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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Research article

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Abstract

Background

The restoration of immune responses is thought as a complementary approach to the nucleos(t)ide analogue (NUC) therapy of chronic HBV infection. The antiviral immunity is negatively regulated by the programmed cell death-1/programmed death ligand-1 (PD-1/PD-L1) axis. Here, the soluble form of PD-L1 (sPD-L1) that represents the amount of PD-L1 expression cells was used as an indicator to investigate the involvement of this axis in chronic HBV infection, especially in the setting of NUC therapy.

Methods

A total of 273 adult patients with chronic HBV infection, regardless of the treatment, and 86 healthy controls were consecutively enrolled. Serum sPD-L1 was measured using an ELISA assay. Its correlations with clinical/virological characteristics were analyzed.

Results

Serum sPD-L1 levels in patients with chronic HBV infection (median 425.2 IQR 245.8-558.6 pg/mL) were significantly higher than those in healthy controls (median 81.69 IQR 54.62-121.1 pg/mL). Among patients at various disease phases, those with immune-tolerant CHB had the lowest sPD-L1 levels (median 205.3 IQR 92.27-340.7 pg/mL). These results indicated that serum sPD-L1 was significantly increased in a manner of two steps from health to infection and from immunotolerance to immunoactivation in chronic HBV infection. Furthermore, the serum sPD-L1 in immune-active CHB was correlated with HBsAg positively, HBV DNA negatively and liver damage marginally. Interestingly, the increased serum sPD-L1 levels were strongly associated with the treatment of NUCs, especially in HBeAg-positive immune-active CHB.

Conclusions

Serum sPD-L1 might be a meaningful indicator to monitor the immune status and disease progression in chronic HBV infection. The correlations of the increased sPD-L1 with HBsAg and NUC treatment suggest that the activated PD-1/PD-L1 axis may explain the rarity of HBsAg seroconversion of NUC therapy and the clinically available checkpoint inhibitors may serve as partners for NUCs to improve their anti-HBV efficacy in the future

Background

Chronic infection of hepatitis B virus (HBV) remains a leading public health problem despite of efficient vaccines and antiviral therapy based on nucleos(t)ide analogues (NUCs) and recombinant interferon-α (rIFNα) [1, 2]. Its major poor prognoses are liver cirrhosis (LC) and hepatocellular carcinoma (HCC). To reduce the risks of LC and HCC, long-term NUC treatment is the mainstream approach. Unfortunately, its hepatitis B surface antigen (HBsAg) clearance or seroconversion is rare and slow [1, 3]. In addition, even

in NUC-treated patients with serum HBV DNA below detection limit, HCC occurrence is only delayed, but eliminated [4, 5]. In contrast, rIFNα, due to its immune-regulating potential, leads to about 10% of eligible patients to lose HBsAg [6]. The reactivation of HBV infection upon immunosuppression occurs in inactive chronic hepatitis B (CHB), and even in patients who resolved an acute HBV infection decades ago [7]. Cytokines produced by T cells are correlated with clinical-virological characteristics in untreated CHB patients [8]. Thus, host immunity is very important to HBV infection control. Regretfully, it is unclear what immune mechanisms limit the HBsAg seroconversion during NUC therapy.

It is well known that the immune checkpoint pathways regulate the optimal host immune responses against transformed or virus-infected cells [9]. One of the immune checkpoint mechanism is the programmed cell death-1(PD-1/CD279)/programmed death ligand1 (PD-L1/CD274) axis. 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 [10]. There are at least three clinically available anti-PD-1 antibodies (pembrolizumab, nivolumab and atezolizumab) to be used to block the PD-1/PD-L1 axis. These antibodies have shown effectual anti-tumor activities and controllable adverse effects [11]. As for HBV infection, CD4 + T cell exhaustion is induced by high PD-1 and LAG-3 expression in CHB [12], and HBsAg is the most abundant viral protein in the liver and peripheral blood of patients with chronic HBV infection. HBsAg impairs immune response by up-regulation of the PD-L1 expression on monocytes [13]. HBV also promotes the expression of PD-L1 on hepatocytes in cell model and transgenic mice, which correlates with the levels of HBsAg, hepatitis B e antigen (HBeAg) and HBV DNA in mouse sera [14]. The blockage of the PD-1/PD-L1 axis using anti-PD-1 antibody can enhance virus-specific immunity in the woodchuck model of HBV infection [15], and nivolumab therapy is well-tolerated and leads to HBsAg decline in most virally suppressed HBeAg-negative patients (a phase Ib study) [16]. Therefore, the blockage of the PD-1/PD-L1 axis may be a feasible way to restore the antiviral immune responses in chronic HBV infection. Regretfully, it is unclear whether the PD-1/PD-L1 axis is involved in limiting the HBsAg seroconversion during NUC therapy.

