Positive Hepatitis B Core Antibody Is Associated With Cirrhosis and Hepatocellular Carcinoma in Nonalcoholic Fatty Liver Disease

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OBJECTIVES: **Previous exposure to hepatitis B virus (HBV) may increase the risk of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C. We aim to study the impact of previous HBV infection on the severity and outcomes of patients with nonalcoholic fatty liver disease (NAFLD).**

METHODS: **This was a multicenter study of 489 patients with biopsy-proven NAFLD and 69 patients with NAFLD-related or cryptogenic HCC. Antihepatitis B core antibody (anti-HBc) was used to detect the previous HBV infection.**

RESULTS: **In the biopsy cohort, positive anti-HBc was associated with lower steatosis grade but higher fibrosis stage. 18.8% and 7.5% of patients with positive and negative anti-HBc had cirrhosis, respectively ($P < 0.001$). The association between anti-HBc and cirrhosis remained significant after adjusting for age and metabolic factors (adjusted odds ratio 2.232; 95% confidence interval, 1.202–4.147). At a mean follow-up of 6.2 years, patients with positive anti-HBc had a higher incidence of HCC or cirrhotic complications (6.5% vs 2.2%; $P = 0.039$). Among patients with NAFLD-related or cryptogenic HCC, 73.9% had positive anti-HBc. None of the patients had positive serum HBV DNA. By contrast, antihepatitis B surface antibody did not correlate with histological severity.**

DISCUSSION: **Positive anti-HBc is associated with cirrhosis and possibly HCC and cirrhotic complications in patients with NAFLD. Because a significant proportion of NAFLD-related HCC may develop in noncirrhotic patients, future studies should define the role of anti-HBc in selecting noncirrhotic patients with NAFLD for HCC surveillance.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/B462

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) affects at least a quarter of the global adult population and is one of the leading causes of cirrhosis and hepatocellular carcinoma (HCC) (1,2). Long considered a disease of affluent countries in the West, NAFLD is increasingly recognized in other parts of the world. A recent meta-analysis suggests that NAFLD also affects around 30% of the Asian population (3).

In Asia, chronic hepatitis B is another common chronic liver disease (4). Although fatty liver is less common in patients with chronic hepatitis B virus (HBV) infection (5,6), patients with both conditions have a higher risk of HCC and mortality (7,8). Metabolic diseases such as diabetes and obesity also increase the risk of cirrhosis and HCC in patients with chronic hepatitis B (9,10). Even after hepatitis B surface antigen (HBsAg) seroclearance, diabetes continues to be associated with increased HCC risk (11).

Along with the high prevalence of chronic hepatitis B in Asia, many patients have evidence of occult or previous HBV infection (12,13). This is characterized by the presence of positive antihepatitis B core antibody (anti-HBc) with or without antihepatitis B surface antibody (anti-HBs) in a patient with negative HBsAg (14). The presence of HBV DNA in the blood, liver, or other

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tissues is referred to as occult HBV infection (15,16). Patients with previous HBV infection can sustain liver injury during the period of active HBV infection. Besides, HBV DNA integration into the host genome can increase the risk of HCC (17). In patients with cryptogenic HCC, intrahepatic HBV DNA or covalently closed circular DNA can be found in most cases (18,19). Similarly, some studies demonstrated an association between positive anti-HBc and HCC in patients with chronic hepatitis C (20), although this was not confirmed by other groups (21,22).

With this background, we aim to study whether positive anti-HBc is associated with more severe liver histology and HCC in patients with NAFLD.

METHODS

Patients

This is a post hoc analysis of 3 prospective cohorts, including consecutive patients with biopsy-proven NAFLD from the Prince of Wales Hospital, Hong Kong (n = 343) and University of Malaya, Malaysia (n = 146), and patients with NAFLD-related or cryptogenic HCC from the Prince of Wales Hospital (n = 69) (23,24). Indications for liver biopsy in the first 2 cohorts included high metabolic burden suggestive of increased risk of advanced liver disease, persistent elevation of serum alanine aminotransferase, and abnormal noninvasive tests of liver fibrosis (25). Patients in all 3 cohorts were aged 18 years or older. We excluded patients with excessive alcohol consumption (more than 30 g/d in men and 20 g/d in women), secondary causes of fatty liver (e.g., use of systemic corticosteroids, methotrexate, and tamoxifen), positive HBsAg or anti-hepatitis C virus antibody, clinical or histological features of other liver diseases, or malignancies at or before baseline. The diagnosis of HCC in the third cohort was based on histology or typical radiological features on dynamic imaging in accordance with the international guidelines (26,27). The study protocol was approved by the local institute review board. All patients provided informed written consent.

Clinical assessment

Within 1 week before liver biopsy, we performed physical examination and anthropometric measurements for all patients. Body mass index (BMI) was calculated as body weight (kilogram) divided by height (in meter) squared. Waist circumference was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body in the horizontal position. Venous blood was taken after overnight fasting for at least 8 hours for renal and liver biochemistry, glucose, hemoglobin A_{1c}, lipids, and virological assays.

All patients were followed annually or more frequently as clinically indicated (23). At each visit, we repeated the anthropometric measurements and biochemical tests, and documented all clinical events.

