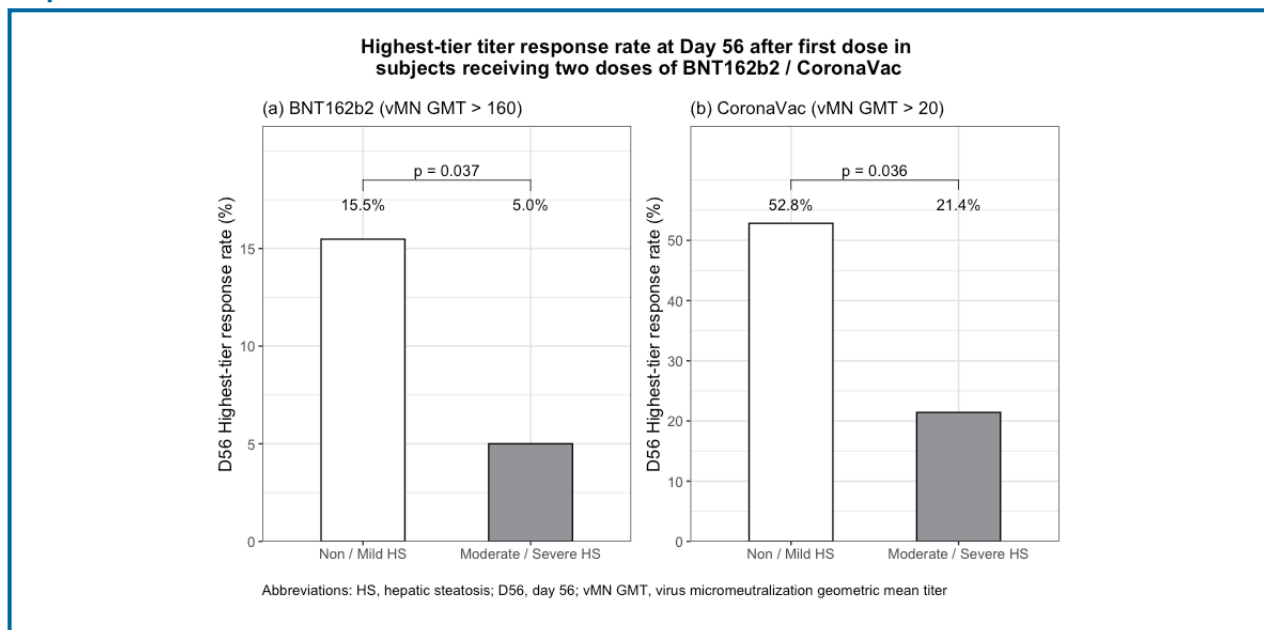**CLINICAL and MOLECULAR HEPATOLOGY**

Original Article

Effect of moderate-to-severe hepatic steatosis on neutralising antibody response among BNT162b2 and CoronaVac recipients

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Graphical Abstract

Highest-tier titer response rate at Day 56 after first dose in subjects receiving two doses of BNT162b2 / CoronaVac

(a) BNT162b2 (vMN GMT > 160)

p = 0.037
15.5%   5.0%

(b) CoronaVac (vMN GMT > 20)

p = 0.036
52.8%   21.4%

Abbreviations: HS, hepatic steatosis; D56, day 56; vMN GMT, virus micromeutralization geometric mean titer

Study Highlights

- There is no difference in the seroconversion rate of neutralising antibody between patients with and without moderate-to-severe hepatic steatosis after two doses of vaccines (for either BNT162b2 or CoronaVac).
- A lower proportion of patients with moderate-to-severe hepatic steatosis achieve a highest-tier humoral response after two doses of vaccines (for either BNT162b2 or CoronaVac).
- There are no serious adverse reactions in COVID-19 vaccine recipients with moderate-to-severe hepatic steatosis.

Background/Aims: Studies of hepatic steatosis (HS) effect on COVID-19 vaccine immunogenicity are lacking. We aimed to compare immunogenicity of BNT162b2 and CoronaVac among moderate/severe HS and control subjects.

Methods: Two hundred ninety-five subjects who received BNT162b2 or CoronaVac vaccines from five vaccination centers were categorized into moderate/severe HS (controlled attenuation parameter ≥268 dB/m on transient elastography) (n=74) or control (n=221) groups. Primary outcomes were seroconversion rates of neutralising antibody by live virus Microneutralization (vMN) assay (titer ≥10) at day21 (BNT162b2) or day28 (CoronaVac) and day56 (both). Secondary outcome was highest-tier titer response (top 25% of vMN titer; cutoff: 160 [BNT162b2] and 20 [CoronaVac]) at day 56.

