

Retrospective Study

Autoantibodies in Chinese patients with chronic hepatitis B: Prevalence and clinical associations

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patients with chronic hepatitis B (CHB).

METHODS: A total of 325 Chinese patients with CHB were enrolled in this retrospective, hospital-based study. Patients with chronic hepatitis C (CHC), autoimmune hepatitis (AIH), or primary biliary cirrhosis (PBC) were included, with healthy donors acting as controls. A panel of autoantibodies that serologically define AIH and PBC was tested by indirect immunofluorescence assay and line immunoassay. The AIH-related autoantibody profile included homogeneous anti-nuclear antibodies (ANA-H), smooth-muscle antibodies, anti-liver kidney microsome type 1, anti-liver cytosolic antigen type 1, and anti-soluble liver antigen/liver pancreas; the PBC-related antibodies were characterized by ANA-nuclear dots/membranous rim-like, anti-mitochondrial antibodies-M2 (AMA-M2), anti-BPO (recombinant antigen targeted by AMA-M2), anti-Sp100, anti-promyelocytic leukemia protein (anti-PML), and anti-gp210. The dichotomization of clustering was used to unequivocally designate the AIH or PBC profiles for each case. Anti-Ro52 antibodies were also tested.

RESULTS: The prevalence of any autoantibody in CHB amounted to 58.2%, which was similar to the 66.2% prevalence in CHC, significantly higher than the 6.7% in the healthy controls ($P < 0.001$), and lower than the 100% found in AIH and PBC ($P = 0.004$ and $P < 0.001$, respectively). There were more anti-PML and anti-gp210 antibodies among the CHB patients than the CHC patients (11.1% vs 0%, $P = 0.003$; 12.6% vs 0%, $P < 0.001$, respectively). The prevalence and titer of AMA, anti-BPO, anti-PML, and anti-gp210 were higher in PBC than in those with CHB. Among the CHB patients, the prevalence of ANA, especially ANA-H, was significantly lower in patients with compensated and decompensated cirrhosis compared with patients without cirrhosis. Thirty-eight cases of hepatocellular carcinoma (HCC) in CHB showed a significant difference

Abstract

AIM: To investigate the prevalence of autoantibodies and their associations with clinical features in Chinese

compared with non-HCC patients in the prevalence of anti-PML (0% *vs* 12.5%, $P = 0.013$). Dichotomization of the autoantibodies revealed that the PBC profile was more prevalent in patients with CHB than in those with CHC, and that it was strongly correlated with both compensated and decompensated cirrhosis. In contrast, the prevalence of the AIH profile was significantly higher in non-cirrhosis patients with CHB than in those with compensated cirrhosis (18.5% *vs* 8.2%, $P = 0.039$). Moreover, the AIH profile was also closely associated with hepatitis B e-antigen positivity.

CONCLUSION: ANA-H could be an indicator of early-stage CHB. Dichotomizing the autoantibody profiles revealed that the PBC profile is strongly associated with cirrhosis in CHB.

Key words: Autoantibodies; Chronic hepatitis B; Autoimmune hepatitis; Primary biliary cirrhosis; Cirrhosis; Hepatocellular carcinoma

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Core tip: We investigated the prevalence of autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC)-related autoantibodies and their associations with clinical features in Chinese patients with chronic hepatitis B. Interestingly, and unexpectedly, we demonstrated that anti-nuclear antibodies (ANA), especially ANA-H, were significantly negatively associated with cirrhosis. Another interesting finding was that the prevalence of anti-promyelocytic leukemia protein antibodies was significantly different between hepatocellular carcinoma (HCC) (0%) and non-HCC patients (12.5%). In terms of analytic methods, for the first time we used an unequivocal dichotomy to cluster the autoantibodies into AIH and PBC profiles to delineate the bias of autoantibody expression for each case. The data showed that the PBC profile was strongly associated with cirrhosis.

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INTRODUCTION

Autoimmunity and autoimmune diseases are frequently present in hepatitis B virus (HBV) and hepatitis C virus (HCV) infections^[1]. Although the prevalence and clinical significance of non-organ-specific autoantibodies (NOSAs), especially anti-nuclear antibodies (ANA), smooth-muscle antibodies (SMA), and anti-liver kidney

microsome type 1 antibodies (anti-LKM-1), have been well documented in HCV infections, this area is still a subject of debate^[2]. For example, the relationship between autoantibodies and some clinical features of viral hepatitis is unclear. Some reports demonstrate that the presence of these autoantibodies is possibly associated with more severe liver damage and cirrhosis, and might be negative prognostic factors for treatment response^[3-6]. However, there are contradictory opinions supported by other studies that NOSAs have no significant impact on disease course, cirrhosis, or therapy outcome^[7-9]. Irrespective of whether the virus-induced production of autoantibodies is an epiphenomenon during the progression of viral hepatitis or a contributor to hepatocellular damage, the putative mechanisms for producing autoantibodies in viral hepatitis are considered to be mainly attributable to molecular mimicry^[10] or polyclonal B cell activation^[11].

