
Blood collection frequency				
Multiple time points	31 (36.9)	16 (34.0)	9 (52.9)	6 (30.0)
Single-time point	53 (63.1)	31 (66.0)	8 (47.1)	14 (70.0)

*Data are medians (interquartile range) or number (%). [a]Other therapies including ursodeoxycholic acid, bezafibrate, bicyclol, diammonium glycyrrhizinate, silibinin capsules, glutathione, and polyene phosphatidylcholine capsules. AIH, autoimmune hepatitis; AILD, autoimmune liver diseases; BMI, body mass index; PBC, primary biliary cirrhosis.

third dose (booster) of inactivated vaccine due to their own concerns and prevention and control policy of COVID-19 in China. Lastly, of the 84 patients, 15 completed booster inactivated vaccination, 31 provided longitudinal blood samples during the observation period (two to four time points; Table 1). The demographic characteristics of patients with AILD are shown in Table 1 and those of the 68 HCs in are shown in Supplementary Table 1. The median age of AILD patients was 54.9 years (IQR 49.3–60.8). Over half the patients were women (84.5%, 71/84). Of the 84 AILD patients, 47 had AIH, 17 patients had PBC, and 20 had AIH + PBC. A total of 34.5% of patients were receiving immunosuppressive therapy, and glucocorticoids were the most used. Liver function indexes were normal or mild elevated in these patients (Supplementary Table 2). Overall, 145 blood samples from the 84 AILD patients were collected, and of them, 31 patients were followed up. The median elapsed time between primary vaccination and blood sampling in AILD patients was 33.0 (IQR 27.0–43.0) days at T1, 92.0 (82.5–98.0) days at T2, 179.5 (170.5–193.8) days at T3, and 35.0 (21.0–40.0) days at T4 between booster vaccination and blood sampling. The control group underwent blood sampling at 246.5 (191.3–274.8) days at T3 after primary vaccination and at 29.0 (28.0–38.8) days at T4 after booster vaccination.

Safety of inactivated SARS-CoV-2 vaccine in patients with AILD

Firstly, we evaluated the general AEs within 7 days after primary and booster vaccination. As shown in Table 2, the overall incidence of AEs was 26.2% (22/84) after primary vaccination, and the most common local and systemic AEs were pain at injection site (7.1%), fatigue (6.0%), and headache (6.0%). After booster vaccination, only two patients reported AEs (13.3%, 2/15). All AEs were mild or moderate (grade 1 or 2). No severe AEs (grade 3 or 4), such as severe thromboembolism and myocarditis were observed in patients with AILD.

Further, we focused to whether inactivated vaccines led to autoimmune response activation or liver inflammatory aggravation. As shown in Figure 1, autoimmune indexes such as ANA, AMA, C4, IgG and IgM were unchanged before and after primary vaccination (Fig. 1A). However, the C3 level was decreased after primary vaccination (1.1 g/L (IQR 1.0–1.2) vs. 1.0 g/L (0.9–1.1), $p<0.01$). Meanwhile, Ig light-chain kappa (κ) and lambda (λ) levels were increased (4.3 (3.4–10.1) g/L vs. 10.5 (8.9–12.7) g/L; 2.6 (2.2–5.5) g/L vs. 6.6 (5.1–7.9) g/L, both $p<0.001$). The results indicate that the autoimmune response was partially activated in AILD patients after primary vaccination. Fortunately, liver function indices such as ALB, ALT, AST, GGT, TB, were unchanged after

Table 2. Adverse events after primary and booster inactivated vaccination in AILD patients

Adverse events	Primary vaccination, *n*=84	Booster vaccination, *n*=15
Overall, within 7 days	22 (26.2%)	2 (13.3%)
Local		
Pain	6 (7.1%)	/
Swelling	2 (2.4%)	/
Redness	/	/
Itch	/	/
Induration	/	/
Systemic		
Muscle pain	/	/
Pruritus	/	/
Rash	/	/
Fatigue	5 (6.0%)	/
Drowsiness	/	/
Dizziness	/	1 (6.7%)
Headache	5 (6.0%)	/
Rhinorrhea	/	/
Laryngeal pain	/	/
Fever	/	/
Chill	/	/
Cough	/	/
Inappetence	/	/
Abdominal pain	/	/
Abdominal distension	/	/
Diarrhea	/	/
Hepatalgia	/	/
Nausea	1 (1.2%)	1 (6.7%)
Chest distress	/	/
Constipation	/	/
Numbness of limb	2 (2.4%)	/
Lower extremity edema	1 (1.2%)	/
Grade 3 and 4	/	/