Despite of the importance to HBV infection, PD-L1 is seldom used to monitor disease progression or to make treatment decision, partially because of the difficulty in measuring due to its intrahepatic expression. A soluble form of PD-L1 (sPD-L1) can be conveniently detected in sera from patients with cancers or viral infections. It is correlated with the amount of PD-L1 expression cells and is thought as a helpful indicator to monitor the immune status and disease progression in HCC and hepatitis C virus infection [17–20]. The serum sPD-L1 levels in CHB were contradictorily reported to be lower or slightly higher than those in healthy controls in some small sample researches where CHB patients were served as controls [18, 19]. Therefore, the significance of the serum sPD-L1 to chronic HBV infection, especially in the setting of NUC therapy, is not completely clarified yet.

In this study, the serum sPD-L1 was found to be significantly increased in patients with chronic HBV infection. Its concentration was correlated with HBsAg positively, alanine aminotransferase (ALT)

marginally and HBV DNA negatively. The serum sPD-L1 levels were also significantly increased in NUC-treated patients. These results may be helpful in seeking for new therapeutic approaches to chronic HBV infection in the future.

Methods

Subjects and samples

Adult patients with chronic HBV infection (positive HBsAg for more than 6 months, regardless of the treatment) were consecutively recruited for this study from the liver clinic of the fifth Affiliated Hospital of Sun Yat-sen University during May 2017 and October 2018. Healthy volunteers (healthy controls, negative HBsAg) were recruited from physical examination center of the same hospital. Patients infected with other hepatitis viruses (A, C, D and E) and human immunodeficiency virus and patients with cardiovascular disease, diabetes, kidney disease, pregnancy or autoimmune disease were excluded. According to recent guidelines of the American Association for the Study of Liver Diseases and others [21, 22], 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 × g for 10 minutes to obtain serum and stored at − 80℃ until use. The study was approved by the Institutional Review Board of the fifth Affiliated Hospital of Sun Yat-sen University.

Serum sPD-L1 Quantification

Serum levels of sPD-L1 in patients and healthy controls were measured using a specific enzyme-linked immunosorbent assay kit (mlbio®, Shanghai, China) according to the manufacture's protocol. The detection limit of the assay was 1.0 pg/mL. Each sample was tested in duplicate.

Serum Viral Marker Assays

Serum HBV DNA was quantified using a real-time polymerase chain reaction assay (COBAS TaqMan, Roche Molecular Diagnostics, Indianapolis, IN) with a lower limit of quantitation of 12 IU/mL and a linear dynamic range of 2.0×10^{1}-1.7×10^{8} IU/mL. HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc (antibody against hepatitis B core antigen) were measured using chemiluminescence assay kits (Roche Diagnostics Gmbh, Mannheim, Germany). HBsAg was quantified with a low limit of 0.05 IU/mL. HBeAg was semi-quantitatively measured and the result was showed as cut-off index (COI).

Routine Blood, Liver Function And α-fetoprotein Assays

White blood cell, red blood cell and platelet (PLT) counts were measured by XN2000 (SYSMEX, Kobe, Japan). Liver function profiles, such as ALT, aspartate aminotransferase (AST), total bilirubin (TBIL) and albumin (ALB), were measured on a 7600-020 (ISE) Automatic Analyzer (HITACHI, Tokyo, Japan). The serum α-fetoprotein (AFP) was regularly measured using electrochemiluminescence immunoassay.

Liver Biopsy

Liver biopsy was conducted in 20 cases of patients (including 15 CHB-IA patients) following standard procedures. The severity of the inflammation (grade) and the degree of fibrosis (stage) were independently determined by two pathologists.

Miscellaneous

Fibrosis estimators, APRI [AST, platelet ratio index = (AST/upper limit of normal) × 100/platelet count] and FIB-4 [age (years) × AST (IU/L)/platelet count (109/L) × ALT (IU/L)$^{1/2}$] were calculated using routine laboratory values [23].

Statistical analysis

Continuous variables are expressed as the median with the interquartile range (IQR). Categorical variables were expressed as numbers and percentages. The significance of differences was analyzed statistically with the Chi-square, Fisher's exact test, or the Mann-Whitney U test, as appropriate. The correlations between sPD-L1 and detection markers were assessed by Spearman's rank correlation test. The data were analyzed using SPSS software (Ver.18, SPSS Inc., Chicago, IL, USA). In all cases, the level of significance was set as $P < 0.05$.

Results

Demographic and clinical characteristics of the subjects in this study

A total of 273 patients with chronic HBV infection and 86 healthy controls were included for analysis. Their demographic and disease characteristics were shown 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 (n = 86)
	CHB-IT (n = 12)	CHB-IA (n = 184)	CHB-IC (n = 36)	LC (n = 28)	HCC (n = 13)	
Gender (M/F)	4/8	135/49	19/17	24/4	10/3	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 (∝mol/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 (10^9/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	-

CHB-IT, immune-tolerant CHB; CHB-IA, immune-active CHB; CHB-IN, inactive CHB; LC, Liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBil, total bilirubin; AFP, α-fetoprotein; PLT, blood platelet; †IQR, interquartile range; ‡ Data expressed in log(IU/mL); § Longer than 4 weeks; *P < 0.05 when compared with healthy controls.

