Serum Soluble Programmed Death Ligand 1 is Correlated with HBsAg Level and Nucleos(t)ide Analogue Therapy in Chronic Hepatitis B

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Citation:

Ke RM, et al. Serum Soluble Programmed Death Ligand 1 is Correlated with HBsAg Level and Nucleos(T)Ide Analogue Therapy in Chronic Hepatitis B. Japanese J Gstro Hepato.

2021; V6(3): 1-9

#Author Contributions:

Ke RM and Dong WX have contributed equally to this work. XMP is the guarantor of the article. XMP and MXH brought the concept; RMK, LJO and WFL collected the data and performed the tests; XMP, MXH and WFL made the statistical analysis and wrote the paper; All co-authors approved the final version of the paper.

Keywords:

HBV; Soluble PD L1; Antiviral immunity; Immune checkpoint; Antiviral therapy; Nucleotide analogues

Abbreviations:

AFP: α-Fetoprotein; ALB: Albumin; ALT: Alanine Amino Transferase; APRI: Aspartate Aminotransferase Platelet Ratio Index; AST: Aspartate Aminotransferase; CHB: Chronic Hepatitis B; COI: Cut-Off Index; HBeAg: Hepatitis B e Antigen; HBsAg: Hepatitis B Surface Antigen; HBV: Hepatitis B Virus; HCC: Hepatocellular Carcinoma; IA: Immune-Active; IC: Inactive; IFN: Interferon; IQR: Interquartile Range; IT: Immune-Tolerant; LC: Liver Cirrhosis; NUCs: Nucleos(t)ide Analogues; PD-1: Programmed Cell Death-1; PD-L1: Programmed Death Ligand 1; PLT: Platelet; sPD-L1: soluble PD-L1; TBIL: Total Bilirubin.

1. Abstract

1.1. Aims: Restoration of immune responses is considered as a complementary approach to Nucleos(t)ide analogue (NUC) therapy for chronic Hepatitis B Virus (HBV) infection. Antiviral immunity is negatively regulated by the Programmed cell Death-1 (PD-1)/Programmed Death Ligand 1 (PD-L1) axis. In the present study, soluble PD-L1 (sPD-L1), which represents the amount of PD-L1+ cells, was used as an indicator to investigate the involvement of the PD-1/PD-L1 axis in chronic HBV infection, particularly in the setting of NUC therapy.

1.2. Methods: A total of 273 adult patients with chronic HBV infection, regardless of treatment, and 86 healthy controls were enrolled. Serum sPD-L1 levels were measured by performing an ELISA. The correlations between sPD-L1 and clinical/virological characteristics were analyzed.

1.3. Results: Serum sPD-L1 levels in patients with chronic HBV infection [median, 425.2; Interquartile Range (IQR), 245.8-558.6] were significantly higher compared with healthy controls (median, 81.69; IQR, 54.62-121.1). Among patients at various disease phases, patients with immune-tolerant Chronic Hepatitis B (CHB) displayed the lowest sPD-L1 levels (median, 205.3; IQR, 92.27-340.7). The results indicated that serum sPD-L1 was significantly increased in two-step manner in chronic HBV infection from health to infection, and from immune tolerance to immune activation. Furthermore, serum sPD-L1 in patients with immune-active CHB was positively correlated with Hepatitis B surface Antigen (HBsAg), negatively correlated with HBV DNA and marginally correlated with liver damage. Interestingly, increased serum sPD-L1 levels were strongly associated with NUC treatment, particularly in HBeAg-positive patients with immune-active CHB.

1.4. Conclusion: The results of the present study collectively suggested that serum sPD-L1 may serve as an indicator to monitor immune status and disease progression in chronic HBV infection. The correlations between increased sPD-L1 levels and HBsAg or NUC treatment suggested that the activated PD-1/PD-L1 axis may pro-

vide an explanation for the rarity of HBsAg seroconversion in NUC therapy. Moreover, clinically available checkpoint inhibitors may serve as partners for NUC therapy to improve anti-HBV efficacy.

2. Introduction

Chronic Hepatitis B Virus (HBV) infection is a leading public health problem despite the development of efficient vaccines and antiviral therapies based on Nucleos(t)ide analogues (NUCs) and recombinant Interferon α (rIFNα) [1,2]. The primary causes for the poor prognosis associated with chronic HBV infection are Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC). To reduce the risk of LC and HCC, long-term NUC treatment is the most common therapeutic strategy; however, Hepatitis B surface Antigen (HBsAg) clearance and seroconversion with long-term NUC treatment is rare and slow [1,3]. In addition, even in NUC-treated patients with serum HBV DNA levels below the detection limit, HCC occurrence is delayed, but not completely eliminated [4,5]. By contrast, due to its immune-regulating potential, rIFNα results in the loss of HBsAg in ~10% of eligible patients [6]. Reactivation of HBV infection upon immunosuppression occurs in inactive Chronic Hepatitis B (CHB), even in patients with resolved acute HBV infections [7]. Therefore, host immunity is important for HBV infection control; however, the immune mechanisms limiting HBsAg seroconversion during NUC therapy are not completely understood.