primary vaccination (Fig. 1B) and after booster vaccination (Fig. 1C). Meanwhile, there were no changes in routine blood indicators after vaccination (Supplementary Fig. 1). In detail, seven patients had mild/moderate increases of liver enzymes (ALT, AST, GGT, etc.) after primary vaccination (Supplementary Table 3). But none of them was judged to be related to vaccine by the investigator after reviewed their past medical and treatment history, due to the aminotransferases increase was related to disease activity, and interruption, or self-modification of treatment regimen. In addition, no one was hospitalized due to this reason. Moreover, we observed four cases of AIH with no AILD before inactivated vaccination by the questionnaire and clinical lab examination. Diagnosis and treatment information are shown in Supplementary Table 4. In brief, no aggravated liver inflammation was seen after primary and booster vaccination in patients with AILD, and one had recurrent abnormal liver function. Taken together, the inactivated vaccines were well tolerated in AILD patients.

Antibody response to inactivated SARS-CoV-2 vaccine

Next, we wanted to observe the dynamic changes of antibody response after primary and booster vaccination in this study. As expected, both the seroprevalence and titers of anti-RBD-IgG declined over time in patients with AILD after primary vaccination (87% vs. 45% positive and 7.45 AU/mL (95% confidence interval (CI): 5.0–9.9) vs. 1.7 AU/mL 95% CI: (0.4–3.0) at T1 and T3, respectively, $p<0.01$) (Fig. 2A). After booster vaccination, almost all the patients had detectable anti-RBD-IgG, and the titer was significantly elevated compared with before booster vaccination (10-fold: 1.7 AU/mL 95% CI: (0.4–3.0) vs. 17.0 AU/mL 95% CI: (8.4–25.7), $p<0.001$). Similar results were observed for NAbs responses (7.4-fold) (Fig. 2B). The

