Characterization of HIV–HBV coinfection in a multinational HIV-infected cohort

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Objective: To understand the HIV–hepatitis B virus (HBV) epidemic from a global perspective by clinically and virologically characterizing these viruses at the time of antiretroviral therapy (ART) initiation in a multinational cohort.

Methods and design: HIV-infected patients enrolled in two international studies were classified as HIV–HBV coinfected or HIV monoinfected prior to ART. HIV–HBV coinfected patients were tested for HBV characteristics, hepatitis D virus (HDV), a novel noninvasive marker of liver disease, and drug-resistant HBV. Comparisons between discrete covariates used χ^2 or Fisher's exact tests (and Jonchkheere–Terpstra for trend tests), whereas continuous covariates were compared using Wilcoxon Rank-Sum Test.

Results: Of the 2105 HIV-infected patients from 11 countries, the median age was 34 years and 63% were black. The 115 HIV–HBV coinfected patients had significantly higher alanine aminotransferase and aspartate aminotransferase values, lower BMI, and lower $CD4^+$ T-cell counts than HIV monoinfected patients (median 159 and 137 cells/μl, respectively, $P = 0.04$). In the coinfected patients, 49.6% had HBeAg-negative HBV, 60.2% had genotype A HBV, and 13% were HDV positive. Of the HBeAg-negative patients, 66% had HBV DNA 2000 IU/ml or less compared to 5.2% of the HBeAg-positive individuals. Drug-resistant HBV was not detected.

Conclusion: Screening for HBV in HIV-infected patients in resource-limited settings is important because it is associated with lower $CD4^+$ T-cell counts. In settings in which HBV DNA is not available, HBeAg may be useful to assess the need for HBV treatment. Screening for drug-resistant HBV is not needed prior to starting ART in settings in which this study was conducted.

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Introduction

Chronic hepatitis B (CH-B), which is the leading cause of end-stage liver disease worldwide, is common in HIV type-1-infected individuals with a prevalence ranging from 5 to 20% in various studies of HIV-infected patients [1–5]. Although coinfection with HIV and hepatitis B virus (HBV) is recognized as being common, there are limited data to provide an international perspective on this epidemic. Such a perspective is especially important as HAART is being introduced worldwide including in resource-limited settings with high HBV endemicity such as Asia and Africa. Because liver disease is a leading cause of non-AIDS death in HIV-infected patients receiving HAART, characterizing HBV prior to HAART is important in order to better understand the scope of the disease and to prioritize treatment needs in resource-limited settings.

Most studies of HIV–HBV coinfected patients have been conducted in countries with low HBV endemicity and have shown a negative effect of HIV on CH-B with decreased hepatitis B e antigen (HBeAg) clearance, high HBV DNA levels, and increased risk for cirrhosis and liver-related death [6,7]. Data from countries with low HBV endemicity are informative, but they represent HBV disease that occurs primarily in adulthood. In contrast, in countries with high HBV endemicity, transmission primarily occurs in infancy or early childhood; thus, in most of these cases, HBV has been present for years prior to HIV infection. It is not known if the worldwide epidemic is similar to what has been reported from countries with low HBV endemicity. One study from Nigeria suggests that this may not be the case because HBV DNA levels were lower than what has been seen in studies from North America and Europe [8]. Thus, data on HIV and HBV characteristics with a more global perspective in these coinfected individuals at the time HAART is initiated are needed.

Two randomized clinical trials of antiretroviral therapy were conducted by the Adult AIDS Clinical Trials Group (ACTG) in diverse resource-limited settings. The first, ACTG A5175, enrolled HIV-infected men and women worldwide to compare different initial HAART regimens [9]. The second, A5208, compared the response of first-line HAART in African women who had received single-dose nevirapine during a prior pregnancy to those who had not received nevirapine [10]. Both of these trials tested individuals for HBV at HAART initiation; thus, they are useful to characterize untreated HIV and HBV coinfection from a multinational perspective.