Immune checkpoint signaling pathways regulate optimal host immune responses against transformed or virus-infected cells [8]. One of the immune checkpoint mechanisms is the Programmed cell Death-1 (PD-1)/Programmed Death Ligand 1 (PD-L1) axis [9,10]. PD-1 is expressed on T-cells, and PD-L1 is expressed on transformed cells, professional antigen-presenting cells and hepatocytes. After antigen stimulation, the PD-1/PD-L1 axis regulates the magnitude and quality of T-cell responses by cellular exhaustion. The chronicity of cancer and persistent infections leads to cellular exhaustion and abrogation of antigen-reactive T cells by providing constant antigen exposure [11,12]. At present, there are at least three clinically available anti-PD-1 antibodies (pembrolizumab, nivolumab and atezolizumab) that can be used to block the PD-1/PD-L1 axis, which display effective antitumor activities and controllable adverse effects [13]. As for HBV infection, CD4+ T cell exhaustion is induced by high PD-1 and lymphocyte-activation gene 3 expression in CHB [14,15], and HBsAg is the most abundant viral protein in the liver and peripheral blood of patients with chronic HBV infection. HBsAg impairs immune responses by upregulating PD-L1 expression on monocytes [16]. HBV also promotes the expression of PD-L1 on hepatocytes in cell models and transgenic mice, which correlates with the levels of HBsAg, Hepatitis B e Antigen (HBeAg) and HBV DNA in mouse sera [17]. Blockage of the PD-1/PD-L1 axis using an anti-PD-1 antibody can enhance virus-specific immunity in the woodchuck model of HBV infection [18]. Moreover, nivolumab therapy is well tolerated and leads to HBsAg decline in most virally suppressed HBeAg-negative patients, as reported in a phase Ib study [19]. Therefore, blockage of the PD-1/PD-L1 axis may serve as a therapeutic strategy to restore antiviral immune responses in chronic HBV infection. However, whether the PD-1/PD-L1 axis is involved in limiting HBsAg seroconversion during NUC therapy is not completely understood.

Despite its importance to HBV infection, PD-L1 is rarely used to monitor disease progression or make treatment decisions, partially because of the difficulty in measuring PD-L1 expression levels due to its intrahepatic expression. Soluble (s)PD-L1 can be conveniently detected in sera from patients with cancer or viral infections. sPD-L1 is correlated with the number of PD-L1+ cells and is considered as a helpful indicator for monitoring immune status and disease progression during HCC and hepatitis C virus infection [20-23]. Serum sPD-L1 levels in CHB have been reported to be lower or slightly higher compared with healthy controls in two small sample studies (n=22 and 30, respectively) where patients with CHB served as controls [21,22]. Therefore, the significance of serum sPD-L1 to chronic HBV infection, especially in the setting of NUC therapy, is not completely understood.

In the present study, serum sPD-L1 was significantly increased in patients with chronic HBV infection. Serum sPD-L1 concentrations were positively correlated with HBsAg, marginally correlated with alanine Aminotransferase (ALT) and negatively correlated with HBV DNA. Moreover, serum sPD-L1 levels were significantly increased in NUC-treated patients. The results of the present study may aid with the identification of novel therapeutic strategies for chronic HBV infection.

3. Materials and Methods

3.1. Subjects and Samples

Adult patients with chronic HBV infection (positive HBsAg for>6 months regardless of treatment) were recruited from the liver clinic of the Fifth Affiliated Hospital of Sun Yat-sen University between May 2017 and October 2018. Healthy volunteers (healthy controls; negative HBsAg) were recruited from the physical examination center of the Fifth Affiliated Hospital of Sun Yat-sen University. The following exclusion criteria were used in the present study: other hepatitis viruses (A, C, D and E), human immunodeficiency virus, cardiovascular disease, diabetes, kidney disease, pregnancy or autoimmune disease. According to recent guidelines provided by the American Association for the Study of Liver Diseases and others [24,25], chronic HBV infection was further classified as follows: Immune-Tolerant CHB (CHB-IT), Immune-Active CHB (CHB-IA), inactive CHB (CHB-IC), LC and HCC. Blood samples were collected from each individual, centrifuged at 4000 x g for 10 min to obtain serum and stored at -80˚C until further analysis. The present study was approved by the Institutional Review Board of the Fifth Affiliated Hospital of Sun Yat-sen University.

