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Impact of hepatitis B virus infection on HIV response to antiretroviral therapy in a Chinese antiretroviral therapy center

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SUMMARY

Background: Co-infection with hepatitis B virus (HBV) and HIV is common in China; however, the impact of HBV on long-term antiretroviral therapy (ART) outcomes has not been fully characterized.
Methods: Patients were classified as being HIV mono-infected (hepatitis B surface antigen (HBsAg)-negative) or HIV/HBV co-infected (HBsAg-positive). The effects of HBV on HIV virological response, changes in CD4 cell counts, hepatotoxicity, and mortality among Chinese patients receiving ART were evaluated.
Results: The HIV/HBV co-infection rate in our cohort was 9.9% (354/3562). Five hundred and fifty HIV mono-infected and 78 HIV/HBV co-infected individuals fulfilled the inclusion criteria. HIV/HBV co-infected individuals were less likely to achieve HIV-RNA suppression and a CD4 increase than HIV mono-infected individuals at 48 months post-ART. Greater hepatotoxicity and a more rapid occurrence of death were observed in HIV/HBV co-infected subjects. HBV-related mortality accounted for 84.2% (16/19) of the total deaths in HIV/HBV co-infected subjects.
Conclusions: HBV co-infection can affect late immunological and virological responses to ART and increase the risk of hepatotoxicity. Mortality due to liver disease was high among HIV/HBV co-infected individuals in this study, despite HBV-active ART. As long as HIV/HBV co-infected persons need anti-HBV therapy, they should be recommended ART that includes agents with activity against both HIV and HBV, regardless of the CD4 cell count level.

1. Introduction

In China, approximately 700 000 people are infected with HIV in a population of more than 1.3 billion.[1] China is a country with one of the highest prevalence rates for hepatitis B surface antigen (HBsAg) seropositivity in the population, with a reported prevalence of 7.18% in 2006.[2] Chronic hepatitis B (CHB) occurs in about 10% of HIV-infected individuals,[3] and it is important to understand the impact of hepatitis B virus (HBV) infection on HIV-RNA suppression, CD4 recovery, and hepatotoxicity during antiretroviral therapy (ART).

Several studies of HIV/HBV co-infected individuals have examined the short-term response to ART.[4–7] However, the impact of CHB on HIV treatment outcomes remains uncertain because of inconsistent reports.[5,8–10] It is unclear whether CHB affects the rate and durability of HIV-RNA suppression and increases in CD4 cell counts during ART, especially in China. Thus, we evaluated the impact of CHB on the outcomes of HIV virological response, CD4 recovery, hepatotoxicity, and mortality among patients at a Chinese ART center.

2. Patients and methods

2.1. Patients

The study included patients from a single center and all patients were screened for HBsAg and hepatitis C virus antibodies (anti-HCV). Patients included in this study fulfilled the following criteria: (1) initiated ART between January 2004 and December 2011; (2) received follow-up prospectively until May 2013 or for a maximum of 60 months; (3) had pre-ART serum samples available for testing; (4) had a minimum of 12 months of follow-up on ART; (5) had results of at least three follow-up laboratory tests available; (6) were negative for anti-HCV; and (7) had not received anti-HBV therapy before ART and/or during ART other than lamivudine (3TC).

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In accordance with Chinese[11] and World Health Organization (WHO)[12] guidelines, the initiation and monitoring of ART was based on clinical and immunological parameters. Individuals with or without HBV received a regimen of stavudine (d4T)/zidovudine (AZT), 3TC, and nevirapine (NVP)/efavirenz (EFV). Liver function was tested at baseline, at weeks 2 and 6, and then every 3 months. CD4 cell counts were determined at baseline, every 3 months for the first year, and then every 6–12 months. The HIV-RNA level was determined at baseline and then yearly.

This study was approved by the ethics committee of Zhongnan Hospital of Wuhan University. Informed consent was obtained from all patients on the collection of demographic data and plasma samples. All experiments were performed in accordance with the ethical standards of the Declaration of Helsinki.

2.2. Laboratory measurements

HBsAg and anti-HCV were tested with a third-generation enzyme immunoassay (EIA) (Shanghai Kehua Biology Company, China). Real-time PCR kits (Shanghai Kehua Biology Company) were used to test HBV-DNA levels; the detection limit was 500 copies/ml. HIV viral load was determined using NucliSens Easy Q HIV-1 v2.0 (bioMérieux, Iyon, France), with a limit of detection of 20 copies/ml. The CD4+ T cell count was determined by flow cytometry (Beckman Coulter Epics XL; Beckman Coulter, Inc., Los Angeles, USA). Liver function was assessed using an automatic biochemical analyzer manufactured by Rili Company (Tokyo, Japan).

