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Original Article

Clinicopathological and Prognostic Value of Programmed Cell Death 1 Expression in Hepatitis B Virus-related Hepatocellular Carcinoma: A Meta-analysis

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Abstract

Background and Aims: The efficacy of targeted programmed cell death 1/programmed death ligand 1 (PD-1/PD-L1) monoclonal antibodies (mAbs) has been confirmed in many solid malignant tumors. The overexpression of PD-1/PD-L1 serves as a biomarker to predict prognosis and clinical progression. However, the role of PD-1 in patients with hepatitis B virus-related hepatocellular carcinoma (HBV-HCC) remains indeterminate. Given that HBV is the most important cause for HCC, this study aimed to investigate the prognostic and clinicopathological value of PD-1 in HBV-HCC via a meta-analysis. ***Methods:*** We searched PubMed, Embase, Scopus, the Cochrane Library, Web of Science and Google Scholar up to January 2021 for studies on the correlation between clinicopathology/prognosis and PD-1 in patients with HBV-HCC. The pooled hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to investigate the prognostic significance of PD-1 expression. The odds ratios (ORs) and 95% CIs were determined to explore the association between PD-1 expression and clinicopathological features. ***Results:*** Our analysis included seven studies with 658 patients, which showed that high PD-1 expression was statistically correlated with poorer overall survival (HR=2.188, 95% CI: [1.262–3.115], $p<0.001$) and disease-free survival (HR=2.743, 95% CI: [1.980–3.506], $p<0.001$). PD-1 overexpression was correlated with multiple tumors (OR=2.268, 95% CI: [1.209–4.257], $p=0.011$), high level of alpha fetoprotein (AFP; OR=1.495, 95% CI: [1.005–2.223], $p=0.047$) and advanced Barcelona Clinic Liver Cancer (BCLC) stage (OR=3.738, 95% CI: [2.101–6.651], $p<0.001$). ***Conclusions:*** Our meta-analysis revealed that the high level of PD-1 expression was associated with multiple tumors, high level of AFP and advanced BCLC stage. It significantly predicted a poor prognosis of HBV-HCC, which suggests that anti-PD-1 therapy for HBV-HCC patients is plausible.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and the sixth leading cause of cancer-related deaths worldwide.[1] Although the development of healthcare and living standards have altered the etiology of HCC, most existing HCC cases are still associated with chronic hepatitis B virus (HBV) infection, especially in Asia and Africa.[2,3] More than half of the patients have missed the opportunity to accept surgical section at first diagnosis, which makes locoregional therapies and adjuvant therapies necessary.[4,5]

Immunotherapy is a promising treatment of malignant tumors and programmed cell death 1/programmed death ligand 1 (PD-1/PD-L1) is the most commonly used target.[6,7] Belonging to the B7-CD28 of the immunoglobulin superfamily, PD-1 is a type I transmembrane glycoprotein, with a molecular weight of 50~55 kDa. It is an important immunosuppressive receptor, mainly expressed in activated T cells, B cells, natural killer cells, monocytes and mesenchymal stem cells. Under physiological conditions, PD-1 recognizes antigens via T cell receptors and regulates the function of peripheral T cells as a part of the immune response modulation to allogenic materials or autoantigens, and prevents immune-related diseases.[8] However, the tumor environment induces the up-regulation of PD-1 molecules in infiltrating T cells. Tumor cells demonstrate a high expression of the PD-1 ligands PD-L1 and PD-L2, which may lead to the continuous activation of the PD-1 pathway in the tumor mi-

Keywords: Hepatocellular carcinoma; Hepatitis B virus; Programmed cell death protein 1; Prognosis; Clinicopathology.

Abbreviations: AFP, alpha fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; CTLA-4, cytotoxic T-lymphocyte antigen 4; DFS, disease-free survival; FDA, Food and Drug Administration; HBV-HCC, hepatitis B virus-related hepatocellular carcinoma; HCC, hepatocellular carcinoma; HRs, hazard ratios; HBV, hepatitis B virus; mAbs, monoclonal antibodies; NOS, Newcastle Ottawa quality assessment scale; ORs, odds ratios; OS, overall survival; PD-1/PD-L1, programmed cell death 1/programmed death ligand 1; TAM, tumor-associated macrophage; TIL, tumor infiltrating lymphocyte; NIL, nontumor infiltrating lymphocytes; TME, tumor microenvironment; TNM, tumor-node-metastasis; Treg, regulatory T cell.

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croenvironment (TME) and the inhibition of T cell function, so that tumor cells can escape immune surveillance.[9,10] PD-L1 monoclonal antibodies (mAbs) can block this pathway and partially restore T cell function, allowing them to play defensive roles in eliminating tumor cells.[11] The efficacy of targeted PD-L1 mAb has been confirmed in breast cancer, melanoma, lung cancer and gastric cancer, wherein it stimulates inherent immune function, prolongs survival time and stabilizes disease progression.[12,13] Additionally, the overexpression of PD-1/PD-L1 has been found in the tumors mentioned above and has served as a biomarker to predict tumor prognosis and clinical progression.[14–16]

In recent years, clinical trials of immunotherapy in HCC have been in full swing but the effects are still controversial.[17] This difference might lie in the complex microenvironment in HCC and the presence of multiple antigens.[18–20] Considering the high infection rate of HBV among HCC patients and the interaction between HBV and the immune system, it is necessary to analyze the relationship between the PD-1/PD-L1 level and progression of HBV-HCC. Moreover, some studies have already suggested that the level of PD-1/PD-L1 could predict the prognosis and clinicopathological characteristics of HBV-HCC patients, while others have argued that there is no correlation.[21–27] Thus, we conducted this analysis to explore the predictive value of PD-1 in HBV-HCC, to better understand the role of the PD-1/PD-L1 pathway with regards to immunotherapy in HBV-HCC patients and to design a more reasonable treatment plan for these patients.