Although there are many studies regarding HCV infections complicated by autoimmune diseases and autoantibodies, few reports are available on HBV-associated autoimmunity. Gregorio *et al*^[12] investigated the effects of interferon- α treatment on the prevalence of a panel of autoantibodies in HBV infection and concluded that autoantibodies are common in HBV infection without being influenced by interferon- α . Another multicenter retrospective study found that 15% of chronic hepatitis B (CHB) patients were positive for at least one autoantibody that was influenced by anti-HBe antibody positivity and that the HBV genotype had no relationship with extra-hepatic manifestations^[13]. Given the striking prevalence of HBV infection in China^[14] and the common presence of autoimmune manifestations in hepatitis B in clinical practice, exploration of the correlation between autoantibodies and disease characteristics has potential clinical implications. More importantly, this type of study may provide valuable clues to guide medication and treatment regimes.

In this study, we evaluated the association of autoantibody profiles that serologically define autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC) with the clinical features, including cirrhosis, hepatitis B e-antigen (HBeAg) status, and hepatocellular carcinoma (HCC), in Chinese CHB patients.

MATERIALS AND METHODS

Subjects and controls

Three hundred twenty-five patients with CHB who had visited the 302 Military Hospital of China between January 2009 and April 2013 were retrospectively analyzed. The diagnosis of CHB was established based on documented hepatitis B surface antigen (HBsAg) status (*i.e.*, HBsAg positive for more than 6 mo). Considering that interferon is a potent inducer of autoimmunity, only those patients who had been previously treated with first-line oral medications were included in this study. Cases co-infected with HCV and/or HIV or that

Table 1 Demographic and clinical features, biochemical parameters and response to antiviral treatment in chronic hepatitis B

All patients (n = 325)	
Male/female	201/124
Age (yr)	43.44 ± 16.19
Associated immune disease ¹ n (%)	8 (2.4)
Cirrhosis	206 (63.4)
Hepatoma	38 (11.7)
Duration of HBsAg pos. n (%)	140 cases
1-5 yr	35 (25.0)
6-10 yr	25 (17.9)
11-15 yr	34 (24.3)
16-20 yr	28 (20.0)
> 20 yr	18 (12.9)
HBeAg ²	147/266 (43.3)
AST (U/L)	145.10 ± 96.79
ALT (U/L)	126.51 ± 100.15
ALP (U/L)	121.08 ± 74.54
GGT (U/L)	73.43 ± 62.52
IgA (g/L)	3.24 ± 2.67
IgG (g/L)	14.73 ± 5.58
IgM (g/L)	1.37 ± 1.32
γ-globulin (g/L)	20.1 ± 7.38
Liver biopsy	31 cases
Staging and grading ³ n (%)	
G1S1	5 (16.1)
G1S2	3 (9.7)
G1S3	1 (3.2)
G2S1	3 (9.7)
G2S2	6 (19.4)
G2S3	2 (6.5)
G2S4	2 (6.5)
G3S2	3 (9.7)
G3S3	4 (12.9)
G4S4	2 (6.5)
Anti-viral treatment response evaluation ⁴ n (%)	72 cases
Virological non-responders	6 (8.3)
Virological responders	66 (91.7)

¹Hashimoto's thyroiditis and type 1 diabetes mellitus: 1 case each; hypothyroidism: 2 cases; probable autoimmune hepatitis: 4 cases; ²Hepatitis B e-antigen statuses were identified in 266 cases; ³The Scheuer system was used to score necroinflammatory activity and fibrosis/cirrhosis; ⁴The virological response was defined as hepatitis B virus DNA concentration of less than 2000 IU/mL at 12 mo after nucleoside/nucleotide therapy. AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl transpeptidase; ALT: Alanine aminotransferase.

also had systemic autoimmune diseases. such as SLE, rheumatoid arthritis, or anti-phospholipid syndrome, were excluded. Among the 325 patients, 206 cases (63.4%) had progressed to cirrhosis and were subdivided into compensated and decompensated cirrhosis groups, accounting for 85 and 121 cases, respectively. In this study, cirrhosis was determined by clinical examination, laboratory tests, ancillary studies (including abdominal ultrasonography), and either gastroscopy or liver biopsy. Compensated and decompensated cirrhosis were distinguished by the absence or presence, respectively, of complications (*e.g.*, jaundice, ascites, and bleeding from esophageal varices). The clinical and biochemical features of the CHB patients are summarized in Table 1.

Seventy-one patients with chronic hepatitis C (CHC), 11 with AIH, and 71 with PBC were enrolled as disease

controls. The diagnosis of CHC was based on the following criteria: (1) abnormal alanine aminotransferase (ALT) $\geq 2 \times$ the normal upper limit for at least 6 mo; (2) positive anti-HCV results from a third-generation ELISA with HCV RNA detected by real-time polymerase chain reaction for at least 6 mo; and (3) exclusion of other causes of liver dysfunction according to clinical, serological, and histological features. AIH was diagnosed based on the scoring systems of the International Autoimmune Hepatitis Group^[15]. Finite AIH was established based on a pre-treatment aggregate score > 15 or post-treatment aggregate score > 17 . PBC was established based on biochemical evidence of cholestasis, anti-mitochondrial antibodies (AMA) positivity, and histological features^[16]. Sixty healthy blood donors, age- and sex-matched with the CHN patients, were recruited as normal controls. All subjects gave informed consent before the collection of sera. The study was authorized by the local ethics committee.

