

Predictors of inflammatory activity in treatment-naïve hepatitis B e-antigen-negative patients with chronic hepatitis B infection

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Abstract

Objective: Liver inflammatory activity staging is critical to guide the treatment of chronic hepatitis B virus (CHB) infection. Here, we aimed to identify practical clinical biomarkers of moderate inflammatory activity in hepatitis B e-antigen (HBeAg)-negative CHB patients.

Methods: Treatment-naïve HBeAg-negative CHB patients who underwent liver biopsy at our hospital from 1 January 2013 to 31 December 2016 were enrolled. Markers of inflammatory activity were analyzed using binary logistic regression. The area under the receiver operator characteristic curve (AUROCC) was used to assess diagnostic accuracy.

Results: A total of 106 HBeAg-negative treatment-naïve CHB patients were enrolled. According to their METAVIR inflammatory scores, 30.2% of patients were in stage $\geq A2$. Total triiodothyronine (TT3) and hepatitis B virus (HBV) DNA levels were predictors of moderate inflammatory activity ($A \geq 2$). The AUROCCs of TT3 and HBV DNA levels were 0.651 and 0.797, respectively. The optimal cut-off values for TT3 and HBV DNA were 1.755 nmol/L and 4.61 log₁₀ IU/mL, respectively.

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Conclusions: A sizable proportion of treatment-naïve HBeAg-negative CHB patients required antiviral treatment (30.2%) after undergoing liver biopsy. TT3 and HBV DNA helps identify patients with moderate inflammatory activity ($A \geq 2$), potentially reducing the need for liver biopsies and helping guide treatment of CHB patients.

Keywords

Chronic hepatitis B, inflammatory activity, thyroid hormone, total triiodothyronine, HBeAg-negative, noninvasive biomarkers

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Introduction

Chronic hepatitis B virus (CHB) infection remains a worldwide public health concern. Currently, about 3.9% of individuals are positive for hepatitis B surface antigen (HBsAg) and nearly 350 million people are infected with hepatitis B virus (HBV).¹ Replication of HBV, liver inflammatory activity and immune injury eventually lead to abnormal connective tissue hyperplasia and fibrosis. Over time, CHB can lead to cirrhosis, liver failure and hepatocellular carcinoma.² Over 600,000 people die each year from late-stage CHB infection-related liver disease.³ According to the latest guidelines,⁴⁻⁶ antiviral therapy should be administered to patients with moderate to severe inflammatory changes. Therefore, it is important to diagnose and stage liver inflammatory changes so that potentially curative treatments can be administered during early-stage disease.

Liver biopsy remains the gold standard for evaluation of liver inflammatory activity and fibrosis staging. However, liver biopsy has some disadvantages: it is invasive and may lead to sampling errors; it may cause bleeding or infection; and it is useless for dynamic surveillance.^{7,8} Thus, many patients wish to avoid this method of examination. Noninvasive markers, such as the

aspartate transaminase-to-platelet ratio index⁹ or the fibrosis-4 index,¹⁰ have also been used to stage fibrosis. However, non-invasive markers of liver inflammatory activity are relatively limited. Li et al. reported that the hepatitis B core antibody (HBcAb) level was associated with inflammatory activity in treatment-naïve CHB patients.¹¹ Age and HBV DNA level may also predict inflammatory activity.¹²

The liver is an important organ for the metabolism, conversion and excretion of thyroid hormones. Therefore, changes in thyroid hormone concentrations, such as hyperthyroidism or hypothyroidism, may occur in conjunction with liver disease.¹³⁻¹⁵ It was reported that thyroid function was a good index of hepatic function, and that it could be used to evaluate severity of liver injury as a prognostic indicator for patients with liver cirrhosis. However, patterns of thyroid hormone abnormalities in patients with liver disease vary markedly. Mansour-Ghanaei et al.¹⁴ reported that serum total triiodothyronine (TT3) concentration was a good index of hepatic function, decreasing in proportion to severity of liver damage in CHB patients. Punekar et al.¹⁶ found that levels of free triiodothyronine (FT3), free tetraiodothyronine (FT4), and thyroid stimulating hormone (TSH) correlated with severity of liver