2.3. Statistical analysis

Baseline characteristics were summarized with the median and interquartile range (IQR) for continuous variables and the proportion for categorical variables. The Chi-square test or Fisher's exact test was used for categorical variables. For measurement data, normal distribution of the measurement data was tested first. If the result was of non-normal distribution, the Wilcoxon test was used for statistical calculations. If the result was of normal distribution, *t*-tests were used. A time to event analysis was performed using Kaplan–Meier survival curves. All tests were two-sided and a p-value of <0.05 was taken as significant. Analyses were performed using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patient demographic data

A total of 3562 newly diagnosed HIV-positive patients were screened routinely for HBsAg and anti-HCV antibody; 354 were HBsAg-positive. In accordance with the inclusion criteria of this study, data from 78 HIV/HBV co-infected patients and 550 HIV mono-infected patients were analyzed (Figure 1).

3.2. Baseline characteristics

Table 1 summarizes the demographic and clinical characteristics of the patient population. Of note, the median liver enzymes at baseline in the HIV/HBV co-infected group were higher compared to the HIV mono-infected group ($p = 0.0001$).

3.3. Risk factors associated with ART effect

Multivariate logistic regression identified HBV co-infection ($p = 0.001$), baseline CD4 cell count <200 cells/mm^3 ($p = 0.021$), baseline HIV-RNA >5000 copies/ml ($p = 0.027$), elevated liver enzymes ($p = 0.031$), clinical cirrhosis ($p = 0.023$), and side effect related to ART ($p = 0.014$) as independent risk factors related to ART effect (Table 2).

3.4. CD4 cell count recovery

Similar CD4 cell count levels were detected in HIV/HBV co-infected subjects and in HIV mono-infected subjects during 36 months post-ART (Table 3). However, the median CD4+ cell count was lower in the HIV/HBV co-infected subjects compared to the HIV mono-infected subjects at 36–48 months (280 cells/ml vs. 333 cells/ml; $p = 0.041$) and 49–60 months (304 cells/ml vs. 363 cells/ml; $p = 0.037$).

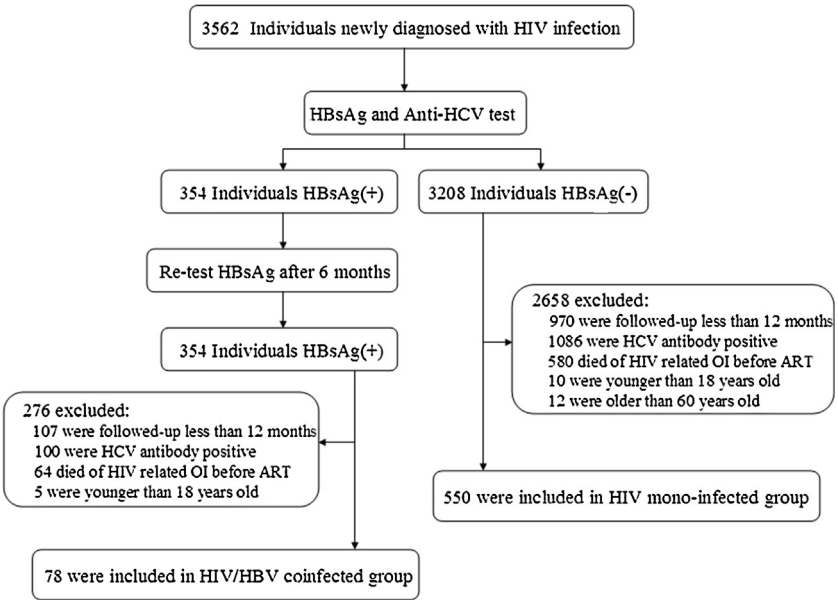

Figure 1. Flow chart of the study subjects (HBsAg, hepatitis B virus surface antigen; anti-HCV, hepatitis C virus antibodies; OI, opportunistic infection).

