ORIGINAL ARTICLE

Hepatotoxicity associated with PD-1 blockade antibodies in cancer patients co-infected with hepatitis B virus

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Abstract

The use of anti-programmed cell death-1 (PD-1) antibodies in treating malignancies is increasing; however, most registered clinical trials on anti-PD-1 antibodies exclude patients infected with hepatitis *B* virus (HBV). This retrospective study aimed to assess hepatotoxicity in cancer patients infected with HBV undergoing anti-PD1 antibody therapy and identify the associated risk factors. A total of 301 cancer patients positive for hepatitis *B* core antibodies (HbcAb) (negative or positive hepatitis *B* surface antigen [HBsAg]) who received PD-1 inhibitors were enrolled. The primary and secondary endpoints were the incidence rate of hepatotoxicity related to PD-1 inhibitor treatment, and risk factors associated with hepatic toxicity, respectively. Of the enrolled analyzed, 16.9% ($n = 51$) developed any grade and 4.7% ($n = 14$) developed grade 3–4 hepatotoxicity, respectively. Higher risk for any-grade hepatotoxicity development was associated with sero-positive HBsAg (OR = 6.30; $P = 0.020$), existence of liver involvement (OR = 2.10; $P = 0.030$), and detectable baseline HBV DNA levels (OR = 2.39; $P = 0.012$). Patients with prophylactic antiviral therapy decreased hazard for the incidence of grade 3–4 hepatotoxicity (OR = 0.10; $P = 0.016$). Our results suggested chronic (HBsAg-positive)/resolved (HBsAg-negative and HBcAb-positive) HBV-infected cancer patients are at an increased risk of hepatotoxicity following PD-1 inhibitor therapy. Cancer patients should be tested for HBsAg/HBcAb prior to the commencement of immune checkpoint inhibitor therapy. For patients with chronic/resolved HBV infection, ALT/AST and HBV DNA should be closely monitored during the whole immunotherapy period.

Keywords PD-1 · Cancer, Hepatitis *B* virus · Hepatotoxicity · Immunotherapy · Safety

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Introduction

Anti-programmed cell death-1 (PD-1) antibody treatment is associated with promising responses across variety of cancer subtypes, including non-small-cell lung cancer [1], small-cell lung cancer [2], Hodgkin lymphoma [3, 4], and gastric cancer [5]. Approval for indications of anti-PD1 antibodies continues to be extended and the use of this agent is increasing. However, the efficacy profile of anti-PD-1 antibodies has been accompanied by a range of associated adverse events. The greater number of patients exposed to anti-PD1 antibody therapy has generated more frequent immune-related adverse events (irAEs).

The liver is one of the most common immunologically affected organs associated with anti- PD-1 immunotherapy [6, 7]. Some studies have reported that the pooled incidence rates of elevated liver transaminase were approximately 8% and 15% for anti-PD-1 antibody treatment alone [8]

and PD-1 inhibitor combined with an ipilimumab regimen, respectively [9]. The incidence rate of hepatitis among patients treated with PD-1 inhibitor plus chemotherapy has been reported to be 2% [10]. Throughout most clinical trials, there has been a low incidence rate of hepatotoxicity attributed to anti-PD-1 antibody treatment.

However, most immunotherapy clinical trials have typically excluded patients with hepatitis *B* virus (HBV) infection. HBV infection remains the most common chronic viral infection, from which approximately 887,000 individuals die from HBV-related liver disease each year and about half of all patients are from China [11]. Among patients with malignancies who receive anti-tumor therapy, HBV infection is considered to be one risk factor for liver toxicity [12, 13]. Severe acute hepatotoxicity can occasionally progress to fulminant hepatic failure and death [14]. Due to exclusion of HBV-infected patients in most registered clinical trials, the safety of anti-PD-1 immunotherapy among this population remains unclear.

To date, there has been limited data exploring the association between HBV infection and liver toxicity induced by anti-PD-1 antibodies. This study aimed to access the prevalence of hepatotoxicity in patients with malignancies infected with HBV undergoing anti-PD-1 antibody therapy. This study also sought to identify potential risk factors for the development of immune-related hepatotoxicity.