Methods

Study participants

The participants were enrolled in one of the following parent ACTG studies: A5175 or A5208. A5175 was a randomized, open-label comparison of once-daily protease inhibitor or nonnucleoside reverse transcriptase inhibitor-containing regimens for HAART-naive HIV-infected men and women [with $CD4^+$ T-cell count <300 cells/μl and alanine aminotransferase (ALT)/aspartate aminotransferase (AST) $<5\times$ upper limit of normal (ULN)] from Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States, and Zimbabwe, as previously described ($n=1571$) [9]. A5208 studied the response to a nevirapine-based initial HAART regimen after peripartum exposure to single-dose nevirapine ($n=745$). Women in A5208 had $CD4^+$ T-cell count less than 200 cells/μl, baseline AST/ALT less than $2.5\times$ the ULN, and came from one of the following African countries: South Africa, Kenya, Zimbabwe, Botswana, Zambia, Malawi, and Uganda, as previously described [10]. All A5175 sites and seven of the 10 A5208 sites received local Institutional Review Board approval for participation in this study. Informed consent was obtained from the study participants in their native language, and the study was also approved by the Johns Hopkins University IRB. Of the sites that participated, Brazil excluded people who had CH-B from enrolling in the parent study; thus, Brazilian participants were not included in prevalence estimates but are included in the HIV monoinfected study group. In addition, four of the 25 US sites, which enrolled 13% of the individuals from the United States, excluded people with CH-B.

Participants were classified as HIV–HBV coinfected if they were hepatitis B surface antigen (HBsAg) positive at the visit prior to randomization and positive for HBeAg, HBV DNA, or hepatitis B e antibody (anti-HBe) at the study entry visit. All others were classified as HIV monoinfected. Data abstracted from the parent study database included the following criteria: age, sex, race, BMI, history of liver disease, hemoglobin, HIV disease stage, HIV RNA, $CD4^+$ T-cell count, ALT, and AST. The HIV–HBV coinfected participants also had the following performed using study entry visit serum: HBeAg, anti-HBe, HBV genotype, HBV DNA, hepatitis D antibody [anti-hepatitis D virus (anti-HDV)], hepatitis C virus (HCV) antibody (anti-HCV), HCV RNA if anti-HCV positive, HBV *Pol* sequencing, and a noninvasive marker of liver disease that correlates with liver disease [11]. This noninvasive marker measures the change in glycosylation of an immunoglobulin G reactive to a specific α-galactose epitope, which is measured by reactivity to the fucose-binding lectin aleuria aurantia lectin (AAL-reactivity). This marker was selected because other readily available markers use parameters such as platelets that are affected by HIV infection.

Laboratory testing

All specimens were stored at -80°C. Serological testing for HBeAg (ETI-EBK Plus; Diasorin, Stillwater Minnesota, USA or Abbott ARCHITECT HBeAg v17.0, Abbott Park, Illinois, USA), anti-HBe (ETI-AB-

EBK Plus or Abbott ARCHITECT Anti HBe v13.0), anti-HDV (ETI-AB-DELTAK-2; Diasorin, Stillwater, Minnesota, USA), and anti-HCV (Ortho HCV v3.0; Ortho Diagnostics, Raritan, New Jersey, USA) were performed according to manufacturer's instructions. HBV DNA testing was done with real-time PCR using either RealART HBV LC PCR v 3.0 (Qiagen, Valencia, California, USA) or Abbott RealTime HBV DNA (Abbott Molecular, Des Plains, Illinois, USA). The highest common lower limit of detection of these assays was 200 IU/ml, which is the value used in the analyses. HBV genotype and drug-resistance mutations were determined by HBV *Pol* sequencing, performed as previously described [12]. HCV RNA was determined with the Abbott RealTime HCV RNA (Abbott Molecular) performed according to manufacturer's instructions. AAL-reactivity was tested as previously described [11] with values greater than 5 representing cirrhosis, values 3–5 representing moderate liver disease, and values of 1–2 representing no or mild liver disease.