disease. Xie¹⁵ reported that upon aggravation of liver injury, levels of TT3, total tetraiodothyronine (TT4) and FT3 gradually decreased in CHB patients. It was reported that thyroid function was a good index of hepatic function. Thus, thyroid function could be used to evaluate the severity of liver injury in CHB patients and as a prognostic indicator for patients with liver cirrhosis.^{14–18} These studies suggested a potential relationship between thyroid function and inflammatory activity during liver disease. To date, no studies have addressed the relationship between thyroid function and liver inflammatory activity, especially based on METAVIR inflammatory scores.

HBV e-antigen (HBeAg)-positive and -negative patients experience different stages over the course of HBV infection. Because of differences in viral replication and biochemical conditions, these patients can have different outcomes.⁵ Therefore, it is important to assess HBeAg-positive and HBeAg-negative CHB patients separately when searching for markers of liver inflammatory activity. Thus, in the present study, we aimed to identify routinely available clinical biomarkers of moderate inflammatory activity (stage $A \geq 2$). We also explored the relationship between thyroid function and liver inflammatory activity in HBeAg-negative patients based on METAVIR inflammatory scores.

Methods

Patients

HBeAg-negative, treatment-naïve CHB patients who underwent liver biopsy and thyroid hormone detection at the First Affiliated Hospital, College of Medicine, Zhejiang University from 1 January 2013 to 31 December 2016 were enrolled. All patients had been HBsAg-positive for at least 6 months prior to study entry. The exclusion criteria were as follows: (1)

coinfection with hepatitis A, C, D, or E virus, human immunodeficiency virus, or other viruses; (2) nonalcoholic fatty liver disease, alcoholic liver diseases, or autoimmune liver diseases; (3) compensated or decompensated liver cirrhosis or hepatocellular carcinoma; (4) tumors other than hepatocellular carcinoma; (5) history of liver transplantation; (6) underlying diseases requiring long-term corticosteroid or immunosuppressive treatments; and (7) incomplete laboratory and clinical data. Demographic, clinical and laboratory data including age, thyroid hormone levels (TT3, TT4, free T3, free T4 and TSH), and liver function, were reviewed and recorded.

This study was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Patient consent and institutional review board approval were waived because of the retrospective nature of the study.

Liver biopsies and the staging of inflammatory changes

The staging of liver inflammatory changes was determined by liver biopsies. Ultrasound was performed to select the most suitable puncture site, and liver biopsies were performed using an 18-gauge biopsy needle. Liver inflammatory staging (A0–A3) was determined using the METAVIR inflammatory score as follows:¹⁹ no histological activity (A0); mild activity (A1); moderate activity (A2); and severe activity (A3). Moderate inflammation was defined as a METAVIR inflammatory score of $A \geq 2$.

Statistical analysis

Statistical analyses were performed using SPSS software, version 19.0 (SPSS, Chicago, IL, USA). Continuous variables were expressed as means \pm standard

deviations or medians with interquartile ranges. Categorical variables were expressed as counts and percentages. The means of continuous variables were compared using Student's t tests or one-way ANOVA for normally distributed data and using Mann-Whitney tests for non-normally distributed data. Differences between categorical variables were assessed using Chi-square or Fisher's exact tests. Predictive indicators of inflammatory stages were analyzed using univariate and multivariate logistic regression analyses. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. Areas under the receiver operating characteristic curves (AUROCCs) were calculated to identify markers of moderate inflammatory activity. All P-values were based on a two-tailed test of significance and values of $P < 0.05$ were considered significant.