Materials and methods

Study design and patients

Cancer patients with seropositive hepatitis *B* core antibodies (HBcAb) who received anti-PD-1 antibody immunotherapy at the Sun Yat-sen University Cancer Center between January 2015 and July 2018 were enrolled in this retrospective study.

Patients with pathologically confirmed advanced cancer (not amenable to curative surgery or local treatment) were eligible for this study. At least one dose of anti-PD-1 single agent or combination therapy was prescribed to each of the participants. Patients were also required to be diagnosed as having a chronic or previous HBV infection (HBcAb-positive with either seropositive or sero-negative hepatitis *B* surface antigen [HBsAg]). The enrolled patients should have complete clinical and follow-up data. Adequate available clinical information, including regularly monitored liver function, was required for the recruited patients over the study period. The selected patients had no evidence of other types of viral hepatitis (e.g., hepatitis *A* virus [HAV], hepatitis *C* virus [HCV], hepatitis *D* virus [HDV], hepatitis *E* virus [HEV], or human immunodeficiency virus [HIV]) infection. Patients who displayed elevated levels of alanine

aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TBR) at the initiation of PD-1 inhibitor-involved therapy were excluded. Data regarding adverse events and laboratory abnormalities were regularly collected from medical records and reviewed by oncologists over the study period. The causality between anti-PD-1 antibody-associated treatment and hepatotoxicity was retrospectively assessed using the WHO-UMC system (Word Health Organization-Uppsala Monitoring Center) based on patient's clinical manifestation, results of CT scans and laboratory examinations. The various causality categories included: certain/definitely; probable/likely; possible; unlikely; conditional/unclassified; and unassessable/unclassifiable [15]. All patients provided written informed consent, and the study protocol was approved by the Sun Yat-Sen University Cancer Center Institutional Review Board (Guangzhou, China).

The primary objective was the total incidence rate of suspected hepatotoxicity (definitely, probably, or possibly) related to treatment with PD-1 inhibitor therapy as judged by an oncologist. Suspected hepatotoxicity was defined as an elevation in the level of aminotransferase (ALT and/or AST, whichever came first). The hepatotoxicity grade was determined using the Common Terminology Criteria for Adverse Events (CTCAE), ver. 5.0. The CTCAE defines grade 1, grade 2, grade 3, and grade 4 toxicity levels of ALT/AST as 1–3 times, 3–5 times, 5–20 times, and over 20 times the upper limit of normal, respectively. In this study, hepatotoxicity was inferred as grade 1 or higher. Severe hepatotoxicity was defined as grade 3–5 change in the level of ALT or AST. If the AST and ALT grades were discordant, the higher of the two was used for classification. The secondary objective was to identify risk factors associated with the development of hepatic toxicity in these patients (Fig. 1).

The level of HBV DNA was assessed using a real-time viral polymerase chain reaction assay (ABI 7900; Applied Biosystems, Foster City, CA, USA). The lower limit of detection for the level of HBV DNA was 10 IU/mL.

Statistical analysis

A Chi-squared test or Fisher's exact test was used to compare the categorical variables between patients with and without hepatotoxicity. A multivariate logistic regression analysis was used to identify predictors of hepatotoxicity. Factors with a *P* value < 0.2 in the univariate analysis in conjunction with strong confounding factors (e.g., age, sex, and body weight) were included in the multivariate analysis. Odds ratio (OR) and adjusted ORs were estimated by univariate and multivariate analyses, respectively. The time taken to reach hepatotoxicity was analyzed using a Kaplan–Meier survival analysis and log-rank test. Statistical significance was defined as $P < 0.05$ (2-tailed). Data were analyzed using

