ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Soluble Programmed Cell Death 1 Protein Is a Promising Biomarker to Predict Severe Liver Inflammation in Chronic Hepatitis B Patients

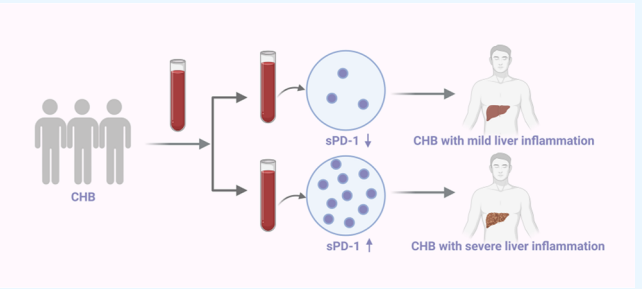
Mingrong Ou,[#] Weiming Zhang,[#] Wen Zhang,[#] Jianmin Guo, Rui Huang, Jian Wang, Jiacheng Liu, Juan Xia, Chao Wu, Yijia Zhu,[*] and Yuxin Chen[*]

Cite This: *ACS Omega* 2024, 9, 16716−16724

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: *Background and Aims*: Liver inflammation is important in guiding the initiation of antiviral treatment and affects the progression of chronic hepatitis B(CHB). The soluble programmed cell death 1 protein (sPD-1) was upregulated in inflammatory and infectious diseases and correlated with disease severity. We aimed to investigate the correlation between serum sPD-1 levels and liver inflammation in CHB patients and their role in indicating liver inflammation. *Methods*: 241 CHB patients who underwent liver biopsy were enrolled. The correlation between sPD-1 levels and the degree of liver inflammation was analyzed. Univariate and multivariate logistic regression analyses were performed to analyze independent variables of severe liver inflammation. Binary logistic regression analysis was conducted to construct a predictive model for severe liver inflammation, and the receiver operating characteristic curve (ROC) was used to evaluate the diagnostic accuracy of the predictive model. *Results*: sPD-1 was highest in CHB patients with severe liver inflammation, which was higher than that in CHB patients with mild or moderate liver inflammation ($P < 0.001$). Besides, sPD-1 was weakly correlated with AST ($r = 0.278$, $P < 0.001$). Multivariable analysis showed that sPD-1 was an independent predictor of severe liver inflammation. The predictive model containing sPD-1 had areas under the ROC (AUROCs) of 0.917 and 0.921 in predicting severe liver inflammation in CHB patients and CHB patients with ALT ≤ 1× upper limit of normal (ULN), respectively. *Conclusions*: Serum sPD-1 level is associated with liver inflammation in CHB patients, and high levels of sPD-1 reflect severe liver inflammation. Serum sPD-1 is an independent predictor of severe liver inflammation and shows improved diagnostic accuracy when combined with other clinical indicators.



INTRODUCTION

Hepatitis B virus (HBV) infection is a heavy burden on global public health. There are 240 million people estimated to suffer from chronic HBV infection.[1] During chronic HBV infection, CHB may progress to different liver diseases, including liver cirrhosis (LC), liver decompensation, and hepatocellular carcinoma (HCC).[2] CHB patients with severe liver inflammation are more susceptible to develop LC, liver decompensation, and HCC. Timely antiviral treatment is essential to impede the deterioration of the disease.[3] Indications for antiviral treatment in CHB patients include the level of HBV replication, the severity of liver inflammation, and liver fibrosis.[1,4] The ALT level is one of the most commonly used indicators to evaluate liver inflammation in patients with CHB.[5] However, several studies have shown that ALT has limited specificity in reflecting liver inflammation, while some CHB patients with normal ALT levels have severe liver inflammation.[6,7] Liver biopsy remains the gold standard for assessing liver pathohistology.[8] However, the invasiveness of liver biopsy has limited its wide application, and many patients refuse to undergo a liver biopsy. Therefore, there is an urgent need to find new biomarkers to assess the degree of liver inflammation and to better guide the initiation of antiviral treatment.

Programmed cell death protein 1 (PD-1) is inducibly expressed on activated T cells and is also expressed on B cells, natural killer (NK) cells, activated monocytes, and dendritic cells (DCs).[9] The expression of PD-1 can be upregulated by inflammatory cytokines such as IFN-α, IL-6, and IL-12.[10,11] When PD-1 binds to its ligand programmed cell death 1 ligand 1 (PD-L1)/programmed cell death 1 ligand 2 (PD-L2), it can suppress the proliferation and effector function of T cells.[12] Under physiological conditions, the PD-1/PD-L1(2) immunosuppressive pathway contributes to the maintenance of

Received: January 23, 2024
Revised: March 9, 2024
Accepted: March 14, 2024
Published: March 29, 2024

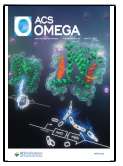


Table 1. Clinical Features of the Enrolled Patients and Patients with Different Stages of Inflammation[a]

variables	total (n = 241)	G < 3 (n = 211)	$G \geq 3$ (n = 30)	P-value
gender, male: female	157/84	141/70	16/14	0.147
age, years	39 (33−48)	39 (33−48)	40.5 (33−51.75)	0.434
platelet $\times 10^9$/L	168 (143−208)	177 (146−214)	147 (111.5−166.5)	<0.001
tbil, umol/mL	11.6 (8.3−15.9)	11.2 (8.1−15.4)	13.55 (10.975−20.275)	0.009
alb, g/L	41.7 (39.2−43.9)	42 (39.5−44)	39.6 (37.65−42.425)	0.022
ALT, U/L	36.60 (22.25−60.55)	32.3 (21.5−55.7)	112.9 (42.8−260.55)	<0.001
AST, U/L	23.60 (19.65−34.80)	22.5 (18.9−29.8)	67.05 (30.85−135.875)	<0.001
GGT, U/L	27.00 (16.25−42.50)	22.9 (15.9−40.5)	56.05 (36.05−95.575)	<0.001
HBsAg, lg IU/mL	3.431 (2.829−4.0815)	3.424 (2.802−4.109)	3.490(2.886−3.9711)	0.863
HBeAg, positive: negative	79/162	60/151	19/11	<0.001
HBV DNA, lg IU/mL	3.892 (2.993−6.552)	3.614 (2.975−5.375)	6.044 (4.124−7.375)	0.003
HBV RNA, lg IU/ml	3.952 (3.205−7.105)	3.819 (3.047−7.041)	6.044 (4.122−7.245)	0.002
sPD-1, pg/mL	131.09 (101.47−195.70)	126.059 (101.284−171.05)	211.01 (164.295−390.629)	<0.001

[a]Tbil, total bilirubin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; and sPD-1, soluble programmed cell death 1 protein.