3.2. Serum sPD-L1 Quantification

Serum sPD-L1 levels in patients and healthy controls were measured using a specific ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd.) according to the manufacturer's protocol. The detection limit of the assay was 1.0 pg/ml. Each sample was tested in duplicate.

3.3. Serum Viral Marker Assays

Serum HBV DNA was quantified by performing reverse transcription-PCR using COBAS TaqMan (Roche Molecular Diagnostics) with a lower limit of quantitation of 12 IU/ml and a linear dynamic range of 2.0x101-1.7x108 IU/ml. HBsAg, anti-HBs, HBeAg, an- ti-HBe and anti-hepatitis B core antigen were measured using chemiluminescence assay kits (Roche Diagnostics GmbH). HBsAg was quantified with a low limit of 0.05 IU/ml. HBeAg was semi-quantitatively measured and the result is presented as a Cut-Off Index (COI).

3.4. Routine Blood, Liver Function and A-Fetoprotein Assays

White blood cell, red blood cell and Platelet (PLT) counts were measured using a Sysmex XN-2000™ Hematology System (Sysmex Corporation). Liver function profiles, including ALT, aspartate Aminotransferase (AST), total bilirubin and Albumin (ALB), were measured using a 7600-020 (ISE) Automatic Analyzer (Hitachi Ltd.). Serum α-Fetoprotein (AFP) was measured using an electrochemiluminescence immunoassay.

3.5. Liver Biopsy

Liver biopsies were performed in 20 patients, including 15 patients with CHB-IA, following standard procedures. The severity of inflammation (grade) and the degree of fibrosis (stage) were independently determined by two pathologists.

3.6. Miscellaneous

Fibrosis estimators, AST platelet ratio index [APRI = (AST/upper limit of normal) x/ 100/platelet count] and Fibrosis-4 (FIB-4) [age (years) x AST (IU/l)/platelet count (109/l) x ALT (IU/l)1/2], were calculated using routine laboratory values [26].

3.7. Statistical Analysis

Continuous variables are presented as the median ± interquartile range. Categorical variables are presented as n (%). The significance of differences was analyzed using the χ^2 test, Fisher's exact test or Mann-Whitney U test, as appropriate. Spearman's rank correlation test was used to assess the correlation between sPD-L1 and detection markers. The association between age/sex and serum sPD-L1 levels in patients with chronic HBV infection was analyzed using two-way Analysis of Variance (ANOVA). Bonferroni correction analyses were conducted when there were multiple comparisons. Statistical analyses were performed using SPSS software (version 18; IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

4. Results

4.1. Demographic and Clinical Characteristics of Patients and Healthy Controls

A total of 273 patients with chronic HBV infection and 86 healthy controls were included in the analysis. The demographic and disease characteristics of the patients and healthy controls are presented in (Table 1). All patients and healthy controls were Chinese.

Table 1: Demographics and disease characteristics of subjects (Continuous variables: median, IQR†)

	Chronic HBV infection					Healthy controls
	CHB-IT (n =12)	**CHB-IA (n =184)**	**CHB-IC n=36**	**LC (n =28)**	**HCC (n =13)**	**(n =86)**
Gender (M/F)	8-Apr	135/49	19/17	24/4	3-Oct	78/8
Age (Years old)	28.0 (25.0-36.5)	37.0 (30.0-47.0)*	37.5 (32.3-44.8)*	47.5 (41.3-55.5)*	58.0 (50.5-63.5)*	24.0 (22.0-26.3)
ALT (U/L)	32.5 (24.5-38.8)*	26.0 (19.0-46.3)*	19.0 (14.0-29.5)	31.0 (23.0-41.5)*	35.0 (28.5-60.0)*	17.0 (12.0-23.3)
AST (U/L)	26.5 (20.0-37.0)*	24.0 (19.0-32.3)*	20.0 (17.0-23.8)	29.5 (24.0-45.8)*	41.0 (30.0-137.5)*	19.0 (17.0-22.3)
ALB (g/L)	40.7 (37.4-43.5)*	44.9 (43.1-47.5)	44.7 (42.5-46.6)	42.5 (6.1-46.1)	38.2 (36.4-40.3)*	44.5 (44.0-47.5)
TBil (mmol/L)	10.9 (7.7-13.05)	12.1 (9.6-17.0)	13.3 (10.9-14.6)	17.4 (11.1-26.1)*	17.5 (11.4-20.2)	12.3 (10.1-16.2)
AFP (ng/mL)	2.8 (1.4-4.3)	2.5 (1.8-3.7)	2.4 (1.5-3.0)	2.5 (1.6-4.4)	6.4 (3.2-2670.2)*	2.6 (1.9-4.3)
PLT (10⁹/L)	241 (190-315)	204 (175-239)*	209(184-236)*	104 (75-176)*	185 (155-276)*	239 (211-277)
HBV DNA (IU/mL)‡	8.23 (8.01-8.23)	1.30 (1.30-3.55)	2.02 (1.3-2.72)	1.30 (1.08-1.30)	2.06 (1.30-4.55)	-
HBsAg (IU/mL)‡	4.23 (3.40-4.72)	3.26 (2.76-3.52)	2.59 (1.67-3.38)	2.91 (2.28-3.26)	2.94 (2.54-3.64)	-
HBeAg						
Positive	12	76	0	6	2	-
Negative	0	108	36	22	11	-
NUC treatment§						
ETV	0	59	0	13	8	-
TDF/TAF	0	52	0	5	1	-
LAM	0	3	0	0	1	-
LdT	0	1	0	1	0	-
ADV	0	5	0	0	0	-