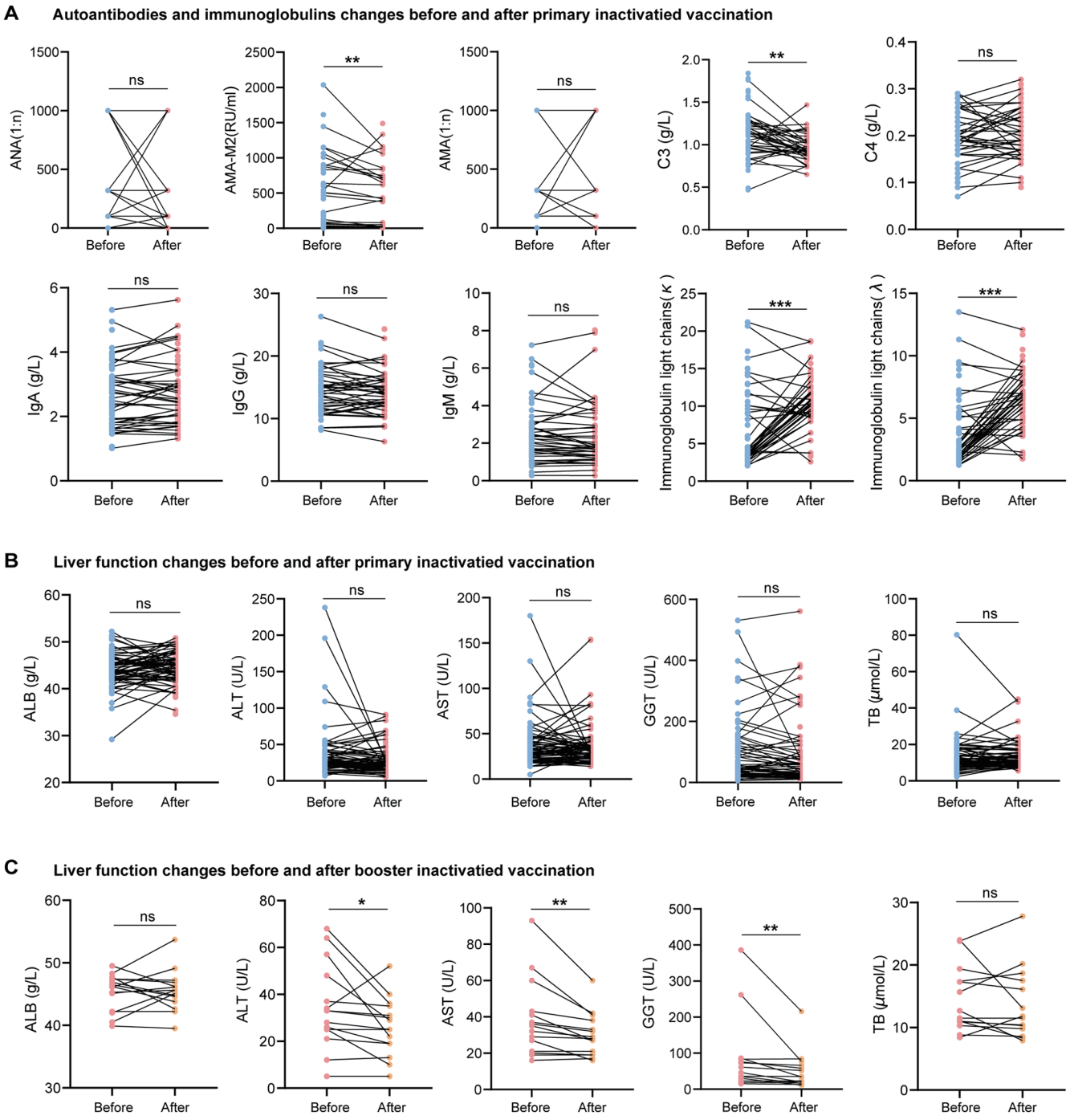

Fig. 1. Autoimmune responses and liver function changes after primary and booster inactivated vaccination in AILD patients. (A) Changes of autoantibodies and immunoglobulins levels before and after primary inactivated vaccination. (B and C) Changes of liver function tests indexes before and after the primary (B) and booster (C) inactivated vaccination in AILD patients. *p<0.05; **p<0.01; ***p<0.001; ns, not significant. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibody; AMA-M2, antimitochondrial antibody-M2; ANA, antinuclear antibody; AST, aspartate aminotransferase; C3, complement 3; C4, complement 4; DB, direct bilirubin; GGT, gamma-glutamyl transferase; HB, hemoglobin; Ig, immunoglobulin; PLT, platelet; RBC, red blood cell; TB, total bilirubin; WBC, white blood cell; κ, kappa; λ, lambda.

two antibodies were highly correlated (Supplementary Fig. 2). Further longitudinal study analysis showed similar but more obvious trends (Fig. 2C, D). Strikingly, both the titers of anti-RBD-IgG and NAbs in AILD patients were increased as high as in the HCs after booster inactivated vaccination (anti-RBD-IgG: 21.1 AU/mL 95% CI: (8.9–33.3) vs. 26.7 AU/mL 95% CI: (16.9–36.5); NAbs: 1.8 AU/mL 95% CI: (0.6–3.0) vs. 1.7 AU/mL 95% CI: (1.1–2.3), both p>0.05) (Fig. 2E, F).

As expected, subgroup analysis showed that the anti-RBD-IgG titer after primary vaccination was lower in patients with