immune homeostasis and peripheral immune tolerance.[13] PD-1 is upregulated in viral infections, such as human immunodeficiency virus (HIV) and HBV infection. PD-1 prevents the virus from killing T cells and induces the persistence of infection.[14]

In addition to its membrane-bound form, PD-1 also exists in the extracellular space in its soluble form. sPD-1 is produced by the activated peripheral blood mononuclear cells (PBMCs) through the expression of alternative spliced PD-1 mRNA transcript PD-1Δex3.[15] Recent studies have shown that sPD-1 is elevated in infectious, inflammatory, and autoimmune diseases and associated with the disease activity.[16−19] In chronic HBV infection, sPD-1 is also elevated and correlates with the levels of liver enzymes and the virological response. Besides, high sPD-1 levels were demonstrated to be associated with a high risk of developing HCC.[20−22] These results suggest that sPD-1 may be associated with disease activity and is involved in disease progression in chronic HBV infections. However, there are few relevant studies, and the ability of serum sPD-1 to predict liver inflammation in CHB patients is still unclear.

In the present study, we detected sPD-1 in the serum of CHB patients and analyzed the association between sPD-1 and liver inflammation, and other clinical parameters. Furthermore, we constructed a predictive model to evaluate the role of serum sPD-1 in predicting liver inflammation in CHB patients.

MATERIALS AND METHODS

Patient Selection. In total, 241 consecutive CHB patients who underwent liver biopsy between November 2017 and March 2022 at the Nanjing Drum Tower Hospital, Nanjing, China, were enrolled in this study. CHB was defined as the persistence of serum hepatitis B surface antigen (HBsAg) for >6 months and significant inflammation necrosis and/or fibrosis (≥G2/S2), as shown in the histological examination of the liver biopsy. The inclusion criterion was CHB patients with detectable HBV DNA at the time of liver biopsy. We excluded patients who (1) were coinfected with other viruses, including hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), and HIV; (2) had HCC; (3) had cardiovascular diseases; (4) had diabetes; (5) had kidney diseases; (6) were pregnant; (7) had autoimmune disease. Whole blood samples were collected via phlebotomy in Gel&Clot Activator serum separator tubes from CHB patients and centrifuged at 3000 rpm for 5 min at room temperature to separate the serum. Sera samples were isolated and stored at −80 °C for further analysis. All enrolled patients were informed of the necessary information concerning this study and provided written informed consent. This study was approved by the Ethics Committee of the Nanjing Drum Tower Hospital.

Laboratory Assay. Routine blood tests, including white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs), were performed using an XN 1000 (SYSMEX, Kobe, Japan). Liver function tests, including ALT, AST, and GGT, were performed using an AU5800 (Beckman Coulter, Inc., Brea, CA). Virological tests, including HBsAg and HBeAg, were performed using commercial immunoassays (Abbott Gmbh & Co. KG). HBV DNA was measured using a real-time fluorescence quantitative polymerase chain reaction (RT-PCR; Aikang Biotechnology Co., Ltd.).

Liver Biopsy. Liver biopsy was performed under ultrasonic guidance and evaluated by two experienced pathologists without clinical information. The histodiagnosis of liver tissues was determined according to the degree of liver inflammation and fibrosis. Liver inflammation and fibrosis were staged according to Scheuer's classification.[23] G0-G1, G2, and G3−G4 were defined as no or mild, moderate, and severe liver inflammation, respectively. Liver fibrosis was categorized as no significant fibrosis (S0−S1), moderate fibrosis (S2−S3), and cirrhosis (S4).

Serum sPD-1 Analysis. In the present study, we used the TOP sPD-1 immunoassay (ETHealthcare, Shanghai, China), an established high-throughput and automated assay, to quantitatively measure sPD-1 levels in the serum of CHB patients. The detection principle and detailed procedures for the TOP sPD-1 immunoassay were described in our previous study.[24]

Statistical Analysis. Data were analyzed using SPSS 25.0 software (SPSS Inc., Chicago, IL). Continuous variables were expressed as median and interquartile range (IQR), and categorical variables were expressed as numbers and percentages. Continuous variables between the two groups were compared using the t-test (normal distribution) or Mann−Whitney U test (nonnormal distribution), and categorical variables were compared using the chi-square test. Correlations between sPD-1 and other parameters were evaluated by Spearman's rank correlation test. Univariate and