Fig. 1 Flowchart depicting patient deposition. PD-1, programmed cell death 1; HBcAb, hepatitis *B* core antibody; anti-HCV, antibody to the hepatitis *C* virus; ALT, alanine aminotransferase; ALT, aspartate aminotransferase; HBsAg, hepatitis *B* surface antigen
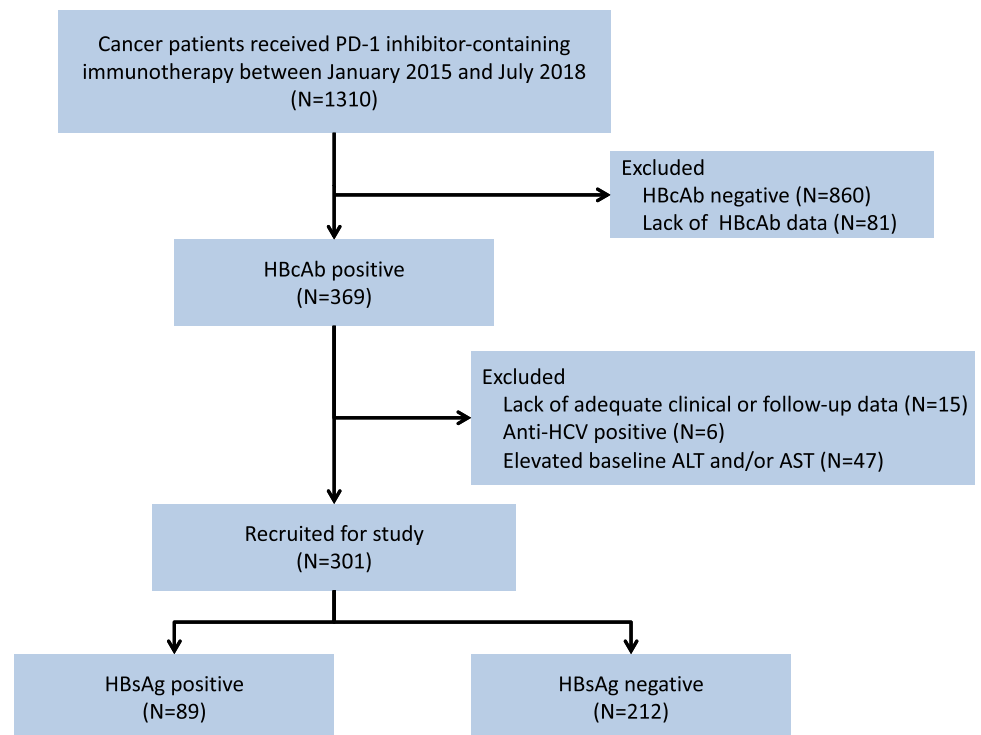
Results

Patients

A total of 348 individuals were screened. Of these, 47 patients were determined to be ineligible due to the following reasons: the pooled values were elevated for both ALT and AST (20 patients), only ALT levels were detected (9 patients), and only AST levels were detected (18 patients) at the initiation of PD-1 immunotherapy, respectively. Consequently, the data from 301 patients treated with anti-PD-1 antibodies were analyzed.

The baseline characteristics of the patients included in this study are listed in Table 1. In a total of 301 HBcAb-positive patients, HBsAg seropositivity and HBeAg seropositivity were found in 89 (29.6%) and 8 (2.7%) patients, respectively. Of the recruited patients, 71.4% ($n=215$) were male and the median age was 52 years old (range: 44–84). The most common tumor types included: nasopharyngeal carcinoma (NPC, $n=84$; 27.9%); non-small-cell lung cancer (NSCLC, $n=66$; 21.9%); melanoma ($n=38$; 12.6%); gastric cancer ($n=24$; 8.0%); hepatocellular carcinoma (HCC, $n=20$; 6.6%); and lymphoma ($n=21$; 7.0%). There were 114 (37.8%) patients who had received more than two lines of therapy prior to receiving anti-PD-1 immunotherapy. During the immunotherapy phase, 92 (30.6%) patients received treatment with the PD-1 inhibitor alone (pembrolizumab, nivolumab, toripalimab, camrelizumab, and sintilimab), whereas 209 (69.4%) patients received combination therapy. The level of HBV DNA was detectable in 24 (8.0%) patients and undetectable in 101 (33.6%) patients. The median titer of the detectable HBV DNA was 6.57×10^2 IU/mL (range: $3.01 \times 10^1 - 1.69 \times 10^5$ IU/mL). There were 63 (20.9%) patients who received prophylactic antiviral therapy, including 20, 42, and 1 for the baseline HBV-DNA status of detectable, undetectable, and unknown status, respectively. Five types of antiviral drugs were used, including entecavir ($n=49$), lamivudine ($n=9$), tenofovir ($n=3$), telbivudine ($n=1$), adefovir ($n=1$).