Severity of liver disease

We performed liver biopsy using 16G (Hong Kong) and 18G (Malaysia) cutting needles. One pathologist at each site (A.W.-H.C., N.R.N.M.) scored the liver specimens according to the nonalcoholic steatohepatitis (NASH) Clinical Research Network system (28). Both assessors were specialists in pathology and had more than 10 years of experience in liver pathology. NASH was defined as the presence of hepatic steatosis, lobular inflammation, and hepatocyte ballooning. Fibrosis was staged on a 5-point system, with F0 = no fibrosis, F1 = perisinusoidal or portal fibrosis, F2 = perisinusoidal and portal/periportal fibrosis, F3 = bridging fibrosis, and F4 = cirrhosis. We previously compared the blinded scores by our pathologists and showed high interobserver agreement for fibrosis stage (κ 0.80–1.00) and steatosis grade (κ 0.71–0.85), and moderate agreement for lobular inflammation (κ 0.44–0.63) and hepatocyte ballooning (κ 0.53–0.75) (29).

In the HCC cohort, the fibrosis stage was determined by histology of the adjacent nontumorous tissue in the resection specimens. For patients who were not candidates for liver resection, we performed vibration-controlled transient elastography using the FibroScan 502 machine (Echosens, Paris, France) and defined advanced chronic liver disease as liver stiffness ≥10 kPa as recommended by the Baveno VI consensus statements with local validation (30,31).

Virological assays

Patients' serum samples were stored at −80 °C and retrieved for virological assays in one batch. HBsAg, anti-HBs, and anti-HBc were tested using enzyme-linked immunosorbent assays (Elecsys, Roche, Basel, Switzerland). Samples tested positive for anti-HBc were further tested for HBV DNA using the TaqMan real-time polymerase chain reaction system (iCycler; Bio-Rad, Hercules, CA). The lower limit of detection for HBV DNA was 20 IU/mL.

Study endpoints

The primary endpoint was the presence of cirrhosis because it was the key disease state linking NAFLD/NASH to liver-related complications. Secondary endpoints include histological NASH, significant (F2-4) fibrosis, liver-related events, and HCC. Liver-related event was a composite of HCC, ascites, spontaneous bacterial peritonitis, incident varices, variceal hemorrhage, hepatic encephalopathy, hepatorenal syndrome, and liver-related deaths.

Statistical analysis

Continuous variables are expressed as mean ± SD or median (interquartile range) and compared using the unpaired *t* test or Mann-Whitney *U* test as appropriate. Categorical variables were compared using the χ^2 test. The binary logistic regression model was used to identify the independent factors associated with F2-4 and F4 diseases. The incidence of liver-related events was shown by the Kaplan-Meier curves and compared between patients with positive and negative anti-HBc by the logrank test. When a patient had more than one liver-related event, we considered the earliest event in the incidence analysis. We used the IBM Statistical Package for Social Sciences version 25 to perform the statistical analysis. A 2-sided *P* value of less than 0.05 was taken as statistically significant.

RESULTS

From July 2006 to July 2017, 500 patients underwent liver biopsy for suspected NAFLD, of whom 489 had confirmed NAFLD (Figure 1). The cohort represents middle-aged patients (age 52 ± 11 years, 54.6% men) with predominantly Chinese ethnicity (77.9%) (Table 1). Most patients had type 2 diabetes and hypertension, and the mean BMI was 28.9 ± 5.1 kg/m^2. In total, 57.1% of patients had NASH, 38.0% had F2-4 fibrosis, and 11.5% had cirrhosis (Table 2).

One hundred seventy (34.8%; 95% confidence interval, 30.5–39.2) patients had positive anti-HBc. Compared with patients with negative anti-HBc, those with positive anti-HBc

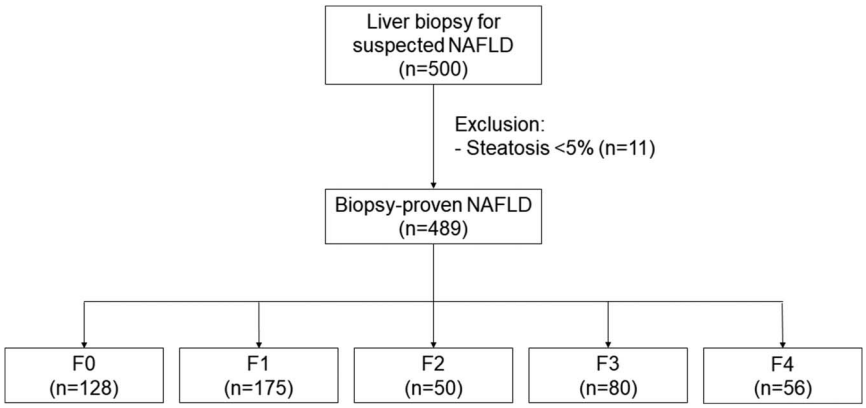

Figure 1. Study participant flow. NAFLD, nonalcoholic fatty liver disease.

