Presence of Liver Inflammation in Asian Patients With Chronic Hepatitis B With Normal ALT and Detectable HBV DNA in Absence of Liver Fibrosis

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Liver biopsies are recommended to exclude significant liver inflammation in patients with chronic hepatitis B (CHB) with elevated HBV DNA but without other indications for antiviral treatment. We aimed to investigate the proportions and determinants of significant inflammation in Asian patients with CHB with detectable HBV DNA. We conducted a cross-sectional study that retrospectively included 581 patients with CHB with detectable HBV DNA who had undergone liver biopsy. Liver inflammation and fibrosis were staged by Scheuer's classification. Significant inflammation and significant fibrosis were defined as G ≥ 2 and S ≥ 2, respectively. There were 179 (30.8%) patients with alanine aminotransferase (ALT) < 1 × upper limit of normal (ULN), 205 (35.3%) patients with ALT 1-2 × ULN, and 197 (33.9%) patients with ALT > 2 × ULN. A total of 397 (68.3%) patients had significant inflammation, and 340 (58.5%) patients had significant fibrosis. Significant inflammation was found in 85% of patients with significant fibrosis and in 44.8% of patients without significant fibrosis. Furthermore, 28.7% of patients with CHB with detectable HBV DNA and normal ALT in the absence of significant fibrosis had significant inflammation. Moderate HBV DNA (5-7 $\log_{10}$ IU/mL) was a risk factor for significant inflammation (odds ratio [OR] 6.929, 95% confidence interval [CI] 2.830-16.966, $P < 0.001$) in patients with CHB with detectable HBV DNA, especially for patients with detectable HBV DNA and normal ALT in the absence of significant fibrosis (adjusted OR 13.161, 95% CI 1.026-168.889, $P = 0.048$). *Conclusion:* A high proportion of CHB patients with detectable HBV DNA and normal ALT in the absence of significant fibrosis have significant liver inflammation. Liver biopsies are recommended to evaluate liver inflammation in such patients, especially for those with moderate HBV DNA. (*Hepatology Communications* 2022;6:855-866).

Chronic hepatitis B (CHB) virus infection is a worldwide public health burden that causes major adverse outcomes, including liver cirrhosis, liver failure, and hepatocellular carcinoma (HCC), all of which can result in considerable liver-related mortality.[1,2] Previous studies have demonstrated that elevated serum hepatitis B virus (HBV) DNA levels are closely related to the occurrence of HCC.[3,4] Antiviral therapy may reduce the risk of adverse events and improve the long-term prognosis of patients with CHB by inhibiting HBV replication.[3-8] Currently, initiation of antiviral treatment depends on the liver

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; CHB, chronic hepatitis B; CI, confidence interval; FIB-4, fibrosis index based on the four factors; GGT, gamma-glutamyl transpeptidase; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IQR, interquartile range; OR, odds ratio; PLT, platelet; ULN, upper limits of normal.

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inflammation degree and fibrosis stage, especially for patients with CHB with detectable HBV DNA but without other indications for antiviral treatment.[9,10]

Over the past two decades, numerous noninvasive tests have been created to assess the liver fibrosis stages with good diagnostic accuracy,[11,12] whereas there is still a lack of noninvasive tests to assess the liver inflammation grades. According to the American Association for the Study of Liver Diseases (AASLD) 2018 Hepatitis B Guidance, in patients with CHB with elevated HBV-DNA levels and without significant fibrosis (noninvasively evaluated), liver biopsy should be considered to exclude significant liver inflammation.[9] Liver biopsy has always been regarded as the golden standard for assessing the liver inflammation.[13] Nevertheless, the invasive nature, diverse complications, and high cost restrict the application of liver biopsy in clinical practice.[12,14,15] Serum alanine aminotransferase (ALT) levels remain the most commonly used laboratory parameter to reflect liver inflammatory activity.[16] However, numerous studies have demonstrated that the severity of liver inflammation is not always consistent with the levels of ALT in patients with CHB.[17,18] Patients with detectable HBV DNA and normal ALT levels still have a probability of moderate-to-severe liver inflammation.[19-21] A multicenter study that included 253 patients with CHB with normal ALT showed that 36.4% of patients had moderate-to-severe inflammation.[22] Zhou et al. also found that 35.7% (35 of 98) of patients with hepatitis B envelope antigen (HBeAg[+]) patients and 36.8% (35 of 95) of patients with HBeAg(–) CHB with normal ALT levels had at least moderate inflammation.[18] However, a recent study has indicated that the probability of significant inflammation can be reduced significantly if the presence of liver fibrosis is ruled out.[23] An ALT level < 2× upper limits of normal (ULN) is reported to be associated with less than 5% probability of significant liver inflammation in patients with CHB without significant fibrosis.[23] However, that study did not evaluate liver inflammation in patients with an HBV-DNA level of less than 2,000 IU/mL, and nearly half of the included patients were of Caucasian origin.[23] There is an intermediate-to-high prevalence of HBV infection in the Asia-Pacific regions, representing three-quarters of patients with chronic HBV worldwide.[24,25] Thus, we aimed to evaluate the proportions and determinants of significant liver inflammation in Asian patients with CHB with detectable HBV DNA.