Statistical analysis

ALT and AST levels were graded according to standard ACTG definitions [13]. Comparisons between groups were made using χ^2 or Fisher's exact test for discrete covariates and Wilcoxon Rank-Sum Test for continuous covariates. For three-level ordinal group comparisons, the Jonchkheere–Terpstra trend test was performed. To test for associations of pretreatment covariates with baseline HBV DNA level, linear regression modeling on the $\log_{10}$ transformed HBV DNA was performed. Pretreatment covariates tested include screening CD4 T-cell count category (<50, 50–199, and 200–299), $\log_{10}$ HIV-1 RNA copies/ml, sex, age, BMI, anti-HDV, HBeAg, anti-HBe, HBV genotype, hemoglobin per 5 g/dl increase, creatinine clearance (Cockcroft–Gault equation), ALT, and AST. *P* values less than 0.05 were considered statistically significant and were not adjusted for multiplicity.

Results

A5175 and A5208 recruited 2316 participants of whom 2105 (90.9%) were enrolled in sites that obtained IRB approval for this substudy. Of these 2105 participants, 115 (5.5%) were HIV–HBV coinfected. There was no significant difference in the prevalence of a positive HBsAg between the sites that did (6.7%) and did not (5.6%) have IRB approval for this substudy. The HIV–HBV coinfection prevalence varied with the highest prevalence in Zimbabwe (11%) and the lowest in Kenya (2.2%) (Table 1). The prevalence of HIV–HBV coinfection across continents was similar with Asia at 5.9%, Africa at 6.7%, Central/South America at 5.1% (excluding Brazil), and North America (United States) at 4.8%. Anti-HCV was positive in six participants who all had undetectable HCV RNA.

The median age of the 2105 participants was 34 years and 39% were men (Table 2). The racial make-up was majority black (62.7%) followed by Asian (17%), white (11.9%), and other (7.4%). The HIV–HBV coinfected participants had more blacks and fewer whites than the HIV monoinfected individuals ($P = 0.07$). The BMIs were lower among the HIV–HBV coinfected (median 21.7) compared to the HIV monoinfected participants (median 22.4, $P = 0.02$); however, the distribution of numbers in each BMI category (underweight, normal, overweight, and obese) was similar.

Liver function and HIV parameters

Given the entry criteria for the parent studies, only seven individuals had grade 3 and 4 elevations of ALT or AST, all of whom were HIV monoinfected. Although ALT and AST values were typically low (median 23 and 29 U/l, respectively), the distributions of these values were both higher among the HIV–HBV coinfected (median ALT 25.5 U/l, median AST 34 U/l) compared to the HIV

Table 1. Distribution of study participants by country.

Country	HIV monoinfected number (% of group)	HIV–HBV coinfected number (% of group)	HIV–HBV coinfected estimated prevalence (%) (95% CI)
Africa			
Botswana	86 (4)	4 (3)	4.4 (1.2–11.0)
Kenya	135 (7)	3 (3)	2.2 (0.5–6.2)
Malawi	265 (13)	24 (21)	8.3 (5.4–12.0)
South Africa	314 (16)	17 (15)	5.1 (3.0–8.1)
Zimbabwe	203 (10)	24 (21)	11.0 (6.9–15.0)
South/Central America			
Brazil	231 (12)	0[a]	N/A
Haiti	94 (5)	6 (5)	6 (2.2–13.0)
Peru	128 (6)	6 (5)	4.5 (1.7–9.5)
North America			
United States	200 (10)	10 (9)	4.8 (2.3–8.6)
Asia			
India	242 (12)	13 (11)	5.1 (2.7–8.6)
Thailand	92 (5)	8 (6)	8.0 (3.5–15.0)

CI, confidence interval; HBV, hepatitis B virus.
[a]Excluded HIV–HBV coinfected patients at enrollment.

Table 2. Characteristics of study participants.