	Chronic HBV infection					Healthy controls (n = 86)
	CHB-IT (n = 12)	CHB-IA (n = 184)	CHB-IC (n = 36)	LC (n = 28)	HCC (n = 13)	
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	-

CHB-IT, immune-tolerant CHB; CHB-IA, immune-active CHB; CHB-IN, inactive CHB; LC, Liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBil, total bilirubin; AFP, α-fetoprotein; PLT, blood platelet; †IQR, interquartile range; ‡ Data expressed in log(IU/mL); § Longer than 4 weeks; *P < 0.05 when compared with healthy controls.

Serum sPD-L1 level rises in patients with chronic HBV infection

The serum sPD-L1 levels were significantly increased in patients with chronic HBV infection (median 425.2 IQR 245.8-558.6 pg/mL) when compared with those in healthy controls (median 81.69 IQR 54.62–121.1 pg/mL) (Fig. 1A). When the phases of chronic HBV infection were taken into account, the serum sPD-L1 levels in each phase were all significantly higher than those in healthy controls (Fig. 1B). In addition, patients in phase of CHB-IT had relatively lower levels of serum sPD-L1 (median 205.3 IQR 92.27–340.7 pg/mL) than those patients in other phases. There was no significant difference among the rest phases of CHB-IA, CHB-IC, LC and HCC though the levels in CHB-IC seemed a little lower (Fig. 1B). These results clearly showed that the serum sPD-L1 levels in chronic HBV infection increased in two major steps from health to infection and from immunotolerance to immunoactivation, respectively.

Correlations of serum sPD-L1 levels with serum HBV markers in patients with CHB-IA

Patients with chronic HBV infection had higher serum sPD-L1 levels than healthy controls, in concordance with the PD-1/PD-L1 axis as a negative regulator in antiviral immunity [9, 10]. Here, we further analyzed the correlations of serum sPD-L1 with serum HBV markers, HBsAg, HBeAg and HBV DNA in patients with CHB-IA. The serum sPD-L1 levels in patients with high levels of HBsAg were much higher (Fig. 2A). They were also found to be positively correlated with HBsAg in Spearman's rank correlation test (Fig. 2B). HBsAg level < 100 IU/mL is thought to be a useful marker to discontinue NUC therapy [24]. The serum sPD-L1 levels in those patients were concordantly very lower (Fig. 2A). However, the serum sPD-L1 was uncorrelated with the status (Fig. 2C) or the COI of HBeAg (Fig. 2D). As for serum HBV DNA, those patients with negative levels (≤ 20 IU/mL) had significantly higher serum sPD-L1 levels than those ones

with high levels (> 2000 IU/mL) (Fig. 2E), and the serum sPD-L1 was negatively correlated with serum HBV DNA load in Spearman's rank correlation test (Fig. 2F).

Correlations of serum sPD-L1 levels with blood liver damage markers in patients with CHB-IA

Since HBV is not directly cytopathic, host immune responses to the virus-infected hepatocytes are believed to mediate liver cell injury [25], suggesting that the PD-1/PD-L1 axis has deep influence on liver damage. For this reason, the correlations of serum sPD-L1 levels with liver damage markers, ALT (Fig. 3A), AST (Fig. 3B), ALB (Fig. 3C), AFP (Fig. 3D) and PLT (Fig. 3E), in CHB-IA patients were analyzed. Among these markers, the serum sPD-L1 was only found to be positively correlated with serum ALB. Since ALT is the most important indicator for hepatitis activity, its correlations with the serum sPD-L1 in HBeAg-positive and in HBeAg-negative CBH-IA were further analyzed. The serum sPD-L1 levels in patients with normal ALT were significantly higher than those with abnormal ALT in HBeAg-positive CBH-IA (Fig. 3F).

Correlations of serum sPD-L1 levels with fibrosis and liver histology in patients with CHB-IA

APRI and FIB-4 are two common estimators of hepatic fibrosis [23]. All patients with CHB-IA were performed the APRI and FIB-4 calculations. The serum sPD-L1 was uncorrelated with APRI (Fig. 4A), but was negatively correlated with FIB-4 (Fig. 4B). The liver histology results were available in 15 CHB-IA patients. The serum sPD-L1 was uncorrelated with the inflammation stage (Fig. 4C) and the fibrosis stage (Fig. 4D) in these patients.