Results: For BNT162b2 (n=228, 77.3%), there was no statistical differences in seroconversion rates (day21: 71.7% vs. 76.6%; day56: 100% vs. 100%) or vMN geometric mean titer (GMT) (day21: 13.2 vs. 13.3; day56: 91.9 vs. 101.4) among moderate/severe HS and control groups respectively. However, lower proportion of moderate/severe HS patients had highest-tier response (day56: 5.0% vs. 15.5%; *P*=0.037). For CoronaVac (n=67, 22.7%), there was no statistical differences in seroconversion rates (day21: 7.1% vs. 15.1%; day56: 64.3% vs. 83.0%) or vMN GMT (5.3 vs. 5.8,) at day28. However, moderate/severe HS patients had lower vMN GMT (9.1 vs. 14.8, *P*=0.021) at day 56 with lower proportion having highest-tier response (21.4% vs. 52.8%, *P*=0.036).

Conclusions: While there was no difference in seroconversion rate between moderate/severe HS and control groups after two doses of vaccine, a lower proportion of moderate/severe HS patients achieved highest-tier response for either BNT162b2 or CoronaVac. **(Clin Mol Hepatol 2022;28:553-564)**

Keywords: COVID-19; SARS-CoV-2; Vaccination; Non-alcoholic fatty liver disease; Liver cirrhosis

INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged into a global health burden. As of March 2022, COVID-19 has affected more than 400 million people and caused nearly 6 million deaths worldwide. Apart from measures such as social distancing, quarantine and iso-lation, vaccination is crucial in preventing infection, severe disease and death.[1]

Chronic liver disease is associated with a higher infection risk and disease severity of COVID-19, especially those with liver cirrhosis.[2] With a global prevalence of 25% for non-alco-holic fatty liver disease (NAFLD),[3] concerns have been raised about COVID-19 vaccination response in this large popula-tion.[4] Since NAFLD is a multisystemic condition that presents

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Abbreviations:
ALT, alanine aminotransferase; aOR, adjusted odds ratio; CAP, controlled attenuation parameter; CI, confidence interval; COVID-19, coronavirus disease 2019; DM, diabetes mellitus; GMT, geometric mean titer; HS, hepatic steatosis; IQR, interquartile range; MAFLD, metabolic-associated fatty liver disease; MRE, magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; vMN, virus Microneutralization

with a wide spectrum of extrahepatic manifestations, such as obesity, diabetes mellitus (DM), cardiovascular diseases, the term metabolic-associated fatty liver disease (MAFLD) has recently been proposed to redefine NAFLD.[5]

Neutralising antibody level is a surrogate marker of vaccine effectiveness[6] and is predictive of protection from symptomatic COVID-19 infection.[7,8] Recently, Wang et al.[9] reported that seroconversion rate of BBIBP-CorV (inactivated vaccine) was 95.5% after two doses of vaccine in 381 NAFLD patients. However, several questions remain unaddressed. First, it did not assess immunogenicity after single dose of vaccine and according to severity of hepatic steatosis (HS). Second, there were no control subjects without HS for comparison. Third, longer-term data (e.g., 6 months after vaccination) were not available. Fourth, studies evaluating the effect of mRNA vaccine and another commonly used inactivated vaccine (CoronaVac) in HS patients are currently lacking.

In Hong Kong, both mRNA and inactivated vaccines are available. Therefore, we aimed to conduct a longer-term prospective cohort study to compare immunogenicity of BNT162b2 and CoronaVac (after both first and second doses) and adverse events in patients with moderate-to-severe HS and control subjects.

MATERIALS AND METHODS

Study design

This is a prospective cohort study recruiting adult COVID-19 vaccine recipients (BNT162b2 and CoronaVac) from five local vaccination centers. Exclusion criteria included age <18 years, transplant patients, patients taking immunosuppressives/chemotherapy, inflammatory bowel disease, other medical diseases (cancer, hematological, rheumatological and autoimmune diseases), those with prior COVID-19 infection (identified from history taking or presence of antibodies to SARS-CoV-2 nucleocapsid protein which are not inducible by BNT162b2 and therefore is an indicator of past infection).

Severity of HS was defined by controlled attenuation parameter (CAP) ≥248 dB/m measured by transient elastography using Fibroscan® (Echosens, Paris, France): mild (CAP 248-267 dB/m), moderate (CAP 268-279 dB/m) and severe (CAP ≥280 dB/m).[10] Since the metabolic and cardiovascular risks were significantly higher in significant fatty liver compared

to mild fatty liver,[11,12] we defined moderate/severe HS group (simplified as "HS" in subsequent sections) in our current study by CAP ≥268 dB/M. An alanine aminotransferase (ALT) level of 40 was used to identify possible non-alcoholic steatohepatitis (NASH).