	HIV monoinfected $N=1990$	HIV–HBV coinfected $N=115$	P
Age (IQR) (years)[a]	34 (29–40)	34 (30–40)	0.53
Male sex (%)	39	47	0.09
Race			0.07
Black (%)	62.1	73.9	
White (%)	12.5	2.6	
Asian (%)	16.9	18.2	
Other (%)	7.5	5.2	
BMI (IQR)[a]	22.4 (20.3–25.3)	21.7 (19.8–24.0)	0.02
ALT (IQR)[a] (U/l)	23 (16.5–35.0)	25.5 (19.0–38.0)	0.03
Normal	1804 (91.0%)	100 (87.0%)	0.14
Mild	154 (7.8%)	11 (9.6%)	
Moderate	20 (1.0%)	4 (3.5%)	
Severe	3 (0.2%)	0 (0.0%)	
Life-threatening	1 (0.01%)	0 (0.0%)	
AST (IQR)[a] (U/l)	28.8 (23.0–38.0)	34 (27.0–44.0)	<0.001
Normal	1703 (85.9%)	86 (74.8%)	0.001
Mild	242 (12.2%)	28 (24.3%)	
Moderate	35 (1.8%)	1 (0.9%)	
Severe	2 (0.1%)	0 (0.0%)	
Life-threatening	1 (0.1%)	0 (0.0%)	
HIV characteristics			
CD4 T-cell count (IQR) (cells/μl)[a]	159 (90–218)	137 (68–210)	0.04
HIV RNA (IQR) (log copies/ml)[a]	5.1 (4.6–5.5)	5.1 (4.6–5.6)	0.23
WHO Stage IV or clinical AIDS (%)	8.6	12.2	0.19

ALT, alanine aminotransferase; IQR, interquartile range.
[a]median values.

monoinfected individuals [median ALT 23 U/l ($P=0.03$), median AST 28.8 U/l ($P<0.001$)] (Table 2).

The median CD4 T-cell count was 157 cells/μl [interquartile range (IQR) 88–218 cells/μl] and was significantly lower in the HIV–HBV coinfected (137 cells/μl, IQR 68–210 cells/μl) than in the HIV monoinfected individuals [159 cells/μl (IQR 90–218 cells/μl, $P=0.04$] (Table 2). However, the two groups did not differ in HIV RNA level (median 5.10 and 5.05 $\log_{10}$ copies/ml, respectively, $P=0.23$). Also, the CD4 T-cell differences between the groups could not be accounted for by differences in HBV prevalence by country (Fig. 1). Consistent with the difference in CD4 T-cell counts, the HIV–HBV coinfected patients had a higher prevalence of clinical AIDS, which was defined as WHO stage IV or history of AIDS, but the difference was not statistically significant (12.2 versus 8.6% in HIV–HBV coinfected and HIV monoinfected, respectively, $P=0.19$).

As a sensitivity analysis, we repeated the above analyses excluding participants from Brazil because they were all HIV monoinfected by that site's inclusion criteria. Comparisons between HIV monoinfected and HIV–HBV coinfected participants were similar with the following exceptions. The proportion of men was significantly higher in the HIV–HBV coinfected (46%) compared to the HIV monoinfected group (36%, $P=0.02$). The difference in the distribution of races between groups was attenuated ($P=0.7$). However, the difference in the ALT distribution between groups was strengthened ($P=0.002$).

Hepatitis B virus characteristics in HIV–hepatitis B virus coinfected patients

From the study entry visit, serum was available for additional HBV testing in the 113 of the 115 HIV–HBV coinfected patients. Approximately, half, 57 of 113 (50.4%), were HBeAg-positive, and this proportion was similar in A5175 (51.8%) and A5208 (46.7%). The median age was 34 years in both the HBeAg-negative and HBeAg-positive individuals and the proportion of women was similar between the HBeAg groups (48 and

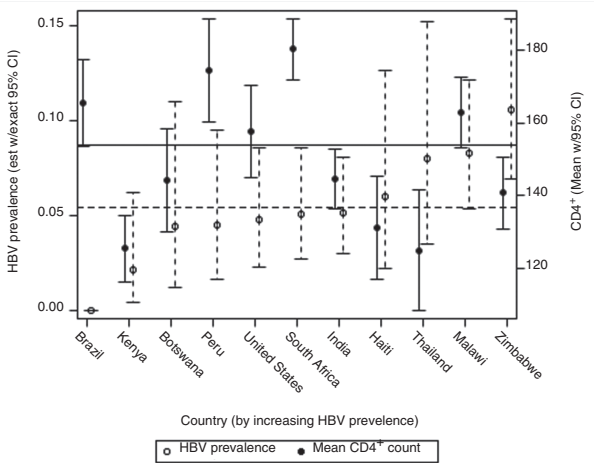


Fig. 1. Distribution of hepatitis B virus prevalence and mean CD4$^+$ T-cell count by country. There is no clear relationship between hepatitis B virus (HBV) prevalence and mean CD4$^+$ T-cell count. CI, confidence interval.