Methods

Literature search strategy

We searched the literature databases to obtain as many related research articles as possible, including the databases of PubMed, Embase, Scopus, the Cochrane Library, Web of Science and Google Scholar. "Hepatitis B virus infection and hepatocellular carcinoma" and "PD-1 or programmed cell death 1 receptor" and "survival or prognosis or clinicopathology" were used as keywords for the search. All articles identified were reported in English and were published before January 2021.

Eligibility criteria

(1) All articles reviewed were reported in English with full-texts. (2) Studies used humans diagnosed with HBV-HCC as subjects. (3) Articles reported the PD-1 level, either in clinical HCC tissues or serum. (4) The correlation between PD-1 expression and prognosis were detected, including survival/disease-free survival (OS/DFS), as well as clinicopathological features. (5) Researches supplied hazard ratio (HR), odds ratio (OR) and their 95% confidence intervals (CIs) or sufficient data to calculate such.

When it came to repetitive studies, we chose to analyze the latest or those with the most comprehensive data.

Data extraction

Two authors performed the data extraction, separately. When it came to disagreements over the information, a third reviewer resolved them. The following data were extracted: name of first author, country, year of publication, method to detect PD-1 expression, cut-off value, survival, clinicopathological parameters (including age, sex, number of tumors, tumor size, liver cirrhosis, alpha fetoprotein [AFP], vascular invasion, Barcelona Clinic Liver Cancer [BCLC] stage, tumor-necrosis-metastasis [TNM] stage, HBV DNA level and Child-Pugh score), ORs with 95% CIs and HRs with 95% CIs. If HRs and 95% CIs were not demonstrated directly in the articles, we used Engauge Digitizer version 4.1 to obtain them from the Kaplan-Meier survival curves; however, this approach may have caused errors due to variation.

Quality assessment

Using a 9-score system of the Newcastle-Ottawa quality assessment scale (NOS),[28] two authors evaluated the quality of articles separately. When it came to discrepancies in the score, authors involved a third reviewer to settle the differences through discussion and analysis. We judged articles according to three aspects: selection, comparability, and outcome assessment. Each article was given a score between 0 to 9 based on these parameters.

Statistical analysis

High or low PD-1 expression was defined according to the cut-off values provided by the original articles. Due to the difference in detection methods, the random effects model was performed regardless of whether the heterogeneity between studies was statistically significant or not. The correlation between PD-1 expression and survival was evaluated based on the combination of HRs and their 95% CIs. If HR was greater than 1, the ratio of patients with poor survival compared to patients with better survival among patients with high PD-1 expression was greater than 1, which indicated higher PD-1 expression correlated with poorer survival. If HR was less than 1, higher PD-1 expression was a protective parameter. ORs and 95% CIs were used to assess the correlation between PD-1 expression and clinicopathological features. In the same vein, ORs greater than 1 meant that among patients with poor clinicopathological characteristics, the ratio of high PD-1 expression patients to low-expression patients was greater than the ratio of high PD-1 expression patients to low-expression patients in patients without adverse clinical characteristics, which meant higher PD-1 expression was correlated with higher malignancy. At last, HRs and ORs were pooled using a meta-analysis.

Sensitivity analysis was performed to evaluate the stability of the results. At each turn, one sample was deleted to observe the role of the individual study on the overall results. The potential publication bias was evaluated using Begg's test, which was also known as the rank correlation test. It was based on Kendall's tau rank correlation test to test the correlation between the standardized effect and the effect variance, namely the correlation between the testing effect and the sample size. Under the null hypothesis that there was no publication bias, the standardized effects could be considered to be independently distributed, and there was no correlation between the standardized effects. All data in the meta-analysis were synthesized using STATA15.0 Software (Stata MP). A p-value less than 0.05 was considered to be statistically significant. All p-values and 95% CIs were two-sided.