Recruited subjects received either BNT162b2 or CoronaVac according to their preference. They received two doses of intramuscular BNT162b2 (0.3 mL) and CoronaVac (0.5 mL) 3 weeks and 4 weeks apart, respectively. Their blood samples were collected at four time-points: (i) before vaccination (baseline), (ii) 21 days (for BNT162b2) or 28 days (for CoronaVac) after first dose, (iii) 56 days after first dose (for both BNT162b2 and CoronaVac), and (iv) 180 days after first dose for BNT162b2 only (CoronaVac data was unavailable at this time point). Live virus Microneutralization (vMN) assay was performed in 96-well plate as described previously (Appendix 1).[13] vMN positivity (seroconversion) was defined as titer ≥10 (31.25 IU/mL).

Subjects were requested to record any systemic and local events daily for 7 days after both first and second dose of vaccine. The severity of adverse events were graded as 1, 2, 3, and 4, according to toxicity grading scale by U.S. Department of Health and Human Services.[14]

The study was approved by the Institutional Review Board of the University of Hong Kong (HKU) and Hong Kong West Cluster (HKWC) of Hospital Authority.

Outcome of interest

Primary outcomes of interest are seroconversion rates at three time points after first dose for BNT162b2 (day21, day56, and day180) and two time points for CoronaVac (day28 and day56).

Secondary outcomes of interest are (i) highest-tier titer response; (ii) overall and individual adverse events after both first and second doses. We defined the top 25% of vMN titer (i.e., above 75 percentile) as highest-tier titer response based on D56 data, while the remainders as having suboptimal humoral immune response. The 25% was an arbitrary cut-off as there is still no international consensus. A cut-off of 160 and 20 antibody tier was adopted for BNT162b2 and CoronaVac, respectively. Top 25% was selected as cut-off as vaccine effectiveness waned by time.[15,16] As Xu et al.[16] has reported a drop of neutralizing antibodies by one natural log unit from the peak at 6 months post infection, protection of vaccine

among patients with higher antibody level is expected to last longer than those with lower antibody level. vMN titer at day 180 after first dose of BNT162b2 was measured to evaluate longer-term effect of BNT162b2.

Exposure of interest

Subjects with moderate or severe HS were grouped as "HS" and those with mild or no HS were regarded as control group. Covariates included age >70, sex, overweight/obesity, smoking, alcohol use, hypertension, DM, liver fibrosis (liver stiffness measurement >6.5 kPa),[17] concomitant renal impairment and past gastrointestinal surgery. Vaccine effectiveness is low until two doses of CoronaVac, and declines with increasing age among those aged >70.[18] Overweight/obesity was defined as body mass index ≥23 according to World Health Organization and National Institutes of Health guidelines for Asians. Obesity may impair the ability to mount a protective immune response to influenza virus,[19] and therefore COVID-19 vaccine immunogenicity. Diabetic patients have lower seropositivity rate of COVID-19 vaccination than healthy population.[20] Serum creatinine level is an useful indicator of metabolic syndrome in HS patients.[21] Renal impairment was defined as creatinine level >106 μmol/L for male and >97 μmol/L for female.[22] Since gut microbiota are involved in immune response to vaccination,[23,24] prior gastrointestinal surgery that could possibly affect gut microbiota was included into analysis.

Sensitivity analysis was carried out by comparing moderate/severe HS subjects fulfilling criteria of MAFLD and control groups (subjects without HS or those with mild HS). The definition of MAFLD[5] was provided in Appendix 2.

Statistical analysis

All statistical analyses were conducted in R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) statistical software. Data is displayed as median (interquartile range [IQR]) for continuous variables, and as number of patients (percentage) for categorical variables. The Mann-Whitney U test was used for two continuous variables, and the chi-square test or Fisher exact test was used for categorical variables to assess the statistical significance between groups. Geometric mean titer (GMT) with 95% confidence interval (CI) was used to express the average vMN titer. A multivariable logistic regression model was used to estimate ad-