52%). By country, participants from Haiti (83%), India (69%), Kenya (67%), and Peru (83%) were predominantly HBeAg-positive, whereas those from Malawi (65%), Thailand (63%), and the United States (80%) were mainly HBeAg negative.

HBV genotype could not be determined for 18 (16%) participants due to low HBV DNA. Among observed genotypes, the most common were A (71.5%) and D (15.8%), but the distribution varied geographically with Africa having 95% genotype A, US with 78% genotype A, Thailand and India with 100% genotype C and D, respectively, and South and Central America with 55% genotype A, 27% genotype F, and 18% genotype E. Genotypes A and C were evenly divided between HBeAg-positive and HBeAg-negative disease, whereas D, E, and F were predominantly in HBeAg-positive participants (Table 3).

Anti-HDV was detected in 15 of 113 (13.3%) HBV coinfected participants, which is within the range of the prevalence reported in HBV monoinfection [14]. Anti-HDV was detected only among participants from Botswana, Kenya, Peru, South Africa, Thailand, or Zimbabwe, with Botswana having the highest relative number of participants (three of four). When stratified by HBeAg status, a similar proportion of the HBeAg-negative and HBeAg-positive participants (14 and 12%, respectively) were anti-HDV positive.

HBV DNA was detected in 91.2% of the 113 coinfected patients and was significantly more common in the HBeAg-positive (98.2%) compared to the HBeAg-negative patients (83.9%, $P = 0.008$) (Table 3). The median HBV DNA was significantly higher in the HBeAg-positive (median 7.96 log IU/ml) compared to the HBeAg-negative patients (median 2.74 log IU/ml, $P < 0.001$). In fact, 66.1% of HBeAg-negative patients had an HBV DNA 2000 IU/ml or less, which is the level above which treatment should be considered in HBeAg-negative patients [15]. In contrast, only 5.2% of HBeAg-positive patients had HBV DNA 2000 IU/ml or less. Only 12% of the HBeAg-positive patients had values below the cutoff recommended by some treatment guidelines for HBeAg-positive monoinfected patients (20 000 IU/ml) [15]. Genotypes C, D, and F had a higher proportion of participants with HBV DNA greater than 20 000 IU/ml compared to the other genotypes ($P = 0.049$).

In univariable analysis with log HBV DNA as the outcome, HBeAg-positive patients had a 3.63 log IU/ml [95% confidence interval (CI) 2.95–4.32 log IU/ml] higher HBV DNA than those who were HBeAg-negative ($P < 0.01$) (Fig. 2a). Those with higher ALT and AST also had higher log HBV DNA values. When compared to patients infected with genotype A, those infected with genotype D had a 1.61 log IU/ml higher HBV DNA (95% CI 0.21–3.01 log IU/ml) and those infected with genotypes C, E, or F had a 1.37 log IU/ml higher HBV DNA (95% CI −0.17 to 2.91 log IU/ml). In a multivariable model, only HBeAg status and AST remained significantly associated with higher HBV DNA (Fig. 2b).

Of the 95 individuals with HBV *Pol* sequencing, none (95% CI 0–3.7%) had mutations in HBV *Pol* that are associated with known resistance to lamivudine, adefovir dipivoxil, or entecavir [16].