Results

Search selection

In the present study, we identified 1,691 articles with the

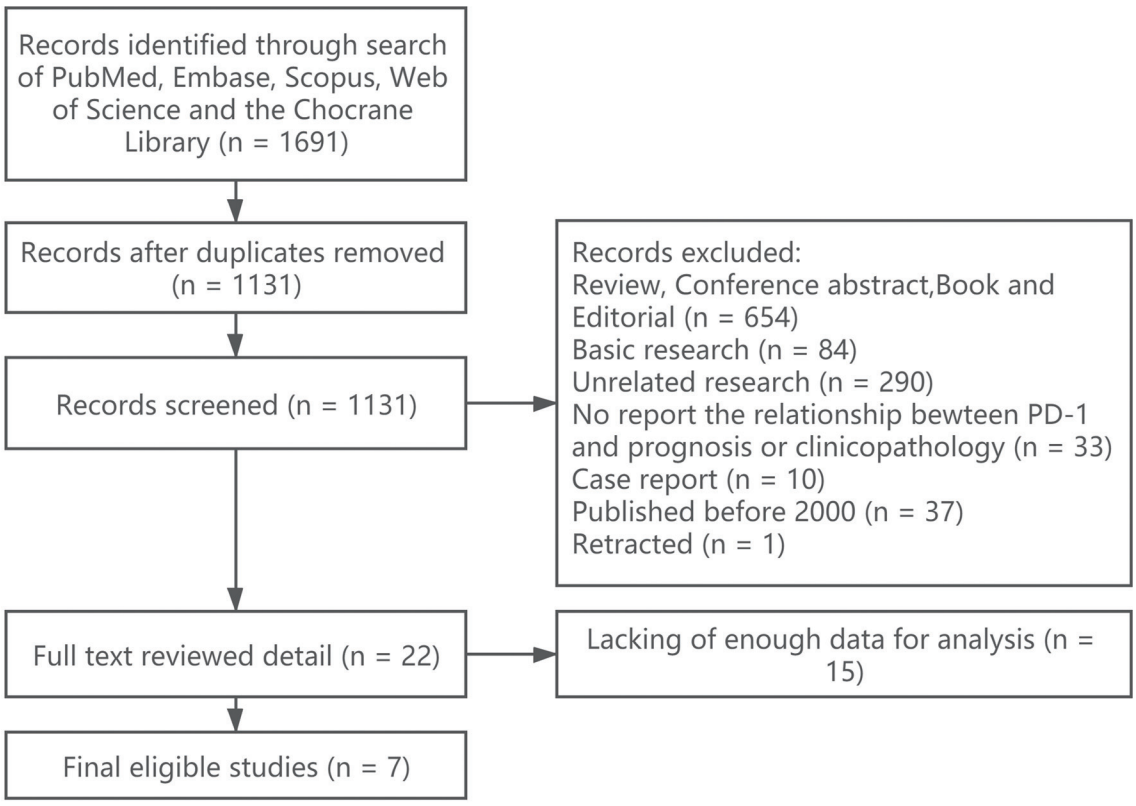

Fig. 1. Flow chart of the study's identification of papers for meta-analysis.

initial searching strategy. Of these studies, we excluded 560 duplicates and deleted another 1,109 records after screening titles or abstracts. After thoroughly reviewing the full texts of 22 potentially eligible articles, 7 trials meeting the inclusion criteria were included in the final analysis. Figure 1 demonstrates the detailed selection process.

Characteristics of the included studies

Table 1 lists the principal features of included studies. All studies were published in the last 20 years. The number of patients was 658 in total, ranging from 40 to 171. Two were cross-sectional studies and the rest were prospective studies. The total seven included studies were all implemented in China.

Among the seven studies, five reported the correlation between survival and PD-1 expression, and some of them also explored the correlation between clinicopathological features and PD-1 expression, while two only examined the latter connection. Some did not directly report HRs and 95% CIs; hence, we calculated these statistics by adopting Kaplan-Meier curves. Heterogeneity existed in the method for detecting PD-1, the cut-off points of high PD-1 and the criteria of other clinical characteristics. We evaluated the study quality using the NOS. The score ranged from 6 to 8 (Table 2), suggesting that the methodology of the studies was relatively reliable.

Survival analysis

OS was measured in three of the seven included studies. A total of 314 patients from these three studies were evaluated to examine the correlation between PD-1 expression and OS. No significant heterogeneity existed among the included studies (χ^2=2.24; p=0.327; I^2=10.6%). Pooled results by random modeling revealed that a high level of circulating PD-1 was related to poor prognosis in term of shorter OS (HR=2.19, 95% CI: 1.26–3.12, p<0.001; Fig. 2A).

Four of the seven included studies reported HR for DFS, including 423 patients. Figure 2B showed no heterogeneity in the included studies (χ^2=3.90; p=0.564; I^2=0%). Pooled results by random model revealed that high PD-1 predicted poorer DFS (HR=2.74, 95% CI: 1.76–3.73, p<0.001), independent of the sample resources. The subgroup analysis showed that circulating PD-1 could significantly predict the DFS (HR=2.65, 95% CI: 1.80–3.50, p<0.001; Fig. 2B), while the PD-1 expressed by tumor infiltrating lymphocytes (TILs) predicted better than the circulating PD-1 in the serum (HR=3.12, 95% CI: 1.40–4.84, p<0.001; Fig. 2B).

Correlation of PD-1 expression with clinical features

Table 3 illustrates the relationship between PD-1 overexpression and clinical parameters. A high level of PD-1 is associated with multiple tumors (OR=2.27, 95% CI: 1.21–4.26, p=0.011; Fig. 3A), advanced BCLC stages (OR=3.74, 95% CI: 2.10–6.65, p<0.001; Fig. 3B) and higher level of serum AFP (OR=1.50, 95% CI: 1.01–2.22, p=0.047; Fig. 4). However, the results of subgroup analysis showed no clinical significance. The other clinical parameters were not statistically associated with the PD-1 overexpression, including age, sex, tumor size, liver cirrhosis, portal vein invasion, vascular invasion, TNM stage and Child-Pugh score (Table 3).