Table 1. Baseline characteristics of patients

	BNT162b2			CoronaVac		
	Moderate/severe HS* (n =60)	Control (n =168)	*P*-value	Moderate/severe HS† (n =14)	Control (n=53)	*P*-value
Age ≥70 years	2 (3.3)	3 (1.8)	0.482	1 (7.1)	2 (3.8)	0.588
Male sex	33 (55.0)	50 (29.8)	<0.001	7 (50.0)	13 (24.5)	0.064
BMI (kg/m²)	27.2 (24.5–29.1)	22.3 (20.2–24.0)	<0.001	26.2 (24.8–26.8)	23.1 (21.0–24.6)	<0.001
Overweight/obesity, BMI ≥23 kg/m²	54 (90.0)	65 (38.7)	<0.001	12 (85.7)	29 (54.7)	0.034
Smoker	8 (13.3)	14 (8.3)	0.260	4 (28.6)	6 (11.3)	0.107
Alcohol	1 (1.7)	7 (4.2)	0.366	0 (0.0)	3 (5.7)	0.362
Hypertension	12 (20.0)	21 (12.5)	0.156	5 (35.7)	6 (11.3)	0.028
Diabetes mellitus	11 (18.3)	6 (3.6)	<0.001	2 (14.3)	2 (3.7)	0.140
Liver stiffness (kPa)	4.8 (4.1–5.9)	4.3 (2.5–5.0)	<0.001	4.5 (3.7–6.1)	4.3 (3.6–5.0)	0.267
Fibrosis, liver stiffness measurement >6.5 kPa	8 (13.3)	11 (6.5)	0.103	3 (21.4)	1 (1.9)	0.006
CAP score (dB/m)	294 (276–314)	217 (196–236)	<0.001	281 (274–305)	218 (202–240)	<0.001

Values are presented as median (interquartile range) and number (%).
HS, hepatic steatosis; BMI, body mass index; CAP, controlled attenuation parameter.
*59 (98.3%) of 60 moderate/severe HS subjects had metabolic-associated fatty liver disease (MAFLD).
†All moderate/severe HS subjects had MAFLD.

justed odds ratio (aOR) of highest-tier titer response with HS as well as all aforementioned covariates. For the purpose of statistical analysis, an MN titer of <10 was considered as 5. A two-sided *P*-value ≤0.05 was considered as statistically significant.

RESULTS

Demographics and baseline characteristics

A total of 295 subjects (moderate/severe HS: 74 [25.1%; 73 fulfilled the criteria of MAFLD] and control group: 221 [74.9%]) were enrolled. The demographics of subjects are shown in Table 1. Two hundred twenty-eight subjects re-

Table 2. Comparison between neutralizing antibody response of patients receiving BNT162b2 and CoronaVac

	BNT162b2	CoronaVac	*P*-value
Whole cohort	228	67	
Seroconversion rate*			
D21 (BNT162b2)/D28 (CoronaVac)	171/227 (75.3)	9/67 (13.4)	<0.001
D56	228/228 (100.0)	53/67 (79.1)	<0.001
D180	156/157 (99.4)	NA	NA
vMN GMT			
D21 (BNT162b2)/D28 (CoronaVac)	13.3 (11.9–14.7)	5.7 (5.2–6.2)	<0.001
D56	98.4 (88.2–110.0)	13.4 (11.2–15.8)	<0.001
D180	40.5 (35.9–45.6)	NA	NA
Moderate/severe HS	60[†]	14[‡]	
Seroconversion rate*			
D21 (BNT162b2)/D28 (CoronaVac)	43/60 (71.7)	1/14 (7.1)	<0.001
D56	60/60 (100.0)	9/14 (64.3)	<0.001
D180	40/40 (100.0)	NA	NA
vMN GMT			
D21 (BNT162b2)/D28 (CoronaVac)	13.2 (10.7–16.2)	5.3 (4.8–5.8)	<0.001
D56	91.9 (75.9–111.1)	9.1 (6.8–12.1)	<0.001
D180	39.3 (31.8–48.9)	NA	NA
Control	168	53	
Seroconversion rate*			
D21 (BNT162b2)/D28 (CoronaVac)	128/167 (76.6)	8/53 (15.1)	<0.001
D56	168/168 (100.0)	44/53 (83.0)	<0.001
D180	116/117 (99.1)	NA	NA
vMN GMT			
D21 (BNT162b2)/D28 (CoronaVac)	13.3 (11.8–15.0)	5.8 (5.3–6.6)	<0.001
D56	101.4 (88.2–115.6)	14.8 (12.2–18.0)	<0.001
D180	41.0 (35.2–47.5)	NA	NA

vMN GMT is presented as mean (95% confidence interval).

D21, day21; D28, day28; D56, day56; D180, day180; vMN GMT, virus microneutralization geo-metric mean titer; NA, not available; HS, hepatic steatosis.

*Seroconversion rate is considered as positive if vMN titer ≥10.

[†]59 (98.3%) of 60 moderate/severe HS subjects had metabolic-associated fatty liver disease (MAFLD).