Table 1. Clinical characteristics of patients with biopsy-proven NAFLD

Characteristics	All	Anti-HBc positive	Anti-HBc negative	*P*
N	489	170	319	
Age (yr)	52 ± 11	56 ± 10	50 ± 11	<0.001
Male sex, n (%)	267 (54.6)	86 (50.6)	181 (56.7)	0.19
Race, n (%)				<0.001
Chinese	381 (77.9)	152 (89.4)	229 (71.8)	
Malay	73 (14.9)	13 (7.6)	60 (18.8)	
Indian	35 (7.2)	5 (2.9)	30 (9.4)	
Diabetes mellitus, n (%)	313 (64.0)	121 (71.2)	192 (60.2)	0.016
Hypertension, n (%)	295 (60.3)	115 (67.6)	180 (56.4)	0.016
Body weight (kg)	77.9 ± 15.6	75.8 ± 14.4	79.0 ± 16.1	0.029
BMI (kg/m^2)	28.9 ± 5.1	28.3 ± 4.2	29.3 ± 5.5	0.040
Waist circumference (cm)	97 ± 13	96 ± 13	97 ± 13	0.31
Men	100 ± 11	99 ± 12	100 ± 11	0.68
Women	93 ± 13	93 ± 13	94 ± 13	0.85
Total bilirubin (mg/dL)	0.75 ± 0.40	0.71 ± 0.37	0.77 ± 0.42	0.11
Albumin (g/dL)	4.4 ± 0.4	4.4 ± 0.3	4.4 ± 0.4	0.050
Alanine aminotransferase (IU/L)	54 (35–86)	47 (28–73)	59 (38–97)	<0.001
Aspartate aminotransferase (IU/L)	34 (24–52)	29 (22–47)	37 (25–55)	0.005
Gamma-glutamyl transpeptidase (IU/L)	61 (36–103)	48 (33–80)	68 (40–115)	<0.001
Creatinine (mg/dL)	0.86 ± 0.25	0.88 ± 0.31	0.85 ± 0.19	0.37
Fasting glucose (mg/dL)	121 ± 39	120 ± 35	122 ± 41	0.56
HbA_{1c} (%)	6.8 ± 1.3	6.9 ± 1.2	6.7 ± 1.4	0.37
Total cholesterol (mg/dL)	183 ± 43	182 ± 51	183 ± 39	0.80
HDL-cholesterol (mg/dL)	56 ± 30	61 ± 40	53 ± 23	0.014
LDL-cholesterol (mg/dL)	107 ± 37	103 ± 36	108 ± 37	0.15
Triglycerides (mg/dL)	124 (97–168)	115 (97–151)	133 (97–177)	0.055
Platelet count (×10^9/L)	240 ± 72	236 ± 66	243 ± 75	0.32
International normalized ratio	1.00 ± 0.40	0.97 ± 0.07	1.01 ± 0.50	0.29

Continuous variables are expressed as mean ± SD or median (interquartile range).
Anti-HBc, anti-hepatitis B core antibody; BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease.

Table 2. Histological features of NAFLD patients

	All	Anti-HBc positive	Anti-HBc negative	*P*
N	489	170	319	
Steatosis grade	2 (1–2)	2 (1–2)	2 (1–2)	0.038
Lobular inflammation	1 (1–2)	1 (1–2)	1 (1–2)	0.44
Hepatocyte ballooning	1 (0–1)	1 (0–1)	1 (0–1)	0.86
Fibrosis stage	1 (0–3)	1 (1–3)	1 (0–3)	0.011
NAFLD activity score	4 (3–5)	4 (3–4)	4 (3–5)	0.10
NASH, n (%)	279 (57.1)	99 (58.2)	180 (56.4)	0.70
F2-4, n (%)	186 (38.0)	76 (44.7)	110 (34.5)	0.027
F4, n (%)	56 (11.5)	32 (18.8)	24 (7.5)	<0.001

Histological scores are expressed as median (interquartile range) and compared by the Mann-Whitney *U* test. NASH is defined as the presence of hepatic steatosis, lobular inflammation and hepatocyte ballooning.
Anti-HBc, anti-hepatitis B core antibody; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

were older, were more likely to be Chinese, and had higher prevalence of diabetes and hypertension but had lower body weight, BMI, liver enzymes, and higher high-density lipoprotein-cholesterol (Table 1). Chinese patients were more likely to have positive anti-HBc than Malays and Indians. Serum HBV DNA was <20 IU/mL in all patients.

Histological features

Patients with positive anti-HBc had lower steatosis grade but higher fibrosis stage than those with negative anti-HBc (Table 2 and Figure 2). The 2 groups had similar lobular inflammation and hepatocyte ballooning grades and NAFLD activity score. Overall, 58.2% of patients with positive anti-HBc and 56.4% of those with negative anti-HBc had NASH ($P = 0.70$). More patients with positive anti-HBc had F2-4 fibrosis (44.7% vs 34.5%; $P = 0.027$) and cirrhosis (18.8% vs 7.5%; $P < 0.001$).