Correlations of serum sPD-L1 levels with antiviral therapy in patients with CHB-IA

Due to the negative effect of the PD-1/PD-L1 axis on antiviral immunity [9, 10], it is comprehensible that the serum sPD-L1 was positively correlated with HBsAg. However, the negative correlation with serum HBV DNA was unexpected. It possibly resulted from the NUC treatment (longer than 4 weeks) in the majority (65.2%, 120/184) of those patients with CHB-IA. NUCs usually achieve a stronger viral suppression without substantial influence on viral antigens [1, 3]. To clarify the influences of NUCs, the patients were classified into treated and untreated patients. The former was further divided into adequate responders (HBV DNA ≤ 100 IU/mL) and inadequate responders (HBV DNA > 100 IU/mL) as reported [26]. The serum sPD-L1 levels in adequate responders were significantly higher than those of untreated patients (Fig. 5A). Furthermore, treated patients (adequate/inadequate responders) also had significantly higher serum sPD-L1 levels than untreated patients in HBeAg-positive CHB-IA (Fig. 5B), which was still truth between adequate and inadequate responders in such patients with normalized ALT (Fig. 5C). In HBeAg-negative CHB-IA, no significant difference was found between treated and untreated patients (Fig. 5D).

Discussion

The PD-1/PD-L1 axis as an immune checkpoint plays key role in the development and maintenance of persistent viral infection. Soluble PD-1 in serum has been found to be a useful indicator for inflammatory and fibrosis severity in CHB [27]. In this study, the serum sPD-L1 was also found to be meaningful in chronic HBV infection. Firstly, the serum sPD-L1 levels in chronic HBV infection were significantly increased in two major steps from health to infection and from immunotolerance to immunoactivation, respectively. Secondarily, the serum sPD-L1 was correlated with HBsAg positively, HBV DNA negatively and liver damage marginally. Finally, NUC treatment was correlated with the increased serum sPD-L1 levels in CHB-IA patients. Since the serum sPD-L1 well represents the expression of PL-L1 on cell membrane [17], these findings suggest that the serum sPD-L1 may be a helpful indicator to monitor the immune statue 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.

The increased serum sPD-L1 levels in chronic HBV infection, especially in the first step from health to infection, are in concordance with the PD-1/PD-L1 axis as a negative immune regulator and HBV-induced the expressions of PD-1 and PD-L1 [9–14]. The positive correlation of the serum sPD-L1 with HBsAg further supports the effect of the PD-1/PD-L1 axis on the chronicity of HBV infection. It is well known that the ideal goal of anti-HBV therapy is the loss or seroconversion of HBsAg. Therefore, the positive correlation with HBsAg also implies that the PD-1/PD-L1 axis inhibitors may help to enhance the HBsAg response of current antiviral therapy. Indeed, anti-PD-1 antibody treatment in clinical trial (a phase Ib study) can significantly decrease HBsAg [16]. In addition, the low level of HBsAg in NUC-treated patients with negative HBV DNA is an indicator to discontinue the therapy [24], or to add on or switch to rIFNα treatment [28]. Thus, the serum sPD-L1 may serve as an additional indicator to make such treatment decisions.

The second step of the increased serum sPD-L1 levels, from immunotolerance (CHB-IT) to immunoactivation (CHB-IA), is in concordance with the positive correlation of the intrahepatic expressions of PD-1 and PD-L1 with liver inflammation in CHB [29, 30]. Thus, the PD-1/PD-L1 axis, after immunoactivation, is served as an adaption of the host defense to minimize immunopathology [31]. Therefore, the anti PD-1 and anti-PD-L1 treatments may aggravate inflammation or liver damage in CHB. Indeed, the 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 [32], and lead to the increase in ALT in patients with advanced cancers in the context with HBV/HCV [33]. On the other hand, the immunoactivation-related increase in the serum sPD-L1 was of no significant sign to return to normal as disease alleviation (CHB-IC), which is in concordance with the abnormal expressions of PD-1 and PD-L1 on T-cells in patients with long-term remission of liver inflammation-necrosis [34]. Thus, checkpoint inhibitors are needed in all phases of CHB and NUC treatment may create a safe opportunity for their usage. Meanwhile, we believe that it is very intelligent to conduct early clinical try of checkpoint inhibitors in virally suppressed HBeAg-negative patients [15].

The most unexpected result in the study was the negative correlation of serum sPD-L1 with HBV DNA load. Together with no significant or marginal correlations with liver damage markers in CHB-IA, their underlying cause was suspected to be the NUC treatment. In CHB-IA, NUC treatment was correlated with significantly higher serum sPD-L1 levels, which was strong and independent on the status of ALT in HBeAg-positive CHB-IA. However, the correlation in HBeAg-negative CHB-IA was not obvious, probably because such NUC-treated patients with adequate response is more like CHB-IC, in which the long-term remission of liver inflammation is trend to decrease the intrahepatic PD-L1 expression [29, 30]. Indeed, the serum sPD-L1 levels showed a downward trend in CHB-IC in this study. In addition, NUCs lacking of direct effect on viral antigens may be its underlying mechanism to be correlated with the increased serum sPD-L1 since viral antigens or proteins such as HBsAg, HBx protein and viral polymerase up-regulate the PD-L1 expression [14, 35].