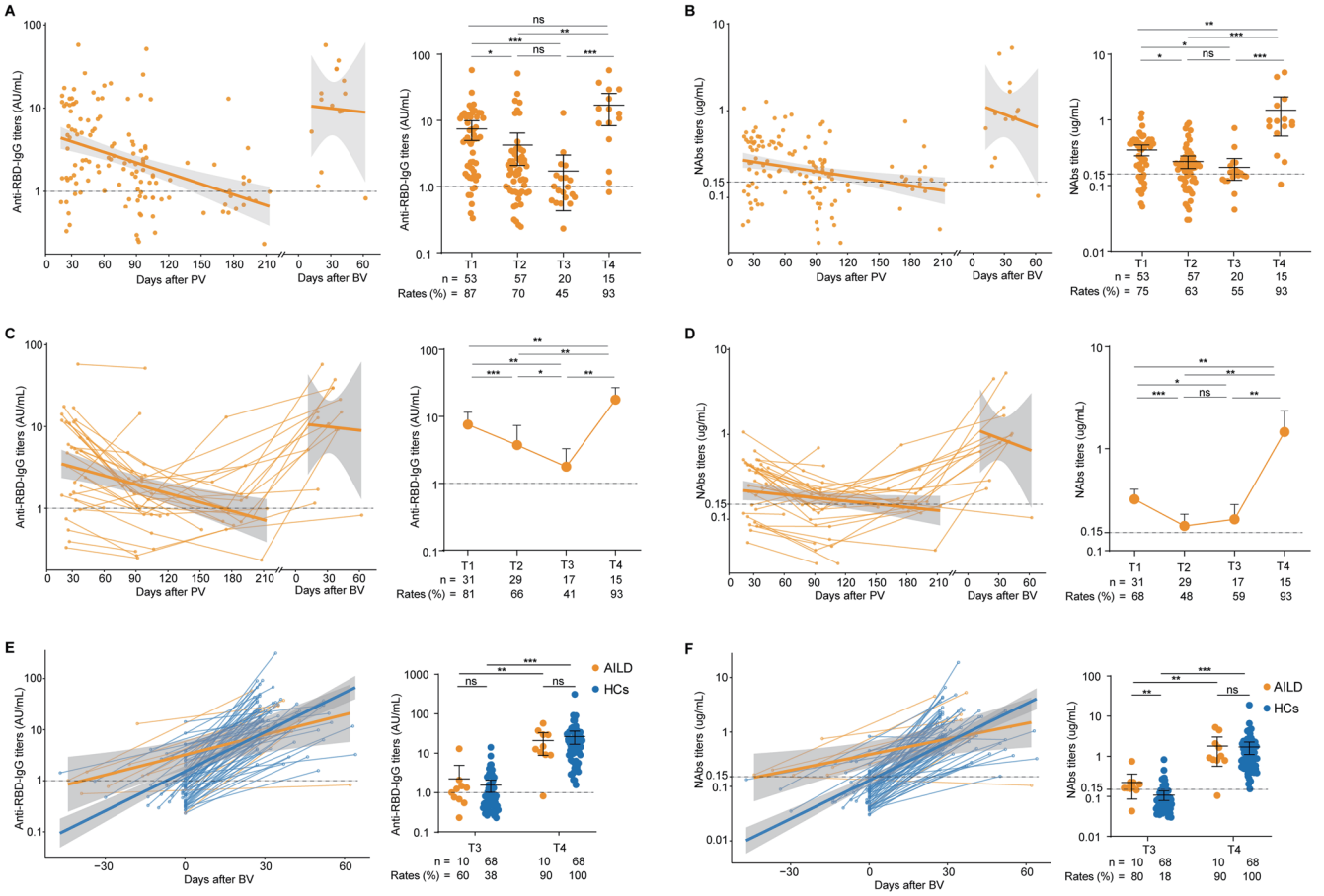

Fig. 2. Antibody responses after primary and booster inactivated vaccinations in AILD patients. (A and B) Cross-sectional changes of Anti-RBD-IgG (A) and NAbs titers (B) over time after primary and booster vaccination in AILD patients. (C and D) Longitudinal changes of Anti-RBD-IgG (C) and NAbs (D) titers in patients over time after primary and booster vaccination in AILD patients. (E and F) Longitudinal changes of Anti-RBD-IgG (E) and NAbs (F) titers in AILD patients and HCs at T3 and T4 point after vaccination. Dotted lines indicate the detection limit for anti-RBD-IgG and NAbs. The trendlines were produced using a linear model fit, and the shaded area showed the 95% CI for each fit. *p<0.05; **p<0.01; ***p<0.001; ns, not significant. AILD, autoimmune liver disease; anti-RBD-IgG, antireceptor-binding-domain IgG; BV, booster vaccination; HCs, healthy controls; NAbs, neutralizing antibodies; PV, primary vaccination.