4.2. Serum Spd-L1 Levels are Increased in Patients with Chronic HBV Infection

Serum sPD-L1 levels were significantly increased in patients with chronic HBV infection (median, 425.2; ITQ, 245.8-558.6) compared with healthy controls (median, 81.69; IQR, 54.62-121.1) (Figure 1A). Serum sPD-L1 levels in each phase of chronic HBV infection were significantly higher compared with healthy controls (Figure 1B). In addition, patients in the CHB-IT phase displayed relatively lower lev-

els of serum sPD-L1 (median, 205.3; IQR, 92.27-340.7) compared with patients in other phases, but its difference with CHB-IC, LC and HCC did not survive Bonferroni correction analyses. There was no significant difference among the CHB-IA, CHB-IC, LC and HCC phases, although levels in the CHB-IC phase were slightly lower (Figure 1B). Since the average age was significantly different among some subgroups of patients with HBV infection and healthy controls (Table I), the influence of age and sex on serum sPD-L1 was analyzed by performing two-way ANOVA (Figure 1C). Neither age (P=0.639) nor sex (P=0.991) were significant determinants of serum sPD-L1 in patients with chronic HBV infection. The results indicated that serum sPD-L1 levels were significantly increased during chronic HBV infection, and the increase occurred in two major steps from health to infection, and from immunotolerance to immunoactivation.