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724

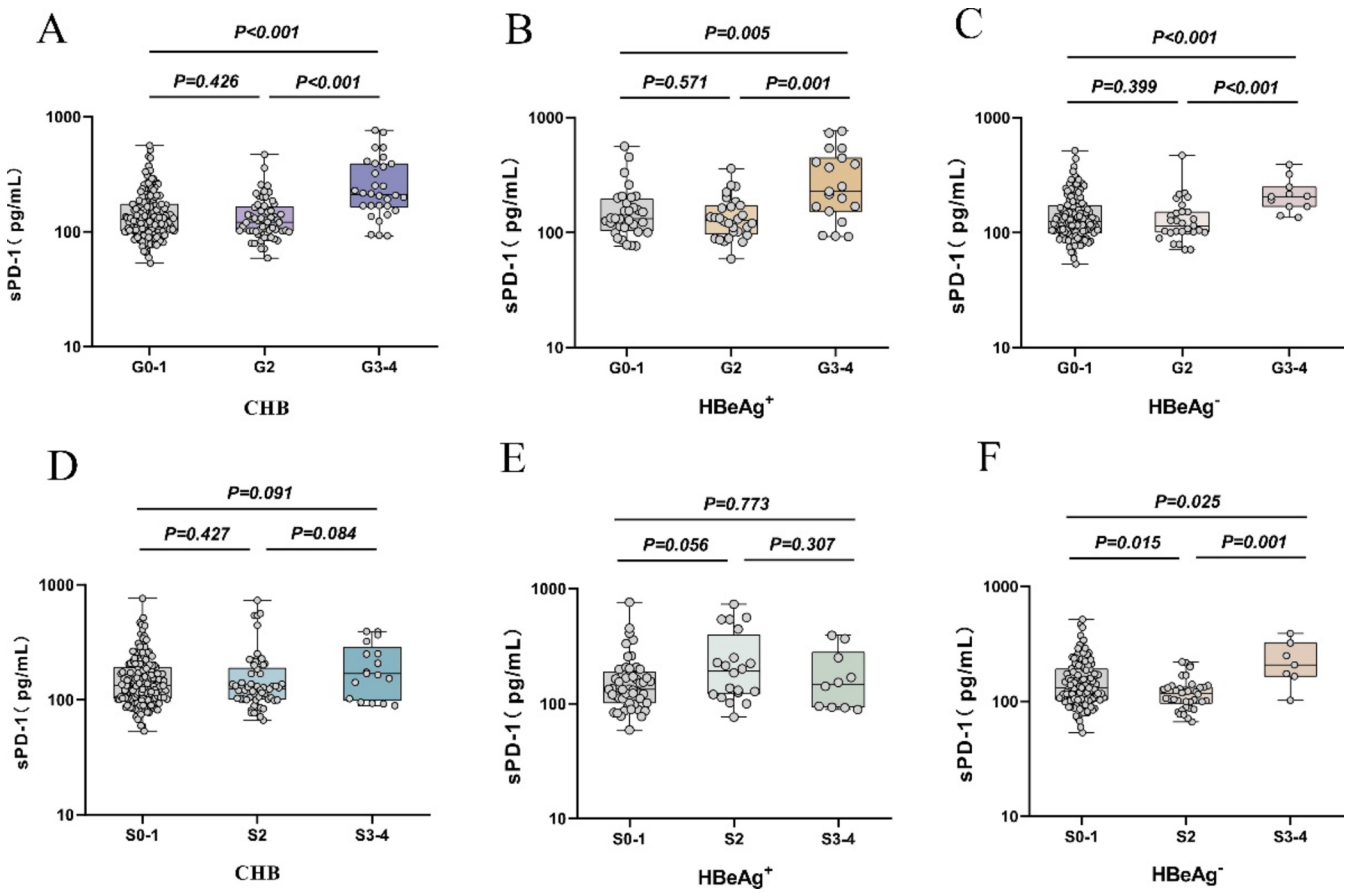


Figure 1. Serum sPD-1 levels in CHB patients with different stages of inflammation and fibrosis. (A) Serum sPD-1 levels in CHB patients at G0−1, G2, and G3−4 stages. (B, C) Serum sPD-1 levels in $HBeAg^+$ and $HBeAg^-$ CHB patients at G0−1, G2, and G3−4 stages. (D) Serum sPD-1 levels in CHB patients at S0−1, S2, and S3−4 stages. (E, F) Serum sPD-1 levels in $HBeAg^+$ and $HBeAg^-$ CHB patients at S0−1, S2, and S3−4 stages. The Mann−Whitney U test was used for comparisons between groups.

multivariate logistic regression analyses were performed to determine the determinants of severe liver inflammation and fibrosis. Binary logistic regression analysis was conducted using the backward (conditional) method. Receiver operating characteristic curves (ROC) were used to evaluate the diagnostic ability of the predictors for liver inflammation. The areas under the ROC curves (AUROCs) and 95% confidence interval (CI) of the AUROC were calculated. All significance tests were two-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and Clinical Features of the Study Cohort. A total of 241 CHB patients who underwent liver biopsy were enrolled for subsequent analysis, including 84 women and 157 men, with a median age of 39 years (range 33−48 years). Among CHB patients, 79 (32.8%) were HBeAg-positive. The serum sPD-1 level in CHB patients was 131.09 (101.47,195.70) pg/mL. The enrolled CHB patients were divided into different groups according to the degree of liver inflammation or fibrosis: 211 (87.6%) CHB patients with $G < 3$ and 30 (12.4%) CHB patients with $G \geq 3$. No significant difference was observed in HBsAg levels ($P = 0.863$) between CHB patients with $G < 3$ and $G \geq 3$. According to the degree of liver fibrosis, 224 (92.9%) cases had $S < 3$, and 17 (7.1%) cases had $S \geq 3$. The detailed demographic and clinical parameters are listed in Table 1.

Serum sPD-1 in CHB Patients at Different Stages of Inflammation and Fibrosis. We combined CHB patients in G3 and G4 into one group due to the small sample size in these two groups, as in the S3 and S4 groups. The serum levels of sPD-1 in CHB patients at different stages of inflammation and fibrosis are shown in Figure 1. The results showed that the serum sPD-1 level was highest in CHB patients at G3 and G4 stages [211.01, (164.30,390.63) pg/mL], which was significantly higher than that in CHB patients at G0-G1 [128.07, (100.98−174.06) pg/mL, $P < 0.001$] and G2 stages [120.97 (100.73−165.37) pg/mL, $P < 0.001$]. However, serum sPD-1 levels were comparable between patients in the G0-G1 and G2 stages ($P = 0.426$). Furthermore, the serum sPD-1 level of CHB patients with $G \geq 3$ was higher than that of CHB patients with $G < 3$ in both HBeAg- and $HBeAg^+$ groups (Figure 1B,C), suggesting that either $HBeAg^+$ or HBeAg- CHB patients with higher serum sPD-1 levels may have severe liver inflammation. Besides, the serum sPD-1 level was positively correlated with the inflammation stage ($r = 0.192$, $P = 0.003$, Figure 2A). In our study, we did not find differences in serum sPD-1 between patients with different stages of fibrosis [S0−S1, 133.03 (102.39−190.96) pg/mL; S2, 126.132 (101.284−196.061) pg/mL; S3−S4, 169.181(98.581−286.977) pg/mL, $P = 0.289$] (Figure 1D−F).

Correlation between Serum sPD-1 Levels and Clinical Parameters. ALT and AST are the most common biochemical indicators used to evaluate liver inflammation,