Table 1. Characteristics of studies included in the meta-analysis

References	Country	Case number	Method	Cut-off	Outcome	Follow-up time
Hsu *et al*.[21]	Taiwan	45	Flow cytometry	Median percentage: 34.6% in CD3+ cells	Clinical characteristics	–
Shi *et al*.[22]	China	56	Flow cytometry Immunohistochemistry	Median percentage: 16.31% in PMBC, 23.55% in NIL, 42.17% in TIL	Stage, DFS	36 months
Zeng at el.[23]	China	141	Flow cytometric	Median percentage in CD8+ cells: 6.55% in stage A, 10.37% in stage B, 18.34% in stage C	Clinical characteristics, OS, PFS	Median of 23 months (6–36 months)
Li *et al*.[24]	China	171	Immunohistochemistry	IHC score median: 5.06 in tumor, 3.07 in tumor adjacent tissues	Clinical characteristics	–
Li *et al*.[25]	China	83	ELISA	10 ng/mL	OS, surgery	Median of 36 (1–77) months
Liu *et al*.[26]	China	40	Flow cytometry	Median percentage: 7.05% in PMBC, 36.57% in NIL, 30.01% in TIL	DFS	30 months
Liu *et al*.[27]	China	122	Flow cytometry staining	Median percentage: 12.8% in CD8+ cells	Clinical characteristics, OS, PFS	Median of 59 weeks (32–84 weeks)

NIL, nontumor infiltrating lymphocytes; TIL, Tumor infiltrating lymphocytes; PBMC, peripheral blood monocyte cell; PFS, progression-free survival.

Publication bias and sensitivity analysis

We used Begg's funnel plot to test potential publication bias. Sensitivity analysis was executed by sequentially omitting each trial one at a time. Supplementary Figure 1 shows the potential publication bias and sensitivity analysis results among the studies involved in the survival analysis. No apparent publication bias for analysis existed (Egger's test: p=0.153 for OS and p=0.202 for DFS; Begg's test: p=0.296 for OS and p=0.452 for DFS). The sensitivity analysis showed that no single trial remarkably altered the pooled results for OS and DFS, which indicated that our estimates were robust and reliable. Besides, there was no significant publication bias in the analysis of clinical features, either (Table 3). Sensitivity analysis demonstrated that deleting any single study did not remarkably affect the pooled ORs for the clinical parameters with significant differences (Supplementary Fig. 2).

Discussion

HCC, considered as a common highly aggressive tumor, has been disturbing the global public health system for its dismal prognosis. Elevated serum HBV DNA level serves as a reliable risk predictor independent of hepatitis B e antigen, serum alanine aminotransferase level, and liver cirrhosis.[29] HBV-HCC has been the focus of research on HCC. Given the high prevalence of HBV in China, it could be profound to provide insight into the relationship between HBV-HCC and cutting-edge immunotherapy.

Since the first PD-1/PD-L1 inhibitors nivolumab and pem-

Table 2. NOS quality assessment of the enrolled studies

References	Selection				Comparability of cohorts on the basis of the design or analysis (study adjusts for ag, se)	Outcome			Total
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study		Assessment of outcome	Was follow-up long enough for outcome to occur	Adequacy of follow up of cohorts	
Hsu *et al*.[21]	–	*	*	–	**	*	*	*	7
Shi *et al*.[22]	*	*	*	–	**	*	*	*	8
Zeng at el.[23]	–	*	*	–	**	*	*	*	7
Li *et al*.[24]	*	*	*	–	**	*	*	*	8
Li *et al*.[25]	*	–	*	–	*	*	*	*	6
Liu *et al*.[26]	*	*	*	–	–	*	*	*	6
Liu *et al*.[27]	*	*	*	–	**	*	*	*	8