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Patients and Methods

STUDY PATIENTS

We conducted a retrospective cross-sectional study that included patients with CHB who had undergone liver biopsy in two medical centers in Jiangsu, China (Nanjing Drum Tower Hospital and Huai'an No. 4 People's Hospital), between November 2004 and October 2020. CHB was diagnosed as the persistent presence of serum hepatitis B surface antigen (HBsAg) for more than 6 months.

The inclusion criterion was CHB with detectable HBV DNA at the time of liver biopsy. The exclusion criteria were as follows: (1) coexistence of HCC and other malignancies; (2) concomitant nonalcoholic fatty liver disease; (3) antiviral therapy before liver biopsy; (4) a history of significant alcohol consumption (defined by >30 g per day in men and >20 g per day in women); and (5) undetectable HBV DNA. Written informed consent was obtained from all of the enrolled patients who underwent liver biopsy. The study was approved by the Ethics Committee of Nanjing Drum Tower Hospital and Huai'an No. 4 People's Hospital. The study was registered at ClinicalTrials.gov (NCT03097952).

DATA COLLECTION AND DEFINITION

Clinical and laboratory parameters of the included patients, including ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), platelet counts (PLT), and the levels of HBsAg, HBeAg, and HBV DNA, were acquired from electronic medical records within 2 weeks before liver biopsies. A total of 504 of 581 (86.7%) patients had laboratory data within 1 week before liver biopsies. The ULN of the ALT level was 35 U/L for men and 25 U/L for women according to the AASLD guidance.[9]

Fibrosis-4 Index (FIB-4) and aspartate aminotransferase to platelet ratio index (APRI) were calculated based on clinical and laboratory parameters measured within 2 weeks before liver biopsies. The computational formulas of FIB-4 and APRI were as follows[26,27]:

$$\text{FIB-4} = \text{age[years]} \times \text{AST[U/L]} / (\text{PLT count}[\times 10^9/\text{L}] \times \text{ALT}^{1/2}[\text{U/L}])$$

$$\text{APRI} = ((\text{AST}\ [\text{U/L}]\ /\text{ULN of AST}) / \text{PLT count}\ [\times 10^9/\text{L}]) \times 100$$

LIVER BIOPSY

Liver biopsies were performed under ultrasonic guidance. All of the liver biopsy specimens were fixed in formalin, embedded in paraffin, and cut into 2-3-μm-thick sections. The sections that were stained with hematoxylin and eosin included no less than six available portal tracts. Two experienced pathologists who were unaware of all of the clinical information evaluated the liver sections. Liver inflammation and fibrosis were staged by Scheuer's classification.[28] G0-G1, G2, and G3-4 were defined as no or mild, moderate, and severe liver inflammation, respectively. Liver fibrosis was categorized into no significant fibrosis (S0-S1), moderate fibrosis (S2-S3), and cirrhosis (S4). Significant inflammation and significant fibrosis were defined as ≥G2 or ≥S2, respectively.

STATISTICAL ANALYSIS

Data were analyzed using SPSS software, version 22.0 (IBM, Armonk, NY). Continuous variables were expressed by median and interquartile range (IQR), and categorical variables were presented as the counts and percentages. For comparison of continuous variables between two groups, *t* test for independent samples (normal distribution), and Mann-Whitney U test (nonnormal distribution) were used. Categorical variables were compared using chi square or Fisher exact test. Univariate and multivariate logistic regression analysis were performed to verify determinants of significant inflammation. All of the significance tests were two-sided, and $P < 0.05$ was recognized as being statistically significant.

Results

PATIENT CHARACTERISTICS

A total of 1,188 patients with CHB who had undergone liver biopsy were enrolled in the study. Of that number, 537 patients with CHB were excluded according to the exclusion criteria. Additionally, 70

patients with detectable HBV DNA also had to be excluded due to insufficient data (Fig. 1). Finally, 581 patients with CHB were eligible for the analysis.

The demographic and laboratory characteristics of patients with CHB with detectable HBV DNA are presented in Table 1. Approximately 68.2% of the patients were of male sex, and the median age of all of the patients was 38.0 (IQR 30.0-46.0) years. A total of 316 (54.4%) patients were HBeAg-negative. ALT levels were < 1 × ULN in 179 (30.8%) patients, 1-2 × ULN in 205 (35.3%) patients, and >2 × ULN in 197 (33.9%) patients. The HBV DNA levels were below 3 $\log_{10}$ IU/mL in 63 (10.8%) patients, 3-5 $\log_{10}$ IU/mL in 190 (32.7%) patients, 5-7 $\log_{10}$ IU/mL in 165 (28.4%) patients, and ≥7 $\log_{10}$ IU/mL in 163 (28.1%) patients. A total of 397 (68.3%) patients had significant inflammation (≥G2), and 340 (58.5%) patients had significant fibrosis (≥S2).