4.3. Correlation Between Serum Spd-L1 Levels and Serum HBV Markers In Patients with CHB-IA

Patients with chronic HBV infection displayed higher serum sPD-L1 levels compared with healthy controls, which was consistent with the PD-1/PD-L1 axis serving as a negative regulator in antiviral immunity [8,9]. The present study further analyzed the correlation between serum sPD-L1 and serum HBV markers, including HBsAg, HBeAg and HBV DNA, in patients with CHB-IA. Serum sPD-L1 levels in patients with high HBsAg levels were much higher (Figure 2A), all of which survived Bonferroni correction analyses. Serum sPD-L1 levels were also weakly positively correlated with HBsAg, as indicated by Spearman's rank correlation test (Figure 2B). A HBsAg level <100 IU/ml is considered as a useful marker to discontinue NUC therapy [27]; therefore, in patients with HBsAg levels <100 IU/mL, serum sPD-L1 levels were lower (Figure 2A). However, serum sPD-L1 was not correlated with the status (Figure 2C) or COI of HBeAg (Figure 2D). As for serum HBV DNA, patients with negative levels (≤20 IU/ml) displayed significantly higher serum sPD-L1 levels compared with patients with high levels (>2,000 IU/ml) (Figure 2E), but it did not survive Bonferroni correction analysis. Concordantly, serum sPD-L1 was weakly negatively correlated with serum HBV DNA load, as determined by Spearman's rank correlation test (Figure 2F).
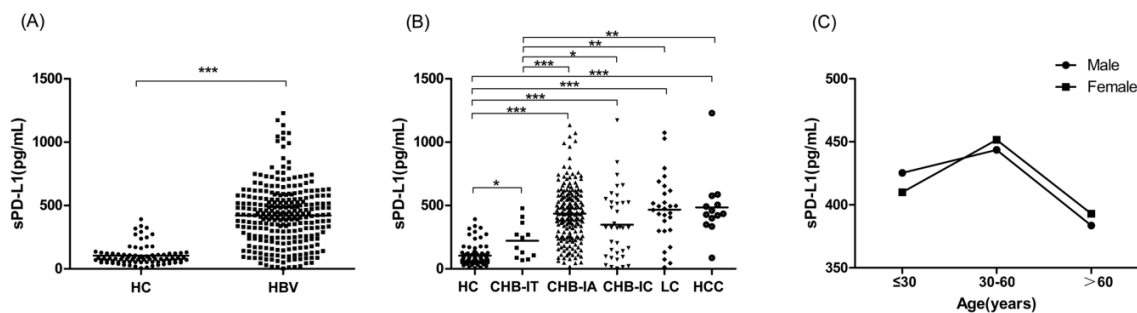
Figure 1: Serum sPD-L1 levels in patients with chronic HBV infection. (A) Serum sPD-L1 levels in healthy controls (HC) and patients with chronic HBV infection (HBV). (B) Serum sPD-L1 levels in various disease stages of chronic HBV infection. (C) Effects of gender and age on the levels of serum sPD-L1 in patients with chronic HBV infection based on two-way ANOVA. HC, healthy controls; CHB, chronic hepatitis B; IT, immune tolerant; IA, immune active; IC, inactive; LC, liver cirrhosis; HCC, hepatocellular carcinoma. *P<0.05, ** P<0.01, *** P<0.001.
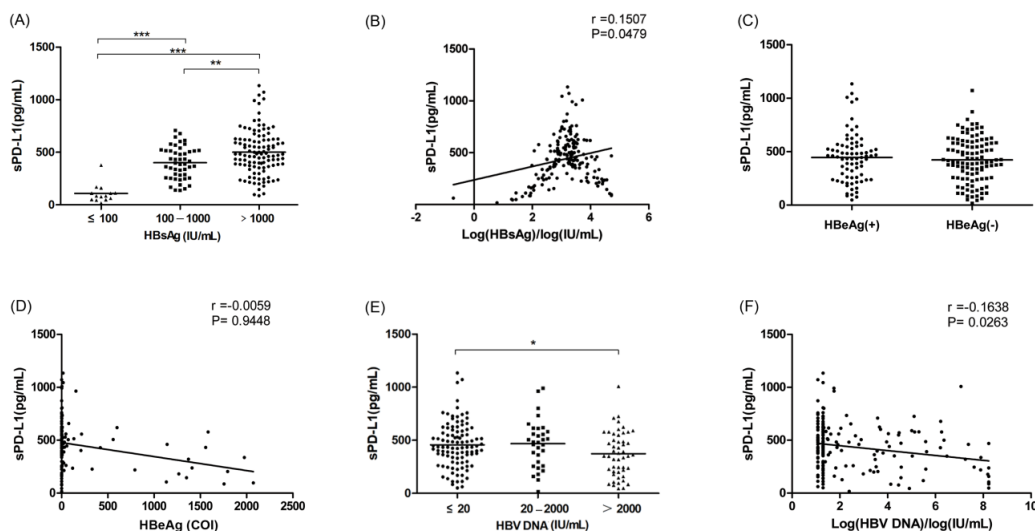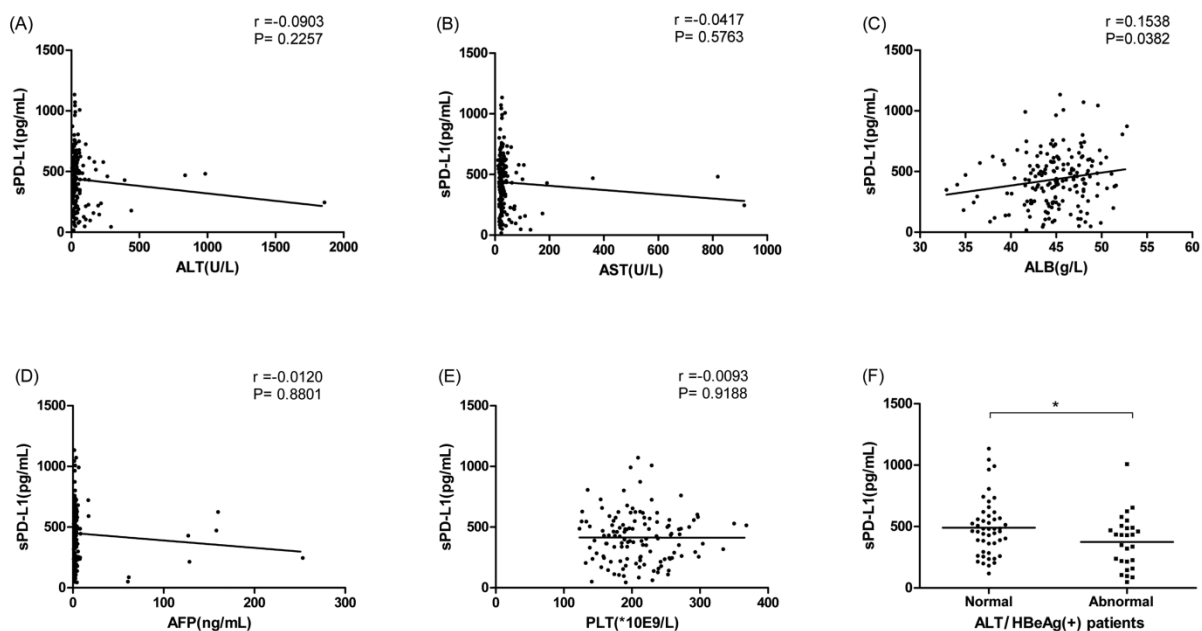