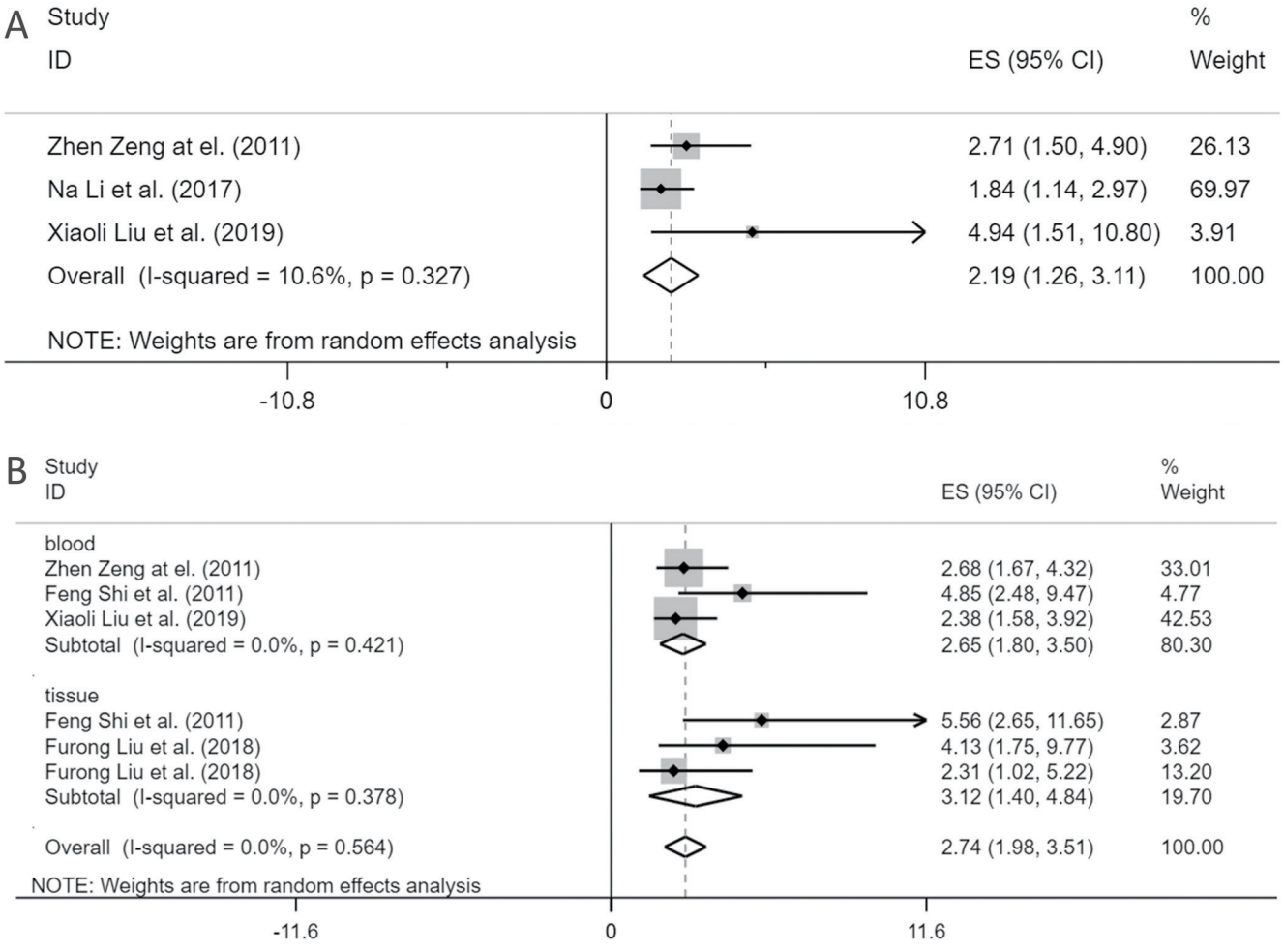

Fig. 2. Forest plot of studies evaluating the association between PD-1 expression and OS (A)/DFS (B) in patients with HBV-HCC.

brolizumab were approved by the Food and Drug Administration (FDA) in 2014, this class has been rapidly developed and was approved for several solid tumors, such as melanoma and Hodgkin lymphoma.[7] PD-1 is a negative regulator of T-cell activation by its mechanism of suppressing T-cell activity at different stages in the immune response when interacting with its two ligands, PD-L1 and PD-L2. When engaged by ligands, PD-1 inhibits kinase signaling pathways through phosphatase activity, rather than T-cell activation as in the physiological condition.[30,31] PD-1/PD-L1 overexpression has been noticed in various solid tumors, and several studies have concluded that the overexpression of PD-1/PD-L1 plays an important role in regulating the T-cell-mediated antitumor response, leading to poor prognosis.[32–34] Although the earliest and most widely recognized predictive biomarker was PD-L1, with several assays approved by FDA, it has not been proven as the definitive biomarker. In the TME with high density of CD8+ tumor infiltrating lymphocytes, PD-1, PD-L1/PD-L2 and cytotoxic T-lymphocyte antigen 4 (i.e. CTLA-4) might predict the prognosis and response to PD-1/PD-L1 blockade as well.[35] In our subgroup analysis, PD-1 expressed by TILs predicted DFS better than the circulating PD-1 in the serum. This may be because the PD-1 expressed by TILs can interact with PD-L1 secreted by tumor cells, leading to immune escape of tumor cells. PD-1 is secreted into serum after being expressed in cells. The PD-1 in TILs has higher predictive efficiency and it is recommended to routinely detect PD-1 in TILs.

There have been several reviews and meta-analyses indicating the relationship between the high level of PD-L1 and worse prognosis in patients with HCC.[36–38] However, two meta-analyses published last year found high expression of PD-1 predicted a better prognosis of HCC patients.[39,40] We had reviewed some literature suggesting that high level of PD-1 is associated with a poor prognosis in HBV-HCC patients, and we decided to conduct a meta-analysis to explore the relationship between the PD-1 expression and prognosis in HBV-HCC. This meta-analysis, presented herein and based on seven studies with 658 patients, showed that the high expression of PD-1 statistically indicated a poor OS and DFS, whether the sample originated from blood or tumors. As for the clinicopathological parameters, our findings suggested that overexpression of PD-1 was significantly associated with multiple tumors, higher level of AFP and advanced BCLC stages of HCC. Chronic liver diseases with longstanding inflammation often induce T cell exhaustion and the appearance of regulatory T cells (i.e. Tregs). PD-1 is an important molecule in the pathway of immune escape of tumors. The increased expression can lead to immune escape of the tumor, leading to an increase of AFP and advanced BCLC stages. A tumor secreting AFP in large amounts is a prognostic sign of larger focus or multifocality, extrahepatic spread, and poor survival. Critelli *et al*.[41] found that fast-growing HCC has poor differentiation and