When stratified by cohort, the difference in fibrosis stage in patients with positive and negative anti-HBc was significant in the Hong Kong cohort ($P = 0.042$), but not in the Malaysian cohort ($P = 0.44$) (see Figure 1, Supplementary Digital Content 1, http://links.lww.com/AJG/B462). When we further stratified the analysis by ethnicity, the difference was observed in Chinese patients ($P = 0.019$), but not in Malays ($P = 0.39$) and Indians ($P = 0.29$).

In a sensitivity analysis of 377 patients with liver biopsy specimens ≥15 mm in length, the fibrosis stage distribution (F0-4) was 31 (22.3%), 44 (31.7%), 17 (12.2%), 20 (14.4%), and 27 (19.4%) in 139 patients with positive anti-HBc and 69 (29.0%), 88 (37.0%), 27 (11.3%), 35 (14.7%), and 19 (8.0%) in 238 patients with negative anti-HBc ($P = 0.020$). Sixty-four (46.0%) patients with positive anti-HBc and 81 (34.0%) patients with negative anti-HBc had F2-4 fibrosis ($P = 0.021$). Twenty-seven (19.4%) patients with positive anti-HBc and 19 (8.0%) patients with negative anti-HBc had cirrhosis ($P = 0.001$).

The hepatitis B vaccine was introduced in 1988 in Hong Kong and 1989 in Malaysia. Because vaccination might reduce the positivity rate of anti-HBc and introduce bias in this study, we further analyzed the results by age stratification. Among 22 patients aged 30 years or younger, 2 (9%) had positive anti-HBc. Neither of the 2 patients with positive anti-HBc had F2-4 fibrosis,

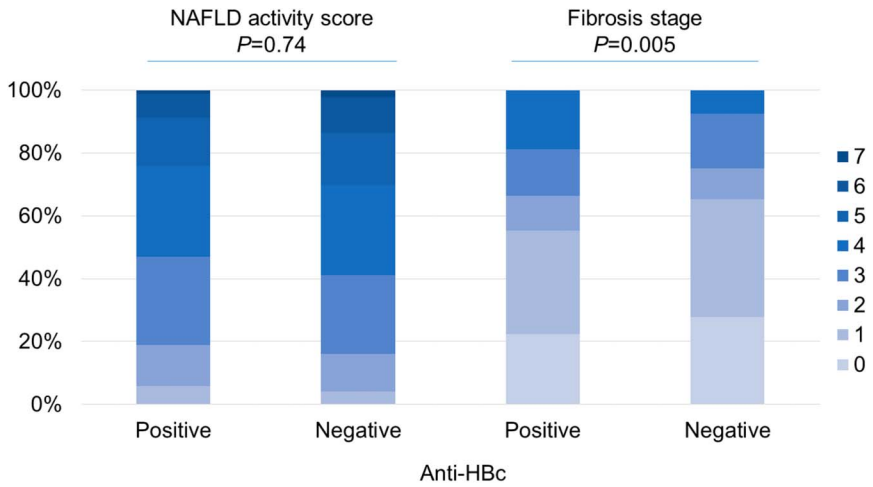

Figure 2. NAFLD activity score and fibrosis stage of patients with NAFLD with positive and negative anti-HBc. The comparisons were by χ^2 test. Anti-HBc, antihepatitis B core antibody; NAFLD, nonalcoholic fatty liver disease.

compared with 4 of 20 (20%) patients with negative anti-HBc ($P = 1.0$). Among 467 patients aged older than 30 years, 168 (36.0%) had positive anti-HBc. Seventy-six of 168 (45.2%) patients with positive anti-HBc had F2-4 fibrosis, compared with 106 of 299 (35.5%) patients with negative anti-HBc ($P = 0.037$). Similarly, 32 of 168 (19.0%) patients with positive anti-HBc had cirrhosis, compared with 23 of 299 (7.7%) patients with negative anti-HBc ($P < 0.001$).

At another age cutoff, among 187 patients aged 50 years or younger, 48 (25.7%) had positive anti-HBc. Thirteen of 48 (27.1%) with positive anti-HBc had F2-4 fibrosis, compared with 34 of 139 (24.5%) patients with negative anti-HBc ($P = 0.72$). Three of 48 (6.3%) patients with positive anti-HBc had cirrhosis, compared with 3 of 139 (2.2%) patients with negative anti-HBc ($P = 0.18$). Among 302 patients aged older than 50 years, 122 (40.4%) had positive anti-HBc. Sixty-three of 122 (51.6%) patients with positive anti-HBc had F2-4 fibrosis, compared with 76 of 180 (42.2%) patients with negative anti-HBc ($P = 0.11$). Twenty-nine of 122 (23.8%) patients with positive anti-HBc had cirrhosis, compared with 21 of 180 (11.7%) patients with negative anti-HBc ($P = 0.005$).

Factors associated with F2-4 fibrosis and cirrhosis

By univariable analysis, positive anti-HBc, older age, diabetes, hypertension, and increased BMI and waist circumference were associated with F2-4 fibrosis (Table 3). By multivariable analysis, older age, diabetes, and increased BMI remained independent factors associated with F2-4 fibrosis.

By univariable analysis, positive anti-HBc, older age, diabetes, hypertension, and increased waist circumference were associated with cirrhosis (Table 3). By multivariable analysis, positive anti-HBc and older age were independent factors associated with cirrhosis.