Liver disease characteristics in HIV–hepatitis B virus coinfected patients

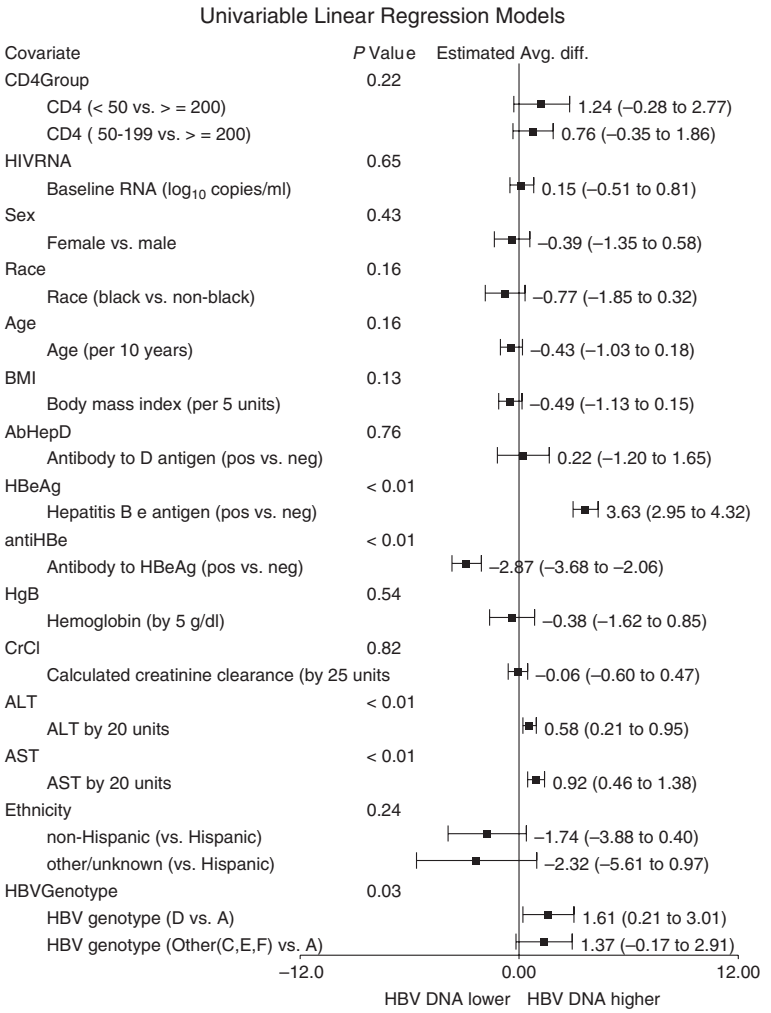
AST values were higher in the HBeAg-positive (median 36.0 U/l) compared to the HBeAg-negative patients (median 31.5 U/l, $P = 0.008$) with a similar relationship in ALT (median 28.0 and 23.0 U/l, respectively, $P = 0.03$) (Table 3). Liver disease was measured by AAL-reactivity in 103 individuals because the samples from India were

Table 3. Hepatitis B virus characteristics in HIV–hepatitis B virus coinfected patients by HBeAg status.

	Overall $N = 113$	HBeAg-negative $N = 56$	HBeAg-positive $N = 57$
Median ALT (IQR)(U/l)*	25.5 (19.0–38.0)	23.0 (17.5–33.0)	28.0 (21.9–49.0)
Median AST (IQR)(U/l)*	34.0 (27.0–44.0)	31.5 (24.0–40.0)	36.0 (29.0–55.0)
Detectable HBV DNA [>200 IU/ml (%)]*	91.2	83.9	98.2
Median HBV DNA (IQR)(log IU/ml)*	5.1 (2.7–8.1)	2.7 (2.3–4.4)	8.0 (5.6–8.6)
HBV genotype (number)[a]			
A	68	33	35
C	5	2	3
D	15	4	11
E	4	1	3
F	3	0	3
AAL reactivity (number)[b]			
1–2	39	18	21
3–5	48	26	22
>5	16	8	8

AAL, aleuria aurantia lectin; ALT, alanine aminotransferase; HBV, hepatitis B virus; IQR, interquartile range.
*$P < 0.05$ comparing HBeAg-negative to HBeAg-positive.
[a]HBV genotype not determined in 15 due to inability to amplify.
[b]Missing in 12 individuals.