Table 3. Relationship between high PD-1 and the clinicopathological features

Characteristic	Studies	Case number	Pooled OR (95% CI)	*p*	Heterogeneity		Model	Publication bias Begg's *p*	References
					I^2	*p*			
Age	3	434	1.148 (0.777–1.696)	0.488	0%	0.761	Random	0.296	23,24,27
Sex (male/female)	3	434	1.018 (0.568, 1.823)	0.952	30%	0.241	Random	1	23,24,27
Number of tumors (multiple/solitary)	2	167	2.268 (1.209, 4.257)	0.011	0%	0.744	Random	1	21,27
Tumor size (>5 cm/≤5 cm)	4	478	1.709 (0.420, 6.959)	0.455	90.60%	<0.001	Random	0.734	21,23,24,27
Liver cirrhosis (present/absent)	2	167	1.522 (0.080–28.893)	0.78	88.30%	0.003	Random	1	21,27
AFP (high/low)	3	434	1.495 (1.005, 2.223)	0.047	0%	0.802	Random	1	23,24,27
Vascular invasion (present/absent)	3	308	0.535 (0.059–4.816)	0.577	91.70%	<0.001	Random	1	21,23,27
BCLC stage (C+D vs. A+B)	2	263	3.738 (2.101, 6.651)	<0.001	0%	0.462	Random	1	23,27
TNM stage (3+4 vs. 1+2)	3	272	2.116 (0.855, 5.237)	0.105	60.50%	0.08	Random	1	21,22,24
HBV DNA level	2	263	0.951 (0.553, 1.635)	0.856	0%	0.901	Random	1	23,27
Child-Pugh score (B/A)	3	357	1.704 (0.893–3.252)	0.106	34.20%	0.219	Random	1	21,23,24

more angiogenesis, characterized by an immunosuppressed microenvironment under local up-regulation of PD-1 and PD-L1, along with elevated AFP, TGF-β, and IL-8. PD-1 may have a predictive value in AFP-nonsecreting HCC.

Among the included studies, two showed that high PD-1 expression was associated with larger tumor size, one showed that high PD-1 expression was associated with smaller tumor size and the results of two others showed that the PD-1 expression and tumor size were not statistically correlated (Supplementary Fig. 3). Two of included studies reported the relationship between PD-1 expression and TNM stage, which both showed that the PD-1 expression and TNM stage were not statistically correlated (Supplementary Fig. 4). Since there were studies that confirmed that the outcome of PD-1 inhibitor may be correlated with tumor size and TNM stage[42,43] and two of the included studies indicated that high PD-1 expression was associated with larger tumor size, we speculated that the results may not be confirmative due to insufficient sample. It required further expansion of the sample to draw an accurate conclusion. The same was true for liver cirrhosis. Neither of the two articles that mentioned the relationship between cirrhosis and PD-1 expression showed the severity of cirrhosis. Most of the patients had low HBV DNA replication (<100 IU/mL). Liu *et al*.[27] analyzed PD-1 level in peripheral blood mononuclear cells rather than TILs, which might not reflect the exact situation of immune infiltration in HCC tissue. Hsu *et al*.[21] provided no information on antiviral therapy or details of cirrhosis. This could explain why such a vital factor of hepatitis B did not significantly correlate with PD-1 expression levels. The incidence of HCC increased with serum HBV DNA level in a dose-response relationship. Participants with persistent elevation of serum HBV DNA level during follow-up had the highest HCC risk.[29] Regular antiviral treatment in some patients may not necessarily increase the expression of PD-1. Grouping HBV-HCC patients according to the amount of HBV replication to analyze the relationship between PD-1 and prognosis can define the predictive value of PD-1 in such patients more clearly.

The expression of PD-1 in T cells can be induced by up-regulated PD-L1 in tumor cells and by other molecules. Some studies have suggested that PD-L1-positive tumor cells have prominent immune cell infiltration in HCC, such as CD3+ TILs (representing overall T cells), CD8+ TILs (representing cytotoxic T cells), and tumor-associated macrophages (i.e. TAMs).[27,37,44,45] This result may support the possibility of a role for an adaptive immune resistance mechanism. Some other research studies have pointed out that PD-1 expression is related to T cell exhaustion. Blocking the PD-1 pathway could reverse this phenotype, to restore anti-tumor immunity.[46–48] In HBV-HCC, the reactivation of oncofetal gene SALL4 by HBV counteracts miR-200c in PD-L1-induced T cell exhaustion. Overexpression of miR-200c antagonizes HBV-mediated PD-L1 expression by targeting the 3′-UTR of the CD274 gene (encoding PD-L1) directly, which reverses antiviral CD8+ T cell exhaustion.[49] Through analysis, we also found that the positive rate of PD-1 was relatively higher than that of PD-L1 in HCC tissue. In view of its high positive ratio, the diversity of detection methods and the stability of the results on tumor prognosis, we believe that PD-1 can be a marker to predict HBV-HCC prognosis. A meta-analysis in patients with pretreated advanced non-small-cell lung cancer indicated a slight benefit from anti-PD-1 compared to that from anti-PD-L1 inhibitors.[50] An indirect comparison in advanced squamous non-small-cell lung cancer showed that, for PD-L1 low/negative patients, pembrolizumab had superior OS (HR=0.43, range: 0.24–0.76; $p<0.01$/HR=0.74, range: 0.40–1.38; $p=0.35$) and better progression-free survival (HR=0.80, range: 0.51–1.26; $p=0.33$/HR=0.46, range: 0.28–0.75; $p<0.01$) than atezolizumab. There has been no study comparing the efficacy between PD-1 inhibitors and PD-L1 inhibitors in