It is well known that the HBsAg clearance of NUC therapy is rare and slow. Recently, entecavir treatment is even found to hinder the HBsAg clearance in HBeAg-negative patients [36], which is in concordance with the correlation of NUC treatment with the increased serum sPD-L1levels and the relationship between serum sPD-L1and HBsAg. Therefore, NUCs may induce the expression of PD-L1, suggesting that the activated PD-1/PD-L1 axis may explain the rarity of HBsAg seroconversion of NUC therapy. Interestingly, the rIFNα treatment also up-regulates PD-L1 expression by STAT3 activation [35]. That means both NUCs and rIFNα are urgent for the PD-1/PD-L1 axis inhibitors as partners to promote their anti-HBV efficacy in the future.

Conclusions

The serum sPD-L1 was significantly increased in chronic HBV infection. It may be a helpful indicator to monitor the immune status and disease progression. Its positive correlations with HBsAg and NUC treatment explain the rarity of HBsAg seroconversion of NUC therapy and vote for the clinical usage of checkpoint inhibitors. Regrettably, NUC treatment had fuzzed up the correlations of serum sPD-L1 with viral and liver damage markers, but it may create a safe opportunity for the usage of checkpoint inhibitors. Nonetheless, the exact clinical significance of the 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 are urgent for more prospective or mechanism studies in the future.

Abbreviation

HBV, hepatitis B virus; NUCs, nucleos(t)ide analogues; IFN, interferon; LC, liver cirrhosis; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; CHB, chronic hepatitis B; PD-1, programmed cell death-1; PD-L1, programmed death ligand1; HBeAg, hepatitis B e antigen; sPD-L1, soluble PD-L1; IT, immune-tolerant; IA, immune-active; IC, inactive; COI, cut-off index; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin; AFP, α-fetoprotein; APRI, aspartate aminotransferase, platelet ratio index; IQR, interquartile range.

Declarations

Ethics and consent to participate

Informed written consent was received from all participants after detailed explanation of the study at the time of blood sampling. The study was approved by the Institutional Review Board of the fifth Affiliated Hospital of Sun Yat-sen University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interest.

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Authors’ contributions

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.

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Figures

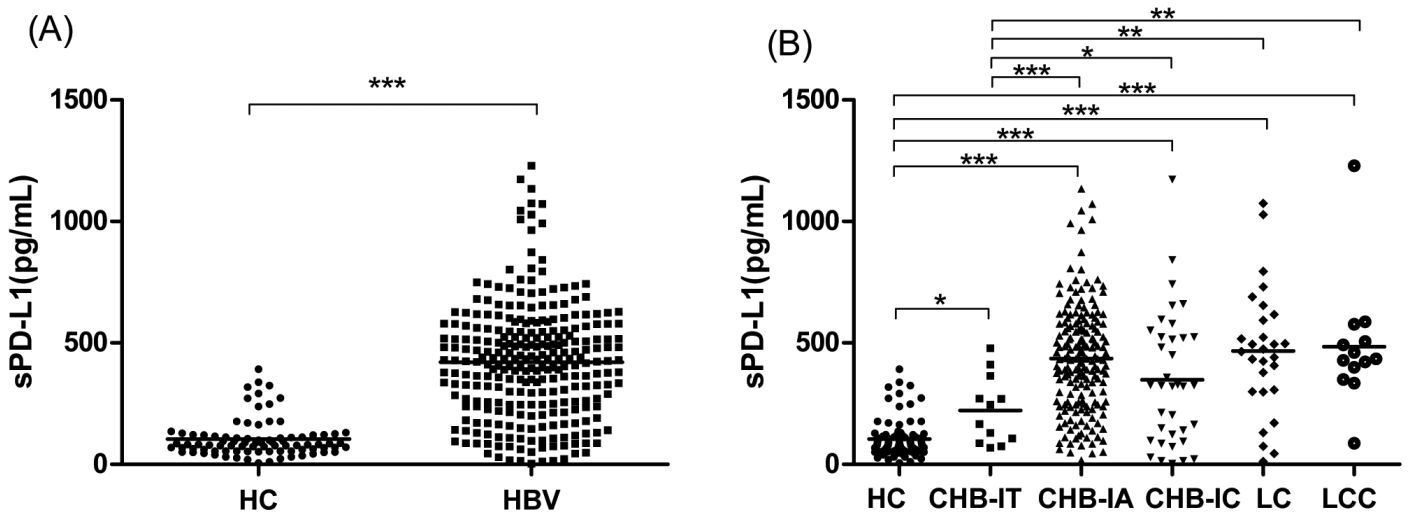

Figure 1

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. 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.