immunosuppressants than in patients without immunosuppressants (3.9 AU/mL (95% CI: 1.7–6.2) vs. 5.9 AU/mL (95% CI: 4.1–7.8), p<0.05). However, the antibody response was not enhanced significantly in patients with immunosuppressants after booster vaccination, and this discrepancy of anti-RBD-IgG titer between the two subgroups was persisted (5.9 AU/mL (95% CI: 2.3–14.0) vs. 22.6 AU/mL (95% CI: 11.1–34.1), p<0.05) (Fig. 3A). A similar trend was observed in NAbs responses (Fig. 3B). Further, patients with PBC, and AIH without immunosuppressants subgroups showed a significantly enhanced antibody responses after booster vaccination (both p<0.01). However, the difference was not observed in AIH with immunosuppressants and AIH + PBC subgroups (Fig. 3B). Of note, two patients (one AIH with immunosuppressants and one AIH + PBC with immunosuppressants) did not mount an antibody response after the primary vaccination. After booster vaccination, the former was induced a *de novo* response, but the latter was nonresponse (Fig. 2C and Fig. 3B). Patients with cirrhosis showed a lower trend of antibody responses than patients without cirrhosis after primary and booster vaccination, but the difference was not significant (Fig. 3C). Subgroup analysis in different vaccine types, similar trend was observed between BBIBP-CorV, Corona-Vac and mixed subgroups (Fig. 3D). In brief, booster inactivated vaccines significantly enhanced antibody responses in AILD patients, but not in patients with immunosuppressive therapy.

B-cell response to inactivated SARS-CoV-2 vaccine

Lastly, we evaluated the changes of immune memory function in AILD patients after primary and booster vaccination. Overall, the frequency and percentage of total B cells of RBD$^+$ MBCs decreased over time after primary vaccination (6.7 (IQR 5.1–10.1) % at T1 vs. 3.9 (2.8–5.6)% at T3, p<0.05), but had an trend for increase after booster vaccination (3.9 (2.8–5.8)% vs. 5.8 (3.2–8.7) %, p>0.05) (Fig. 4A). Further longitudinal study analysis showed a similar but more obvious trend (p<0.05) (Fig. 4B). To better understand the functional phenotypes of the RBD$^+$ MBCs, we further classed the RBD$^+$ MBCs into four subsets:[22] resting MBCs (rMBCs), activated MBCs (actMBCs), atypical MBCs (atyMBCs), and intermediate MBCs (intMBCs). The gating strategy and representative results are shown in Supplementary Figure 3. After primary vaccination, the frequencies of rMBCs and intMBCs decreased over time. On the contrary, the frequency of atyMBCs increased (20.3 (13.9–25.7)% vs. 29.5 (21.2–35.2)%, p<0.05). Interestingly, the frequency of atyMBCs had a decreasing trend (29.5 (21.2–35.2)% vs. 23.3 (18.8–31.0)%, p>0.05) (Fig. 4C) and the frequency of rMBCs and