Results

Associations between histological inflammatory activity and clinical and laboratory data

We screened 380 treatment-naïve CHB patients who underwent liver biopsy from 1 January 2013 to 31 December 2016 in our hospital. In total, 274 patients were excluded for various reasons (Figure 1). A total of 106 treatment-naïve HBeAg-negative CHB patients (59 men and 47 women) were enrolled. Their mean age was 41.62 years. According to their METAVIR inflammatory scores, 74 patients (69.8%) were in stage A0 or A1, 26 patients (24.5%) were in stage A2, and 6 patients (5.7%) were in stage A3. We compared laboratory indicators between patients in stages A0/A1 and A2/A3. We found that patients in stages A2/A3

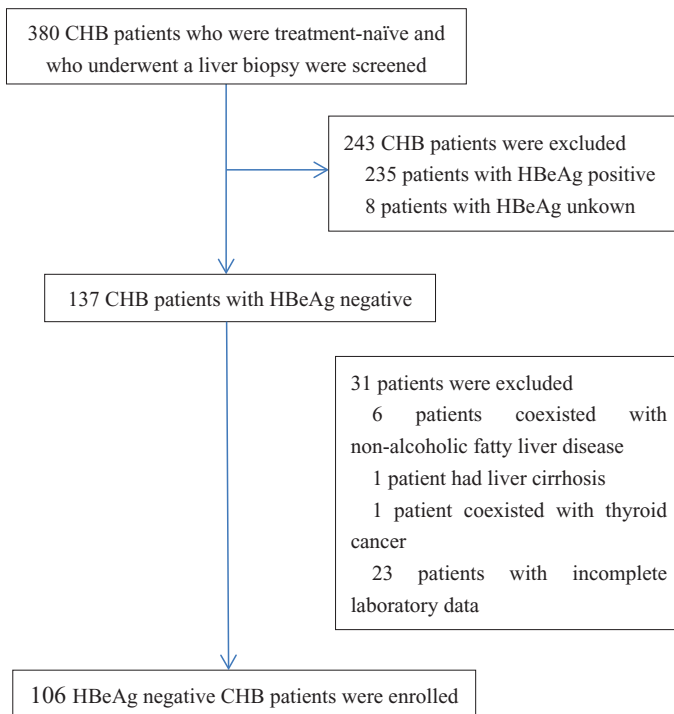


Figure 1. Flow chart of patient selection in this study.

Table 1. Associations between histological inflammatory activity stages and clinical and laboratory data.

	Total (n = 106)	A < 2 (n = 74)	A ≥ 2 (n = 32)	P
Age (years)	41.62 ± 9.052	41.39 ± 9.550	42.16 ± 7.895	0.692
Sex (M/F)	59/47	40/34	19/13	0.613
Albumin (g/L)	45.10 (42.20–49.10)	46.20 (42.38–49.40)	43.40 (41.23–47.40)	0.070
Globulin (g/L)	27.90 ± 6.199	27.79 ± 5.943	28.14 ± 6.848	0.790
ALT (U/L)	26.00 (16.00–44.25)	22 (15–33.75)	40.00 (26.75–50.00)	0.003
AST (U/L)	26.99 ± 10.734	24.16 ± 7.668	33.53 ± 13.732	<0.001
TB (U/L)	13.66 ± 6.031	13.68 ± 6.120	13.63 ± 5.917	0.969
DB (U/L)	4.00 (3.00–6.00)	4.00 (3.00–5.25)	4.00 (3.00–6.00)	0.132
ALP (U/L)	63.00 (53.00–80.75)	64.50 (54.00–83.25)	59.50 (51.25–73.50)	0.128
ChE (U/L)	8302.28 ± 2064.948	8505.09 ± 1942.597	7833.28 ± 2287.128	0.125
GGT (U/L)	17.00 (12.75–25.50)	15.50 (12.00–23.50)	21.50 (15.50–32.00)	0.013
HBV DNA (log ₁₀ IU/mL)	4.44 ± 1.355	3.97 ± 0.926	5.51 ± 1.578	<0.001
HBsAg (log ₁₀ IU/mL)	3.00 ± 0.699	2.91 ± 0.745	3.19 ± 0.540	0.059
HBcAb (log sample/cutoff ratio)	1.08 ± 0.063	1.08 ± 0.062	1.07 ± 0.065	0.811
Total T4 (nmol/L)	109.56 ± 21.964	106.74 ± 19.801	116.08 ± 25.451	0.071
Total T3 (nmol/L)	1.70 ± 0.360	1.63 ± 0.263	1.87 ± 0.486	0.013
Free T4 (pmol/L)	14.63 ± 2.071	14.58 ± 1.949	14.74 ± 2.357	0.709
Free T3 (pmol/L)	4.70 (4.34–5.19)	4.67 (4.31–5.15)	4.82 (4.35–5.43)	0.162
TSH (mIU/L)	2.22 ± 1.458	2.34 ± 1.414	1.95 ± 1.543	0.209