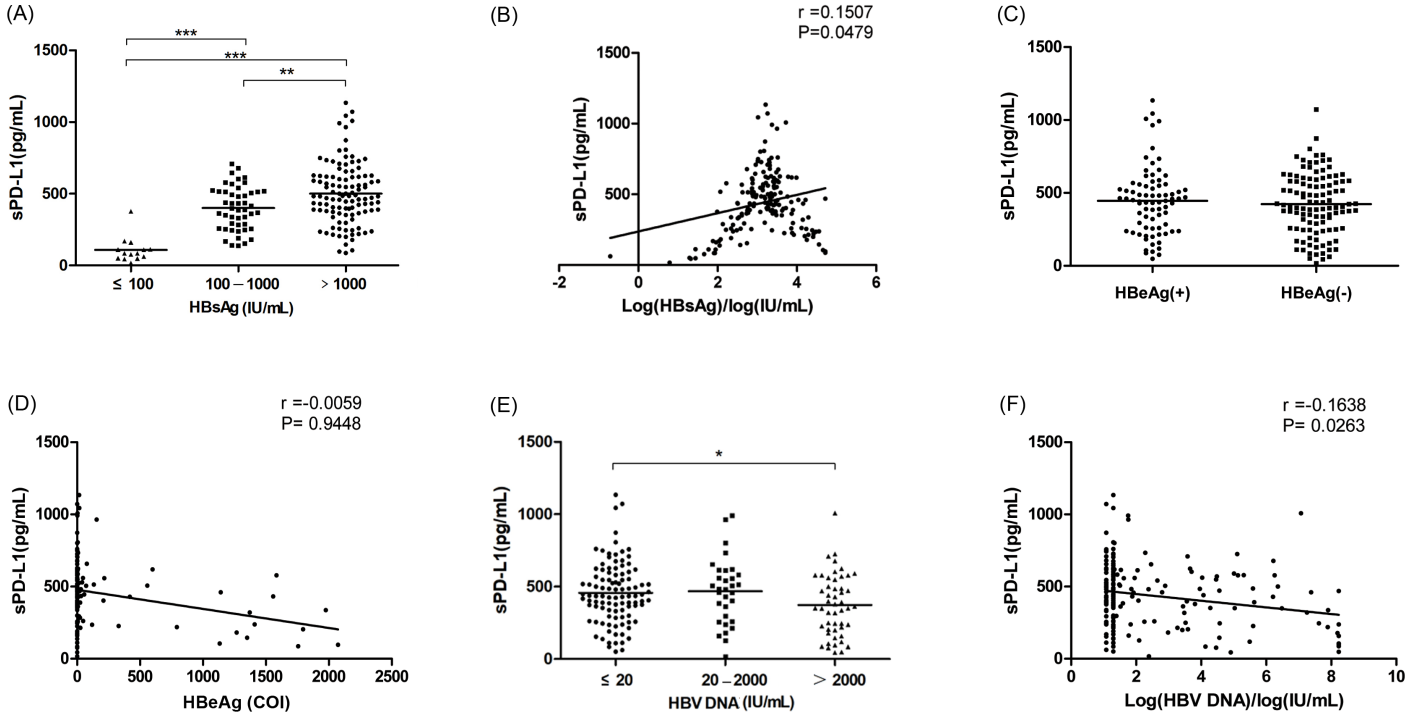

Figure 2

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.