Autoantibody test

The sera to be investigated were diluted 1:100 in PBS-Tween (pH 7.2) just before examination. Indirect immunofluorescence assays (IFAs) using the multiple-substrate panel of HEp-2 cells, rat liver, and rat stomach (EUROIMMUN AG, Lübeck, Germany) were used to detect ANA and SMA. A serum titer of 1:100 or higher was considered to be a positive result in the present study. Titers of positive reactions were determined at 1:320, 1:640 and 1:1000 serum dilutions. ANA positivity was subdivided into 3 clusters [*i.e.*, nuclear homogeneous (ANA-H), nuclear dots/membranous rim-like (ANA-DM) and other (ANA-other)] based on the specific fluorescence patterns. For ANA-other, fluorescence patterns such as centromeres, nuclear speckles, and nucleoli were included. The line immunoassay (EUROIMMUN AG) was used to test the IgG autoantibody panel for 8 different specificities [AMA-M2, anti-BPO (the recombinant antigen of the E2 subunits derived from the 2-oxo-acid dehydrogenase complex targeted by AMA-M2)^[17], anti-Sp100, anti-promyelocytic leukemia protein (anti-PML), anti-gp210, anti-LKM-1, anti-liver cytosolic antigen type 1 (anti-LC-1), anti-soluble liver antigen/liver pancreas (anti-SLA/LP)] and for anti-Ro52 antibodies. The incubation was performed on EUROBlotMaster (EUROIMMUN AG). The results were evaluated using EUROLineScan (EUROIMMUN AG). The positive results were graded into 3 levels from weak to strong (*i.e.*, “+”, “++”, and “+++”).

Statistical analysis

For continuous variables, between-groups differences were compared using Student's *t* test or the Mann-Whitney *U* test. For categorical variables, the χ^2 test or Fisher's exact test was used to compare the prevalence between groups when appropriate. SPSS 15.0 (SPSS Inc., Chicago, IL, United States) was used to perform all statistical analyses. Two-sided *P* values less than 0.05 were considered statistically significant.

Table 2 Prevalence of the autoantibodies in chronic hepatitis B compared with chronic hepatitis C, autoimmune hepatitis, primary biliary cirrhosis, and healthy controls *n* (%)

	CHB (<i>n</i> = 325)		CHC (<i>n</i> = 71)		AIH (<i>n</i> = 11)		PBC (<i>n</i> = 71)		HC (<i>n</i> = 60)
	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.
Any autoantibody	189 (58.2)	0.211	47 (66.2)	0.004	11 (100.0)	< 0.001	71 (100.0)	< 0.001	4 (6.7)
ANA (IFA)	76 (23.4)	0.353	13 (18.3)	0.044	6 (54.5)	< 0.001	47 (66.2)	< 0.001	2 (3.3)
SMA (IFA)	10 (3.1)	1.000	2 (2.8)	1.000	0 (0.0)	0.220	0 (0.0)	0.373	0 (0.0)
AMA-M2 (LIA)	22 (6.8)	0.322	2 (2.8)	1.000	1 (9.1)	< 0.001	60 (84.5)	0.033	0 (0.0)
Anti-BPO (LIA)	23 (7.1)	0.124	1 (1.4)	1.000	1 (9.1)	< 0.001	60 (84.5)	0.034	0 (0.0)
Anti-Sp100 (LIA)	8 (2.5)	0.360	0 (0.0)	1.000	0 (0.0)	< 0.001	11 (15.5)	0.616	0 (0.0)
Anti-PML (LIA)	36 (11.1)	0.003	0 (0.0)	0.615	0 (0.0)	< 0.001	30 (42.3)	0.003	0 (0.0)
Anti-gp210 (LIA)	41 (12.6)	< 0.001	0 (0.0)	0.372	0 (0.0)	< 0.001	35 (49.3)	0.001	0 (0.0)
Anti-LKM-1 (LIA)	2 (0.6)	1.000	1 (1.4)	0.190	1 (9.1)	1.000	0 (0.0)	1.000	0 (0.0)
Anti-LC-1 (LIA)	10 (3.1)	0.707	1 (1.4)	0.810	1 (9.1)	0.901	3 (4.2)	0.373	0 (0.0)
Anti-SLA/LP (LIA)	7 (2.2)	0.360	0 (0.0)	1.000	0 (0.0)	0.555	3 (4.2)	0.602	0 (0.0)
Anti-Ro52 (LIA)	91 (28.0)	0.015	10 (14.1)	< 0.001	10 (90.9)	< 0.001	36 (50.7)	< 0.001	3 (5.0)

$P < 0.05$, CHB *vs* CHC, CHB *vs* AIH, CHB *vs* PBC, and CHB *vs* HC, respectively. CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; HC: Health controls; ANA: Anti-nuclear antibodies; AMA: Anti-mitochondrial antibodies; SMA: Smooth-muscle antibodies; anti-PML: Anti-promyelocytic leukemia protein; anti-LKM-1: Anti-liver kidney microsome type 1; anti-LC-1: Anti-liver cytosolic antigen type 1; anti-SLA/LP: Anti-soluble liver antigen/liver pancreas; IFA: Indirect immunofluorescence assay; LIA: Line immunoassay.