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724

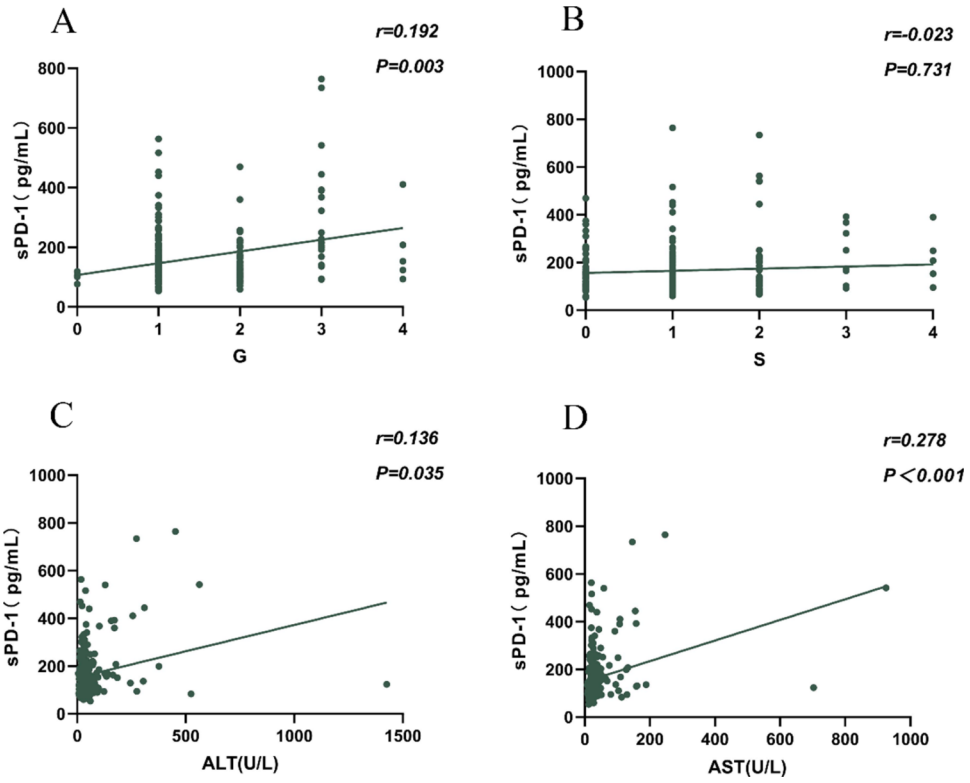


Figure 2. Association between serum sPD-1 levels, inflammation and fibrosis stages, and ALT and AST levels. (A, B) The association between serum sPD-1 levels and the inflammation and fibrosis stages. (C, D) Correlation between serum sPD-1, ALT, and AST levels. Spearman's rank correlation test was used to assess correlations between sPD-1 levels and other parameters.

Table 2. Univariable and Multivariable Analyses of Severe Liver Inflammation ($G \geq 3$) in Enrolled CHB Patients[a]

	univariate		multivariate	
variable	OR (95% CI)	*P*	OR (95% CI)	*P*
age	1.019(0.982,1.056)	0.320		
gender				
female	1.000		1.000	
male	0.567(0.262,1.228)	0.150		
platelet $\times 10^9$/L	0.986(0.978,0.995)	0.002	0.978(0.965,0.991)	0.001
TBil, umol/mL	1.018(0.998,1.037)	0.074		
ALB, g/L	0.996(0.945,1.051)	0.896		
ALT, U/L	1.013(1.007,1.019)	<0.001		
AST, U/L	1.037(1.024,1.050)	<0.001	1.025(1.010,1.041)	0.001
GGT, U/L	1.030(1.017,1.042)	<0.001	1.030(1.012,1.048)	0.001
HBsAg, lg IU/mL	1.028(0.714,1.481)	0.882		
HBeAg				
negative	1.000		1.000	
positive	4.347(1.952,9.680)	<0.001	2.825(0.796,10.025)	0.108
HBV DNA, lg IU/mL	1.323(1.105,1.584)	0.002		
HBV RNA, lg IU/mL	1.305(1.073,1.587)	0.008		
sPD-1, pg/mL	1.008(1.005,1.012)	<0.001	1.007(1.001,1.012)	0.013

[a]Tbil, total bilirubin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; and sPD-1, soluble programmed cell death 1 protein.

hepatocyte damage, and disease fluctuation. Correlation analysis showed that serum sPD-1 levels were positively associated with ALT (r = 0.136, P = 0.035) and AST (r = 0.278, P < 0.001) levels (Figure 2C,D). There was no obvious correlation between serum sPD-1 and other clinical parameters, including PLT, HBsAg, and HBV DNA. Our data suggested that serum sPD-1 levels may be a potential novel biomarker that is distinct from other traditional biomarkers such as ALT, AST, and HBV DNA.

sPD-1 Is an Independent Predictor of Severe Liver Inflammation. Logistic regression analysis was performed to analyze the independent predictors of severe liver inflammation in CHB patients. Univariate logistic regression analysis showed that sPD-1 (odd ratio (OR), 1.008; 95%CI, 1.005−1.012; P < 0.001), PLT (OR, 0.986; 95%CI, 0.978−0.995; P =

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724

Table 3. Univariable and Multivariable Analyses of Severe Liver Inflammation ($G \geq 3$) in HBeAg-Positive CHB Patients[a]

	univariate		multivariate	
variable	OR (95% CI)	P	OR (95% CI)	P
age	1.032(0.961,1.109)	0.385		
gender				
female	1.000		1.000	
male	0.722(0.233,2.236)	0.573		
platelet $\times 10^9$/L	0.972(0.957,0.987)	<0.001	0.976(0.954,0.998)	0.033
TBil, umol/mL	1.010(0.991,1.030)	0.287		
ALB, g/L	1.037(0.956,1.126)	0.382		
ALT, U/L	1.010(1.004,1.016)	0.001		
AST, U/L	1.029(1.013,1.045)	<0.001	1.036(1.012,1.061)	0.003
GGT, U/L	1.047(1.021,1.072)	<0.001		
HBsAg, lg IU/mL	0.283(0.128,0.628)	0.002	0.209(0.067,0.649)	0.007
HBV DNA, lg IU/mL	0.851(0.638,1.135)	0.272		
HBV RNA, lg IU/mL	0.840(0.613,1.150)	0.276		
sPD-1, pg/mL	1.008(1.003,1.012)	0.001	1.008(1.001,1.015)	0.018

[a]Tbil, total bilirubin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; and sPD-1, soluble programmed cell death 1 protein.

Table 4. Univariable and Multivariable Analyses of Severe Liver Inflammation ($G \geq 3$) in HBeAg-Negative CHB Patients[1]

	univariate		multivariate	
variable	OR (95% CI)	P	OR (95% CI)	P
age	1.114(1.039,1.193)	0.002	1.164(1.016,1.334)	0.029
gender				
female	1.000		1.000	
male	0.215(0.055,0.844)	0.028		
platelet $\times 10^9$/L	0.993(0.980,1.006)	0.287	0.978(0.965,0.991)	0.001
TBil, umol/mL	1.028(0.945,1.119)	0.522		
ALB, g/L	0.958(0.896,1.026)	0.219		
ALT, U/L	1.018(1.006,1.031)	0.004	1.021(1.000,1.042)	0.051
AST, U/L	1.043(1.019,1.068)	<0.001		
GGT, U/L	1.022(1.009,1.035)	0.001		
HBsAg, lg IU/mL	0.935(0.506,1.729)	0.831		
HBV DNA, lg IU/mL	1.975(1.168,3.337)	0.011		
HBV RNA, lg IU/mL	3.587(1.569,8.201)	0.002	2.434(1.124,5.270)	0.024
sPD-1, pg/mL	1.008(1.002,1.013)	0.009	1.019(1.004,1.034)	0.011

[1]Tbil, total bilirubin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; and sPD-1, soluble programmed cell death 1 protein.