The patients were divided into two groups according to the fibrosis stage. Patients with significant fibrosis were older than those without significant fibrosis (39 years vs. 36 years; $P = 0.045$). The proportion of patients with ALT < 1 × ULN was higher in patients without significant fibrosis than in those with significant fibrosis (41.9% vs. 22.9%; $P < 0.001$) (Table 1). The proportion of elevated GGT levels was higher in patients with significant fibrosis than in patients without significant fibrosis (42.9% vs. 18.7%; $P < 0.001$). The proportions of patients with HBV DNA < 3 $\log_{10}$ IU/mL, 3-5 $\log_{10}$ IU/mL, 5-7 $\log_{10}$ IU/mL, and ≥7 $\log_{10}$ IU/mL were 8.8%, 30.6%, 39.4%, and 21.2%, respectively, in patients with significant fibrosis, and 13.7%, 35.7%, 12.9%, and 37.8%, respectively, in patients without significant fibrosis. The APRI and FIB-4 values were higher in patients with significant fibrosis. The proportions of patients with moderate inflammation and severe inflammation were also higher among patients with significant fibrosis than among those without significant fibrosis (Table 1).

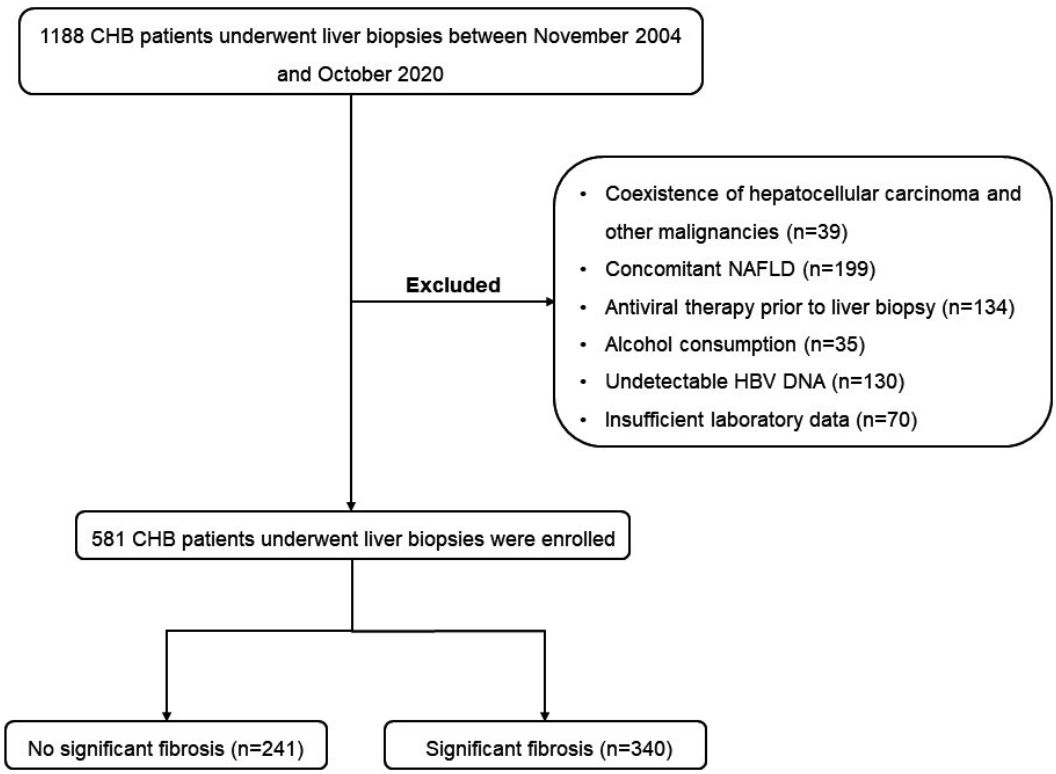

FIG. 1. Flow chart describing the recruitment of patients.

TABLE 1. DEMOGRAPHIC AND EPIDEMIOLOGIC CHARACTERISTICS OF PATIENTS WITH CHB WITH AND WITHOUT SIGNIFICANT FIBROSIS

Characteristics (n [%] or median [IQR])	Total (n = 581)	No Significant Fibrosis (n = 241)	Significant Fibrosis (n = 340)	*P* Value
Age (years)	38.0 (30.0, 46.0)	36.0 (29.0, 45.0)	39.0 (30.0, 47.0)	0.045
Age range				
≥40	260 (44.8)	96 (39.8)	164 (48.2)	0.045
Gender				
Male	396 (68.2)	158 (65.6)	238 (70.0)	0.258
ALT				<0.001
<ULN	179 (30.8)	101 (41.9)	78 (22.9)	
1-2 × ULN	205 (35.3)	87 (36.1)	118 (34.7)	
>2 × ULN	197 (33.9)	53 (22.0)	144 (42.4)	
AST (U/L)	34.0 (24.0, 57.8)	28.0 (21.0, 40.0)	41.0 (27.0, 69.0)	<0.001
GGT (U/L)	29.2 (17.0, 66.0)	22.0 (15.0, 38.0)	39.0 (21.0, 84.0)	<0.001
GGT (U/L)				<0.001
<50	390 (67.1)	196 (81.3)	194 (57.1)	
≥50	191 (32.9)	45 (18.7)	146 (42.9)	
PLT ($\times 10^9$/L)	167.0 (131.0, 207.0)	188.0 (155.5, 225.5)	153.0 (112.5, 191.8)	<0.001
HBsAg ($\log_{10}$ IU/mL)	3.4 (2.5, 4.0)	3.6 (2.6, 4.3)	3.2 (2.4, 3.8)	0.001
HBeAg status				0.855
Negative	316 (54.4)	130 (53.9)	186 (54.7)	
Positive	265 (45.6)	111 (46.1)	154 (45.3)	
HBV DNA ($\log_{10}$ IU/mL)				<0.001
<3	63 (10.8)	33 (13.7)	30 (8.8)	
3-5	190 (32.7)	86 (35.7)	104 (30.6)	
5-7	165 (28.4)	31 (12.9)	134 (39.4)	
≥7	163 (28.1)	91 (37.8)	72 (21.2)	
APRI	0.52 (0.32, 1.06)	0.37 (0.27, 0.56)	0.74 (0.41, 1.55)	<0.001
FIB-4	1.25 (0.77, 1.94)	0.92 (0.66, 1.39)	1.51 (0.93, 2.75)	<0.001
Inflammation activity				<0.001
No or mild inflammation (G0-G1)	184 (31.7)	133 (55.2)	51 (15.0)	
Moderate inflammation (G2)	246 (42.3)	99 (41.1)	147 (43.2)	
Severe inflammation (G3-G4)	151 (26.0)	9 (3.7)	142 (41.8)	