Data are expressed as means ± standard deviations or medians (interquartile range).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase; ChE, Cholinesterase; TB, total bilirubin; DB, direct bilirubin; GGT, gamma-glutamyl transpeptidase; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBsAg, hepatitis B virus surface antigen; HBcAb, hepatitis B virus core antibody; T4, tetraiodothyronine; T3, triiodothyronine; TSH, thyroid stimulating hormone.

had higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), HBV DNA and TT3 than patients in stages A0/A1 ($P=0.003$, $P<0.001$, $P=0.013$, $P<0.001$, and $P=0.013$, respectively). The characteristics of patients are shown in Table 1.

Univariate and multivariate binary logistic regression to identify predictors of moderate inflammatory activity

Based on the differences in ALT, AST, GGT, HBV DNA, and TT3 levels between patients in stages A0/A1 and A2/A3 (Table 1), we analyzed those markers using binary logistic regression. We found that ALT, AST, HBV DNA, and TT3 levels

were associated with moderate inflammatory activity ($P=0.003$, $P<0.001$, $P<0.001$, and $P=0.005$, respectively). We further analyzed these markers using multivariate binary logistic regression. We found that HBV DNA (OR 1.902, 95% CI 1.344–2.692, $P<0.001$) and TT3 levels (OR 5.831, 95% CI 1.117–30.447, $P=0.005$) were markers of moderate inflammation (Table 2).

Correlations between serum thyroid hormone levels and liver inflammatory activity

Different thyroid hormones displayed different changes at each inflammatory stage. Serum levels of TT3, FT4, and FT3 displayed increases from stage A0/1 to A2

Table 2. Logistic regression analysis of factors associated with inflammatory activity ($A \geq 2$) in e-antigen-negative patients with chronic hepatitis B virus infection.

	Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P
ALT (U/L)	1.037 (1.012–1.063)	0.003	0.980 (0.936–29.329)	0.383
AST (U/L)	1.093 (1.041–1.147)	<0.001	1.044 (0.985–1.106)	0.143
GGT (U/L)	1.013 (0.995–1.032)	0.168		
Total T3 (nmol/L)	8.308 (1.870–36.921)	0.005	5.831 (1.117–30.447)	0.037
HBV DNA (log ₁₀ IU/mL)	1.967 (1.411–2.743)	<0.001	1.902 (1.344–2.692)	<0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; HBV DNA, Hepatitis B virus deoxyribonucleic acid; T3, triiodothyronine; OR, odds ratio; 95% CI, 95% confidence interval.

Table 3. Thyroid hormone levels in e-antigen-negative patients with chronic hepatitis B virus infection at different inflammatory activity stages.

	A0/A1 (n = 74)	A2 (n = 26)	A3 (n = 6)	P
Total T4 (nmol/L)	106.74 ± 19.801	115.95 ± 25.048	116.68 ± 29.641	0.132
Total T3 (nmol/L)	1.63 ± 0.263	1.88 ± 0.499*	1.81 ± 0.462	0.006
Free T4 (pmol/L)	14.58 ± 1.949	14.93 ± 2.581	13.92 ± 0.394	0.526
Free T3 (pmol/L)	4.72 ± 0.513	5.02 ± 0.838	4.76 ± 0.551	0.098
TSH (mIU/L)	2.34 ± 1.414	2.01 ± 1.676	1.69 ± 0.798	0.405

Data are expressed as means ± standard deviations.