Anti-HBs and NAFLD

Two hundred thirty-three patients from the Hong Kong cohort were also tested for anti-HBs, among whom 144 (61.8%; 95% confidence interval, 55.2–68.1%) were anti-HBs positive. Compared with patients with negative anti-HBs, those with positive anti-HBs were less likely to have hypertension and had lower triglyceride level (see Table 1, Supplementary Digital Content 1, http://links.lww.com/AJG/B462). As expected, more patients with positive anti-HBs had positive anti-HBc than those with negative anti-HBs (62.5% vs 12.4%; $P < 0.001$). Because anti-HBs could represent previous HBV infection or hepatitis B vaccination, we stratified the analysis by age. Among patients aged 30 years or younger, 2 of 7 (29%) with positive anti-HBs also had positive anti-HBc, compared with 0 of 4 (0%) with negative anti-HBs ($P = 0.49$). Among patients aged older than 30 years, 88 of 137 (64.2%) with positive anti-HBs also had positive anti-HBc, compared with 11 of 85 (12.9%) with negative anti-HBs ($P < 0.001$).

Patients with positive and negative anti-HBs had a similar degree of histological steatosis, lobular inflammation, hepatocyte ballooning, fibrosis, and NAFLD activity score (see Table 2, Supplementary Digital Content 1, http://links.lww.com/AJG/B462). There was also no significant difference in the proportion of patients with NASH, F2-4 fibrosis, and cirrhosis.

Among patients with positive anti-HBc, those with positive anti-HBs had lower fibrosis stage than those with negative anti-HBs (see Table 3, Supplementary Digital Content 1, http://links.lww.com/AJG/B462). However, the 2 groups had similar steatosis, lobular inflammation, hepatocyte ballooning, and NAFLD activity scores. There was also no difference in the proportion of patients with NASH. On the other hand, patients with positive anti-HBs were less likely to have F2-4 fibrosis (37% vs 73%; $P = 0.047$) and cirrhosis (13% vs 55%; $P = 0.004$).

Anti-HBc and liver-related events

At a mean follow-up of 6.2 ± 2.8 years, 18 patients developed liver-related events, including 4 patients with ascites, 11 with incident varices, 2 with variceal hemorrhage, 1 with hepatic encephalopathy, and 4 with HCC (see Table 4, Supplementary Digital Content 1, http://links.lww.com/AJG/B462). Eleven of 170 (6.5%) patients with positive anti-HBc and 7 of 319 (2.2%) patients with negative anti-HBc had liver-related events ($P = 0.039$) (Figure 3a). All 4 patients with HCC had positive anti-HBc, with HCC diagnosed at the age of 54, 60, 75, and 77 years. One patient had F3 fibrosis and 3 had cirrhosis.

Sixteen of the 17 patients who developed liver-related events had F2-4 fibrosis at the time of liver biopsy. When the analysis was restricted to patients with F2-4 fibrosis, liver-related events developed in 10 of 76 (13.2%) patients with positive anti-HBc and 6 of 110 (5.5%) patients with negative anti-HBc ($P = 0.14$) (Figure 3b).

Table 3. Factors associated with F2-4 and F4 disease

	F2-4				F4			
Factors	OR (95% CI)	*P*	aOR (95% CI)	*P*	OR (95% CI)	*P*	aOR (95% CI)	*P*
Positive anti-HBc	1.536 (1.050–2.247)	0.027	1.221 (0.800–1.866)	0.36	2.850 (1.617–5.023)	<0.001	2.232 (1.202–4.147)	0.011
Age (yr)	1.054 (1.035–1.074)	<0.001	1.038 (1.016–1.060)	0.001	1.090 (1.056–1.126)	<0.001	1.077 (1.039–1.117)	<0.001
Male sex	0.768 (0.532–1.108)	0.16	0.824 (0.540–1.256)	0.37	0.880 (0.504–1.536)	0.65	0.959 (0.519–1.774)	0.90
Diabetes mellitus	3.755 (2.439–5.781)	<0.001	2.599 (1.610–4.198)	<0.001	3.804 (1.756–8.240)	0.001	1.884 (0.794–4.468)	0.15
Hypertension	2.566 (1.726–3.815)	<0.001	1.502 (0.954–2.364)	0.079	2.134 (1.132–4.025)	0.019	0.941 (0.450–1.971)	0.87
BMI (kg/m^2)	1.051 (1.010–1.094)	0.014	1.063 (1.005–1.125)	0.033	1.031 (0.984–1.081)	0.20	1.051 (0.989–1.117)	0.11
Waist circumference (cm)	1.023 (1.007–1.038)	0.004	1.003 (0.981–1.025)	0.78	1.026 (1.006–1.048)	0.012	1.015 (0.989–1.041)	0.27

Anti-HBc, anti-hepatitis B core antibody; aOR, adjusted odds ratio, BMI, body mass index, CI, confidence interval; OR, odds ratio.