[‡]All moderate/severe HS subjects had MAFLD.

ceived BNT162b2 (HS: 60 [26.3%] and control: 168 [73.7%]), while 67 subjects received CoronaVac (HS: 14 [20.9%] and control: 53 [79.1%]). Among BNT162b2 recipients, 27 (16.1%) of 168 subjects in control group had mild HS, and 13 (21.7%) of 60 subjects with moderate/severe HS had raised ALT. Among CoronaVac recipients, 11 (20.8%) of 53 subjects in control group had mild HS, and five (35.7%) of 14 subjects with moderate/severe HS had raised ALT.

The median age was similar between two groups, for either BNT162b2 (HS: 51.1 years vs. control: 50.9 years; P=0.242) or CoronaVac recipients (HS: 53.9 vs. control: 53.3 years; P=0.677). There were more males in the HS group than controls among both BNT162b2 recipients (55.0% vs. 29.8%; P<0.001) and CoronaVac recipients (50.0% vs. 24.5%; P=0.064). There was a higher proportion of HS patients being overweight/obese compared to controls (BNT162b2 group: 90% vs. 38.7%, P<0.001; CoronaVac group: 85.7% vs. 54.7%, P=0.034).

HS-associated comorbidities including hypertension, diabetes, alcohol intake were also included in Table 1. For BNT162b2 recipients, there were more HS patients who had DM compared to controls (18.3% vs. 3.6%, P<0.001). For CoronaVac recipients, there were more HS patients who had hypertension compared to controls (35.7% vs. 11.3%, P=0.028), and a higher proportion of HS patients had fibrosis than con-

trols (21.4% vs. 1.9%, P=0.006). There were no significant difference in proportion of smokers (BNT162b2 group: 13.3% vs. 8.3%, P=0.260; CoronaVac group: 28.6% vs. 11.3%, P=0.107) and alcohol users (BNT162b2 group: 1.7% vs. 4.2%, P=0.366; CoronaVac group: 0% vs. 5.7%, P=0.362) among HS and control groups.

Comparison of vaccine immunogenicity between BNT162b2 and CoronaVac recipients

All subjects had vMN titer lower that of the detection limit. Table 2 shows that the seroconversion rate of BNT162b2 was higher than CoronaVac after both first dose (75.3% vs. 13.4%, P<0.001) and second dose (day56) (100% vs. 79.1%, P<0.001) for the whole cohort.

Among HS group, seroconversion rate of BNT162b2 was higher than CoronaVac after both first dose (71.7% vs. 7.1%, P<0.001) and second dose (100% vs. 64.3%, P<0.001). vMN GMT of BNT162b2 was higher than that of CoronaVac after both first dose (13.2; 95% CI, 10.7–16.2 vs. 5.3; 95% CI, 4.8–5.8; P<0.001) and second dose (91.9; 95% CI, 75.9–111.1 vs. 9.1; 95% CI, 6.8-12.1; P<0.001).

Among control group, seroconversion rate of BNT162b2 was higher than CoronaVac after both first dose (76.6% vs. 15.1%, P<0.001) and second dose (100% vs. 83%, P<0.001).

Table 3. Neutralizing antibody responses of patients receiving BNT162b2 (n=228)

	Moderate/severe HS (n=60)	Control (n=168)	P-value
Seroconversion rate*			
D21	43/60 (71.7)	128/167 (76.6)[†]	0.442
D56	60/60 (100.0)	168/168 (100.0)	1.000
D180	40/40 (100.0)[†]	116/117 (99.1)[†]	1.000
Highest-tier response rate[‡]			
D56 Highest-tier response rate	3/60 (5.0)	26/168 (15.5)	0.037
D180 Highest-tier response rate	0/40 (0.0)	3/117 (2.6)	0.570
vMN GMT			
D21	13.2 (10.7–16.2)	13.3 (11.8–15.0)	0.841
D56	91.9 (75.9–111.1)	101.4 (88.2–115.6)	0.675
D180	39.3 (31.8–48.9)	41.0 (35.2–47.5)	1.000

Fifty-nine (98.3%) of 60 moderate/severe HS subjects had MAFLD.
vMN GMT is presented as mean (95% confidence interval).
HS, hepatic steatosis; D21, day21; D56, day56; D180, day180; vMN GMT, virus microneutralization geometric mean titer.
*Seroconversion rate is considered as positive if vMN titer ≥10.
[†]Subjects with missing data are excluded.
[‡]Highest-tier response is defined as vMN titer >160.

vMN GMT of BNT162b2 was higher than that of CoronaVac after both first dose (13.3; 95% CI, 11.8–15.0 vs. 5.8; 95% CI, 5.3–6.6; $P<0.001$) and second dose (101.4; 95% CI, 88.2–115.6 vs. 14.8; 95% CI, 12.2–18.0; $P<0.001$).