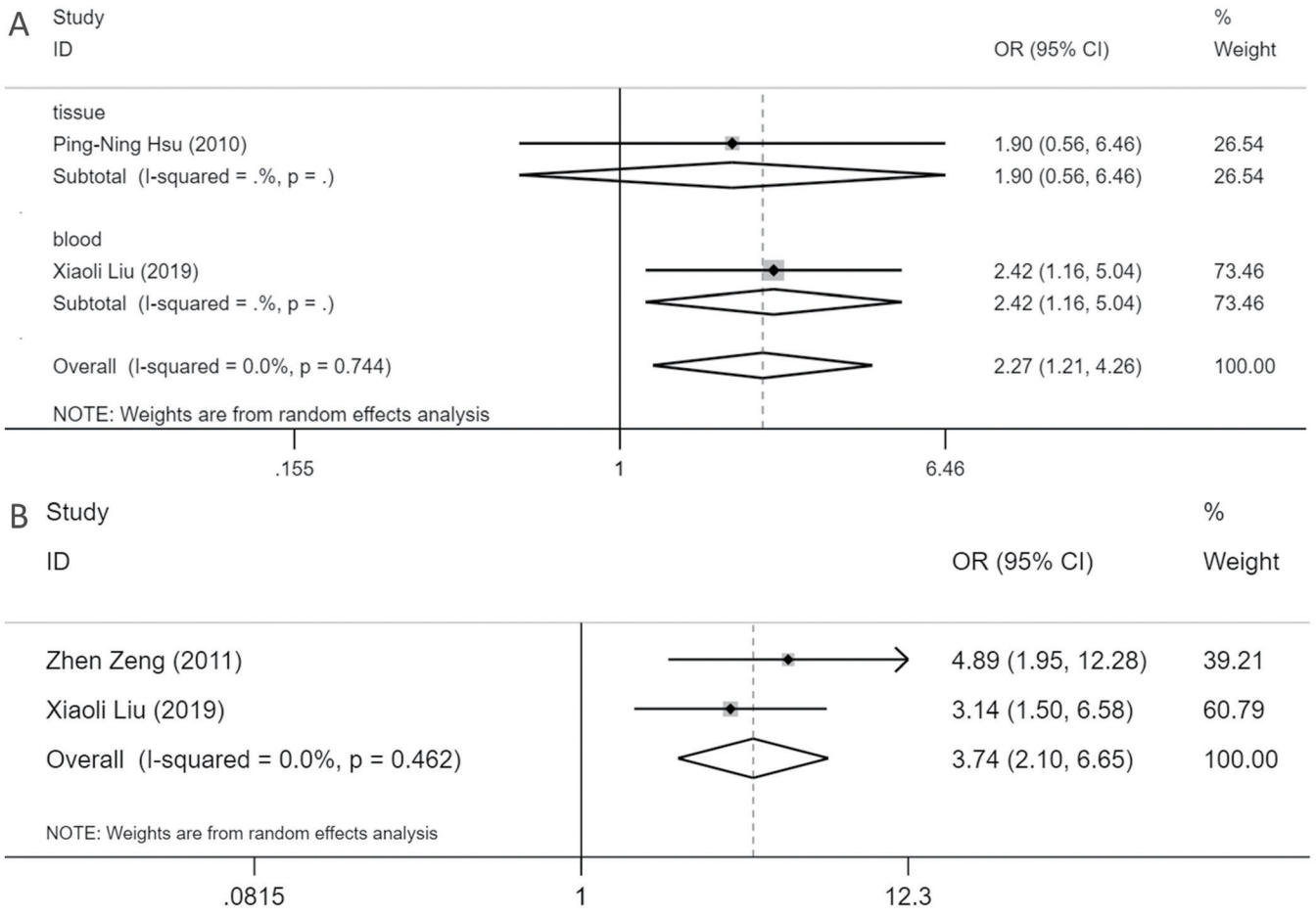

Fig. 3. Forest plot of studies evaluating the association between PD-1 expression and tumor numbers (A)/BCLC stage (B) in patients with HBV-HCC.

HCC patients thus far. More clinical trials are required to determine which target is better for HBV-HCC, PD-1, or PD-L1. In our analysis, PD-1 had shown good predictive efficacy.

Han *et al*.[44] pointed out that PD-1 expression in tumors was statistically related to the level in serum. The higher expression of PD-1 in tumors, the higher concentration of PD-1 in serum. The results of our analysis support this view. The PD-1 expression levels in tumor and in serum were consistent in predicting prognosis and clinical parameters. However, due to different detection methods and grouping levels, the relationship between PD-1 and survival may be inaccurate. Considering this point, though the heterogeneity was low, we still carried out the subgroup analysis to assure whether both of the detection methods could predict the prognosis.

To our knowledge, our study is the first meta-analysis focused on the prognostic value of PD-1 expression in HBV-HCC patients specifically. The results are different from those in all HCC patients regardless of HBV.