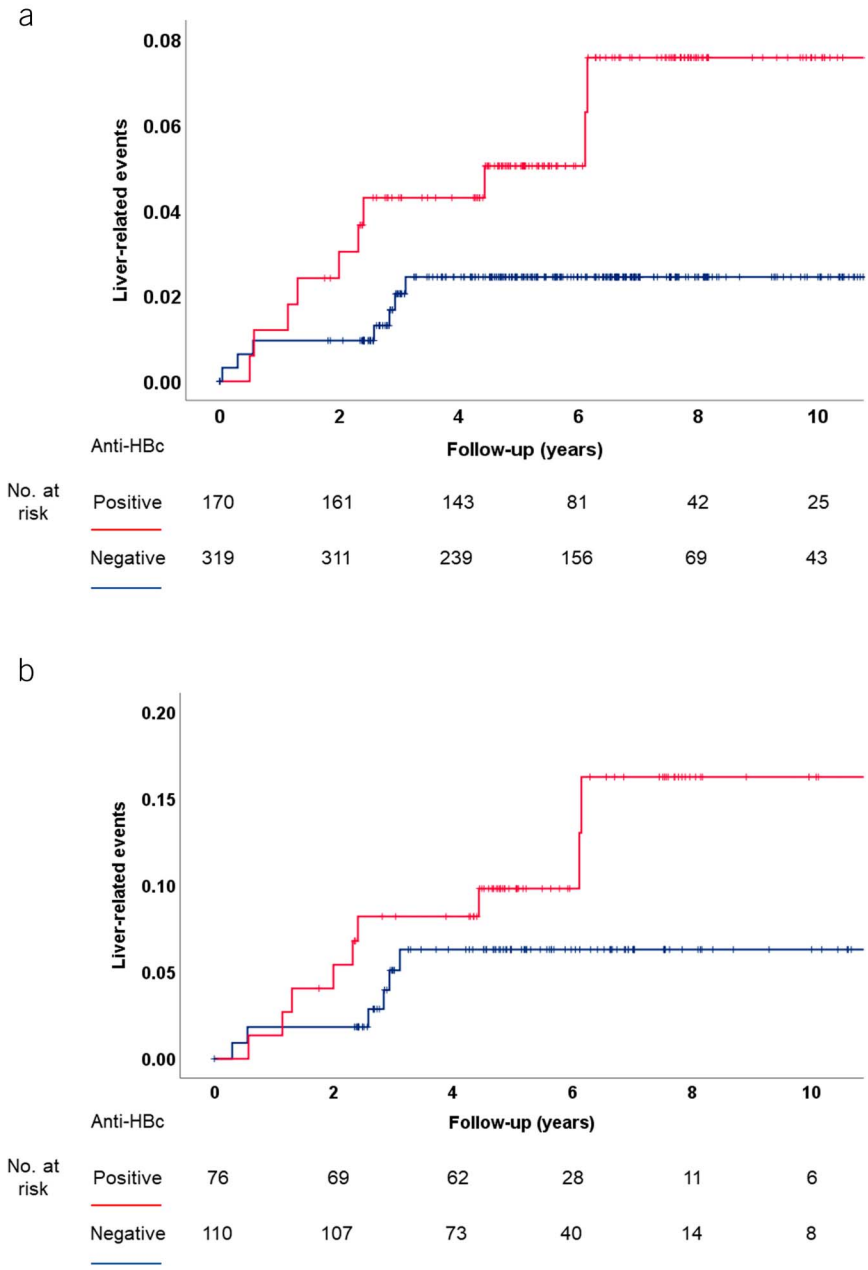

Figure 3. Liver-related events in patients with positive and negative anti-HBc (**a**) entire cohort ($P = 0.039$ by logrank test), (**b**)subgroup of patients with F2-4 fibrosis ($P = 0.14$ by logrank test). Anti-HBc, anti-hepatitis B core antibody.

Because most cohort studies of NAFLD are underpowered for HCC, we analyzed another cohort of 69 patients with NAFLD-related or cryptogenic HCC. Among the HCC patients, those with positive and negative anti-HBc had similar age, sex distribution, prevalence of diabetes and hypertension, anthropometric measurements, and biochemical parameters (see Table 5, Supplementary Digital Content 1, http://links.lww.com/AJG/B462). Among 59 patients who underwent liver resection, the adjacent nontumorous tissue showed F2-4 fibrosis in 28 (48%) patients and cirrhosis in 14 (24%). Eight patients who did not have surgery underwent vibration-controlled transient elastography examination. The median liver stiffness was 22.9 kPa (interquartile range 11.9–45.0); all but one patient had liver stiffness ≥10 kPa suggestive of advanced chronic liver disease. Sixty-seven (97%) patients had Child-Pugh class A and 2 (3%) had Child-Pugh class B disease.

Fifty-one of 69 (73.9%) patients with NAFLD-related or cryptogenic HCC had positive anti-HBc, which was significantly higher than the positivity rate in noncirrhotic patients (138/433 [31.9%]) and cirrhotic patients (32/56 [57.1%]) in the liver biopsy cohort ($P < 0.001$) (Figure 4a). When the analysis was repeated in patients aged older than 30 years to avoid confounding by hepatitis B vaccination, positive anti-HBc was found in 51 of 68 (75.0%) patients with HCC, 136 of 412 (33.0%) patients with F0-3 fibrosis, and 32 of 55 (58.2%) patients with cirrhosis ($P < 0.001$) (Figure 4b).