Figure 2: Correlations of serum sPD-L1 levels with serum viral markers in patients with CHB-IA. (A) Serum sPD-L1 levels in patients with low, medium and high levels of HBsAg. (B) Correlation of serum sPD-L1 and HBsAg. (C) Serum sPD-L1 and the status of HBeAg. (D) Correlation of serum sPD-L1 with HBeAg. (E) Serum sPD-L1 levels in patients with low, medium and high loads of HBV DNA. (F) Correlation of serum sPD-L1 with HBV DNA loads. *P<0.05, ** P<0.01, *** P<0.001.

4.4. Correlation Between Serum Spd-L1 Levels and Blood Liver Damage Markers in Patients with CHB-IA

Since HBV is not directly cytopathic, host immune responses to virus-infected hepatocytes have been reported to mediate liver cell injury [28], suggesting that the PD-1/PD-L1 axis has an influence on liver damage. Therefore, the correlation between serum sPD-L1 levels and liver damage markers, including ALT (Figure 3A), AST (Figure 3B), ALB (Figure 3C), AFP (Figure 3D) and PLT (Figure 3E), in patients with CHB-IA patients was analyzed. Among the markers, serum sPD-L1 was only positively correlated with serum ALB. Since ALT is the most important indicator of hepatitis activity, its correlation with the serum sPD-L1 in patients with HBeAg-positive and HBeAg-negative CBH-IA was further analyzed. In patients with HBeAg-positive CBH-IA, serum sPD-L1 levels in patients with normal ALT levels were significantly higher compared with patients with abnormal ALT levels (Figure 3F).

Figure 3: Correlations of serum sPD-L1 levels with blood liver damage markers in patients with CHB-IA. Correlations of serum sPD-L1 with alanine ami- notransferase (ALT) (A), aspartate aminotransferase (AST) (B), albumin (ALB) (C), α-fetoprotein (AFP) (D) and blood platelet (PLT) (E). (F) Serum sPD-L1 levels in patients with normal and abnormal ALT in HBeAg-positive CBH-IA. *$P<0.05$.
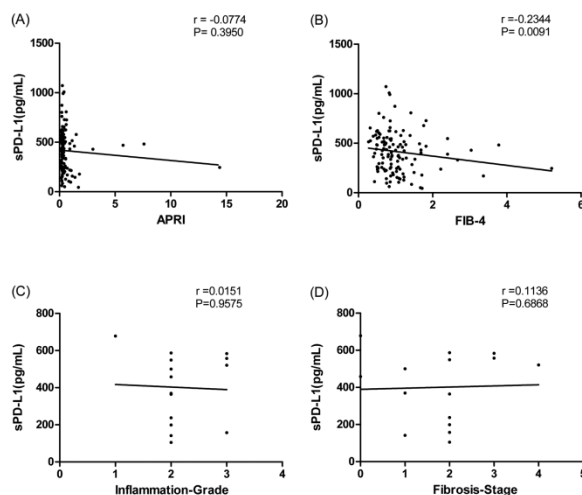
4.5. Correlation Between Serum Spd-L1 Levels, Fibrosis and Liver Histology in Patients with CHB-IA

APRI and FIB-4 are two common estimators of hepatic fibrosis [26]. The APRI and FIB-4 calculations were performed in all patients with CHB-IA. Serum sPD-L1 was not correlated with APRI (Figure 4A), but was negatively correlated with FIB-4 (Figure 4B). The liver histology results were available for 15 patients with CHB-IA. Serum sPD-L1 was not correlated with inflammation stage (Figure 4C) or fibrosis stage (Figure 4D) in patients with CHB-IA.