Development of any grade hepatotoxicity and risk factors

The proportion (number) of all subjects with suspected hepatotoxicity was associated with anti-PD-1 antibody-involved therapy was 16.9% ($n=51$) (Table 1). Hepatotoxicity occurred at a median of 10.57 weeks (95%CI: 7.15–13.99 weeks) following the initiation of a PD-1 inhibitor. The median time to resolution was 4 weeks (95%CI: 3.05–4.95 weeks). Eight patients (grade 3, $n=7$; grade 2, $n=1$) experienced treatment delay due to hepatic AEs and successfully went back on immunotherapy after resolution. Five patients with grade 3–4 hepatotoxicity permanently discontinued PD-1 inhibitor treatment. Eight patients received

Stata software (SPSS Inc., Chicago, Illinois, version 22.0, USA).

Table 1 Baseline characteristics of the included patients (*n* = 301)

	No. of patients (%)	No. of any-grade hepatotoxicity (%)	P^a	No. of grade 3–4 hepatotoxicity (%)	P^a
Total	301 (100%)	51 (16.9%)		14 (4.7%)	
Age (years, range 44–84)			0.813		0.365
> 52	143 (47.5)	25 (17.4)		5 (3.5)	
≤ 52	158 (52.5)	26 (16.5)		9 (5.7)	
Gender			0.409		0.226
Male	215 (71.4)	34 (15.8)		8 (3.7)	
Female	86 (28.6)	17 (19.8)		6 (7.0)	
Body weight(kg)			0.813		0.848
> 60	143 (47.5)	25 (17.5)		7 (4.9)	
≤ 60	158 (52.5)	26 (16.5)		7 (4.4)	
History of alcoholism			0.900		0.091
Yes	49 (16.3)	8 (16.3)		0 (0.0)	
No	252 (83.7)	43 (17.1)		14 (5.6)	
[b]*HTN/DM/CVD*			0.858		0.334
Yes	39 (13.0)	7 (17.9)		3 (7.7)	
No	262 (87.0)	44 (16.8)		11 (4.2)	
Liver cirrhosis			0.790		0.030
Yes	21 (7.0)	4 (19.0)		3 (13.6)	
No	280 (93.0)	47 (16.8)		11 (3.9)	
Hepatocellular carcinoma			0.320		0.023
Yes	20 (6.6)	5 (25.0)		3 (15.0)	
No[c]	281 (93.4)	46 (16.4)		11 (3.9)	
HBsAg status			0.757		0.004
Seropositive	89 (29.6)	16 (18.0)		9 (10.0)	
Seronegative	212 (70.4)	35 (16.5)		5 (23.6)	
HBeAg status			0.116		0.006
Seropositive	8 (2.7)	3 (37.5)		2 (25%)	
Seronegative	293 (97.3)	48 (16.4)		12 (4.1)	
ECOG performance status			0.227		0.665
> 1	32 (10.6)	3 (9.4)		1 (3.1)	
≤ 1	269 (89.4)	48 (17.8)		13 (4.8)	
Lines of treatment			0.464		0.694
> 2	114 (37.8)	17 (14.9)		6 (5.2)	
≤ 2	187 (62.1)	34 (18.2)		8 (4.3)	
Liver involvement[d]			0.022		0.016
Yes	122 (40.5)	28 (23.0)		10 (8.1)	
No	179 (59.5)	23 (12.8)		4 (2.2)	
Antiviral therapy[e]			0.527		0.472
Yes	63 (20.9)	9 (14.3)		4 (6.3)	
No	238 (79.1)	42 (17.6)		10 (4.2)	
Baseline HBV DNA level			0.026		0.000
Undetectable[f]	101 (33.6)	9 (8.9)		4 (4.0)	
Detectable	24 (8.0)	6 (25.0)		5 (20.8)	
Unknown	176 (58.4)	36 (20.5)		5 (2.8)	
Treatment modality			0.141		0.307
Combined therapy[g]	92 (30.6)	20 (21.7)		6 (6.5)	
Monotherapy[h]	209 (69.4)	31 (14.8)		8 (3.8)	

[a]Determined using a χ2 test

[b]Abbreviations: *HTN* hypertension; *DM* diabetes mellitus; *CVD* cardiovascular disease