Table 1
Baseline characteristics of the study population

Characteristic	HIV mono-infected (n = 550)	HIV/HBV co-infected (n = 78)	p-Value
At treatment initiation			
Age, years, median (IQR)	45 (26–56)	44 (23–55)	0.268
Male, n (%)	289 (52.5)	41 (52.6)	0.998
HIV transmission route, n (%)			
Heterosexual contact	358 (65.1)	49 (62.8)	0.694
Unsafe blood transfusion	85 (15.5)	13 (16.7)	0.783
Commercial plasma donation	61 (11.1)	9 (11.5)	0.906
Homosexual contact	25 (4.5)	4 (5.1)	0.818
Injection drug use	21 (3.8)	3 (3.8)	0.990
Follow-up time between diagnosis and start of ART, months, median (IQR)	3.0 (1–8.8)	3.0 (1–9.1)	0.990
CD4 cell count when diagnosed with HIV infection, $\times 10^6$/l, median (IQR)	103 (50–270)	127 (61–265)	0.190
Nadir CD4 cell count, $\times 10^6$/l, median (IQR)	100 (36–252)	108 (30–240)	0.192
HIV-RNA level, $\log_{10}$ copies/ml, median (IQR)	4.5 (3.8–5.0)	4.5 (3.9–4.7)	0.872
AIDS-defining events, n (%)	247 (44.9)	36 (46.2)	0.836
Clinical cirrhosis, n (%)	0 (0)	2 (2.6)	0.0001
Liver enzymes, U/l, median (IQR)			
ALT	30 (21–47)	63 (40–92)	0.0001
AST	30 (23–41)	47 (33–64)	0.0001
Serum HBV-DNA positive rate, n (%)	-	36 (46.2)	-
Serum HBeAg positive rate, n (%)	-	10 (12.8)	-
Initial treatment regimen, n (%)			
AZT or d4T, 3TC, and NVP	458 (83.3)	58 (74.4)	0.054
AZT or d4T, 3TC, and EFV	92 (16.7)	20 (25.6)	-
AZT, 3TC, and NVP or EFV	237 (43.1)	34 (43.6)	0.934
d4T, 3TC, and NVP or EFV	313 (56.9)	44 (56.4)	-

HIV, human immunodeficiency virus; HBV, hepatitis B virus; IQR, interquartile range; ART, antiretroviral therapy; AIDS, acquired immunodeficiency syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B virus e antigen; AZT, zidovudine; d4T, stavudine; 3TC, lamivudine; NVP, nevirapine; EFV, efavirenz.

3.5. HIV-RNA suppression

Similar HIV-RNA levels were detected in HIV/HBV co-infected and HIV mono-infected subjects during 36 months post-ART (Table 4). However, the proportion of patients with detectable HIV-RNA was higher in HIV/HBV co-infected subjects after receiving ART for 37–48 months (p = 0.002) and 49–60 months (p = 0.005).

The proportions of second-line ART usage were 10.9% (60/550) in HIV mono-infected patients and 12.8% (10/78) in HIV/HBV co-infected patients. All second-line regimens in this study comprised tenofovir (TDF)–3TC–lopinavir/ritonavir (LPV/r).

3.6. Hepatotoxicity and other side effects

During the observation period, the proportion of patients with hepatotoxicity was greater in those with HIV/HBV co-infection than in those with HIV mono-infection (Table 5). Among the patients using NVP who experienced hepatotoxicity, 30% were switched to EFV.

3.7. HBV replication and hepatitis flares

The proportions of HIV/HBV co-infected subjects with detectable HBV-DNA and hepatitis flares at different durations of ART are shown in Table 6. There were significant differences among multiple groups (p = 0.0001).

3.8. Deaths and HBV-related end-stage liver diseases

Nineteen out of 78 HIV/HBV co-infected subjects (24.4%) died during the follow-up period, compared with 28 out of 550 HIV mono-infected subjects (5.1%). The 19 cases died at a median of 52.4 months post-ART (IQR 48.9–56.0 months); in comparison, the 28 cases died at a median of 58.0 months post-ART (IQR 56.3–59.6 months). A more rapid occurrence of death was observed in HIV/HBV co-infected subjects (p = 0.0001, Figure 2).

Among the HIV/HBV co-infected subjects, 11 died of liver cirrhosis, three died of hepatocellular carcinoma (HCC), two died of fulminant hepatitis, two died of AIDS, and one died of tuberculosis (TB). HBV-related mortality was the most common cause of death (16/19, 84.2%).

Table 2
Risk factors associated with ART effect

Related factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Age >45 years	0.978 (0.631–1.223)	0.867	-	-
Male	1.926 (0.741–2.014)	0.430	-	-
HIV acquisition route	4×10^9 (0–∞)	0.995	-	-
HBV co-infection	1.547 (1.168–1.987)	0.002	2.362 (1.522–3.542)	0.001
Baseline CD4 <200 cells/μl	2.783 (1.474–4.236)	0.021	2.323 (1.284–3.503)	0.011
Baseline HIV-RNA >5000 copies/ml	2.128 (1.323–3.201)	0.032	1.497 (1.188–2.783)	0.024
Elevated liver enzymes	3.205 (1.222–4.563)	0.012	2.704 (1.890–2.961)	0.031
Clinical cirrhosis	1.204 (1.117–1.655)	0.038	1.378 (1.203–1.664)	0.023
Side effect related to ART	1.636 (1.290–2.316)	0.003	1.270 (1.113–1.729)	0.013

ART, antiretroviral therapy; OR, odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; HBV, hepatitis B virus.