Table 3 Autoantibody titers in chronic hepatitis B compared with chronic hepatitis C, autoimmune hepatitis, primary biliary cirrhosis, and healthy controls

	CHB (<i>n</i> = 325)		CHC (<i>n</i> = 71)		AIH (<i>n</i> = 11)		PBC (<i>n</i> = 71)		HC (<i>n</i> = 60)	
	Pos. cases	Mean rank ¹	Pos. cases	Mean rank	Pos. cases	Mean rank	Pos. cases	Mean rank	Pos. cases	Mean rank
ANA	76	66.79 ^a	13	50	6	97.5	47	83.57	2	100.5
SMA	10	6.20	2	8	0	-	0	-	0	-
AMA-M2	22	23.61 ^d	2	11	1	11.0	60	51.71	0	-
Anti-BPO	23	22.85 ^d	1	10.5	1	10.5	60	51.81	0	-
Anti-Sp100	8	8.44	0	-	1	5.0	11	12.50	0	-
Anti-PML	36	29.54 ^b	0	-	3	19.0	30	43.15	0	-
Anti-gp210	41	28.49 ^d	0	-	2	26.75	35	53.13	0	-
Anti-LKM-1	2	1.50	1	4	1	4.0	0	-	0	-
Anti-LC-1	10	9.45	1	7	3	10.17	3	7.00	0	-
Anti-SLA/LP	7	6.00	0	-	3	8.17	3	8.17	0	-
Anti-Ro52	91	68.89 ^b	10	50.9	4	94.25	36	91.89	4	30.5

¹Kruskal-Wallis H test; ^a $P < 0.05$, Mann-Whitney *U* test *vs* the autoantibody titers with PBC; ^b $P < 0.01$, Mann-Whitney *U* test *vs* the autoantibody titers with PBC; ^d $P < 0.01$ Mann-Whitney *U* test *vs* the autoantibody titers with PBC; "-": Not indicated; $P < 0.05$ was considered significant. CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; HC: Health controls; ANA: Anti-nuclear antibodies; AMA: Anti-mitochondrial antibodies; SMA: Smooth-muscle antibodies; anti-PML: Anti-promyelocytic leukemia protein; anti-LKM-1: Anti-liver kidney microsome type 1; anti-LC-1: Anti-liver cytosolic antigen type 1; anti-SLA/LP: Anti-soluble liver antigen/liver pancreas; IFA: Indirect immunofluorescence assay; LIA: Line immunoassay.

RESULTS

Prevalence of autoantibody specificities in the CHB group compared with CHC, AIH, PBC, and healthy control groups

At least one autoantibody was present in 58.2% of the CHB patients. This prevalence was similar to that for CHC (66.2%), significantly lower than that for AIH ($P = 0.004$) and PBC ($P < 0.001$), and significantly higher than that for healthy controls (HC) ($P < 0.001$) (Table 2). Among the autoantibodies tested, anti-PML, anti-gp210, and anti-Ro52 exhibited a significantly higher prevalence in CHB compared with CHC (11.1% *vs* 0%, $P = 0.003$; 12.6% *vs* 0%, $P < 0.001$; 28.0% *vs* 14.1%, $P = 0.015$, respectively). The difference in the autoantibody

positivity between CHB and AIH was small, except that ANA and anti-Ro52 showed a lower prevalence in CHB (23.4% *vs* 54.5%, $P = 0.044$; 28.0% *vs* 90.9%, $P < 0.001$, respectively). The prevalence of ANA, AMA-M2, anti-BPO, anti-Sp100, anti-PML, anti-gp210, and anti-Ro52 in PBC was significantly higher than in CHB ($P < 0.001$). Significant differences existed between the CHB patients and HC with respect to ANA, AMA-M2, anti-BPO, anti-Sp100, anti-PML, anti-gp210, and anti-Ro52 positivity.

Titers of autoantibody specificities in CHB compared with the CHC, AIH, PBC, and HC groups

As shown in Table 3, there were significant differences between the autoantibody titers for CHB and PBC. The autoantibodies for which the titers were significantly

Table 4 Prevalence of the autoantibodies in non-cirrhosis, compensated cirrhosis, and decompensated cirrhosis chronic hepatitis B groups *n* (%)

	Non-cirrhosis (<i>n</i> = 119)		Compensate cirrhosis (<i>n</i> = 85)		Decompensated cirrhosis (<i>n</i> = 121)	
	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.	<i>P</i> value
ANA	39 (32.8)	0.006	16 (18.8)	0.027	21 (17.4)	0.787
SMA	1 (0.8)	0.393	3 (3.5)	0.131	6 (5.0)	0.882
AMA-M2	9 (7.6)	0.410	4 (4.7)	0.971	9 (7.4)	0.427
Anti-BPO	4 (3.4)	0.010	11 (12.9)	0.248	8 (6.6)	0.122
Anti-Sp100	2 (1.7)	1.000	2 (2.4)	0.694	4 (3.3)	1.000
Anti-PML	10 (8.4)	0.293	11 (12.9)	0.311	15 (12.4)	0.908
Anti-gp210	16 (13.4)	0.540	9 (10.6)	0.960	16 (13.2)	0.569
Anti-LKM-1	1 (0.8)	1.000	1 (1.2)	0.496	0 (0.0)	0.413
Anti-LC-1	3 (2.5)	1.000	3 (3.5)	1.000	4 (3.3)	1.000
Anti-SLA/LP	2 (1.7)	0.702	3 (3.5)	1.000	2 (1.7)	0.688
Anti-Ro52	33 (27.7)	0.629	21 (24.7)	0.628	37 (30.6)	0.356