Comparison of vaccine immunogenicity between moderate/severe HS and control groups among BNT162b2 recipients

Table 3 shows the humoral immune response among 228 BNT162b2 recipients. Fifty-nine (98.3%) of 60 subjects with moderate/severe HS had MAFLD. At day21, there was no sig-nificant difference in seroconversion rate among HS and control group (71.7% vs. 76.6%, $P=0.442$) or the vMN GMT (13.2 vs. 13.3, $P=0.841$). At day56, all vaccinees achieved seroconversion with a similar vMN GMT (91.9 vs. 101.4, $P=0.675$). However, there was a lower proportion of vaccinees having highest-tier titer response among the HS than control groups (5.0% vs. 15.5%, $P=0.037$). At day180, more than 99% remained seropositive with decreasing of vMN GMT from 91.9 to 39.3 in HS and from 101.4 to 41.0 in control group, and there was no longer significant difference in terms of highest-tier titer response rate (0% vs. 2.6%, $P=0.570$). Supplementary Table 1 shows similar results by comparing MAFLD and

Table 4. Factors associated with highest-tier neutralizing antibody response at day 56 among BNT162b2 recipients on multivariable analysis

	Adjusted OR	95% CI	P-value
Age, ≥70 years	<0.001	–	0.995
Male sex	1.62	0.62–4.05	0.312
Moderate/severe HS*	0.24	0.05–0.87	0.047
Hypertension	0.23	0.01–1.29	0.173
Diabetes mellitus	1.13	0.06–8.36	0.915
Overweight/obesity, BMI ≥23 kg/m^2	0.94	0.37–2.28	0.891
Alcohol use	<0.001	–	0.994
Smoking	0.32	0.02–1.86	0.300
Fibrosis	1.09	0.16–4.69	0.913
Past gastrointestinal surgery	3.25	0.64–13.92	0.121
Renal impairment	<0.001	–	0.993

OR, odds ratio; CI, confidence interval; HS, hepatic steatosis; BMI, body mass index.
*59 (98.3%) of 60 moderate/severe HS subjects had metabolic-associated fatty liver disease.

Table 5. Neutralizing antibody responses of patients receiving CoronaVac (n=67)

	Moderate/severe HS (n=14)	Control (n=53)	P-value
Seroconversion rate*			
D28	1/14 (7.1)	8/53 (15.1)	0.438
D56	9/14 (64.3)	44/53 (83.0)	0.125
Highest-tier response rate[†]			
D56 Highest-tier response rate	3/14 (21.4)	28/53 (52.8)	0.036
vMN GMT[†]			
D28	5.3 (4.8–5.8)	5.8 (5.3–6.6)	0.420
D56	9.1 (6.8–12.1)	14.8 (12.2–18.0)	0.021

All moderate/severe HS subjects had MAFLD.
vMN GMT is presented as mean (95% confidence interval).
HS, hepatic steatosis; D28, day28; D56, day56; D180, day180; vMN GMT, virus microneutralization geometric mean titer.
*Seroconversion rate is considered as positive if vMN titer ≥10.
[†]Highest-tier response is defined as vMN titer ≥20.

control groups.

On univariate analysis, the OR of highest-tier titer response to BNT162b2 with HS was 0.29 (95% CI, 0.07–0.86). On multivariable analysis, HS was the only independent factor associated with this outcome (aOR, 0.24; 95% CI, 0.05–0.87) (Table 4).

Comparison of vaccine immunogenicity between moderate/severe HS and control groups among CoronaVac recipients

Table 5 shows the humoral immune response among 67 CoronaVac recipients. All moderate/HS subjects fulfilled criteria of MAFLD. At day28, there was no significant difference in seroconversion rate among HS and control group (7.1% vs. 15.1%, P=0.438) or the vMN GMT (5.3 vs. 5.8, P=0.420). At day56, all vaccinees achieved similar seroconversion rate among HS and control group (64.3% vs. 83.0%, P=0.125), but HS has lower vMN GMT than control group (9.1 [95% CI, 6.8–12.1] vs. 14.8 [95% CI, 12.2–18.0], P=0.021). There was a lower proportion of vaccinees having highest-tier titer response among the HS than control groups (21.4% vs. 52.8%; P=0.036). Data of vMN GMT at day180 was not available for CoronaVac vaccinees.

On univariate analysis, OR of highest-tier titer response to CoronaVac with HS was 0.24 (95% CI, 0.05–0.88). Multivariable analysis did not reveal an association between HS and highest-tier antibody titer response.