*Compared with A0/A1, $P < 0.05$.

Abbreviations: T4, tetraiodothyronine; T3, triiodothyronine; TSH, thyroid stimulating hormone.

and decreases from stage A2 to A3. With increasing inflammatory grade, serum levels of TT4 increased and serum levels of TSH decreased. However, only the difference in TT3 level was significant among the A0/A1, A2 and A3 inflammatory stages ($P = 0.006$). The mean levels of TT3 in patients in the A0/A1, A2 and A3 inflammatory stages were 1.63 ± 0.263 nmol/L, 1.88 ± 0.499 nmol/L, and 1.81 ± 0.462 nmol/L, respectively. With increasing inflammatory grade, TT3 first increased and then decreased. Levels of TT3 in the A3 inflammatory stage were also higher than those in the A0/A1 inflammatory stage. However, only the difference between the A0/A1 stage and the A2 stage was significant ($P = 0.002$) (Table 3, Figure 2).

AUROCcs of serum HBV DNA and TT3 levels for prediction of moderate inflammation

The AUROCC of serum TT3 level was 0.651 (95% CI 0.531–0.771). The optimal cut-off was 1.755 nmol/L, with a sensitivity of 56.3% and a specificity of 67.6%. The AUROCC of serum HBV DNA level was 0.797 (95% CI 0.694–0.899). The optimal cut-off was 4.61 log₁₀ IU/L, with a sensitivity of 75.0% and a specificity of 77.0%.

The 106 patients were divided into a normal ALT group (76 cases, 18 cases with $A \geq 2$) and an elevated ALT group (30 cases, 14 cases with $A \geq 2$). In the elevated ALT group, the AUROCcs of serum TT3 and HBV DNA levels for predicting

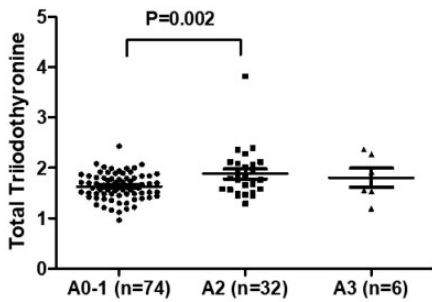


Figure 2. Total triiodothyronine levels in patients with e-antigen-negative chronic hepatitis B virus infection at different inflammatory activity stages.

moderate inflammation were 0.714 and 0.817, respectively. In the normal ALT group, the AUROCCs of serum TT3 and HBV DNA levels for predicting moderate inflammation were 0.596 and 0.744, respectively.

Discussion

The predominant type of CHB infection is HBeAg-negative. In this stage, the infection may remain in a low/nonreplicative phase (inactive chronic HBV carrier status) or may progress to an active phase.^{20,21} It is important to distinguish between these two phases. Only observation is needed for patients with inactive carrier status, while treatment is needed for HBeAg-negative CHB infections. A series of biochemical (ALT and AST level) and virological (HBV DNA level) changes occur in HBeAg-negative CHB infection.²² Serum levels of ALT and AST, which are enzymes released by hepatocytes in response to liver injury, usually reflect the degree of liver damage and liver inflammatory activity. However, some patients do not have noticeably elevated ALT and AST levels in this stage. Therefore, it is important to analyze other markers to assess the degree of liver injury and inflammatory activity. Liver biopsy is the gold standard for staging inflammation. However, the limitations of

liver biopsy prevent its wide application. Thus, it is important to identify routine laboratory indicators predictive of liver inflammatory activity. It was reported that thyroid function was a good index of hepatic function, and that levels of thyroid hormones could be used as indicators to evaluate severity of liver injury in CHB patients.^{14–18} Several studies have suggested a potential relationship between thyroid function and inflammatory activity in liver disease.