4.6. Correlation Between Serum Spd-L1 Levels and Antiviral Therapy in Patients with CHB-IA

Due to the negative effect of the PD-1/PD-L1 axis on antiviral immunity [8,11], it was hypothesized that serum sPD-L1 was positively correlated with HBsAg. Therefore, the negative correlation between serum sPD-L1 and serum HBV DNA was unexpected. A possible explanation is that the correlation resulted from NUC treat-ment (>4 weeks, 34.5 months in average) in the majority (65.2%; 120/184) patients with CHB-IA. NUCs usually achieve a stronger viral suppression without substantial influence on viral antigens [1,3]. To clarify the influence of NUCs, patients were classified into treat-ed and untreated groups. Treated patients were further divided into adequate responders (HBV DNA ≤100 IU/ml) and inadequate re-sponders (HBV DNA >100 IU/ml) as previously reported [29]. Se-rum sPD-L1 levels in adequate responders were significantly higher compared with untreated patients (Figure 5A), all of which survived Bonferroni correction analyses. Furthermore, treated patients (ad-equate/inadequate responders) displayed significantly higher serum sPD-L1 levels compared with untreated patients in patients with HBeAg-positive CHB-IA (Figure 5B), which was also observed be-tween adequate and inadequate responders in patients with normal-ized ALT (Figure 5C). In patients with HBeAg-negative CHB-IA, no significant difference was observed between treated and untreated patients (Figure 5D).

Figure 4: Correlations of serum sPD-L1 levels with fibrosis and liver histology in patients with CHB-IA. Correlations of serum sPD-L1 with AST, platelet ratio index (APRI) (A), FIB-4 (B), inflammation grade (C) and fibrosis stage (D).
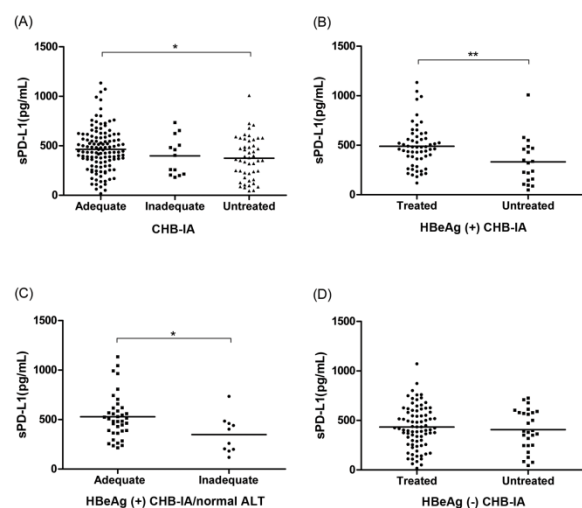
Figure 5: Correlations of serum sPD-L1 levels with NUCs treatment in patients with CHB-IA. (A) Serum sPD-L1 in patients with adequate (HBV DNA ≤ 100 IU/mL) responders, inadequate (HBV DNA > 100 IU/mL) responders and untreated patients. (B) Serum sPD-L1 levels in treated (adequate/inade-quate responders) and untreated patients in HBeAg-positive CHB-IA. (C) Serum sPD-L1 levels in adequate and inadequate responders in HBeAg-positive patients with normal ALT. (D) Serum sPD-L1 levels in treated and untreated patients in HBeAg-negative CHB-IA. *$P<0.05$, ** $P<0.01$.

5. Discussion

The PD-1/PD-L1 axis as an immune checkpoint serves a key role in the development and maintenance of persistent viral infection. sPD-1 in serum has been identified as a useful indicator for inflammatory and fibrosis severity in CHB [30,31]. In the present study, the results suggested that serum sPD-L1 may also be a useful indicator for chronic HBV infection. Firstly, serum sPD-L1 levels in chronic HBV infection were significantly increased in two major steps from health to infection, and from immunotolerance to immunoactivation. Secondly, serum sPD-L1 was weakly positively correlated with HBsAg, negatively correlated with HBV DNA and marginally correlated with liver damage. Finally, NUC treatment was correlated with in-creased serum sPD-L1 levels in patients with CHB-IA. Since serum sPD-L1 represents the expression of PL-L1 on cell membrane [20], the results of the present study indicated that serum sPD-L1 might serve as a helpful indicator to monitor immune status and disease progression. The activated PD-1/PD-L1 axis may explain the rarity of HBsAg seroconversion of NUC therapy and NUCs are urgent for the checkpoint inhibitors as complementary partners to treat chronic HBV infection efficiently in the future.

Increased serum sPD-L1 levels in chronic HBV infection, especially in the first step from health to infection, are in agreement with the latest report [32], the PD-1/PD-L1 axis serving as a negative im-mune regulator and HBV-induced expression of PD-1 and PD-L1

[8-17]. The positive correlation between serum sPD-L1 and HBsAg further supports the effect of the PD-1/PD-L1 axis on the chronicity of HBV infection. The ideal goal of anti-HBV therapy is the loss or seroconversion of HBsAg. Therefore, the positive correlation between serum sPD-L1 and HBsAg suggested that PD-1/PD-L1 axis inhibitors may enhance the HBsAg response of current antiviral therapy. For example, anti-PD-1 antibody treatment in a phase Ib clinical trial has been reported to significantly decrease HBsAg [19]. In addition, low level HBsAg in NUC-treated patients with negative HBV DNA is used as an indicator to discontinue therapy [27], or to add on or switch to rIFNα treatment [33]. Therefore, serum sPD-L1 may serve as an additional indicator to make treatment decisions.