[c]Including nasopharyngeal carcinoma (n = 84), non-small-cell lung cancer (*n* = 66), melanoma (*n* = 38),

Table 1 (continued)

gastric cancer ($n=24$), colorectal cancer ($n=8$), lymphoma (n=21), urothelial carcinoma ($n=5$), esophageal cancer ($n=5$), gallbladder carcinoma ($n=1$), spongiocytoma ($n=1$), germinoma ($n=1$), small-cell carcinoma of the vagina ($n=1$), Breast cancer ($n=4$), soft tissue sarcoma ($n=4$), small-cell lung cancer ($n=4$), tumor of unknown original lesion ($n=3$), neuroendocrine tumor ($n=3$), pancreatic cancer ($n=2$), kidney cancer ($n=2$), cervical cancer ($n=2$), head and neck tumor ($n=2$)

[d]Malignant lesions in the liver, including HCC and liver metastases of non-HCC tumors

[e]Including entecavir (n=49), lamivudine ($n=9$), tenofovir ($n=3$), telbivudine ($n=1$), adefovir ($n=1$)

[f]HBV DNA < 10 IU/mL

[g]including PD-1 inhibitor plus chemotherapy (n=50), targeted agent (osimertinib [$n=1$], bevacizumab [$n=3$], ramucirumab [$n=3$], rituximab [$n=1$], nimotuzumab [$n=1$], sunitinib [$n=1$], axitinib [$n=1$], Ibrutinib [$n=1$], apatinib [$n=1$], cabozantinib [$n=1$], lenvatinib [$n=4$], vemurafenib [$n=1$], pazopanib [$n=1$], SHR7390 [$n=2$]), chemotherapy plus targeted agent ($n=10$), and ipilimumab ($n=7$)

[h]Including pembrolizumab, nivolumab, toripalimab, camrelizumab, sintilimab

Abbreviations: *ECOG* Eastern Cooperative Oncology Group; *HBV* hepatitis B virus; HBeAg hepatitis B e antigen; *HBsAg* hepatitis B surface antigen

steroids treatment (prednisone 0.5–2 mg/kg, tapering over 3–6 weeks) for hepatic AEs. No death was attributed to treatment-related hepatotoxicity.

Causality in these patients was considered as certain in 20/51 (37.3%), likely in 16/51 (31.3%) and possible in 15/51 (29.4%) patients. The prevalence of hepatotoxicity in combination therapy group was greater than that in monotherapy, with no statistical significance (21.7% vs. 14.8%, $P=0.141$). Liver involvement and the levels of baseline HBV DNA were found to be related to hepatotoxicity occurrence (Table 1). The univariate analysis indicated that existed liver involvement existing (OR: 2.02; 95% CI: 1.10–3.71; $P=0.023$) and detectable baseline levels of HBV DNA (OR: 3.41; 95% CI: 1.08–10.76; $P=0.037$) were independent risk factors for the development of hepatotoxicity. The multivariate analysis demonstrated that patients with HBsAg seropositivity had a 6.30-fold increased incidence for hepatotoxicity compared with those without (95% CI: 1.33–29.80; $P=0.020$). Liver involvement ($P=0.030$) and baseline levels of HBV DNA ($P=0.012$) also significantly increased risks for the development of hepatotoxicity (Table 2).

Development of severe hepatotoxicity (grade 3/4) and risk factors

The incidence of grade 3–4 hepatotoxicity related to PD-1 inhibitor-involved therapy was estimated at 14/301 (4.7%). The existence of liver cirrhosis, diagnosis of primary hepatocellular carcinoma, seropositive HBsAg, seropositive HBeAg, existence of liver involvement, and detectable baseline HBV DNA levels were significantly associated with a higher incidence of severe hepatotoxicity (Table 1).