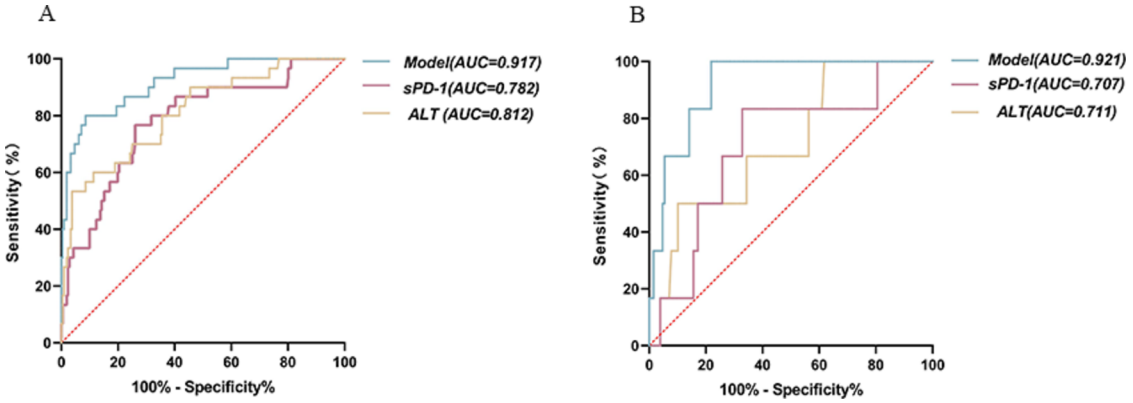


Figure 3. Comparison of AUC between different independent predictive parameters (sPD-1, ALT) and the model. (A) Comparison of AUC between sPD-1, ALT, and the model in all enrolled CHB patients. (B) Comparison of AUC between sPD-1, ALT, and the model in CHB patients with normal ALT levels.

0.002), ALT (OR, 1.013; 95%CI, 1.007−1.019; $P < 0.001$), AST (OR, 1.037; 95%CI, 1.024−1.050; $P < 0.001$), GGT (OR, 1.030; 95%CI, 1.017−1.042; $P < 0.001$), HBeAg-positive (OR, 4.347; 95%CI, 1.952−9.680; $P < 0.001$), HBV DNA (OR, 1.323; 95%CI, 1.105−1.584; $P = 0.002$), and HBV RNA (OR, 1.305; 95%CI, 1.073−1.587; $P = 0.008$) were associated

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724

with severe liver inflammation ($G \geq 3$). These indicators were then selected for the multivariate analysis. The results showed that serum sPD-1 (OR, 1.007; 95%CI, 1.001−1.012; P = 0.013), PLT (OR, 0.978; 95%CI, 0.965−0.991; P = 0.001), AST (OR, 1.025; 95%CI, 1.010−1.041; P = 0.001), and GGT (OR, 1.030; 95%CI, 1.012−1.048; P = 0.001) were independent predictors of severe liver inflammation (Table 2). Besides, we further divided CHB patients into HBeAg- and HBeAg+ groups and carried out logistics regression analysis for patients with $G \geq 3$. Our results showed that the serum sPD-1 level was still an independent predictor of severe liver inflammation in both HBeAg- and HBeAg+ CHB patients (Tables 3 and 4).

Construction of the Predictive Model and Comparison with Individual Predictors. Based on the multivariate logistic regression results, we combined sPD-1, AST, GGT, PLT, and HBeAg levels to construct a novel predictive model to predict severe liver inflammation. The model (M) is as follows:

$$M = 1/[1 + \exp-(0.025 \times \mathrm{AST} + 0.029 \times \mathrm{GGT} + 0.007 \times \mathrm{sPD} - 1 + 1.039 \times \mathrm{HBeAg} - \mathrm{positive(Yes} = 1; \mathrm{No} = 0) - 0.022 \times \mathrm{PLT} - 2.347)]$$

ROC analysis showed that the predictive model had AUROCs of 0.917 (95%CI, 0.863−0.971) for predicting severe liver inflammation, which further improved the predictive performance compared with individual predictors such as sPD-1[0.782 (95% CI,0.692−0.871)] and ALT [0.812 (95% CI,0.726−0.897)] (Figure 3). In addition, we also evaluated the predictive performance of the model in CHB patients with ALT ≤ 1× ULN. In the present study, there were 134 patients with ALT ≤ 1× ULN, of which 6 patients were diagnosed with severe liver inflammation by liver biopsy. The AUROCs of the predictive model, sPD-1, and ALT levels were 0.921 (95% CI,0.851−0.991), 0.707 (95% CI,0.503−0.911), and 0.711 (95% CI,0.517−0.905), respectively. In CHB patients with ALT ≤ 1× ULN, the predictive model still had excellent performance in predicting severe liver inflammation, and its AUROC was higher than that of sPD-1 and ALT alone.

DISCUSSION

During HBV infection, the host antiviral immune response can cause hepatocyte damage and liver inflammation while eliminating the virus. Besides, viral proteins and nucleic acids also contribute to hepatocyte damage and liver inflammation. Persistent liver inflammation promotes the disease progression from CHB to cirrhosis and HCC.[25−28]

Several studies have shown that sPD-1 expression is upregulated in viral infections, such as HCV and HIV, and autoimmune diseases including rheumatoid arthritis and autoimmune hepatitis. In these diseases, sPD-1 was associated with disease activity and progression.[16−18,29] In chronic HBV infection, the expression of sPD-1 is also increased and correlates with liver disease progression.[21,30,31] In our study, the results showed that serum sPD-1 was highest in CHB patients with severe liver inflammation ($G \geq 3$), and higher than in CHB patients with mild (G0) and moderate (G2) liver inflammation. Zhou et al. also found that the levels of sPD-1 in CHB patients with moderate-to-severe liver inflammation were higher than those in patients with mild inflammation.[31] Besides, we also found that serum sPD-1 was positively correlated with the inflammatory stages. Our results suggest that serum sPD-1 is associated with liver inflammation in CHB patients and higher serum sPD-1 levels may indicate severe inflammation in the liver of CHB patients. Our results showed that serum sPD-1 had a weak positive correlation with AST and ALT levels in CHB patients, which may result from the different production mechanisms between sPD-1 and ALT and AST levels in the condition of liver inflammation in CHB patients. This weak correlation may indicate the uniqueness of sPD-1 in reflecting liver inflammation and may shed new light on understanding liver inflammation. A previous study showed that serum sPD-1 levels were higher in CHB patients with moderate-to-severe liver fibrosis than in those with mild fibrosis.[31] However, in our study, there was no significant association between PD-1 and hepatic fibrosis, which may be due to the different distribution of patients with hepatic fibrosis in the two studies. The correlation between serum sPD-1 levels and liver fibrosis in CHB patients should be validated in a large clinical cohort study.