RELATIONSHIP BETWEEN ALT LEVELS AND INFLAMMATORY ACTIVITY IN PATIENTS WITHOUT SIGNIFICANT FIBROSIS

The distribution of ALT levels between patients with and without significant inflammation is given in Table 2. Regardless of HBeAg status and age categories, the rates of significant inflammation were much higher among patients with ALT levels > 2 × ULN. Surprisingly, about 30% of patients with CHB with detectable HBV DNA and normal ALT had significant inflammation in different HBeAg status and age categories.

RELATIONSHIP BETWEEN ALT LEVELS AND INFLAMMATORY ACTIVITY WITH FIBROSIS PROGRESSION

Of 197 patients with ALT levels > 2 × ULN, 40 (75.5%) patients without significant fibrosis, 85 (95.5%) with moderate fibrosis, and 55 (100%) with liver cirrhosis had significant inflammation. Thirty-nine (44.8%) patients without significant fibrosis, 61 (73.5%) with moderate fibrosis, and 35 (100%) with liver cirrhosis had significant inflammation among patients with ALT 1-2 × ULN. Among patients with normal ALT levels, moderate-to-severe liver

TABLE 2. RELATIONSHIP BETWEEN ALT LEVELS AND INFLAMMATORY ACTIVITY IN PATIENTS WITH CHB WITHOUT SIGNIFICANT LIVER FIBROSIS UNDER DIVERSE SUBSECTIONS

	No Significant Inflammation (n = 133)	Significant Inflammation (n = 108)	*P* Value
HBeAg status			
HBeAg-negative (n = 130)			<0.001
ALT < ULN (n = 65)	47 (72.3)	18 (27.7)	
ALT 1-2 × ULN (n = 42)	28 (66.7)	14 (33.3)	
ALT > 2 × ULN (n = 23)	6 (26.1)	17 (73.9)	
HBeAg-positive (n = 111)			0.001
ALT < ULN (n = 36)	25 (69.4)	11 (30.6)	
ALT 1-2 × ULN (n = 45)	20 (44.4)	25 (55.6)	
ALT > 2 × ULN (n = 30)	7 (23.3)	23 (76.7)	
Age			
Age < 40 (n = 145)			<0.001
ALT < ULN (n = 51)	36 (70.6)	15 (29.4)	
ALT 1-2 × ULN (n = 57)	28 (49.1)	29 (50.9)	
ALT > 2 × ULN (n = 37)	8 (21.6)	29 (78.4)	
Age ≥ 40 (n = 96)			0.012
ALT < ULN (n = 50)	36 (72.0)	14 (28.0)	
ALT 1-2 × ULN (n = 30)	20 (66.7)	10 (33.3)	
ALT > 2 × ULN (n = 16)	5 (31.2)	11 (68.8)	

inflammation was found in 29 (28.7%) patients without significant fibrosis, 34 (59.7%) with moderate fibrosis, and 19 (90.5%) with cirrhosis. Regardless of fibrosis stage, the rates of significant inflammation were much higher among patients with ALT levels > 2 × ULN (Fig. 2).

Similar trends were found among patients with different HBeAg status (Supporting Fig. S1), HBV DNA levels (Fig. 3), and age categories (Supporting Fig. S2). It should be noted that the proportion of significant inflammation was highest in patients with moderate HBV DNA levels (5-7 $\log_{10}$ IU/mL), with a decreasing trend in patients with higher and lower levels of HBV DNA (Fig. 3).