$P < 0.05$, non-cirrhosis *vs* compensated cirrhosis, non-cirrhosis *vs* decompensated cirrhosis, and compensated *vs* decompensated cirrhosis, respectively. ANA: Anti-nuclear antibodies; AMA: Anti-mitochondrial antibodies; SMA: Smooth-muscle antibodies; anti-PML: Anti-promyelocytic leukemia protein; anti-LKM-1: Anti-liver kidney microsomes type 1; anti-LC-1: Anti-liver cytosolic antigen type 1; anti-SLA/LP: Anti-soluble liver antigen/liver pancreas; IFA: Indirect immunofluorescence assay; LIA: Line immunoassay.

Table 5 Anti-nuclear antibodies fluorescence patterns and cirrhosis in chronic hepatitis B: Comparison of the non-cirrhosis, compensated cirrhosis and decompensated cirrhosis groups *n* (%)

	Non-cirrhosis ¹ (<i>n</i> = 117)		Compensated cirrhosis (<i>n</i> = 85)		Decompensated cirrhosis ² (<i>n</i> = 119)	
	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.	<i>P</i> value
ANA-H	32 (27.4)	0.001	7 (8.2)	< 0.001	8 (6.7)	0.683
ANA-DM	1 (0.9)	0.403	3 (3.5)	0.223	5 (4.2)	1.000
ANA-other ³	4 (3.4)	0.396	6 (7.1)	0.767	6 (5.0)	0.546

¹Two cases showed the combination of ANA-H/ANA-DM and ANA-DM/ANA-other fluorescence patterns, thus these two cases were excluded from this group; ²Two cases showed the combination of ANA-H/ANA-DM and ANA-DM/ANA-other fluorescence patterns, thus these two cases were excluded from this group; ³Fluorescence patterns such as centromeres, nuclear speckles, and nucleoli were included. $P < 0.05$, non-cirrhosis *vs* compensated cirrhosis, non-cirrhosis *vs* decompensated cirrhosis, and compensated *vs* decompensated cirrhosis, respectively, was considered significant. ANA: Anti-nuclear antibodies; ANA-H: ANA-homogeneous; ANA-DM: ANA-dots or membrane.

lower in CHB than in PBC included ANA ($P < 0.05$), AMA-M2 ($P < 0.001$), anti-BPO ($P < 0.001$), anti-PML ($P < 0.01$), anti-gp210 ($P < 0.001$), and anti-Ro52 ($P < 0.01$). Notably, with the exception of ANA, these antibodies are specific biomarkers for PBC.

Prevalence of autoantibodies in the non-cirrhosis, compensated cirrhosis and decompensated cirrhosis groups of CHB

The prevalence of ANA was highest in non-cirrhosis patients and showed significant differences compared with both compensated and decompensated cirrhosis (32.8% *vs* 18.8%, $P = 0.006$; 32.8% *vs* 17.4%, $P = 0.027$, respectively) (Table 4). ANA-H was the main type of ANA positivity in the three groups. ANA-H showed the highest prevalence in non-cirrhosis patients, which was significantly different from the findings for either compensated cirrhosis (27.4% *vs* 8.2%, $P = 0.001$) or decompensated cirrhosis (27.4% *vs* 6.7%, $P < 0.001$) (Table 5). In contrast, anti-BPO antibodies were most frequently present in compensated cirrhosis patients (12.9%), significantly more often than the 3.4% prevalence in non-cirrhosis patients ($P = 0.01$) (Table

4). There was no significant difference between the compensated and decompensated cirrhosis groups in terms of autoantibody positivity.

Prevalence of autoantibodies in the non-HCC and HCC groups in CHB

As Table 6 shows, the prevalence of anti-PML in non-HCC was 12.5%, which was significantly higher compared with its absence in HCC ($P = 0.013$). Regarding the other autoantibodies, there were no significant differences between these two groups.

Dichotomization of the autoantibody panels into AIH and PBC profiles and the clinical associations

Since the autoantibodies in the present study mainly characterize the serological diagnoses of AIH and PBC, we categorized these antibodies into AIH and PBC profiles, as shown in Table 7. For each CHB patient, if the autoantibodies detected belonged exclusively to the AIH or PBC profile, the profile was designated unequivocally. If a case presented multiple autoantibodies that were representative of both the AIH and PBC profiles, the case was not included.