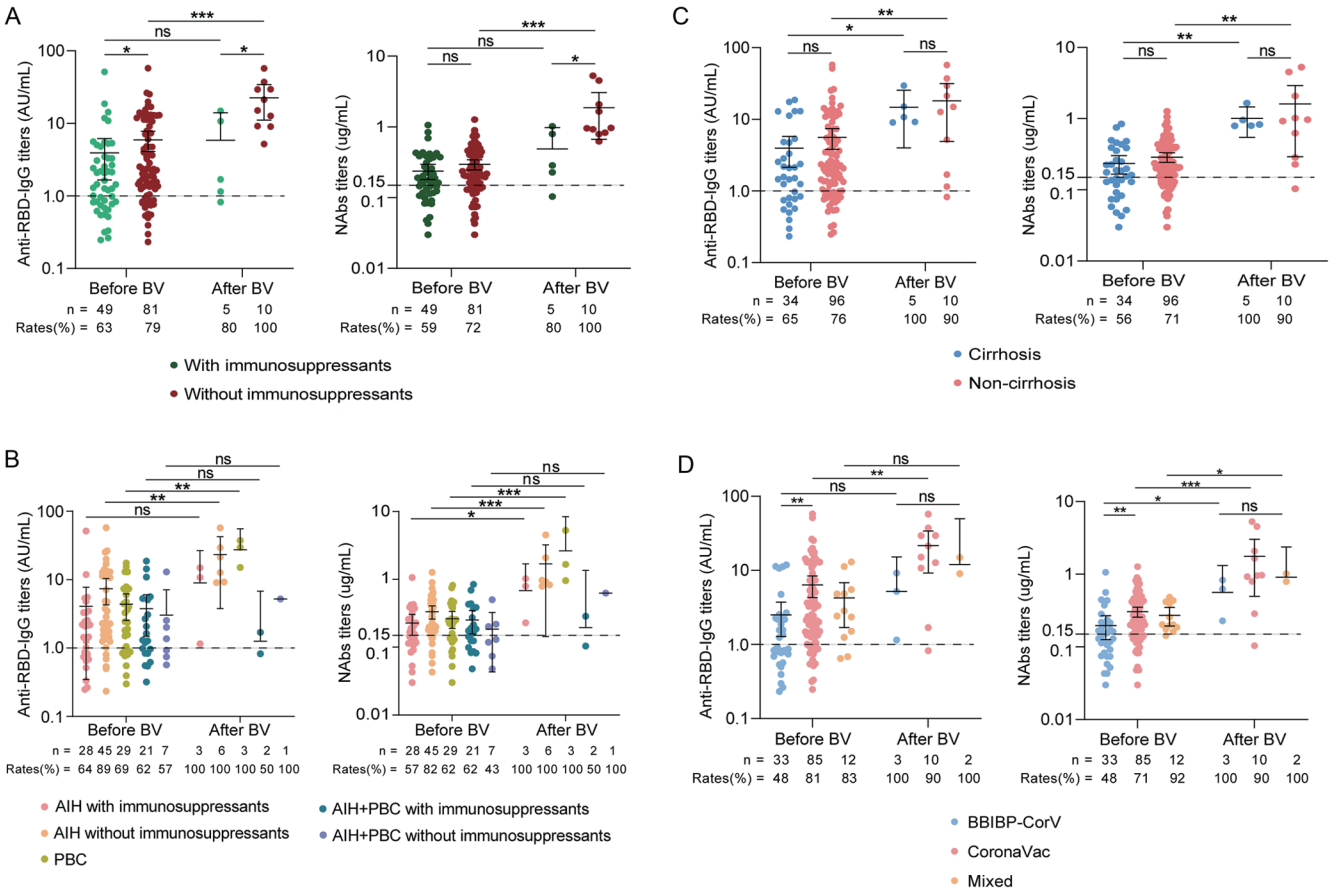

Fig. 3. Subgroup analysis of antibody responses in AILD patients after booster vaccination. (A) The anti-RBD-IgG (left panel) and NAbs (right panel) titers after booster vaccination in patients with and without immunosuppressants. (B) The anti-RBD-IgG (left panel) and NAbs (right panel) titers after booster vaccination in patients with and without cirrhosis. (C) The anti-RBD-IgG (left panel) and NAbs (right panel) titers after booster vaccination in patients with AIH and PBC/PSC. (D) The anti-RBD-IgG (left panel) and NAbs (right panel) titers in AILD patients after different inactivated vaccines. Dotted lines indicate the detection limit for anti-RBD-IgG and NAbs. *p<0.05; **p<0.01; ***p<0.001; ns, not significant. AIH, autoimmune hepatitis; AILD, autoimmune liver disease; anti-RBD-IgG, antireceptor-binding-domain IgG; BV, booster vaccination; NAbs, neutralizing antibodies; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