RISK FACTORS FOR SIGNIFICANT LIVER INFLAMMATION

The risk factors for significant inflammation of patients with CHB with detectable HBV DNA were analyzed (Table 3). The multivariate logistic regression analysis revealed that HBV DNA levels (3-5 $\log_{10}$ IU/mL [OR 2.058, 95% CI 1.059-4.001, P = 0.033],

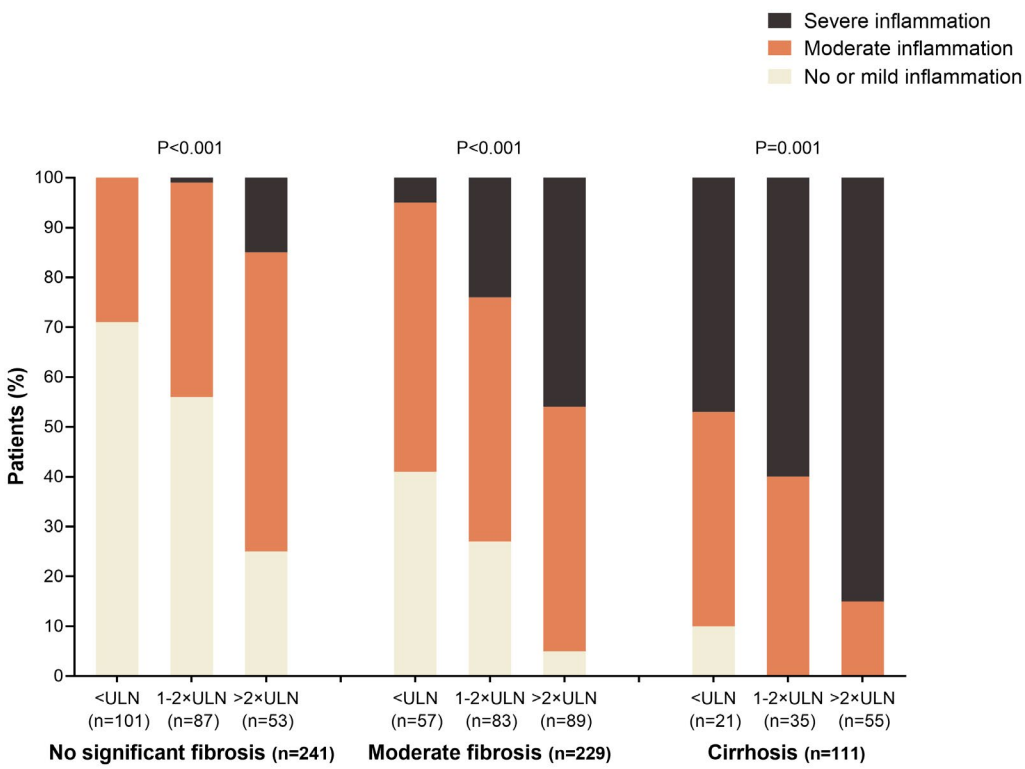

FIG. 2. Relationship between ALT levels and inflammatory activity with fibrosis progression among patients with CHB with detectable HBV DNA levels.