In the univariate analysis, liver cirrhosis (OR: 4.08; 95%CI: 1.04–15.93; $P=0.043$), primary hepatocellular carcinoma (OR: 4.33; 95%CI: 1.10–17.00; $P=0.036$), liver involvement (OR: 3.91; 95%CI: 1.20–12.76, $P=0.024$), seropositive HBsAg (OR: 4.66; 95%CI: 1.51–14.32; $P=0.007$), seropositive HBeAg (OR: 7.81; 95%CI:

1.42–42.79, $P=0.018$), and detectable baseline HBV DNA (OR, 6.38; 95%CI: 1.57–25.98, $P=0.010$) levels were independent risk factors for grade 3–4 hepatotoxicity development. None of the significant aforementioned risk factors for grade 3–4 hepatotoxicity were identified in the multivariate analysis. The administration of antiviral therapy was found to be a significant beneficial factor by a 90% reduction in the risk for the incidence of grade 3–4 hepatotoxicity ($P=0.016$) (Table 2).

Discussion

This study reports a large cohort and systematic analysis emphasizing liver toxicity in cancer patients with chronic or resolved HBV infection who were treated with anti-PD-1 immunotherapy. Our findings showed that the rate of any grade and grade 3–4 hepatotoxicity in this population were 16.9% and 4.7%, respectively. Significant risk factors for the development of hepatotoxicity included the existence of liver involvement, HBsAg seropositivity, and detectable baseline HBV DNA levels. Our data indicate that HBV infection may not be a contraindication to treatment with an PD-1 inhibitors regimen. Moreover, these findings may provide clinically relevant information for this patient population while receiving PD-1 inhibitor treatment by enhancing the awareness of its hepatic profiles.

Anecdotally, hepatic profiles are infrequent but common immune-related adverse events. Hepatotoxicity occurs in 5–10% (of which 1–2% is grade 3) of patients during therapy with PD-1 inhibitor at the approved doses as single agents [16]. Available data reported from large-scale phase III clinical trials of the administration of a single anti-PD-1 antibody showed that the pooled incidence of grade 1–4/3–4 hepatic adverse events was reported to be 10.7%/3.8% and 7%/1% by checkmate-227 [9] and keynote-042 [17], respectively. Most participants included in these trials were negative for HBV infection. Currently, our results indicate 16.9%/4.7%

Table 2 Risk factors for any-grade and grade 3–4 hepatotoxicity development

Covariate	Any-grade hepatotoxicity				Grade 3–4 hepatotoxicity			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Age (years)		0.834				0.370		
> 52	1.07 (0.58–1.95)				0.60 (0.20–1.83)			
≤ 52	1				1			
Gender		0.400				0.233		
Male	0.76 (0.40–1.45)				0.52 (0.17–1.53)			
Female	1				1			
Body weight(kg)		0.813				0.848		
> 60	1.08 (0.59–1.97)				1.11 (0.38–3.25)			
≤ 60	1				1			
History of alcoholism		0.900				0.997		
Yes	0.95 (0.42–2.17)				0.00 (0.00–NA)			
No	1				1			
HTN/DM/CVD		0.858				0.341		
Yes	1.08 (0.45–2.61)				1.90 (0.51–7.14)			
No	1				1			
Liver cirrhosis		0.790		0.492		0.043		0.682
Yes	1.17 (0.37–3.62)		0.47 (0.05–4.13)		4.08 (1.04–15.93)		1.88 (0.09–38.43)	
No	1		1		1		1	
Hepatocellular carcinoma		0.325		0.437		0.036		0.892
Yes	1.70 (0.59–4.92)		2.30 (0.28–18.83)		4.33 (1.10–17.00)		1.23 (0.06–25.438)	
No	1		1		1		1	
HBsAg status		0.757		0.020		0.007		0.123
Sero-positive	1.11 (0.58–2.13)		6.30 (1.33–29.80)		4.66 (1.51–14.32)		5.80 (0.62–54.20)	
Sero-negative	1		1		1		1	
HBeAg status		0.134		0.096		0.018		0.080
Sero-positive	3.06 (0.71–13.25)		4.15 (0.78–22.18)		7.81 (1.42–42.79)		6.83 (0.80–58.66)	
Sero-negative	1		1		1		1	
ECOG performance status		0.237				0.667		
> 1	0.48 (0.14–1.63)				0.64 (0.08–5.02)			
≤ 1	1				1			
Lines of treatment		0.464				0.694		
> 2	0.79 (0.42–1.49)				1.24 (0.42–3.68)			
≤ 2	1				1			
Liver involvement		0.023		0.030		0.024		0.162