According to CHB guidelines,^{4–6} patients should be considered for antiviral therapy when they have moderate to severe inflammation ($A \geq 2$). Therefore, accurate staging of moderate inflammatory activity is important. In this study, we aimed to identify markers of moderate inflammatory activity in HBeAg-negative CHB patients who did not reach antiviral treatment standards⁴ based on their METAVIR inflammatory scores. In this study of 106 treatment-naïve HBeAg-negative CHB patients, 32 patients (30.2%) had reached stage 2 inflammation and above as shown by liver biopsy resulted. These data indicated that a significant number of treatment-naïve HBeAg-negative CHB patients had developed moderate inflammation.

Previous studies revealed that HBcAb¹¹ and age, in combination with HBV DNA,¹² could predict inflammatory activity in treatment-naïve CHB patients. Additionally, some correlations were observed between thyroid hormone levels and liver diseases.^{13–15} Therefore, we assessed age, liver function (ALT, AST, alkaline phosphatase, cholinesterase, total bilirubin, direct bilirubin, GGT, HBV DNA, HBsAg, and HBcAb), and thyroid hormones (TT4, TT3, FT4, FT3, and TSH) in our search for markers of moderate inflammation. We found that serum levels of ALT, AST, HBV DNA, and TT3 were higher in patients with inflammatory scores $\geq A2$. However, HBV DNA and TT3 levels were

the only markers of moderate inflammation based on multivariable logistic regression.

High HBV DNA levels are predictive of liver damage in CHB patients.⁵ HBV DNA level is also a risk factor for cirrhosis and hepatocellular carcinoma.²³ Ormeci et al.¹² determined that HBV DNA viral loads between 2000 and 20,000 IU/mL predicted the requirement for therapeutic intervention in HBeAg-negative CHB patients. In agreement with the present study, HBV DNA was a predictor of inflammatory activity. The previous study indicated that histologically moderate inflammatory activity should be suspected in patients with HBV DNA viral loads $>4.61 \log_{10}$ IU/mL, and that close surveillance of HBV DNA and ALT levels were needed in these patients. Antiviral treatment may be required if HBV DNA and ALT levels remain elevated.

Clinical measurements of thyroid hormones typically include TT4, TT3, FT4, FT3, and TSH. The liver is an important organ for the metabolism, conversion and excretion of thyroid hormones. During liver injury, thyroid dysfunction will occur.¹⁴⁻¹⁸ However, the patterns of thyroid hormone abnormalities vary markedly among patients. Bano et al.¹³ found that higher TSH levels were a risk factor for fibrosis in patients with nonalcoholic fatty liver disease. Mansour-Ghanaei et al.¹⁴ found that TT3 levels decreased with the severity of liver damage. Xie¹⁵ reported that with aggravation of liver injury in CHB patients, TT3, TT4, and FT3 levels gradually decreased. In our study, no patients had underlying thyroid diseases. We found that with increasing inflammatory grade (from stage A0/A1 to A2) TT3 level increased; however, TT3 levels decreased from stage A2 to A3. TT3 levels were also analyzed as a marker of inflammation stage \geq A2 by univariate and multivariate logistic regression. We found that serum TT3 level had good predictive performance, especially

for patients with elevated ALT level. All the above findings suggest that inflammatory stage A2 represents a vital turning point in the progression of inflammation. When serum TT3 reaches >1.755 nmol/L, histologically moderate inflammatory activity should be suspected in HBeAg-negative CHB patients. T3 is converted by hepatocytes from T4. When the liver experiences inflammatory activity, this conversion process will be affected. The mechanisms underlying the differential alteration of thyroid hormone levels in early and late stages of inflammation require further investigation.

Our study had several limitations. First, we analyzed only 106 cases. Second, this was a retrospective study, and indicators of liver biopsy were not uniform. Third, we did not follow-up the patients described here. We will address these drawbacks in future studies.