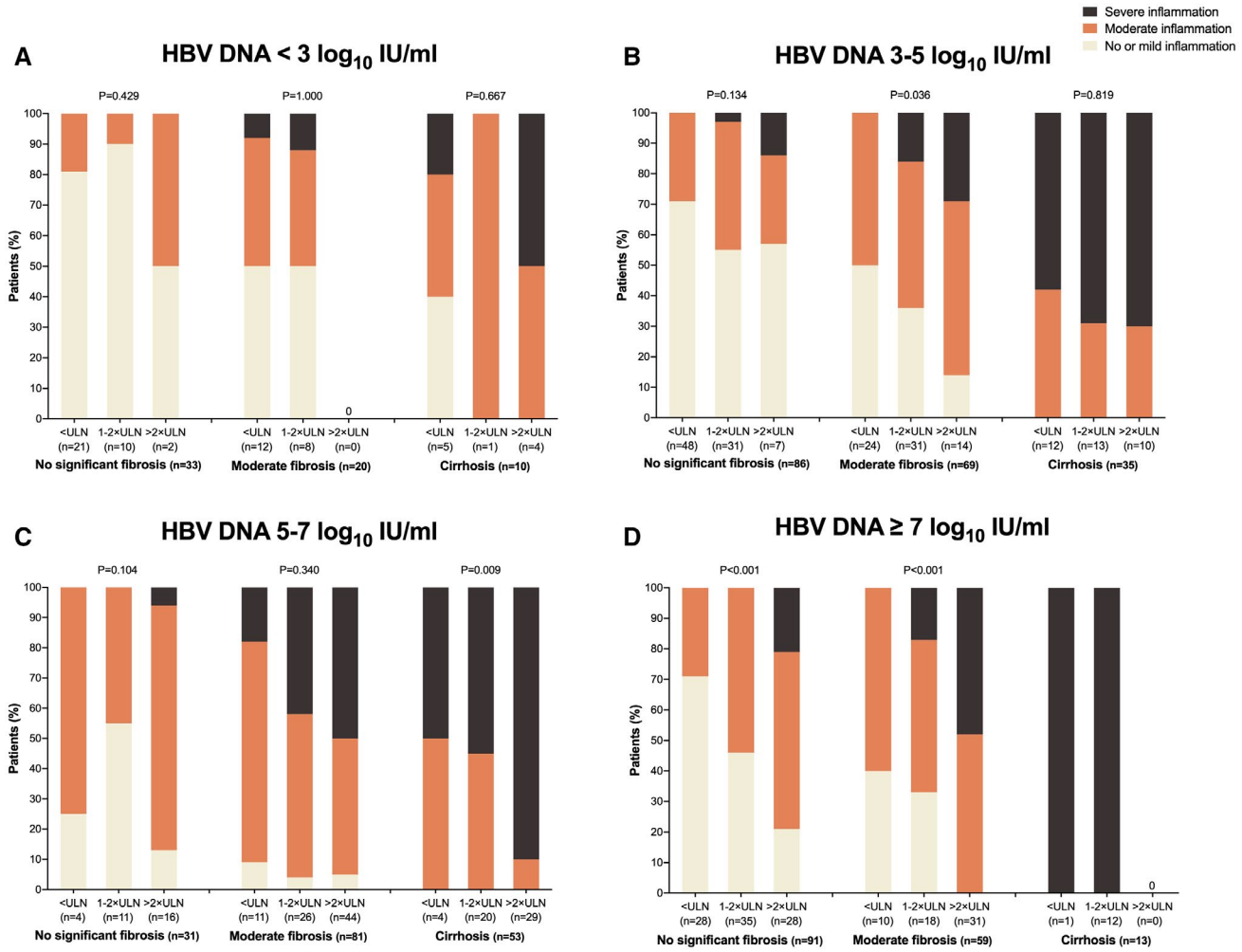

FIG. 3. Relationship between ALT levels and inflammatory activity with fibrosis progression among patients with CHB with detectable HBV DNA levels with different HBV DNA levels. (A) Patients with HBV DNA < 3 $\log_{10}$ IU/mL. (B) Patients with HBV DNA 3-5 $\log_{10}$ IU/mL. (C) Patients with HBV DNA 5-7 $\log_{10}$ IU/mL. (D) Patients with HBV DNA ≥ 7 $\log_{10}$ IU/mL.

5-7 $\log_{10}$ IU/mL [OR 6.929, 95% CI 2.830-16.966, P < 0.001], ≥7 $\log_{10}$ IU/mL [OR 2.947, 95% CI 1.278-6.795, P = 0.011]), ALT > 2 × ULN (OR 4.562, 95% CI 2.209-9.423, P < 0.001), GGT ≥ 50 U/L (OR 2.829, 95% Cl 1.499-5.339, P = 0.001), and significant fibrosis (OR 4.955, 95% CI 3.137-7.824, P < 0.001) were independent risk factors for significant inflammation in patients with CHB with detectable HBV DNA.

Further logistic regression analysis was performed in different subgroups. HBV DNA levels (5-7 $\log_{10}$ IU/mL [OR 5.229, 95% CI 1.445-18.930; P = 0.012], ≥7 $\log_{10}$ IU/mL [OR 3.350, 95% CI 1.096-10.236; P = 0.034]) and ALT > 2 × ULN (OR 4.076, 95% CI 1.694-9.807; P = 0.002) were the independent risk factors for significant inflammation in patients with CHB with detectable HBV DNA in the absence of significant fibrosis (Supporting Table S1) in the multivariate logistic regression analysis. Significant fibrosis (OR 4.088, 95% CI 2.304-8.214; P < 0.001) and moderate serum HBV DNA (5-7 $\log_{10}$ IU/mL) (OR 12.602, 95% CI 2.257-70.370; P = 0.004) were the independent risk factors for significant inflammation in patients with CHB with detectable HBV DNA and normal ALT levels in the multivariate logistic regression analysis (Supporting Table S2).

Moderate serum HBV DNA levels (5-7 $\log_{10}$ IU/mL) (OR 12.750, 95% CI 1.034-157.144; P = 0.047) was the single risk factor for significant inflammation in patients with CHB with detectable HBV DNA

TABLE 3. UNIVARIATE AND MULTIVARIATE ANALYSIS OF RISK FACTORS FOR SIGNIFICANT LIVER INFLAMMATION IN PATIENTS WITH CHB WITH DETECTABLE HBV DNA

	Univariate		Multivariate	
Variable	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age (years)				
<40	Reference			
≥40	0.861 (0.606, 1.223)	0.403		
Gender				
Female	Reference			
Male	1.354 (0.936, 1.959)	0.108		
HBsAg ($\log_{10}$ IU/mL)	0.888 (0.729, 1.080)	0.234		
HBeAg status				
Negative	Reference			
Positive	1.737 (1.213, 2.488)	0.003	1.234 (0.701, 2.169)	0.466
HBV DNA ($\log_{10}$ IU/mL)				
<3	Reference			
3-5	2.234 (1.246, 4.008)	0.007	2.058 (1.059, 4.001)	0.033
5-7	19.000 (8.876, 40.673)	<0.001	6.929 (2.830, 16.966)	<0.001
≥7	3.469 (1.893, 6.357)	<0.001	2.947 (1.278, 6.795)	0.011
ALT				
<ULN	Reference			
1-2 × ULN	2.281 (1.511, 3.445)	<0.001	1.453 (0.897, 2.354)	0.129
>2 × ULN	12.525 (7.029, 22.319)	<0.001	4.562 (2.209, 9.423)	<0.001
AST (U/L)	1.010 (1.005, 1.016)	<0.001	0.999 (0.997, 1.001)	0.371
PLT ($\times 10^9$/L)	0.993 (0.990, 0.996)	<0.001	0.998 (0.994, 1.002)	0.262
GGT (U/L)				
<50	Reference			
≥50	6.639 (3.968, 11.107)	<0.001	2.829 (1.499, 5.339)	0.001
Fibrosis status				
No significant fibrosis	Reference			
Significant fibrosis	6.978 (4.719, 10.320)	<0.001	4.955 (3.137, 7.824)	<0.001

and normal ALT in the absence of significant fibrosis (Supporting Table S3). After adjusting for age, gender, and HBeAg status, moderate serum HBV DNA level (5-7 $\log_{10}$ IU/mL) (adjusted OR 13.161, 95% CI 1.026-168.889, P = 0.048) remained the independent risk factor for significant inflammation in patients with CHB with detectable HBV DNA and normal ALT in the absence of significant fibrosis (Supporting Table S3).