However, there are several limitations inherent to our study's design. First, for some studies, we used the Engauge Digitizer to calculate HRs, which may cause bias. Second, the number of studies was insufficient. There were only three studies that investigated the correlation between PD-1 expression and OS and four that focused on the correlation between PD-1 and DFS. All studies originated from China, which may have limited the data extrapolation. Third, the grouping criteria of PD-1 level and other parameters of included studies varied. Different standards may increase bias. Finally, though our analysis upon prognosis in HBV-HCC showed the opposite result from the study in HCC, the relationship of PD-1 expression was not associated with HBV infection. The mechanism between PD-1 and HBV needs further study.

Conclusions

Our meta-analyses revealed that PD-1 expression was significantly correlated with shorter OS, DFS, higher level of AFP, multiple tumors and advanced BCLC stage of HBV-HCC. Based on the included studies, we found that PD-1 expressed in tumors and blood could reflect the immune status of patients, thereby increasing the reliability of the results. We can preliminarily assume that HBV enhances the expression of PD-1 through certain mechanisms and leads to poor prognosis of HBV-HCC patients. The prognostic role of PD-1 in HBV-HCC and the mechanism of how HBV mediated PD-1 expression still demand further investigation.

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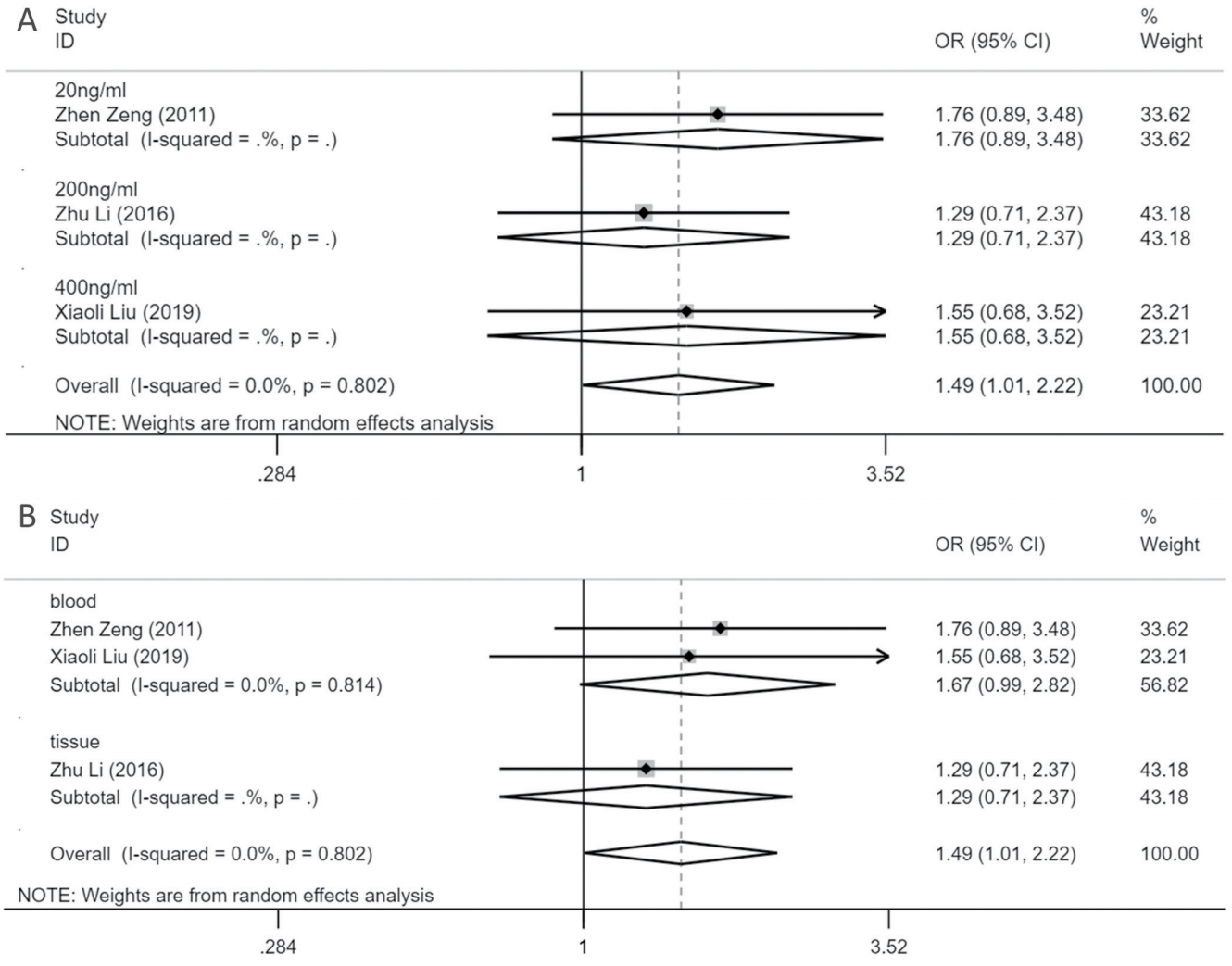

Fig. 4. Forest plot of studies evaluating the association between PD-1 expression and AFP levels in patients with HBV-HCC. A and B are two subgroup analyses respectively based on PD-1 resources and different AFP levels.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (RZ, CL), drafting of the manuscript (ZYZ, SRL), acquisition of data (ZYZ, SRL), critical revision of the manuscript (LBX), study supervision (RZ). All authors have contributed to the revision of the manuscript and approved the submitted version.

Data sharing statement

All data are available upon request.

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