The second step of increased serum sPD-L1 levels, from Immune Tolerance (CHB-IT) to Immune Activation (CHB-IA), is in agreement with the positive correlation between intrahepatic expressions of PD-1/PD-L1 and liver inflammation in CHB [34,35]. Therefore, the PD-1/PD-L1 axis, after immune activation, serves as an adaption of the host defense to minimize immunopathology [36]. Therefore, anti-PD-1 and anti-PD-L1 treatments may aggravate inflammation or liver damage in CHB. Indeed, immune checkpoint inhibitor (nivolumab or pembrolizumab) therapy is associated with a broad array of immune-related toxicities, including life-threatening immune-mediated hepatitis in patients without infection of hepatitis virus [37], leading to increased ALT levels in patients with advanced cancer in the context with HBV/HCV [38]. On the other hand, immune activation-related increases in serum sPD-L1 levels were not return to normal as disease alleviation (CHB-IC), which was consistent with the abnormal expressions of PD-1 and PD-L1 on T cells in patients with long-term remission of liver inflammation-necrosis [39]. Thus, checkpoint inhibitors are required in all phases of CHB, and NUC treatment may create a safe opportunity for their use. Therefore, it is very intelligent that the first clinical trial of checkpoint inhibitors was conducted in virally suppressed HBeAg-negative patients [18].

The most unexpected result in the present study was the negative correlation between serum sPD-L1 and HBV DNA load. Together with no significant or marginal correlations with liver damage markers in CHB-IA, the underlying cause was suspected to be NUC treatment. In CHB-IA, NUC treatment was correlated with significantly higher serum sPD-L1 levels, which was strong and independent of the status of ALT in HBeAg-positive CHB-IA. However, the correlation in HBeAg-negative patients with CHB-IA was not obvious, probably because NUC-treated HBeAg-negative patients with adequate responses were more like CHB-IC, in which the long-term remission of liver inflammation decreases intrahepatic PD-L1 expression [34,35]. Indeed, serum sPD-L1 levels displayed a downward trend in CHB-IC in the present study. In addition, NUCs lacking a direct effect on viral antigens may be the mechanism underlying the correlation with increased serum sPD-L1, since viral antigens or proteins, such as HBsAg, HBx protein and viral polymerase, upregulate PD-L1 expression [17,40].

HBsAg clearance of NUC therapy is rare and slow. Recently, entecavir treatment was reported to hinder HBsAg clearance in HBeAg-negative patients [41], which is in agreement with the correlation between NUC treatment and increased serum sPD-L1 levels, and the relationship between serum sPD-L1 and HBsAg. Therefore, NUCs may lead to increase in the expression of PD-L1, then activation of PD-1/PD-L1 axis and at last decrease in immune clearance of HBsAg, which may explain the rarity of HBsAg seroconversion of NUC therapy. In addition, such unexpected effect may be underlying mechanism of NUC treatment to increase HBV integration and clonal hepatocyte expansion [42]. Interestingly, rIFNα treatment also upregulates PD-L1 expression via STAT3 activation [40]. Therefore, both NUCs and rIFNα are required as partners for PD-1/PD-L1 axis inhibitors to promote their anti-HBV efficacy.

Serum sPD-L1 was significantly increased in chronic HBV infection, thus may serve as an indicator to monitor immune status and disease progression. The weak positive correlations between serum sPD-L1 and HBsAg or NUC treatment provided a potential explanation for the rarity of HBsAg seroconversion in NUC therapy and provided evidence for the clinical use of checkpoint inhibitors. NUC treatment minimized the correlation between serum sPD-L1 and viral/liver damage markers, but may create a safe opportunity for the use of checkpoint inhibitors due to no worry of severe liver damage. Nonetheless, no antiviral activity of PD-1/PD-L1 blockade in terms of months is found in NUC-treated patients with HBV-relate HCC [43]. So, the exact clinical significance of serum sPD-L1, the involvement of the PD-1/PD-L1 axis in antiviral therapy and the prospect of checkpoint inhibitors in chronic HBV infection requires further investigation.

6. Conclusion

Our results collectively suggested that serum sPD-L1 may serve as an indicator to monitor immune status and disease progression in chronic HBV infection. The correlations between increased sPD-L1 levels and HBsAg or NUC treatment suggested that the activated PD-1/PD-L1 axis may provide an explanation for the rarity of HBsAg seroconversion in NUC therapy, and clinically available checkpoint inhibitors may serve as partners for NUC therapy to improve anti-HBV efficacy.

7. Acknowledgements

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