The severity of liver inflammation is one of the indicators for initiating antiviral treatment for chronic HBV infection.[1,4] Liver enzymes including ALT and AST are commonly used to reflect the activity of liver inflammation.[5] However, about 20% of CHB patients with normal ALT levels have significant liver inflammation.[33] Liver biopsy is the gold standard for the diagnosis of liver inflammation,[8] but the invasiveness of liver biopsy procedure limits its application for mass screening. Therefore, it is necessary to identify new noninvasive markers of liver inflammation. Recently, several emerging novel noninvasive biomarkers have been reported for liver inflammation and fibrosis, including Golgi protein-73(GP73), chitinase 3-like 1(CHI3L1), and pro-peptides of type III collagen (Pro-C3). First, GP73 is a novel type II Golgi-localized integral membrane protein and is considered as an optimal biomarker for liver inflammation and fibrosis among HBV patients with normal or slightly elevated ALT levels. Nevertheless, the sensitivity and specificity of gp73 for liver inflammation were 43.59 and 97.18%, respectively. At the same time, the sensitivity and specificity of gp73 for liver fibrosis were 30.70 and 96.23%, respectively.[34] Additionally, CHI3L1 is a fibroblast growth factor associated with liver fibrosis in patients with chronic liver diseases.[35] Serum CHI3L1 had high diagnostic efficiency for liver fibrosis staging, with a sensitivity and specificity of 80.00 and 71.05%, respectively, which might serve as a diagnostic biomarker for liver fibrosis in patients with HBeAg-negative CHB.[36] Besides, Pro-C3 is a potential biomarker for liver fibrosis with robust evidence of an association of PRO-C3 with increasing liver fibrosis.[37,38] In our study, our established predictive model achieved superior diagnostic efficiency for liver inflammation in patients with CHB, with a sensitivity of 80.00% and a specificity of 91.50%, which might serve as a new biomarker for liver inflammation and fibrosis among CHB patients. We used logistic regression to evaluate the role of sPD-1 as a potential biomarker for predicting severe liver inflammation in CHB patients. Multivariate analysis showed that the serum sPD-1 level was an independent predictor of severe liver inflammation ($G \geq 3$) in CHB patients. In patients with chronic HBV infection, serum HBeAg is associated with viral replication, inflammation, disease activity, and response to antiviral therapy.[32] Therefore, we further analyzed CHB patients with different HBeAg statuses, which revealed that serum sPD-1 in CHB patients

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724

with $G \geq 3$ was higher than that in CHB patients with $G < 3$ and was an independent predictor of severe liver inflammation in both HBeAg- and HBeAg+ CHB patients. These results indicate that serum sPD-1 is an independent risk factor for severe liver inflammation in CHB patients, and that patients with higher serum sPD-1 levels had a higher risk of severe liver inflammation. In CHB patients, researchers have found that liver enzymes (ALT, AST, and GGT), viral parameters (HBsAg, HBeAg, anti-HBc), and PLT were also independently associated with liver inflammation.[39−43] Consistent with these studies, we also found that AST, GGT, and PLT were independent predictors of liver inflammation in CHB patients.

Individual parameters are affected by patients' demographics and other factors. We combined sPD-1, AST, GGT, HBeAg, and PLT levels to construct a predictive model for liver inflammation in CHB patients. Compared to a single parameter, the predictive model improved the predictive ability for liver inflammation (AUROC = 0.917, 95%CI, 0.863−0.971). In order not to miss the optimal opportunity to receive therapeutic intervention, it is of great significance to monitor liver inflammation in CHB patients with normal ALT levels. In total, 134 patients in our study cohort had normal ALT levels. In this subset of patients, the predictive model also showed good predictive performance (AUROC = 0.921, 95% CI, 0.851−0.991) using liver biopsy as the gold standard for the diagnosis of liver inflammation. These results suggest that serum sPD-1 can be used as a predictive biomarker for severe liver inflammation, and its predictive performance was further improved when it was combined with other indicators.

Our study had some limitations. The CHB patients enrolled in the present study were relatively small; furthermore, only 30 CHB patients were with $G \geq 3$. At the time of this study, we focused on the ability of sPD-1 to reflect liver inflammation in the early stages of liver damage; CHB patients with severe liver inflammation ($G \geq 3$) were not our primary focus. Therefore, a large-scale cohort including CHB patients with varying degrees of liver inflammation would be desirable for further confirmation of the findings in our study.

In conclusion, our results suggest that serum sPD-1 is associated with the degree of liver inflammation in CHB patients, and high levels of serum sPD-1 levels reflect severe liver inflammation. Besides, we constructed a model based on the noninvasive biomarkers, sPD-1, AST, GGT, HBeAg, and PLT, which have good performance in predicting CHB patients with severe liver inflammation regardless of their ALT levels and may help to decide whether to initiate antiviral treatment.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

■ AUTHOR INFORMATION

Corresponding Authors

Yuxin Chen − *Department of Laboratory Medicine, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, Jiangsu 210008, China*; orcid.org/0000-0001-5955-687X; Phone: 025-83106666; Email: yuxin.chen@nju.edu.cn

Yijia Zhu − *Department of Laboratory Medicine, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, Jiangsu 210008, China*; Phone: 025-83106666; Email: 1015292337@qq.com

Authors

Mingrong Ou − *Department of Laboratory Medicine, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, Jiangsu 210008, China*

Weiming Zhang − *Department of Laboratory Medicine, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

Wen Zhang − *Department of Laboratory Medicine, Joint Institute of Nanjing Drum Tower Hospital for Life and Health, College of Life Science, Nanjing Normal University, Nanjing, Jiangsu 210008, China*

Jianmin Guo − *ET HealthCare, Shanghai 201203, China*

Rui Huang − *Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

Jian Wang − *Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

Jiacheng Liu − *Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

Juan Xia − *Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

Chao Wu − *Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c00780

Author Contributions

#M.O., W.Z., and W.Z. contributed equally to this work. Study conception and design (Y.X.C., Y.J.Z., C.W.); acquisition of data; analysis, and interpretation of data (M.R.O., W.M.Z., R.H., J.M.G.); drafting of the manuscript (M.R.O., W.M.Z., W.Z.); statistical analysis and analysis of data (M.R.O., J.C.L., J.X.); and study supervision; critical revision of the manuscript for important intellectual content (Y.X.C., Y.J.Z., C.W., J.X.).

Notes

The authors declare no competing financial interest.

Ethics Statements Written informed consent was obtained from each patient. This research protocol has been approved by the Medical Ethics Committee of Nanjing Drum Tower Hospital (ethics number: 2008022). This work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

■ ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Program (project number 2023YFC2309100), National Science Foundation of China (Grant numbers 92269118, 92269205), Nanjing Important Science & Technology Specific Projects (project number 2021-11005), and Scientific Research Project of Jiangsu Health Commission (M2022013).