Table 6 Prevalence of the autoantibodies in the non-hepatocellular carcinoma and hepatocellular carcinoma chronic hepatitis B groups *n* (%)

	Non-HCC (<i>n</i> = 287)		HCC (<i>n</i> = 38)	<i>P</i> value
	Pos.	Pos.	Pos.	
ANA	68 (23.7)	8 (21.1)	0.718	
SMA	10 (3.5)	0 (0.0)	0.613	
AMA-M2	19 (6.6)	3 (7.9)	1.000	
Anti-BPO	22 (7.7)	1 (2.6)	0.423	
Anti-Sp100	8 (2.8)	0 (0.0)	0.603	
Anti-PML	36 (12.5)	0 (0.0)	0.013	
Anti-gp210	37 (12.9)	4 (10.5)	0.879	
Anti-LKM-1	2 (0.7)	0 (0.0)	1.000	
Anti-LC-1	10 (3.5)	0 (0.0)	0.613	
Anti-SLA/LP	6 (2.1)	1 (2.6)	1.000	
Anti-Ro52	82 (28.6)	9 (23.7)	0.528	

ANA: Anti-nuclear antibodies; AMA: Anti-mitochondrial antibodies; SMA: Smooth-muscle antibodies; anti-PML: Anti-promyelocytic leukemia protein; anti-LKM-1: Anti-liver kidney microsome type 1; anti-LC-1: Anti-liver cytosolic antigen type 1; anti-SLA/LP: Anti-soluble liver antigen/liver pancreas.

The AIH profile was significantly more prevalent in CHB than in the HC (13.2% *vs* 0%, *P* = 0.003), and was actually similar to the prevalence in CHC (Table 8). For the PBC profile, the prevalence in CHB was notably higher than in CHC and the HC (21.8% *vs* 2.8%, *P* < 0.001; 21.8% *vs* 0%, *P* < 0.001, respectively), but lower than that in PBC (21.8% *vs* 88.7%, *P* < 0.001) (Table 8).

We further analyzed the correlation of the autoantibody profiles with the cirrhosis states in CHB (Table 9). The AIH profile was less prevalent in cirrhosis, especially compensated cirrhosis, than in non-cirrhosis (8.2% *vs* 18.5%, *P* = 0.039). In contrast, the prevalence of the PBC profile in both compensated and decompensated cirrhosis was as high as 25.9% and 26.4%, respectively, both of which were significantly higher than the 14.3% prevalence found in non-cirrhosis (*P* = 0.038 and *P* = 0.019, respectively). No significant difference was found between compensated and decompensated cirrhosis.

In addition, the AIH profile was more prevalent in the HBeAg positive group than in the HBeAg negative group (15.6% *vs* 5.9%, *P* = 0.012) (Table 10). Although the prevalence of the PBC profile was higher in the HBeAg negative group than in the HBeAg positive group, the difference did not reach statistical significance.

DISCUSSION

We investigated the prevalence and clinical significance of the autoantibodies used to serologically define AIH and PBC in Chinese patients with chronic HBV infection. HBV, the prototypical member of the family *Hepadnaviridae*, is not directly cytopathic to hepatocytes. The extent and outcomes of HBV infection are largely dependent on the quality and diversity of the induced immune responses. Patients with chronic HBV infections tend to develop an activated humoral response with

Table 7 Dichotomization of autoantibodies into autoimmune hepatitis and primary biliary cirrhosis profiles by the specific autoantibody panels

	Autoantibody panel
AIH profile	ANA-H (IFA)
	SMA (IFA)
	Anti-LKM-1 (LIA)
	Anti-LC-1 (LIA)
	Anti-SLA/LP (LIA)
PBC profile	ANA-DM (IFA)
	AMA-M2 (LIA)
	Anti-BPO (LIA)
	Anti-Sp100 (LIA)
	Anti-PML (LIA)
	Anti-gp210 (LIA)

AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; ANA: Anti-nuclear antibodies; SMA: Smooth-muscle antibodies; anti-PML: Anti-promyelocytic leukemia protein; anti-LKM-1: Anti-liver kidney microsome type 1; anti-LC-1: Anti-liver cytosolic antigen type 1; anti-SLA/LP: Anti-soluble liver antigen/liver pancreas. ANA-H: ANA-homogeneous; ANA-DM: ANA-dots or membrane; IFA: Indirect immunofluorescence assay; LIA: Line immunoassay.

type 2 T helper (Th2) cells producing IL-4, IL-5, and IL-10, which promote antibody production rather than viral clearance^[18]. Similarly, both AIH and PBC are characterized by immune-mediated injury to parenchymal liver cells and biliary ducts, respectively, and thus related NOSA (such as ANA, SMA, and AMA) play a crucial role in the accurate classification of AIH and PBC^[19].

In the present study, 58.2% of CHB and 66.2% of CHC cases were positive for at least one autoantibody in our study; both rates were significantly higher than that for the healthy subjects but significantly lower than those in AIH and PBC. These findings had no counterpart for comparison, but they were to some degree comparable with the reported prevalence of 66% for SMA in HCV infection^[20]. SMA and anti-LKM-1 in CHB showed a lower prevalence than the previously found rates of 7%^[13] and 2%^[13], respectively. ANA was detected by IFA at a higher frequency (23.4%) compared with the previous finding of 3% in CHB sera^[13], but it was comparable with observations of 35%^[3] and 32%^[4] positivity in CHC patients. AMA-M2, as the serological hallmark of PBC, was present in 6.8% of CHB patients, which contrasted with its absence in another study^[13] but was in accordance with the 8% positivity found in 237 CHC patients with extrahepatic manifestations^[21].