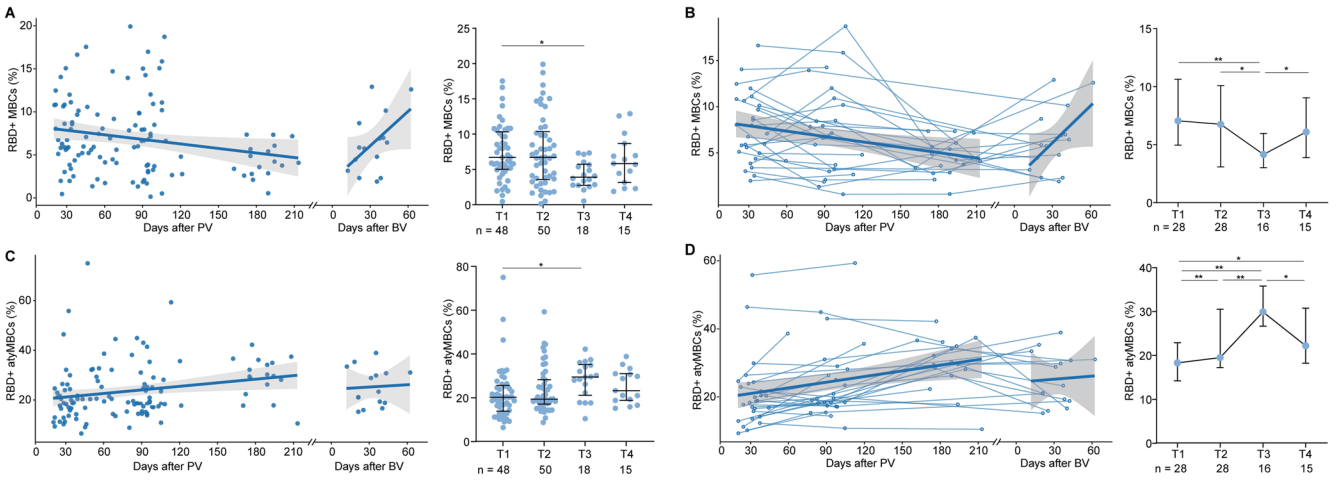

Fig. 4. RBD-specific memory B cells responses after primary and booster vaccination in patients with AILD. (A and B) Cross-sectional analysis (A) and longitudinal analysis (B) of frequency of RBD$^+$ MBCs over time after primary and booster vaccination in AILD patients. (C and D) Cross-sectional analysis (C) and longitudinal analysis (D) of frequency of atyMBCs over time after primary and booster vaccination in AILD patients. The trendlines were produced using a linear model fit, and the shaded area showed the 95% CI for each fit. *p<0.05; **p<0.01. AILD, autoimmune liver disease; atyMBCs, atypical MBCs; BV, booster vaccination; MBCs, memory B cells; RBD, receptor binding domain; PV, primary vaccination.

intMBCs increased after booster vaccination (Supplementary Fig. 4A–C). Further longitudinal study analysis showed more obvious trend in atyMBCs ($p<0.05$) (Fig. 4D) and other subsets (Supplementary Fig. 4D, F). Considering the function of atyMBCs,[23] our results indicated that booster inactivated vaccination might restore partly the damaged immune memory function in AILD patients.

Discussion

In this prospective observational study, we focused on the safety and humoral responses in AILD patients after primary and booster inactivated vaccination. The main findings of this study are: (1) inactivated vaccines were safe for AILD patients; (2) booster dose of inactivated vaccine significantly enhanced the antibody responses in AILD patients without immunosuppressants; (3) booster vaccination partially repaired the impaired immune memory function in AILD patients. Therefore, booster dose of inactivated vaccine is recommended for patients with AILD.

The SARS-CoV-2 vaccine might induce autoimmune responses or aggravate autoimmune diseases.[24–26] Several instances of people who developed AIH after mRNA or adenovirus vector vaccines have been reported.[3–8] Hence, the safety profile in AILD patients after primary and booster inactivated vaccination was evaluated in this study. The overall occurrence of AEs in AILD patients after primary vaccination was 26.2%, which was lower than that reported in previous studies of patients with severe liver disease (33.3%),[27] but was higher than that in chronic hepatitis B patients (14.1%).[21] Interestingly, the total incidence of AEs after booster vaccination was significantly decreased (13.3%). A similar phenomenon was reported in a previous study.[28] Moreover, although IgG is the main indicator of AIH immune activity, C3 is also used to evaluate immunoinflammatory activity in some autoimmune diseases (such as primary glomerulonephritis, and systemic lupus erythematosus nephritis). When liver function is impaired, complement synthesis is affected, and C3 can also decrease. Our results showed the level of C3 was decreased after primary vaccination, which indicated that the autoimmune response might be partially activated in AILD patients. However, significant vaccine-related elevation of liver enzymes levels was not observed. The reason may be that different type of vaccines were used and most patients are receiving treatment in this study. Altogether, both the primary and booster inactivated vaccines were safe in patients with AILD.