REFERENCES

(1) Lampertico, P.; Agarwal, K.; Berg, T.; et al. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J. Hepatol.* **2017**, *67* (2), 370−398, DOI: 10.1016/j.jhep.2017.03.021.

(2) Trépo, C.; Chan, H. L.; Lok, A. Hepatitis B virus infection. *Lancet* **2014**, *384* (9959), 2053−2063.

(3) Russo, F. P.; Rodríguez-Castro, K.; Scribano, L.; Gottardo, G.; Vanin, V.; Farinati, F. Role of antiviral therapy in the natural history of hepatitis B virus-related chronic liver disease. *World J. Hepatol.* **2015**, *7* (8), 1097−1104.

(4) Terrault, N. A.; Lok, A.; McMahon, B. J.; Chang, K. M.; Hwang, J. P.; Jonas, M. M.; et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* **2018**, *67* (4), 1560−1599.

(5) Kim, W. R.; Flamm, S. L.; Di Bisceglie, A. M.; Bodenheimer, H. C. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* **2008**, *47* (4), 1363−1370.

(6) Alam, S.; Ahmad, N.; Mustafa, G.; Shrestha, A.; Alam, A. K.; Khan, M. Evaluation of normal or minimally elevated alanine transaminase, age and DNA level in predicting liver histological changes in chronic hepatitis B. *Liver Int.* **2011**, *31* (6), 824−830.

(7) Zhou, J.; Song, L.; Zhao, H.; Yan, L.; Ma, A.; Xie, S.; et al. Serum hepatitis B core antibody as a biomarker of hepatic inflammation in chronic hepatitis B patients with normal alanine aminotransferase. *Sci. Rep.* **2017**, *7* (1), No. 2747, DOI: 10.1038/s41598-017-03102-3.

(8) Piccinino, F.; Sagnelli, E.; Pasquale, G.; Giusti, G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J. Hepatol.* **1986**, *2* (2), 165−173.

(9) Nishimura, H.; Agata, Y.; Kawasaki, A.; Sato, M.; Imamura, S.; Minato, N.; et al. Developmentally regulated expression of the PD-1 protein on the surface of double-negative (CD4-CD8-) thymocytes. *Int. Immunol.* **1996**, *8* (5), 773−780.

(10) Terawaki, S.; Chikuma, S.; Shibayama, S.; Hayashi, T.; Yoshida, T.; Okazaki, T.; Honjo, T. IFN-α directly promotes programmed cell death-1 transcription and limits the duration of T cell-mediated immunity. *J. Immunol.* **2011**, *186* (5), 2772−2779.

(11) Austin, J. W.; Lu, P.; Majumder, P.; Ahmed, R.; Boss, J. M. STAT3, STAT4, NFATc1, and CTCF regulate PD-1 through multiple novel regulatory regions in murine T cells. *J. Immunol.* **2014**, *192* (10), 4876−4886.

(12) Latchman, Y. E.; Liang, S. C.; Wu, Y.; Chernova, T.; Sobel, R. A.; Klemm, M.; et al. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101* (29), 10691−10696.

(13) Fukasawa, T.; Yoshizaki, A.; Ebata, S.; Nakamura, K.; Saigusa, R.; Miura, S.; et al. Contribution of Soluble Forms of Programmed Death 1 and Programmed Death Ligand 2 to Disease Severity and Progression in Systemic Sclerosis. *Arthritis Rheumatol.* **2017**, *69* (9), 1879−1890.

(14) Reignat, S.; Webster, G. J.; Brown, D.; Ogg, G. S.; King, A.; Seneviratne, S. L.; et al. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. *J. Exp. Med.* **2002**, *195* (9), 1089−1101.

(15) Nielsen, C.; Ohm-Laursen, L.; Barington, T.; Husby, S.; Lillevang, S. T. Alternative splice variants of the human PD-1 gene. *Cell. Immunol.* **2005**, *235* (2), 109−116.

(16) Wang, D.; Zhou, D.; Du, Q.; Liang, Q.; Wang, Q.; Fang, L.; et al. Aberrant production of soluble inducible T-cell co-stimulator (sICOS) and soluble programmed cell death protein 1 (sPD-1) in patients with chronic hepatitis C. *Mol. Med. Rep.* **2013**, *7* (4), 1197−1202.

(17) Zilber, E.; Martin, G. E.; Willberg, C. B.; Fox, J.; Nwokolo, N.; Fidler, S.; Frater, J. Soluble plasma programmed death 1 (PD-1) and Tim-3 in primary HIV infection. *Aids* **2019**, *33* (7), 1253−1256.

(18) Aarslev, K.; Dige, A.; Greisen, S. R.; Kreutzfeldt, M.; Jessen, N.; Vilstrup, H.; et al. Soluble programmed death-1 levels are associated with disease activity and treatment response in patients with autoimmune hepatitis. *Scand. J. Gastroenterol.* **2017**, *52* (1), 93−99.

(19) Wang, J.; Zheng, X.; Jiang, Y.; Jia, H.; Shi, X.; Han, Y.; et al. Soluble Programmed Cell Death Protein 1 and Its Ligand: Potential Biomarkers to Predict Acute Kidney Injury After Surgery in Critically Ill Patients. *J. Inflammation Res.* **2022**, *15*, 1995−2008.

(20) Cheng, H. Y.; Kang, P. J.; Chuang, Y. H.; Wang, Y. H.; Jan, M. C.; Wu, C. F.; et al. Circulating programmed death-1 as a marker for sustained high hepatitis B viral load and risk of hepatocellular carcinoma. *PLoS One* **2014**, *9* (11), No. e95870.

(21) Huyen, P. T. M.; Dung, D.; Weiß, P. J.; Hoan, P. Q.; Giang, D. P.; Uyen, N. T.; et al. Circulating level of sPD-1 and PD-1 genetic variants are associated with hepatitis B infection and related liver disease progression. *Int. J. Infect. Dis.* **2022**, *115*, 229−236.

(22) Tan, N.; Luo, H.; Kang, Q.; Pan, J.; Cheng, R.; Xi, H.; et al. High levels of soluble programmed death-1 are associated with virological response in chronic hepatitis B patients after antiviral treatment. *Virus Res.* **2022**, *309*, No. 198660.

(23) Scheuer, P. J. Classification of chronic viral hepatitis: a need for reassessment. *J. Hepatol.* **1991**, *13* (3), 372−374.

(24) Zhang, J.; Chen, L.; Xu, Q.; Tao, Y.; Pan, J.; Guo, J.; et al. An automated, rapid fluorescent immunoassay to quantify serum soluble programmed death-1 (PD-1) protein using testing-on-a-probe biosensors. *Clin. Chem. Lab. Med.* **2022**, *60* (7), 1073−1080.