Discussion

Significant liver inflammation is an important indication for initiating antiviral therapy in patients with CHB because it has been considered an important risk factor for the development of liver cirrhosis and HCC.[9] In spite of normal ALT levels, antiviral therapy may still be recommended for patients with CHB with detectable HBV DNA and moderate-to-severe inflammation.[9] Thus, evaluating liver inflammatory activity is important for deciding antiviral treatment for patients with CHB with detectable HBV DNA.[9,10]

In the present study, we analyzed the relationship between serum ALT levels and liver inflammatory activity among patients with CHB with detectable HBV DNA. With the increasing fibrosis stages, the proportion of significant inflammatory activity rose remarkably in patients with ALT > 2 × ULN. Regardless of HBeAg status, HBV DNA levels, or age categories, the proportion of patients with CHB with significant inflammation increased with the stages of liver fibrosis. In patients with normal ALT levels, many patients with

moderate fibrosis or cirrhosis had moderate-to-severe liver inflammation. The results are consistent with the previous studies. For example, according to Sonneveld et al., significant liver inflammation activity was found in 34% of patients with CHB with significant liver fibrosis and in only 9.5% of patients without significant liver fibrosis.[23] Another study, which enrolled 227 patients with viral hepatitis with normal or mildly elevated ALT, revealed that liver fibrosis stage was the most significant risk factor for significant inflammation.[29] Currently, noninvasive tests, such as transient elastography, have good diagnostic accuracy for excluding significant fibrosis in patients with CHB.[11,30] Thus, the probability of significant inflammation is reduced if the presence of liver fibrosis can be ruled out.

Surprisingly, our results revealed that 28.7% patients with detectable HBV DNA and normal ALT levels in the absence of significant fibrosis still had significant inflammation. We analyzed the association between ALT levels and significant inflammatory activity in patients with nonsignificant fibrosis, moderate fibrosis, and cirrhosis across subgroups divided by HBeAg status, age, and HBV DNA levels. The results indicated that at least 1 in 5 patients with CHB with detectable HBV DNA and normal ALT levels in the absence of significant fibrosis had significant inflammation under diverse subgroups.

However, a recent retrospective study by Sonneveld et al. has suggested that the probability of significant inflammation was very low (<5%) among patients with CHB with elevated HBV DNA (>2,000 IU/mL) and ALT < 2 × ULN in the absence of fibrosis.[23] Another study, which enrolled 504 patients with CHB with normal ALT, showed that the proportions of patients with significant liver fibrosis and nonsignificant liver fibrosis were 25.4% and 74.6%, respectively. Of note, the nonsignificant liver fibrosis group had a lower proportion of significant liver inflammation than the significant liver fibrosis group (5.8% vs. 32.8%).[31] Moreover, Wei et al. found that 39.7% patients with significant liver fibrosis had significant liver inflammation, whereas the percentage was only 7.1% in patients without significant liver fibrosis in a cohort of 186 patients with CHB.[32] However, the levels of HBV DNA and ALT were not limited in these studies.[31,32] The discrepant findings may be interpreted in four aspects. First, not all of the patients were antiviral treatment-naïve in the study by Sonneveld et al., and antiviral treatment may have affected liver inflammation.[33,34] However, we excluded patients who had received antiviral therapy before liver biopsy. Second, the HBV DNA levels differed among different studies. We included patients with detectable HBV DNA levels instead of HBV DNA > 2,000 IU/mL because low-level viremia (<2,000 IU/mL) is associated with a relatively high risk of HCC.[31,35] Third, in the current study, only Asian patients with CHB were included. Asian ethnicity has been shown to be a risk factor for significant inflammatory activity compared with Caucasian patients.[23] In addition, the discrepancy of liver histology scoring systems may also be an important reason for discrepant results among the studies. A cutoff of G2 by Scheuer classification instead of A2 by METAVIR classification was used to define the significant inflammation in our study. Thus, a more lenient definition of significant inflammation might have been used.