Next, we evaluated dynamic changes of antibody responses after primary and booster vaccination in AILD patients. After primary vaccination, the titers of anti-RBD-IgG and NAbs declined over time, which was consistent with a previous study of BNT162b2 Vaccine in HCs.[29] After booster vaccination, both antibody titers significantly increased, and were higher than those 1 month after primary vaccination, which was similar to previous results in healthy individuals.[30–32] This indicated the immune memory was stimulated after the booster dose of inactivated vaccine in AILD patients.[33] Notably, both the anti-RBD-IgG and NAbs titers in AILD patients were elevated as high as a control group of health care workers after booster vaccination. A recent study[15] and our previous data[14] have shown the inadequate immune responses at early stage after primary vaccination in AILD patients. Therefore, this result indicated that booster vaccination reversed the poor antibody responses of patients with AILD. Unfortunately, booster vaccination did not significantly enhance the antibody responses in patients receiving immunosuppressants, which differed from the results in immunocompromised patients given an mRNA booster dose.[34] The vaccine type may contribute to this discrepancy. Similarly, the lower antibody titers in patients with immunosuppressants than in patients without immunosuppressants were observed after the booster vaccination.

Although several previous studies have shown weakened antibody responses to COVID-19 vaccines in patients with cirrhosis,[35–38] the finding is still controversial[39] Because some studies found no differences in the humoral responses of cirrhotic and noncirrhotic patients.[40,41] In this study, the antibody responses in the two groups were similar (data not shown), which may be explained by the small sample size or different vaccine types.

Lastly, we found that the frequency of aytMBCs increased significantly over time after primary vaccination in AILD patients. AtyMBCs are short-lived activated cells with low binding to the spike protein of SARS-CoV-2[23] and was also increased in patients with common variable immunodeficiency,[42] solid tumors,[43] and severe liver diseases.[27] This indicated that the immune memory function was damaged over time in patients with AILD. Interestingly, the percentage of atyMBCs was decreased after booster vaccination, which suggests that booster vaccination may have partially restored impaired immune memory.

Our study had some limitations. First, the sample size was relatively small. Because COVID-19 patients were unwilling or unable to come to the hospital to take part in the trial, enrollment and follow-up were difficult. Second, the observation period after booster vaccination was short, which hindered extended analysis of changes of the immune response longer than 6 months after booster vaccination. Third, T-cell responses after primary and booster vaccination in AILD patients were not analyzed. A larger and well-designed study is needed in future. Despite some of these limitations, we believe our findings are still important and meaningful to clinicians. In conclusion, our results indicate that the inactivated SARS-CoV-2 vaccine was well tolerated in patients with AILD, regardless of primary or booster vaccination. Booster vaccination significantly enhanced the antibody responses of AILD patients without immunosuppressants and may partially recover immune memory function.

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Conflict of interest

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

Author contributions

Participated in the conception and design of this study (DC, DZ, HR), project manager and coordinated patient recruitment (DC), coordinated the serological analysis (MC, MP), performed patient recruitment (YW, TH, GZ, LA, QP, YZ, QZ, NL, DZ, DC, PH), performed antibody testing (DX, GZ), acquisition, analysis, or interpretation of data (ZC, YW, HL), drafting of the manuscript (ZC, YW), obtained funding for the study (PH, MP, MC HR). All the authors contributed to the critical review and final approval of the manuscript. All authors were responsible for the decision to submit the manuscript.

Ethical statement

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University and in accordance with the ethical guidelines of the Declaration of Helsinki (Ratification No. 94/2021). Written informed consent was obtained from all participants. This study has been registered at ClinicalTrials.gov (NCT05007665).

Data sharing statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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