(a)



(b)

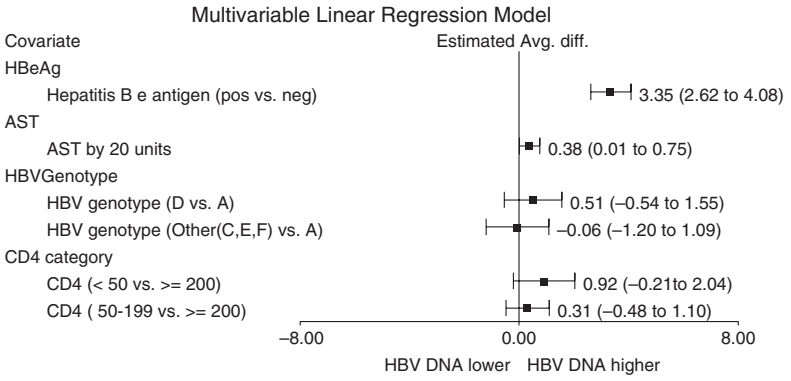


Fig. 2. Univariable and multivariable linear regression models with log hepatitis B virus (HBV) as the outcome (a) Univariable model. Hepatitis B e antigen (HBeAg)-positive status was associated with a 3.63 log IU/ml higher HBV DNA, anti-HBe-positive status was associated with a 2.87 log IU/ml lower HBV DNA, both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were associated with higher HBV DNA levels, and non-A genotype HBV was associated with higher HBV DNA levels. (b) Multivariable model. The associated variables from the univariable models were included in this model and CD4 cell count was forced into the model. Anti-HBe was not included because it is collinear with HBeAg status. HBeAg-positive status was associated with a significantly higher HBV DNA level (3.35 log IU/ml higher compared to HBeAg-negative individuals). AST was also associated with higher HBV DNA level.

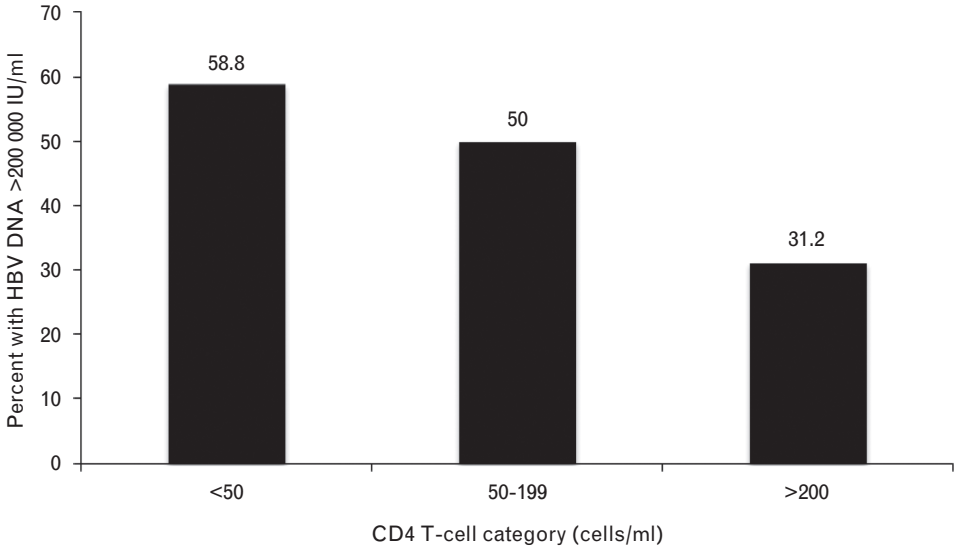


Fig. 3. Frequency (in percentage) of individuals with hepatitis B virus DNA more than 200 000 IU/ml by $CD4^+$ T-cell category. Individuals with CD4 T cells less than 50 cells/μl had the highest proportion with HBV DNA more than 200 000 IU/ml.

not available for this assay. No or mild liver disease was present in 39 (37.8%), moderate liver disease in 48 (46.6%), and cirrhosis in 16 participants (15.5%). All 16 with cirrhosis were from A5175, 15 of whom were black. The 16 with AAL values consistent with cirrhosis (>5) trended toward a lower albumin (3.29 g/dl) compared to the 87 without cirrhosis (3.62 g/dl)($P = 0.06$); however, none of these 16 participants had clinical signs or symptoms of liver disease.