(25) Wu, J.; Han, M.; Li, J.; Yang, X.; Yang, D. Immunopathogenesis of HBV Infection. *Adv. Exp. Med. Biol.* **2020**, *1179*, 71−107.

(26) Zeng, C.; Wang, Y. L.; Xie, C.; Sang, Y.; Li, T. J.; Zhang, M.; et al. Identification of a novel TGF-β-miR-122-fibronectin 1/serum response factor signaling cascade and its implication in hepatic fibrogenesis. *Oncotarget* **2015**, *6* (14), 12224−12233.

(27) Shi, Y.; Wang, J.; Wang, Y.; Wang, A.; Guo, H.; Wei, F.; et al. A novel mutant 10Ala/Arg together with mutant 144Ser/Arg of hepatitis B virus X protein involved in hepatitis B virus-related hepatocarcinogenesis in HepG2 cell lines. *Cancer Lett.* **2016**, *371* (2), 285−291.

(28) Yang, G.; Wan, P.; Zhang, Y.; Tan, Q.; Qudus, M. S.; Yue, Z.; et al. Innate Immunity, Inflammation, and Intervention in HBV Infection. *Viruses* **2022**, *14* (10), No. 2275, DOI: 10.3390/v14102275.

(29) Xu, L.; Jiang, L.; Nie, L.; Zhang, S.; Liu, L.; Du, Y.; Xue, J. Soluble programmed death molecule 1 (sPD-1) as a predictor of interstitial lung disease in rheumatoid arthritis. *BMC Immunol.* **2021**, *22* (1), No. 69, DOI: 10.1186/s12865-021-00460-6.

(30) Xia, J.; Huang, R.; Chen, Y.; Liu, Y.; Wang, J.; Yan, X.; et al. Profiles of serum soluble programmed death-1 and programmed death-ligand 1 levels in chronic hepatitis B virus-infected patients with different disease phases and after anti-viral treatment. *Aliment. Pharmacol. Ther.* **2020**, *51* (11), 1180−1187.

(31) Zhou, L.; Li, X.; Huang, X.; Chen, L.; Gu, L.; Huang, Y. Soluble programmed death-1 is a useful indicator for inflammatory and fibrosis severity in chronic hepatitis B. *J. Viral Hepatitis* **2019**, *26* (7), 795−802.

(32) Kramvis, A.; Kostaki, E. G.; Hatzakis, A.; Paraskevis, D. Immunomodulatory Function of HBeAg Related to Short-Sighted Evolution, Transmissibility, and Clinical Manifestation of Hepatitis B Virus. *Front. Microbiol.* **2018**, *9*, No. 2521, DOI: 10.3389/fmicb.2018.02521.

(33) Kennedy, P. T.; Sandalova, E.; Jo, J.; Gill, U.; Ushiro-Lumb, I.; Tan, A. T.; et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. *Gastroenterology* **2012**, *143* (3), 637−645.

(34) Wei, M.; Xu, Z.; Pan, X.; Zhang, X.; Liu, L.; Yang, B.; Chen, Y. Serum GP73 - An Additional Biochemical Marker for Liver Inflammation in Chronic HBV Infected Patients with Normal or Slightly Raised ALT. *Sci. Rep.* **2019**, *9* (1), No. 1170.

(35) Yan, L.; Deng, Y. Q.; Zhou, J. Y.; Zhao, H.; Wang, G. Q.; China HepB-Related Fibrosis Assessment Research Group. Serum YKL-40 as a biomarker for liver fibrosis in chronic hepatitis B patients with normal and mildly elevated ALT. *Infection* **2018**, *46*, 385−393, DOI: 10.1007/s15010-018-1136-2.

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724

(36) Li, Y.; Li, C.; Zhang, L.; Hu, W.; Luo, H.; Li, J.; Qiu, S.; Zhu, S. Serum CHI3L1 as a diagnostic marker and risk factor for liver fibrosis in HBeAg-negative chronic hepatitis B. *Am. J. Transl. Res.* **2022**, *14* (6), 4090−4096.
(37) Erhardtsen, E.; Rasmussen, D. G. K.; Frederiksen, P.; Leeming, D. J.; Shevell, D.; Gluud, L. L.; Karsdal, M. A.; Aithal, G. P.; Schattenberg, J. M. Determining a healthy reference range and factors potentially influencing PRO-C3 - A biomarker of liver fibrosis. *JHEP Rep.* **2021**, *3* (4), No. 100317.
(38) Mak, A. L.; Lee, J.; van Dijk, A. M.; Vali, Y.; Aithal, G. P.; Schattenberg, J. M.; Anstee, Q. M.; Brosnan, M. J.; Zafarmand, M. H.; Ramsoekh, D.; Harrison, S. A.; Nieuwdorp, M.; Bossuyt, P. M.; Holleboom, A. G. Systematic Review with Meta-Analysis: Diagnostic Accuracy of Pro-C3 for Hepatic Fibrosis in Patients with Non-Alcoholic Fatty Liver Disease. *Biomedicines* **2021**, *9* (12), No. 1920, DOI: 10.3390/biomedicines9121920.
(39) Zeng, D. W.; Dong, J.; Jiang, J. J.; Zhu, Y. Y.; Liu, Y. R. Ceruloplasmin, a reliable marker of fibrosis in chronic hepatitis B virus patients with normal or minimally raised alanine aminotransferase. *World J. Gastroenterol.* **2016**, *22* (43), 9586−9594.
(40) Li, Q.; Zhou, Y.; Huang, C.; Li, W.; Chen, L. A novel diagnostic algorithm to predict significant liver inflammation in chronic hepatitis B virus infection patients with detectable HBV DNA and persistently normal alanine transaminase. *Sci. Rep.* **2018**, *8* (1), No. 15449.
(41) Li, Q.; Huang, C.; Xu, W.; Hu, Q.; Chen, L. A simple algorithm for non-invasive diagnosis of significant liver histological changes in patients with CHB and normal or mildly elevated alanine transaminase levels. *Medicine* **2019**, *98* (28), No. e16429.
(42) Chen, S.; Huang, H. Clinical Non-invasive Model to Predict Liver Inflammation in Chronic Hepatitis B With Alanine Aminotransferase $\leq$ 2 Upper Limit of Normal. *Front. Med.* **2021**, *8*, No. 661725.
(43) Xie, Y.; Yi, W.; Zhang, L.; Lu, Y.; Hao, H. X.; Gao, Y. J.; et al. Evaluation of a logistic regression model for predicting liver necroinflammation in hepatitis B e antigen-negative chronic hepatitis B patients with normal and minimally increased alanine aminotransferase levels. *J. Viral Hepatitis* **2019**, *26* (1), 42−49.

https://doi.org/10.1021/acsomega.4c00780
ACS Omega 2024, 9, 16716−16724