The PBC-specific autoantibodies anti-Sp100, anti-PML, and anti-gp210 were observed for the first time in 2.5%, 11.1%, and 12.6% of CHB patients, respectively, with anti-PML and anti-gp210 being significantly more prevalent than in the HC. All 3 antibodies were absent in the CHC group, possibly because of the small sample size. Compared with PBC, anti-PML and anti-gp210 showed significantly lower prevalences and titers in CHB. This might imply that these autoantibodies were mere “by-passers” rather than true pathogenic antibodies. Anti-

Table 8 Autoimmune hepatitis and primary biliary cirrhosis profiles in chronic hepatitis B compared with chronic hepatitis C, autoimmune hepatitis, primary biliary cirrhosis, and healthy controls *n* (%)

	CHB (<i>n</i> = 325)		CHC (<i>n</i> = 71)		AIH (<i>n</i> = 11)		PBC (<i>n</i> = 71)		HC (<i>n</i> = 60)
	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.	<i>P</i> value	Pos.
AIH profile	43 (13.2)	0.655	8 (11.3)	0.083	4 (36.4)	0.655	8 (11.3)	0.003	0 (0)
PBC profile	71 (21.8)	< 0.001	2 (2.8)	0.954	3 (27.3)	< 0.001	63 (88.7)	< 0.001	0 (0)

P < 0.05, CHB *vs* CHC, CHB *vs* AIH, CHB *vs* PBC, and CHB *vs* HC, respectively. CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; HC: Health controls.

Table 9 Autoimmune hepatitis and primary biliary cirrhosis profiles in the non-cirrhosis, compensated cirrhosis, and decompensated cirrhosis chronic hepatitis B groups *n* (%)

	Non-cirrhosis (<i>n</i> = 119)		Compensated cirrhosis (<i>n</i> = 85)		Decompensated cirrhosis (<i>n</i> = 121)	
	Pos.	P1	Pos.	P2	Pos.	P3
AIH profile	22 (18.5)	0.039	7 (8.2)	0.133	14 (11.6)	0.436
PBC profile	17 (14.3)	0.038	22 (25.9)	0.019	32 (26.4)	0.928

P < 0.05, non-cirrhosis *vs* compensated cirrhosis, non-cirrhosis *vs* decompensated cirrhosis, and compensated *vs* decompensated cirrhosis, respectively. AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis.

Table 10 Autoimmune hepatitis and primary biliary cirrhosis profiles based on hepatitis B e-antigen status in chronic hepatitis B *n* (%)

	HBeAg pos. (<i>n</i> = 147)	HBeAg neg. (<i>n</i> = 119)	<i>P</i> value
AIH profile	23 (15.6)	7 (5.9)	0.012
PBC profile	24 (16.3)	29 (24.4)	0.102

P < 0.05, HBeAg positive group *vs* HBeAg negative group. AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; HBeAg: Hepatitis B e-antigen.

gp210, which targets a component of the nuclear pore complex, is present in approximately 25% of patients with PBC^[22] and produces a membranous/rim-like pattern on HEP-2 cells by IFA^[23]. It strongly correlates with more active and severe liver disease in PBC^[24-26] and represents a hepatic failure-type progression in PBC^[27]. In the context of CHB, however, the presence of anti-gp210 did not appear to associate with progressive liver damage.

Anti-LC-1, anti-SLA/LP, and anti-LKM-1 are disease-specific serological markers for AIH^[28]. We detected anti-LC-1 and anti-SLA/LP in 3.1% and 2.2% of patients, respectively, higher prevalences than that for anti-LKM-1 (0.6%) in CHB patients. The prevalence of anti-LC-1 in CHB was lower than the 12% in HCV infection^[29]. We supposed that these autoantibodies were present but possibly transient in viral hepatitis, which was most likely only an epiphenomenon accompanying inflammation because the AIH profile showed strong correlation with non-cirrhosis (*i.e.*, the early stage of CHB).

Interestingly, in terms of the correlation between autoantibodies and cirrhosis in CHB, the positivity of ANA (particularly ANA-H, which is the typical ANA

pattern of AIH) was more prevalent in non-cirrhosis patients than in cirrhosis patients. When cirrhosis progressively develops, more and more parenchymal tissues are replaced by fibrotic tissue. In this setting, the autoantigens that are involved in the pathogenesis of AIH and PBC become deficient or less accessible to the immune system. Because autoantibody production is generally “autoantigen-driven”, non-cirrhosis patients may produce more ANA than those with cirrhosis or later-stage chronic hepatic diseases. This observation contrasted with certain previous CHC findings, supporting the notion that ANA is associated with more advanced cirrhosis or the severity of liver disease^[3,6], whereas this observation was in agreement with the findings from other reports^[8,9]. Notably, however, similar to ANA, AIH-associated ANA-H also showed higher prevalence in the non-cirrhosis group than in the cirrhosis group, which agreed with the AIH profile distribution in these groups. In contrast, the prevalence of PBC-associated ANA-DM was lower in the non-cirrhosis patients than in the cirrhosis patients, which was consistent with the PBC profile distribution in these groups. Thus, AIH- and PBC-associated autoantibodies may play different roles in the progression of CHB, but the underlying mechanisms need to be elucidated in future studies.