Table 3
Median CD4 cell count at different times after the initiation of ART, by HBV infection group

Duration of ART (months)	HIV mono-infected		HIV/HBV co-infected		*p*-Value
	CD4 tested, *n*	Cells/μl, median (IQR)	CD4 tested, *n*	Cells/μl, median (IQR)	
3–6	546	195 (80–263)	78	191 (78–260)	0.958
7–12	546	227 (127–302)	78	264 (133–300)	0.411
13–24	483	279 (150–338)	76	317 (162–362)	0.301
25–36	434	287 (214–377)	70	307 (220–385)	0.639
37–48	385	333 (246–380)	58	280 (210–354)	0.041
49–60	357	363 (296–421)	50	304 (218–325)	0.037

ART, antiretroviral therapy; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IQR, interquartile range.

Table 4
Comparison of HIV-RNA positive rates after the initiation of ART, by HBV infection group

Duration of ART (months)	HIV mono-infected		HIV/HBV co-infected		*p*-Value
	Number tested	Number with detectable HIV-RNA (%)	Number tested	Number with detectable HIV-RNA (%)	
3–12	546	108 (19.8%)	78	13 (16.7%)	0.515
13–24	483	95 (19.7%)	76	13 (17.1%)	0.599
25–36	434	98 (22.6%)	70	19 (27.1%)	0.401
37–48	385	81 (21.0%)	58	23 (39.7%)	0.002
49–60	357	84 (23.5%)	50	21 (42.0%)	0.005

HIV, human immunodeficiency virus; ART, antiretroviral therapy; HBV, hepatitis B virus.

4. Discussion

In this Chinese ART center, HBV co-infection did not affect the early response to ART. However, the immune and virological responses to ART at 48 months post-ART were poorer in HIV/HBV co-infected subjects. Furthermore, we demonstrated that HBV co-infection increased the risk of ART-related hepatotoxicity, and HBV-related mortality was the most common cause of death after ART.

The current guidelines for the treatment of HIV/HBV co-infected persons recommend that patients who need treatment for HBV receive ART in advance.[13] Even so, the baseline levels of CD4 and HIV-RNA did not differ between mono- and co-infected patients in this study. The potential reason for this is that although HBV is highly endemic in China, HBV testing has not been carried out widely. Most individuals in this study did not know their HBV infection status until they started ART.

Other studies have assessed the impact of hepatitis B on the response to ART and have reported different findings.[5,8,9] CD4 recovery was poorer and the HIV-RNA levels were higher in patients with CHB at 48 months post-ART. The results of this study do not completely disagree with those of other studies, because similar results were found for the same duration of ART. Differences in results may be attributable to the different ART regimens, the duration of ART, the proportion of hepatitis B e antigen (HBeAg), and HBV disease characteristics. The finding that liver damage was more common in the HIV/HBV co-infected group may be accounted for by the greater virological failure in co-infected patients after 48 months, because adjustments to medication or withdrawal related to liver damage can decrease compliance.

CHB is associated with liver enzyme level elevations in ART recipients.[14,15] The high rate of hepatotoxicity in those with HBV co-infection is a challenge during ART. The usage of NVP has been found to be a contributing factor. Another explanation relates to immune recovery inflammatory syndrome. A further possible explanation is that some HBV co-infected patients develop 3TC-resistant hepatitis B due to long-term 3TC monotherapy.[16,17] According to some reports, d4T can induce abnormal liver tests both in mice[18] and in adult *Erythrocebus patas* monkeys.[19] Similarly, multivariate logistic regression defined the use of d4T (odds ratio 7.1, 95% confidence interval 1.0–54.5, $p = 0.05$) as an independent risk factor for aggravation of hepatitis in HIV/HCV co-infected patients.[20] The use of d4T was common in China until August 2013; 56.9% of subjects in this study received d4T-based ART. The high usage of d4T may have been another cause of the liver toxicity seen in this study.