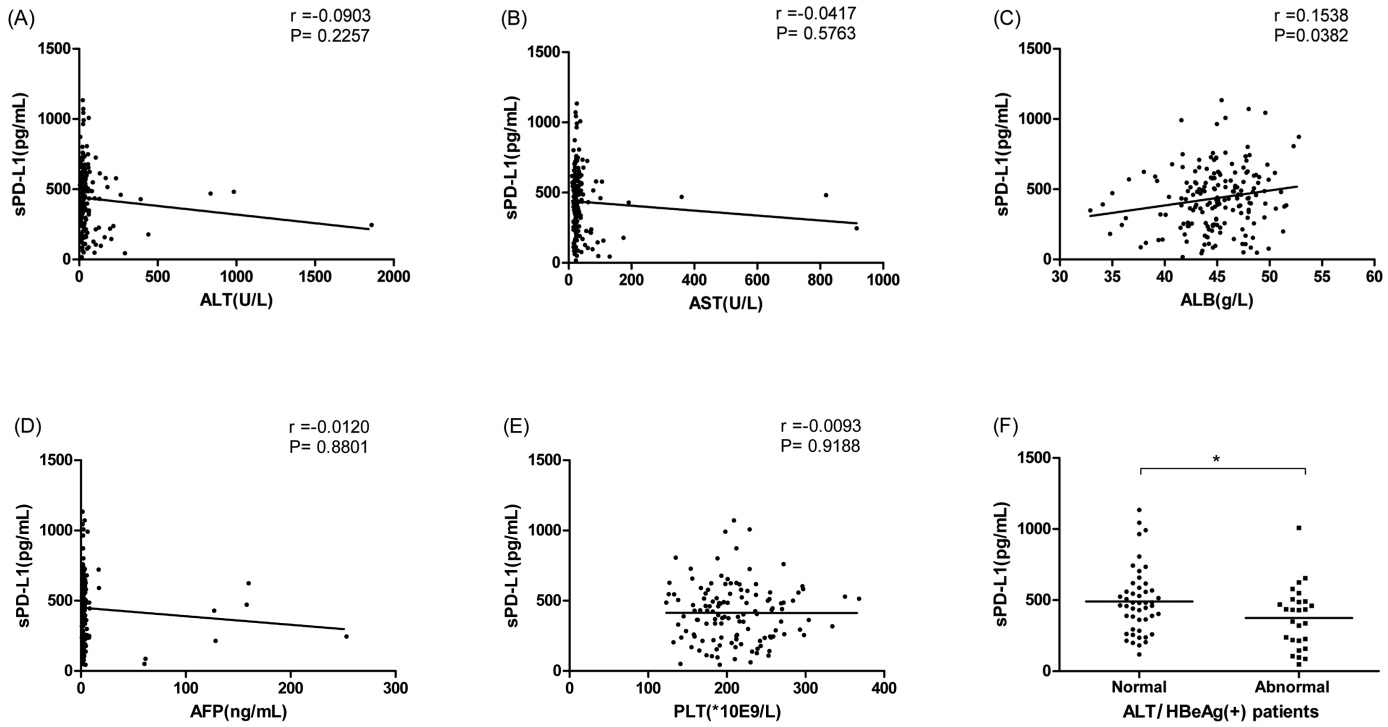

Figure 3

Correlations of serum sPD-L1 levels with blood liver damage markers in patients with CHB-IA. Correlations of serum sPD-L1 with alanine aminotransferase (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.