Patients with CHB are well known to be at increased risk of developing cirrhosis and HCC. The present finding that the prevalence of anti-PML was significantly higher in non-HCC than in HCC patients had not been previously reported. Anti-PML antibodies target the components of nuclear bodies and are specific for PBC. The known link between anti-PML and tumors could be that the antigen targeted by anti-PML antibodies is a transformation and growth-suppressing protein in promyelocytic leukemia cells^[30]. The finding that anti-PML

appeared to correlate closely with non-HCC patients with CHB was either a coincidence or a new discovery, and thus requires validation in the future.

Because autoantibodies with distinct specificities commonly occur in groups or combinations and evolve with time, such as in SLE^[31], comprehensively pinpointing the correlations between individual autoantibodies and clinical characteristics could be difficult. In the attempt to delineate the bias of autoantibody expression for each CHB case, we clustered the autoantibodies into AIH and PBC profiles. In doing so, we found that the PBC profile dominated among the patients with cirrhosis, especially decompensated cirrhosis, rather than among those without cirrhosis. In contrast, the AIH profile appeared to be mainly present in patients without cirrhosis. The differential expression of the AIH-profile and the PBC profile in the subgroups of CHB could, to some degree, reflect the differential role played by disease-specific autoantibodies.

HBeAg status can distinguish two types of CHB. HBeAg-positive CHB is linked to high-level HBV replication. Spontaneous seroconversion from HBeAg-positive to antibody (anti-HBe)-positive is accompanied by a reduction in HBV replication and clinical improvement. Patients with HBeAg-negative chronic HBV infection, in which precore or core-promoter gene mutations preclude or reduce the synthesis of HBeAg, tend to have progressive liver injury, fluctuating ALT activity, and lower levels of HBV DNA than HBeAg-positive patients. In this study, HBeAg appeared to have no relationship with the presence of autoantibodies, although there is evidence showing that autoantibodies, including ANA, SMA, anti-nucleosome, and anti-LKM, are related to HBeAg precore gene mutation in CHB^[13]. However, HBeAg-positivity may be strongly correlated with the AIH profile.

Anti-Ro52 antibodies are commonly present in patients with autoimmune liver diseases, such as PBC or autoimmune hepatitis type 1 (AIH-1), and anti-Ro52 alone or in association with anti-SLA/LP is associated with poorer prognosis in AIH-1^[32]. The findings of this study showed, expectedly, that the prevalence of anti-Ro52 was markedly higher in CHB patients than in the HC, but significantly lower than in AIH and PBC. Therefore, anti-Ro52 appeared to be more closely associated with authentic autoimmune diseases, but possibly related to a lesser degree with virus-induced hepatic inflammation.

In conclusion, we demonstrated that AIH- and PBC-related autoantibodies were commonly present in patients with CHB, and that ANA, especially ANA-H, may correlate with milder hepatic disease. In the dichotomous clustering, the PBC profile was more common in CHB than in CHC and could be strongly associated with the severity of CHB. To obtain solid and valuable information about the association between autoantibodies and the clinical features of CHB, follow-up studies are necessary.

COMMENTS

Background

Autoantibodies and autoimmune manifestations are commonly present in viral hepatitis. Many studies have investigated the correlation between autoimmunity and clinical features in viral hepatitis, but have obtained contradictory findings. To address the clinical implications of autoantibodies in viral hepatitis, the correlations between autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC)-related autoantibodies and clinical features were investigated in Chinese patients with chronic hepatitis B (CHB).

Research frontiers

In terms of the clinical implications of autoantibodies in viral hepatitis, the researches focused on the correlations between the presence of non-organ-specific autoantibodies and clinical features such as disease course, prognosis, and treatment response.

Innovations and breakthroughs

The prevalence of AIH- and PBC-related autoantibodies and their associations with clinical features in Chinese patients with CHB were investigated. Interestingly, anti-nuclear antibodies (ANA), especially ANA-H, were significantly negatively associated with cirrhosis. Another interesting finding was that the prevalence of anti-promyelocytic leukemia protein antibodies was significantly different between the hepatocellular carcinoma (HCC) (0%) and non-HCC patients (12.5%). For the first time we used an unequivocal dichotomy to cluster the autoantibodies into AIH and PBC profiles in order to delineate the bias of autoantibody expression for each case. The data showed the PBC profile was strongly associated with cirrhosis.

Applications

This study suggests that the presence of ANA could correlate with early-stage CHB, and that the PBC-related autoantibody profile could be an indicator of cirrhosis.

Terminology

Hepatitis virus B (HBV) is the leading cause of chronic liver diseases, affecting approximately 400000 people worldwide. Carriers of HBV are at high risk of developing cirrhosis and HCC, accounting for up to half of cirrhosis and HCC cases.

Peer review

This is an interesting manuscript with a large number of patients and a novel analysis of variables (dichotomization of autoantibody profiles). The findings could be useful for clinical practice.

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