Co-infection with HIV and HBV may lead to accelerated hepatic disease progression with higher rates of liver cirrhosis and liver-related mortality compared with HBV mono-infection. In this study, HBV-related mortality accounted for 84.2% of deaths in the HIV/HBV co-infected patients. Despite the advent of ART, liver-related mortality has become the leading cause of non-AIDS-related death in HIV-infected patients in China, whereas cancer has become the first cause of mortality in Western countries.[21,22] The fact that HBV infection is serious in China may account for this difference.

Table 5
Proportion of patients who experienced a hepatotoxicity event after the initiation of ART, by HBV infection group

	HIV mono-infected	HIV/HBV co-infected	*p*-Value
LFT tested, sum number × frequency	5880	946	-
Sum number × frequency with elevated ALT or AST (%)	546 (9.3%)	212 (22.4%)	0.0001
Hepatotoxicity grade[a]			
1	343 (5.8%)	120 (12.7%)	0.0001
2	189 (3.2%)	66 (7.0%)	0.0001
3	11 (0.2%)	16 (1.7%)	0.0001
4	3 (0.05%)	10 (1.1%)	0.0001

ART, antiretroviral therapy; HBV, hepatitis B virus; HIV, human immunodeficiency virus; LFT, liver function tests; ALT alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

[a] Hepatotoxicity grade 1: 1 × ULN < ALT or AST ≤ 2.5 × ULN; hepatotoxicity grade 2: 2.5 × ULN < ALT or AST ≤ 5 × ULN; hepatotoxicity grade 3: 5 × ULN <ALT or AST ≤ 10 × ULN; hepatotoxicity grade 4: ALT or AST > 10 × ULN.

Table 6
Comparison of the proportion of HIV/HBV co-infected subjects with detectable HBV-DNA and hepatitis flares with different durations of ART

Duration of ART (months)	Number tested	Number with detectable HBV-DNA (%)[a]	Number with hepatitis flares (%)[b]
3–12	78	4 (5.1)	4 (5.1)
13–24	76	7 (9.2)	3 (3.9)
25–36	70	13 (18.6)	1 (1.4)
37–48	58	20 (34.5)	6 (10.3)
49–60	50	20 (40.0)	2 (4.0)

HIV, human immunodeficiency virus; HBV, hepatitis B virus; ART, antiretroviral therapy.
[a] $p = 0.0001$.
[b] $p = 0.0001$.

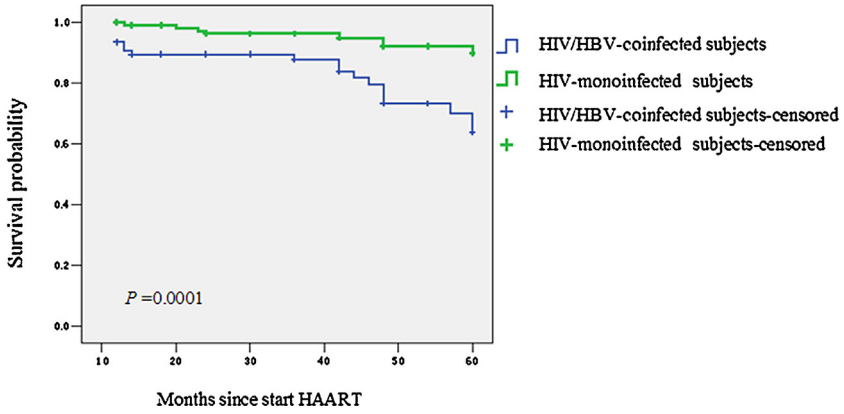

Figure 2. Survival probability in HIV/HBV co-infected patients and HIV mono-infected patients who initiated ART. Green line: patients without HBV infection; blue line: patients with HBV co-infection.

There are several limitations that should be noted. First, since TDF was only used as a second-line regimen in China before 2012, none of the patients in this study received TDF therapy. Second, since NVP was more widely used than EFV due to its cheaper price in China, we were unable to compare the differences between NVP and EFV on hepatotoxicity during ART. Third, HBsAg alone is insufficient to predict hepatotoxicity. Fourth, data on alcohol intake, body mass index, TB therapy, and the use of herbal medications, which are important to identify the etiology of alanine aminotransferase (ALT) elevations, were incomplete.

In summary, this study demonstrates that HBV can affect the long-term ability to respond to ART in terms of HIV-RNA suppression and immunological recovery at 48 months post-ART. Of note, the occurrences of hepatotoxicity and mortality were high among HBV co-infected patients. Our data suggest the careful use of D-drugs and NVP, still common in resource-limited countries, in HBV co-infected patients. Moreover, for HIV/HBV co-infected patients, it is important to use ART that includes two agents with activity against both HIV and HBV.

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Conflict of interest: All authors report no potential conflicts.

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