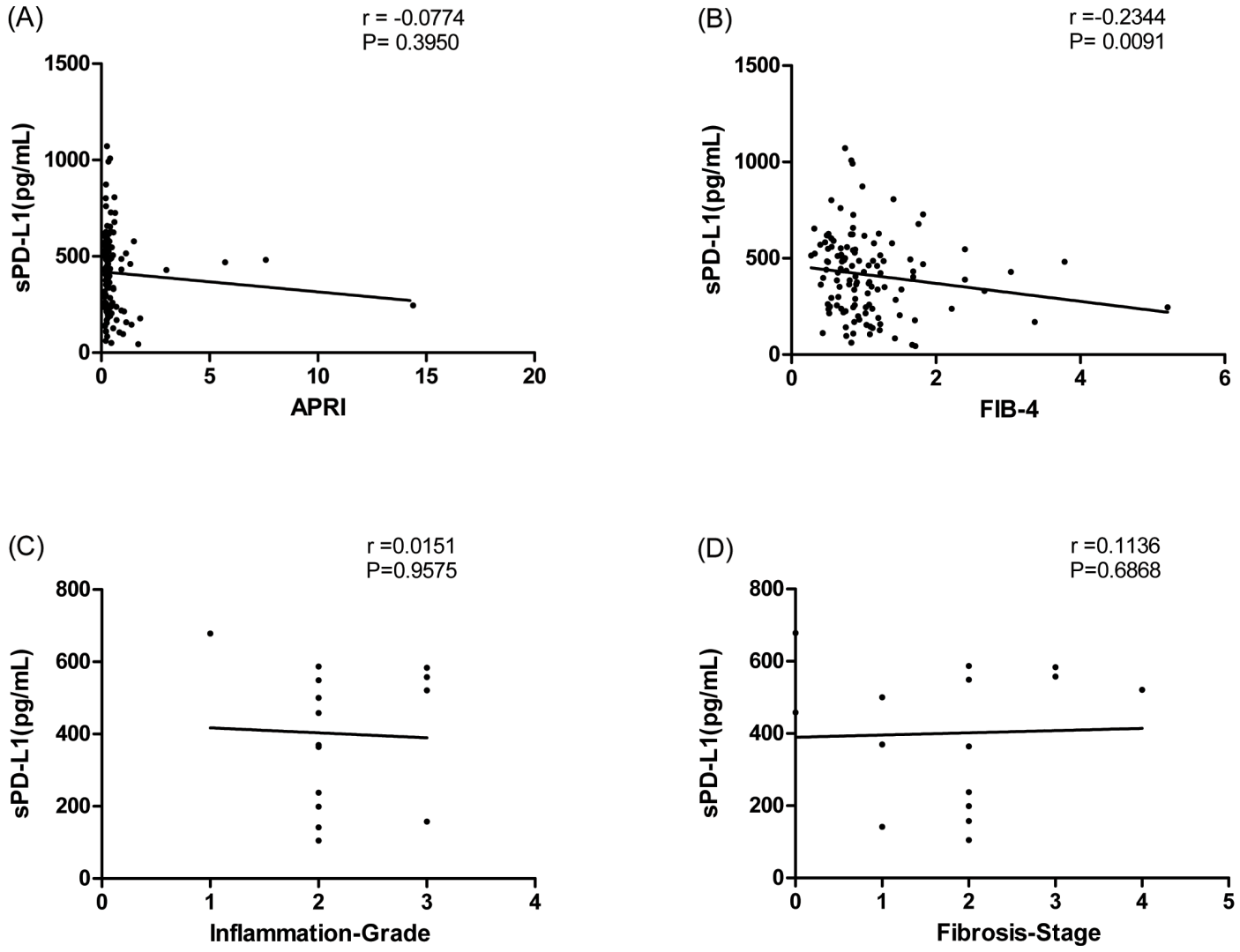

Figure 4

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).

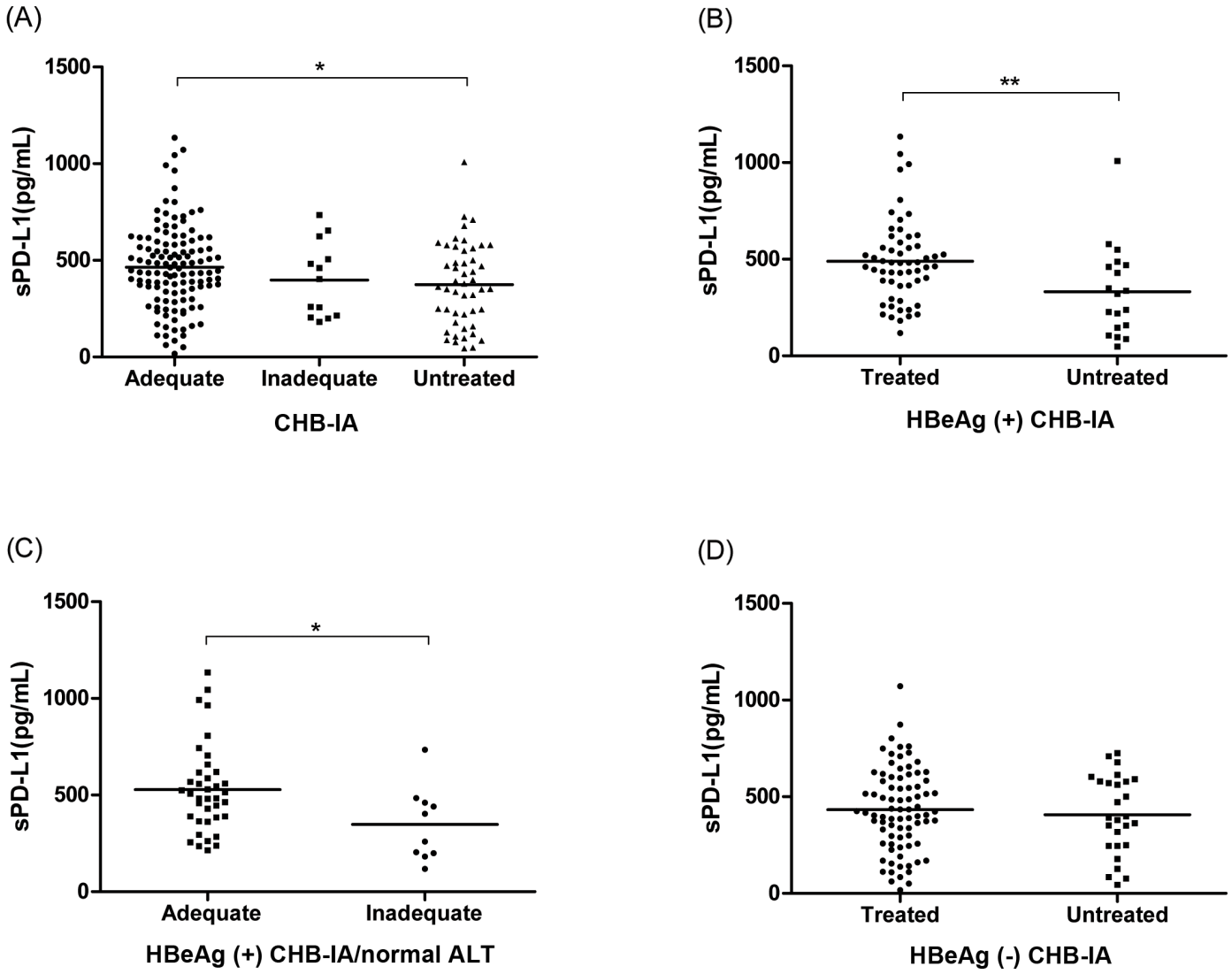

Figure 5

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/inadequate 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.