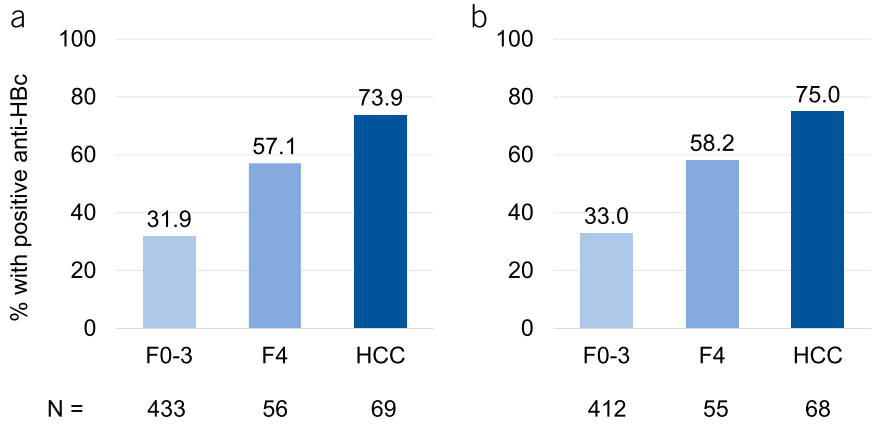

Figure 4. Proportion with positive anti-HBc among patients with NAFLD with F0-3, F4 and HCC. (**a**) Entire cohort, (**b**) subgroup of patients aged older than 30 years. $P < 0.001$ for both comparisons by χ^2 test. Anti-HBc, anti-hepatitis B core antibody; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease.

DISCUSSION

In this large cohort of patients with biopsy-proven NAFLD, we show that positive anti-HBc was associated with cirrhosis independent of age and metabolic factors. Patients with positive anti-HBc also had a higher incidence of liver-related events, and 3-quarters of patients with NAFLD-related or cryptogenic HCC had positive anti-HBc.

Most but not all studies in the past have found an association between positive anti-HBc and HCC in patients with chronic hepatitis C (18,20–22,32,33). Several reasons may explain the discrepant results. First, there is substantial variation in the epidemiology of chronic hepatitis B across regions (4). This would affect the prevalence of anti-HBc positivity in parallel. Second, positive anti-HBc may have different meanings in different settings. In Asia, mother-to-child transmission is the most common route to transmission, and most people acquire HBV during infancy or early childhood. They would then go through a relatively long period of infection before losing HBsAg, and there may be accumulating liver injury during this period (34). By contrast, parenteral transmission during adulthood is more common in Western countries. Such patients typically go through a short period of acute hepatitis B and become HBsAg negative and anti-HBc/anti-HBs positive. In these 2 situations, the duration of active infection and liver injury would be very different. Besides, one of the bigger negative studies was based on the data from the Hepatitis C Antiviral Long-term Treatment against Cirrhosis Trial that included patients with advanced fibrosis or cirrhosis who had failed interferon-based treatment before (22). The advanced liver disease would dominate the clinical outcome, negating the impact of previous HBV infection.

In our study, the proportion of patients with positive anti-HBc was the lowest in noncirrhotic NAFLD, intermediate in cirrhotic NAFLD, and highest in NAFLD-related or cryptogenic HCC (Figure 4). This is in line with the previous studies showing positive HBV markers in patients with cryptogenic HCC (18,19). In previous studies, patients with cryptogenic cirrhosis were more likely to have diabetes and obesity than those with cirrhosis secondary to chronic viral hepatitis, suggesting that burnt-out NASH is an important cause of cryptogenic cirrhosis (35,36). Although it is tempting to consider NASH-related cirrhosis and cryptogenic cirrhosis as the same disease, one should remember that cryptogenic cirrhosis may be caused by other etiologies. In a study using the United Network for Organ Sharing data, patients with NASH were more likely to have type 2 diabetes and obesity and be white than those with cryptogenic cirrhosis (37). In HBV endemic areas, previous HBV infection and NASH would both contribute to the development of cryptogenic cirrhosis and HCC, and our study suggests that the former may alter the natural history of NAFLD.

Although all of our patients had negative serum HBV DNA, a few reasons may explain the association between anti-HBc positivity and HCC. First, in all chronic liver diseases, the clinical outcome is determined not only by the current but also past disease activity. Patients with active hepatitis in the past could still accumulate more liver injury with increased risk of HCC. Second, even with negative serum HBV DNA, patients with positive anti-HBc often have detectable HBV DNA and/or covalently closed circular DNA in the liver (19). Furthermore, HBV DNA integration into the host genome is a common event in patients with chronic hepatitis B, and this would almost certainly persist after HBsAg seroclearance. HBV DNA integration is one of the recognized mechanisms for HCC development (38).