Safety

After 1st dose vaccine

One hundred ninety (83.3%) BNT162b2 recipients and 36 (53.7%) CoronaVac recipients reported adverse events within 7 days of first dose of vaccine (Supplementary Table 2). All adverse events were mild (grade 1 and 2) and self-limiting. The most common local and systemic adverse events were injection site pain (78.1% for BNT162b2 and 35.8% for CoronaVac) and fatigue (30.7% BNT162b2 and 28.4% for CoronaVac).

Among BNT162b2 recipients, HS group showed higher rate of systemic reaction than control group (35 [58.3%] vs. 65 [38.7%], P=0.008). There was no significant difference in frequency of total adverse events and local reaction between HS and control groups (any adverse event: 50 [83.3%] vs. 140 [83.3%], P=1.0; local reaction: 47 [78.3%] vs. 133 [79.2%],

P=0.892).

Among CoronaVac recipients, HS group showed lower rate of any adverse event (4 [28.6%] vs. 32 [60.4%], P=0.034) and local reactions (2 [14.3%] vs. 24 [45.3%], P=0.034) than control group. There was no significant difference in systemic adverse events between the two groups (systemic events: 3 [21.4%] and 22 [41.5%], P=0.167).

After 2nd dose vaccine

One hundred ninety-four (85.1%) BNT162b2 recipients and 30 (44.8%) CoronaVac recipients reported adverse events within 7 days of second dose of vaccine (Supplementary Table 2). All adverse events were mild (grade 1 and 2) and self-limiting. The most common local and systemic adverse events were injection site pain (76.8% for BNT162b2 and 29.9% for CoronaVac) and fatigue (42.1% BNT162b2 and 14.9% for CoronaVac).

Among BNT162b2 recipients, there was no significant difference in frequency of adverse events between HS and control groups (any adverse event: 51 [85%] vs. 143 [85.1%], P=0.982; local reaction: 44 [73.3%] vs. 135 [80.4%], P=0.256; systemic reaction: 36 [60%] vs. 92 [54.8%], P=0.483).

Among CoronaVac recipients, HS group showed lower rate of any adverse event (3 [21.4%] vs. 27 [50.9%], P=0.048) and local reactions (0 [0%] vs. 21 [39.6%], P=0.003) than control group. Lower rate of local adverse events in HS group was observed with injection site pain (0 [0%] vs. 20 [37.7%], P=0.007). There was no significant difference in systemic adverse events between the two groups (systemic events: 3 [21.4%] and 16 [30.2%], P=0.518).

DISCUSSION

This prospective cohort study demonstrates that BNT162b2 is more immunogenic than CoronaVac in terms of neutralising antibody response. While there was no difference in the seroconversion rate between moderate/severe HS and control groups for either BNT162b2 or CoronaVac, a lower proportion of the former group achieved a highest-tier humoral response after two doses of vaccines. Similar findings were observed when MAFLD patients with moderate/severe HS were compared with controls.

It has been suggested that patients with chronic liver disease display immune dysfunction in which predisposes them

to infections, organ inflammatory damage and poor response to vaccinations.[24] Even though patients with chronic liver disease are at a high risk of morbidity and mortality from COVID-19,[2] there are few studies that have assessed the efficacy of SARS-CoV-2 vaccination in patients with HS. Wang et al.[9] has reported a reassuring result of 95.5% patients with HS showing detectable neutralizing antibody level after two doses of inactivated vaccine. However, there was no control group for comparison, and the vaccine used was an inactivated vaccine (BBIBP-CorV). There was no data on mRNA vaccine and CoronaVac (another inactivated vaccine).

Diagnosis of NAFLD was made by either liver biopsy or clinical findings without proportion specified, and immunogenicity according to the severity of HS was not measured. Moreover, immunogenicity after single dose of vaccine, and longer-term data were not evaluated. In correlation with the recent study, our study further evaluated the effectiveness of CoronaVac as well as BNT162b2 in vaccine immunogenicity with HS. Our study is unique in a few different ways. First, we assessed the vaccine immunogenicity after both the first and second doses of vaccination. Second, we used live virus for analysis which is the gold standard for analysing vaccine humoral response,[25] as compared to surrogate virus neutralization test which only correlates well with live virus of 0.7–0.8.[13] Third, our study recruited a homogenous patient population as HS was defined consistently by CAP measured by transient elastography, as compared to Wang et al.'s study[9] in which HS was identified by either liver biopsy or clinical findings. The proportion of patients undergoing liver biopsy was not specified, and identification of HS from clinical findings alone may lead to potential misclassification of HS status. CAP measurement also allowed us to investigate vaccine immunogenicity according to severity of HS. It is noteworthy that presence of NASH was only identified by raised ALT in our study, although there is no optimal ALT level to predict NASH and advanced fibrosis, and a low normal ALT does not fully exclude the presence of steatohepatitis.[26,27]