Table 2 (continued)

| Covariate | Any-grade hepatotoxicity | | | | Grade 3–4 hepatotoxicity | | | |
| | Univariate analysis | | Multivariate analysis | | Univariate analysis | | Multivariate analysis | |
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Yes	2.02 (1.10–3.71)		2.10 (1.08–4.10)		3.91 (1.20–12.76)		2.64 (0.68–10.29)	
No	1		1		1		1	
Antiviral therapy		0.528				0.475		0.016
Yes	0.78 (0.36–1.70)				1.55 (0.47–5.10)		0.10 (0.014–0.65)	
No	1				1		1	
Baseline HBV DNA level		0.032		0.012		0.003		0.080
Detectable[a]	3.41 (1.08–10.76)	0.037	2.39 (0.67–8.53)	0.181	6.38 (1.57–25.98)	0.010	7.49 (1.28–43.72)	0.025
Unknown	2.63 (1.21–5.71)	0.015	8.09 (1.87–34.93)	0.005	0.71 (0.19–2.70)	0.615	1.60 (0.19–13.61)	0.668
Undetectable	1		1		1		1	
Treatment modality		0.143		0.241		0.312		0.515
Combined therapy	1.60 (0.85–2.98)		1.49 (0.77–2.88)		1.75 (0.59–5.20)		1.51 (0.44–5.25)	
Monotherapy	1		1		1		1	

[a]HBV DNA < 10 IU/mL

Abbreviations: OR odd ratio; *CI* confidence interval; *HTN* hypertension; *DM* diabetes mellitus; *CVD* cardiovascular disease; *ECOG* Eastern Cooperative Oncology Group; *HBV* hepatitis B virus; *HBeAg* hepatitis B e antigen; *HBsAg* hepatitis B surface antigen

for 1–4/3–4 hepatotoxicity in HBV-infected cancer patients undergoing anti-PD-1 immumotherapy, which is higher than previously reported. To our knowledge, HBV infection is not cytopathic and does not trigger immunomediated necro-inflammatory liver damage. In patients with chronic HBV infection (CHB), an inadequate HBV-specific *T* cell response can trigger substantial non-antigen-specific cellular infiltration, amplifying the level of liver damage through bystander *T* cells [18, 19]. Even among patients resolved HBV (RHB), most individuals may have detectable HBV DNA in the liver, and some also have detectable HBV DNA in the serum [20]. Potential liver damage by HBV may be the primary and most rational explanation for the higher incidence rate of liver toxicity compared with that in most registered clinical trials excluding viral hepatitis patients. Furthermore, HBV reactivation is a another potential complication of anti-PD-1/PD-L1 therapy in HBV-infected patients with cancers, which could occur in both CHB and RHB populations and result in hepatitis [21]. Our previous work reported a 5.3% incidence rate of HBV reactivation among 114 CHB cancer patients who developed HBV reactivation with abnormal liver function [22]. In addition, immune checkpoint inhibitor (ICI) combination therapy has been reported to induce a greater number of hepatic profiles than a single agent [23]. In our

study, 30.6% patients received combination therapy while 69.4% received monotherapy. Either all grade or severe grade hepatotoxicity, the incidence rate caused by the former was higher than that by the latter (grade 1–5: 21.7% vs. 14.8%; Grade ≥ 3:6.5% vs. 3.8%), although the outcomes were of no significant differences.

So far, there has been a lack of data regarding the risk factors of liver toxicity in cancer patients with HBV infection receiving immunotherapy. The current finding suggests that when receiving anti-PD-1 therapy, patients with positive HBsAg were at a much higher risk of developing hepatotoxicity than those with negative HBsAg and positive HBcAb by 4.66 times (*P* = 0.020). The persistent presence of HBsAg establishes the diagnosis of CHB, which, as part of its natural course, may lead to cirrhosis, liver failure, and/or HCC. Negative HBsAg and positive HBcAb indicates a previous exposure to HBV infection (resolved HBV); the majority of this population recovered from acute HBV infection earlier in life and anti-HBs titers have waned to undetectable levels. In RHB patients, the risk of liver dysfunction, cirrhosis or HCC due to HBV is minimal [24]. It has been wildly observed that CHB patients are in a high risk of HBV reactivation when receiving anticancer therapy [24]. For RHB patients, covalently closed circular DNA remains and is