The identification of predictive factors of significant inflammation would be very useful to help health care practitioners in the assessment of patients who may have higher risk of significant inflammation. Age, Asian ethnicity, ALT > 2 × ULN, AST level, and the presence of significant fibrosis were reported to be risk factors for significant inflammation in patients with CHB with serum HBV DNA > 2,000 IU/mL.[23] We also found that ALT > 2 × ULN and the presence of significant fibrosis were independent risk factors for significant inflammation in Asian patients with CHB with detectable HBV DNA. Previous studies have shown that serum GGT levels are independently associated with significant liver inflammation in patients with CHB.[36-39] In the present study, elevated GGT levels were also identified as the risk factor for the presence of significant inflammation in patients with CHB with detectable HBV DNA. As previously reported, age is significantly associated with liver inflammation activity in patients with CHB.[36,40-42] However, other recent studies have found no significant association between age and liver inflammation activity in patients with CHB.[31,43-45] Our results show that age was not associated with significant inflammation in either the univariate analysis or in the multivariate analysis. Different races, HBeAg levels, HBsAg levels, HBV DNA loads, as well as the discrepancy of scoring systems of liver inflammation may partially explain the inconsistent results on the association between age and liver inflammation activity among different studies. Thus, the association

between age and liver inflammation activity in patients with CHB deserves further investigation.

One important finding is that serum HBV DNA level, especially for moderate serum HBV DNA level (5-7 $\log_{10}$ IU/mL), was the risk factor for significant inflammation in patients with CHB with detectable HBV DNA despite normal ALT and/or in the absence of significant fibrosis. A retrospective cohort study demonstrated that the highest HCC risk occurred in patients with CHB with ALT < 2 × ULN and with moderate serum HBV DNA levels of 6-7 $\log_{10}$ IU/mL.[46] Significant liver inflammation is an important risk factor for developing HCC in patients with CHB.[47,48] Thus, our finding revealing that patients with CHB with moderate serum HBV DNA levels had a high risk of significant inflammation may partially explain the highest HCC risk in patients with CHB with moderate serum HBV-DNA levels and without significant ALT elevation reported by Kim et al.[46] Of note, we found that about 20% patients with normal ALT, HBV DNA < 3 $\log_{10}$ IU/mL, and without significant fibrosis had significant liver inflammation. Further analysis revealed that most (81.0%, 17 of 21) of these patients were HBeAg-negative, which suggests that initiating antiviral treatment is necessary to reduce long-term risk of adverse events. A retrospective study including 550 patients with HBeAg-negative CHB with normal and minimally increased ALT showed that 36.7% of the patients presented liver necroinflammation (histological activity index ≥4), whereas the rate of patients with liver necroinflammation in those with HBV DNA < 3 $\log_{10}$ copy/mL was 29.3%.[49] Thus, significant liver inflammatory activity may exist despite relatively low HBV DNA levels.

There were several limitations in our study. First, our study is retrospective in nature, and the data were obtained from two medical centers, which might have caused selection bias. Second, our cross-sectional study lacks the long-term follow-up data on ALT levels. Third, we could not perform stratified analysis according to ethnicities and HBV genotypes. However, the most common genotypes in Asian patients infected with HBV are HBV genotypes B and C.[50] Fourth, normal ALT levels were based on a single value in the present study. Because the longitudinal data were not available, we could not define patients with CHB as having "persistently normal ALT levels." Thus, more studies with longitudinal follow-up data are needed to assess liver inflammation in patients with CHB with fluctuating ALT levels. Fifth, current CHB treatment guidelines are often not specific about what grade of inflammation is "significant" enough to warrant antiviral therapy in the absence of other treatment indications. The AASLD hepatitis B guidance suggests using a cutoff of METAVIR A2 grade for antiviral treatment indications.[9] However, Scheuer and METAVIR scoring systems are not directly interchangeable. Due to the discrepancy of scoring systems, the evaluation results of liver inflammatory activities might be different, and the percentage of patients with CHB with "significant" inflammation based on the criterion of a cutoff of G2 by Scheuer classification might be higher than that based on the cutoff of A2 by METAVIR system as a more lenient definition of "significant inflammation" was used in our study. However, the Scheuer classification is also widely used for the evaluation of liver histology, and the cutoff of G2 has widely been used as the definition of "significant inflammation" in patients with CHB.[21,43,51-56] The Chinese CHB guidelines also recommend the use of a cutoff of G2 by Scheuer classification for antiviral treatment indications.[57] Because this was a retrospective study and the patients were included from two medical centers during a relatively long period of time, we were not able to re-assess the biopsies in these patients by the METAVIR system. Thus, further studies are needed to compare the proportions of patients with CHB with significant inflammation between a cutoff of G2 by Scheuer scoring system and a cutoff of A2 inflammation according to the METAVIR system. Moreover, whether the cutoff of G2 by Scheuer system may be too lenient for antiviral treatment indications in patients with CHB deserves further investigation. Finally, moderate HBV DNA level was identified as an independent risk factor for significant inflammation in patients with CHB with detectable HBV DNA and normal ALT in the absence of significant fibrosis. However, the 95% CI was wide (95% CI 1.026-168.889) and the *P* value was just below 0.05, which may be due to the small sample size.

In conclusion, a substantial proportion of Asian patients with CHB with detectable serum HBV DNA levels may present significant liver inflammation despite normal ALT levels and the absence of

significant fibrosis. Liver biopsies are recommended to evaluate the liver inflammation in Asian patients with CHB with detectable HBV DNA and normal ALT in the absence of significant fibrosis, especially for those with moderate serum HBV DNA levels. However, more prospective studies with a larger sample size are needed to validate our findings.

DATA AVAILABILITY STATEMENT

The data that support the study findings are available upon reasonable request from the corresponding authors (R.H. and C.W.).

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