In conclusion, this study suggested that a sizable proportion of treatment-naïve HBeAg-negative CHB patients require antiviral treatment (30.2%) after undergoing liver biopsy. Serum levels of HBV DNA and TT3 were independent predictors of moderate inflammatory activity ($A \geq 2$). Furthermore, patients with HBV DNA viral loads $>4.61 \log_{10}$ IU/mL and TT3 >1.755 nmol/L are more likely to require antiviral treatment. The use of this predictive score based on serum HBV DNA viral load and TT3 levels could potentially reduce the need for liver biopsies and help guide clinical management of treatment-naïve HBeAg-negative CHB patients.

Authors' contributions

Study design: Jianhua Hu, Yong Wang, Gongying Jiang, Lanjuan Li; patient care: Meifang Yang, Xuan Zhang, Hong Zhao; data collection: Jie Zheng, Tuxiang Chen, Zhiping Chen; data analysis: Jianhua Hu, Meifang Yang; paper writing: Jianhua Hu, Yong Wang, Gongying Jiang, Lanjuan Li.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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References

1. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: A modelling study. *Lancet Gastroenterol Hepatol* 2018; 3: 383–403.
2. Ogawa E, Yeo YH, Dang N, et al. Diagnosis rates of chronic hepatitis B in privately insured patients in the United States. *JAMA Netw Open* 2020; 3: e201844.
3. Liaw YF and Chu CM. Hepatitis B virus infection. *Lancet* 2009; 373: 582–592.
4. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: A 2015 update. *Hepatol Int* 2016; 10: 1–98.
5. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; 67: 370–398.
6. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; 67: 1560–1599.
7. Schuppan D and Kim YO. Evolving therapies for liver fibrosis. *J Clin Invest* 2013; 123: 1887–1901.
8. Rockey DC, Caldwell SH, Goodman ZD, et al. Liver biopsy. *Hepatology* 2009; 49: 1017–1044.
9. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518–526.
10. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43: 1317–1325.
11. Li MR, Lu JH, Ye LH, et al. Quantitative hepatitis B core antibody level is associated with inflammatory activity in treatment-naïve chronic hepatitis B patients. *Medicine (Baltimore)* 2016; 95: e4422.
12. Ormeci A, Aydin Y, Sumnu A, et al. Predictors of treatment requirement in HBeAg-negative chronic hepatitis B patients with persistently normal alanine aminotransferase and high serum HBV DNA levels. *Int J Infect Dis* 2016; 52: 68–73.
13. Bano A, Chaker L, Plompen EPC, et al. Thyroid function and the risk of nonalcoholic fatty liver disease: The Rotterdam study. *J Clin Endocrinol Metab* 2016; 101: 3204–3211.
14. Mansour-Ghanaei F, Mehrdad M, Mortazavi S, et al. Decreased serum total T3 level in hepatitis B and C related cirrhosis by severity of liver damage. *Ann Hepatol* 2012; 11: 667–671.
15. Xie DW. Serum level of thyroid hormone in patients with type B hepatitis. *Journal of Xinxiang Medical College* 2012; 29: 365–366.
16. Punekar P, Sharma AK and Jain A. A study of thyroid dysfunction in cirrhosis of liver and correlation with severity of liver disease. *Indian J Endocrinol Metab* 2018; 22: 645–650.
17. Ding WJ, Wang MM, Wang GS, et al. Thyroid function is associated with non-alcoholic fatty liver disease in chronic hepatitis B-infected subjects. *J Gastroenterol Hepatol* 2015; 30: 1753–1758.

18. Wu D, Rao Q, Chen W, et al. Development and validation of a novel score for fibrosis staging in patients with chronic hepatitis B. *Liver Int* 2018; 38: 1930–1939.
19. Bedossa P and Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289–293.
20. Funk ML, Rosenberg DM and Lok AS. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. *J Viral Hepat* 2002; 9: 52–61.
21. Hadziyannis SJ and Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: Natural history and treatment. *Semin Liver Dis* 2006; 26: 130–141.
22. Papatheodoridis GV, Manolakopoulos S and Archimandritis AJ. Current treatment indications and strategies in chronic hepatitis B virus infection. *World J Gastroenterol* 2008; 14: 6902–6910.
23. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; 295: 65–73.