capable of replicating in the liver of individuals with this serologic profile. This population is also potentially at risk for HBV reactivation but the incidence is much lower [24]. Detectable baseline HBV DNA was also reported to be a risk factor for the incidence of hepatotoxicity in our study. The level of HBV DNA is a direct measurement of the viral load, which demonstrates the replication activity of the virus and has been recognized as a risk factor for HBV reactivation in previous studies [25]. Worthy of note, in a recent retrospective study evaluating the safety of ICIs in cancer patients with HBV infection, the authors demonstrated that hepatitis flare (alanine aminotransferase > 2 times of the upper limit of normal) occurred in 39.3% HBsAg-positive and 30.4% HBsAg-negative patients, and CHB or RHB had no impact on the emergence of hepatic flare [26]. However, the primary endpoint, criteria of patient enrollment and the type of ICIs performed in this study are quite different from ours. The natural bias of retrospective study could also give rise to heterogeneous conclusions. Nevertheless, both studies delivered clinically relevant and nonoverlapping information to the area of ICI application in HBV-involved cancer patients. Hepatotoxicity related to PD-1 inhibitors in this population deserves greater vigilance and further study, especially in patients with positive HBsAg.

Our result suggested liver involvement as another potential risk factor for the development of hepatotoxicity while receiving ICIs. In accordance with the finding above, subgroup analysis from CheckMate 017 and 057 demonstrated that rates of treatment-related hepatic AEs were slightly higher in nivolumab-treated NSCLC patients with liver metastases than in the overall study population (all hepatic events: 10% vs. 6%; grade 3–4 hepatic events: 2% vs. 1%) [27]. Liver involvements of malignancy could directly reduce the volume of functional healthy liver or induce intrahepatic and extrahepatic biliary obstruction [28]. Humoral and immunological factors associated with cancer may also increase cholestasis and inflammatory damage in the liver [28]. Assessment of liver function is a fundamental part of work-up for patients with liver involvements before initiation of anti-PD-1 immunotherapy.

Another relevant finding is that antiviral prophylaxis substantially reduced the risk of developing severe hepatic profiles, suggesting that some of hepatic profiles in our study are HBV-related hepatotoxicity. Moreove, we previously declared that a lack of antiviral prophylaxis was a risk factor for HBV-related hepatitis (OR: 13.44; $P = 0.019$) among HBsAg-positive cancer patients receiving ICIs [22]. According to these data and the current guidance [24], we suggest all HBsAg-positive patients receive effective antiviral drugs before and during anti-PD-1 treatment. For patients with resolved HBV infection, considering the low risk of HBV reactivation, treatment can begin on prophylaxis, or ALT, HBV DNA, and HBsAg can be carefully monitored with the intent for on-demand anti-HBV therapy [24]. Entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide, are the recommended drugs.

One limitation of this study is that most of the recruited patients only provided test results for HBV DNA levels at the initiation of immunotherapy, as few patients were monitored for HBV DNA levels throughout the treatment period, particularly patients who had previously been infected with HBV. This lack of monitoring regarding the changes in HBV DNA levels may result in an impairment in the timely discovery of viral reactivation and initiation of relevant treatment. Another limitation was the relatively small sample size included in the present study. We found that several virological factors were of significance in the univariate analysis, which were determined to be false-negative factors in the multivariate analysis, which may have led to the concealment of critical risk factors. In addition, causality between anti-PD-1 antibody treatment and hepatotoxicity was mainly assessed retrospectively based on available clinical and laboratory data, which may lead to some bias.

In summary, the findings of the present study indicate that anti-PD-1 therapy has acceptable safety in patients with chronic or resolved HBV infection and cancers. Although the development of adverse events in the liver is more frequent in this population compared to HBV-negative patients, the hepatic adverse events induced by PD-1 inhibitors therapy are considered manageable. Thus, cancer patients should be tested for HBsAg/HBcAb prior to the commencement of immune checkpoint inhibitor therapy. For patients with chronic or resolved HBV infection, physicians should closely monitor the levels of ALT/AST and HBV DNA, as well as consider the proper use of preemptive antiviral drugs throughout the entire immunotherapy period.

Declarations

Conflict of interest The authors declare no conflict of interest that pertain to this work.

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