To our knowledge, our study is the first to compare the immunogenicity of both inactivated virus and mRNA vaccines among patients with and without moderate/severe HS. We found no difference in the seroconversion rate or vMN GMT after either first or second dose between the HS and control groups, regardless of the vaccine platform. Although the level of antibody or neutralizing activity required to confer protection against future infection is currently not well defined,

a higher level of antibodies is likely associated with a higher level of protection against future infections.[7,28] To further evaluate the correlation between HS and vaccine immunogenicity, we compared the proportion of highest-tier titer vaccine responders between HS and control groups. It is observed that a lower proportion of HS patients achieved the highest-tier titer response for both BNT162b2 and CoronaVac. Various mechanisms have been proposed, in particular for inactivated vaccine, including links through hyperglycemia, insulin resistance,[29] obesity, gut microbiota imbalance,[30] and alterations in innate immunity.[31] Nevertheless, the effect of DM and overweight/obesity was adjusted for in the multivariable analysis in our study. Specifically, for BNT162b2, although the seroconversion rate sustained at day180, the vMN GMT decreased at day180, and the significant difference in the proportion of highest-tier titer vaccine response rate between HS and control groups was no longer observed, which is likely due to underpower. This shows the waning effect of vaccine immunogenicity with time. COVID-19 re-infection was reported 3 to 5 months after initial infection due to diminishing serum neutralizing antibody levels.[32,33] A recent study showed progressive waning of vaccine effectiveness against COVID-19 infection among BNT162b2 recipients, from 92% at 15–30 days to 47% at 121–180 days, and subsequently to 23% from day211 onwards.[34] Data on the immunogenicity at day180 for CoronaVac recipients are not available in our study. Further long-term follow-up studies with larger sample size are needed to illustrate the influence of HS on the long-term vaccine immunogenicity.

Regarding safety, COVID-19 vaccines were well tolerated with mild and self-limiting adverse events. There were difference in safety profile after 1st dose and 2nd dose vaccine for different vaccine platforms. After 1st dose vaccine, HS group showed higher rate of systemic adverse events than control group among BNT162b2 recipients. The underlying mechanism was not well understood but it could be related to circulating concentrations of inflammatory cytokines. After 2nd dose vaccine, adverse events were generally similar between HS and control groups, except for the difference in local reactions of CoronaVac recipients between HS and control groups. It could be explained by higher subcutaneous tissue thickness in patients with HS that serve as a protective barrier to localized pain.[35]

Several limitations of our study should be noted. First, the sample size was relatively small considering the potential in-

teraction between different vaccine platforms and HS severity. In particular, there were only 14 subjects with moderate/severe HS in CoronaVac recipients. Further study with a larger sample size will allow for better evaluation. Second, although HS was defined by CAP measured by transient elastography, magnetic resonance elastography (MRE) with high diagnostic accuracy of 0.9 was still remained as the most sensitive non-invasive modality in diagnosing HS.[36,37] Third, vaccine-induced cellular immunity against SARS-CoV-2 was not investigated. It is proposed that vaccine-induced T-cell response may protect against severe infection despite seronegativity,[7] via suppressing viral replication and producing long-term memory of the immune system.[38] Fourth, data on neutralizing antibody against mutant viruses like Delta or Omicron are not available.

While there was no difference in the seroconversion rate between subjects with or without moderate/severe HS after two doses of either BNT162b2 or CoronaVac, a lower proportion of moderate/severe HS patients achieved a highest-tier humoral response for both types of vaccines. Whether HS is an independent risk factor for poorer vaccine immunogenicity warrants further investigation.

Authors' contribution

Drs. Ka-Shing Cheung and Lok-Ka Lam were involved with study concept and design; acquisition of data; analysis and interpretation of data; drafting of manuscript; Drs. Rex Wan Hin Hui, Xianhua Mao, Ruqi R Zhang and Kwok-Hung Chan were involved with analysis and interpretation of data; and critical revision of the manuscript for important intellectual content. Profs. Wai-Kay Seto and Man-Fung Yuen were involved with the study concept and design; analysis and interpretation of data; drafting of manuscript; critical revision of the manuscript for important intellectual content; and study supervision. The corresponding author had full access to all data, and was fully responsible for the data integrity and statistical analysis. All authors revised the manuscript and approved the final version of this article.

Conflicts of Interest

The authors have no conflicts to disclose.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

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https://doi.org/10.3350/cmh.2022.0082   http://www.e-cmh.org