It is also noteworthy that patients with positive anti-HBc had lower body weight and BMI, higher high-density lipoprotein-cholesterol, and a trend toward lower triglycerides (Table 1). This suggests that the metabolic factors were the main drivers of liver disease in patients with negative anti-HBc, whereas both metabolic factors and previous HBV infection accounted for liver injury in patients with positive anti-HBc. The latter group thus had more severe liver fibrosis and worse outcomes despite being less obese. Nonetheless, patients with positive anti-HBc were also more likely to have diabetes mellitus. In previous studies, diabetes was strongly associated with cirrhosis and HCC in patients with NAFLD (39,40). On the other hand, patients with positive anti-HBc also tended to have lower triglyceride level. Although the mechanism is poorly understood, chronic HBV infection is consistently associated with low triglycerides (5,41). The clinical impact of the differential metabolic changes in patients with positive anti-HBc deserves further studies.

Compared with previous studies, our study is unique in providing comprehensive histological and clinical outcome data. In particular, positive anti-HBc was associated with cirrhosis even after adjustment for age and metabolic factors. Because hepatitis B vaccination can prevent not only chronic hepatitis B but also occult HBV infection, age would be an important confounder in studies on this topic (42). Therefore, we performed analysis by both multivariable logistic regression and stratification and confirmed the association between anti-HBc and cirrhosis in the older population. Furthermore, during the longitudinal follow-up, patients with positive anti-HBc had higher risk of not only HCC but also cirrhotic complications (Figure 3).

It is intriguing to note that anti-HBs was not associated with histological severity of patients with NAFLD. Among patients with positive anti-HBc, the presence of positive anti-HBs was associated with less severe fibrosis and a lower risk of cirrhosis. Because few patients had positive anti-HBc but negative anti-HBs, we recommend caution in interpreting this subgroup analysis. There are however 3 potential explanations of the unexpected results. First, in patients with spontaneous HBsAg seroclearance, those with positive anti-HBs are less likely to have HBsAg reversion (43). Anti-HBs is thus a marker of immune control and is associated with a lower risk of disease progression. Second, positive anti-HBs is more often seen in patients with acute hepatitis B than those with chronic hepatitis B and HBsAg seroclearance. The former would have a much shorter disease duration and less liver injury. Third, anti-HBs can reflect both previous HBV infection and vaccination. Vaccinated patients are protected against infection and liver injury even if they are exposed to HBV later in life.

Our study has the strengths of a large sample size, multicenter design, and a long duration of follow-up. It also has a few limitations. First, although our study included Asian patients of different racial background, our findings should be confirmed outside Asia. Second, the use of stored serum samples might explain why none of the patients tested positive for HBV DNA. Nonetheless, other than the rare condition of HBV variants with decreased HBsAg production or altered HBsAg epitopes, most patients with anti-HBc have undetectable or low serum HBV DNA levels (15). Our study also focused on host antibodies rather than viral nucleic acids. Finally, although we excluded current viral hepatitis and excessive alcohol consumption, some patients with cryptogenic cirrhosis might have liver diseases other than NASH.

In conclusion, positive anti-HBc is associated with cirrhosis and possibly HCC and cirrhotic complications in Chinese patients with NAFLD. Because NAFLD-related HCC may develop in noncirrhotic patients (44,45), future studies should define the role of anti-HBc in selecting noncirrhotic patients with NAFLD for HCC surveillance.

CONFLICTS OF INTEREST

Guarantor of the article: Vincent Wai-Sun Wong, MD.

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Potential competing interests: W.K.C., G.L.-H.W., and S.M. have served as speakers for Echosens. H.L.-Y.C. has served as an advisory committee member for AbbVie, Aligos, Aptorum, Arbutus, ContraVir, Intellia, Janssen, Gilead, Medimmune, Roche, Vaccitech, VenetoRx, Vir Biotechnology, and GRAIL; and as a speaker for AbbVie, Gilead, and Roche. V.W.-S.W. has served as an advisor/consultant for 3V-BIO, AbbVie, Allergan, Echosens, Gilead Sciences, Janssen, Novartis, Novo Nordisk, Perspectum Diagnostics, Pfizer and Terns; a speaker for Bristol-Myers Squibb, Echosens, Gilead Sciences, and Merck; and received an unrestricted grant from Gilead Sciences for fatty liver research. The other authors report no conflict of interests.

Study Highlights

WHAT IS KNOWN

- ✓ NAFLD affects at least 25% of the global adult population. It is one of the leading causes of cirrhosis and HCC in Western countries.
- ✓ 30%–50% of NAFLD-related HCCs occur in patients without cirrhosis.
- ✓ Previous and occult HBV infection, as evidenced by positive anti-HBc, may increase the risk of HCC in patients with chronic hepatitis C.

WHAT IS NEW HERE

- ✓ NAFLD patients with positive anti-HBc were more likely to have significant fibrosis and cirrhosis than those with negative anti-HBc.
- ✓ The association between anti-HBc and cirrhosis remained significant after adjusting for age, sex, and metabolic conditions.
- ✓ Positive anti-HBc was found in 32% of NAFLD patients with F0-3 fibrosis, 57% of patients with NAFLD-related cirrhosis, and 74% of those with NAFLD-related or cryptogenic HCC.
- ✓ Anti-HBs did not correlate with histological severity of NAFLD.

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