HIV disease characteristics in HIV–hepatitis B virus coinfected patients

Because the HIV–HBV coinfected patients had lower $CD4^+$ T-cell counts than the HIV monoinfected patients, we further explored the relationship between HBV DNA and $CD4^+$ T-cell counts among the coinfected patients. The median $CD4^+$ T-cell count did not differ by HBeAg status. To determine if $CD4^+$ T-cell count was associated with HBV DNA, the $CD4^+$ T-cell count was stratified into three groups of less than 50, 51–199, and more than 200 cells/μl. The proportion of patients with HBV DNA more than 200 000 IU/ml decreased significantly as $CD4^+$ T-cell count increased ($P = 0.04$) (Fig. 3).

Discussion

This is the first multinational study of HIV–HBV coinfected individuals characterizing both HBV and HIV parameters at the time of HAART initiation. Several notable findings include an association between HBV coinfection and lower $CD4^+$ T-cell count especially those with high HBV DNA levels, the low HBV DNA levels in the majority of HBeAg-negative patients, and the absence of drug-resistant HBV. Such characterization of HBV in HIV-infected patients at the time of HAART initiation advances our understanding of HBV in this setting in order to focus treatment efforts.

The association of lower $CD4^+$ T-cell counts with HIV–HBV coinfection was first noted in a Nigerian study, but it was not clear if that finding was universal or specific to Nigeria [8]. This is the first multinational study demonstrating that HIV–HBV coinfected patients have lower $CD4^+$ T-cell counts than HIV monoinfected patients. If the findings were region-specific, then the association would have been diminished with the diverse countries included in this study. This finding is also robust because it was detected despite the fact that study entry criteria for the parent studies required a $CD4^+$ T-cell count less than 300 cells/μl (for A5175) or 200 cells/μl (for A5208). Furthermore, this is the first study to demonstrate that the HIV–HBV coinfected patients with $CD4^+$ T-cell counts less than 50 cells/μl were more likely to have high levels of HBV replication (HBV DNA >200 000 IU/ml) than were those with higher $CD4^+$ T-cell counts. One possible mechanism for this association is that CH-B may lead to immune activation, which increases $CD4^+$ T-cell apoptosis. This association is not due to differences in geographical distribution of $CD4^+$ T-cell counts among countries because those with higher HBV prevalence did not recruit patients with lower $CD4^+$ T-cell counts.

The low levels of HBV DNA in a large proportion of individuals was unexpected because HIV-HBV coinfection is associated with higher HBV DNA in patients from areas of the world with low HBV endemicity [6]. In our

study, 16% of the HBeAg-negative patents had an undetectable HBV DNA, and an additional 50% had a HBV DNA less than 2000 IU/ml, which is the level above which treatment is considered. In contrast, the majority of HBeAg-positive patients (75%) had detectable HBV DNA levels that were more than 200 000 IU/ml. These findings are supported by the multivariable analysis demonstrating that HBeAg-positive status was the only factor associated with higher HBV DNA levels. The low HBV DNA in this cohort is not simply due to exclusion of patients with higher ALT or AST levels, as studies from Nigeria and South Africa that did not exclude such patients also show low HBV DNA in a substantial proportion of HIV–HBV coinfected patients [8,17]. In the Nigerian study, these low levels were also found primarily in HBeAg-negative patients, but HBeAg was not determined in the other study. Taken together, these studies suggest that determining HBeAg may provide a strategy to prioritize the need for HBV treatment in HIV-infected individuals when HBV DNA assays are not available in resource-limited settings.

In our study, no participant had pre-existing drug-resistant mutations in the majority population of the quasispecies. In two prior Japanese studies of therapy-naive individuals, the prevalence of drug-resistant mutants using population sequencing was 0 and 1.6% [18,19]. One of these studies used a second assay that could detect as little as 0.001% of mutant virus and found that 11% of patients had a drug-resistant virus in the minority population [18]. However, whether such low levels of virus lead to HBV treatment failure is not known. One study found lamivudine-resistant strains in 10 of 20 HIV–HBV coinfected patients prior to therapy, but the pattern of mutations was identical in the majority of the tested samples suggesting that contamination may explain this high prevalence [20]. Our study does not support the need for HBV drug-resistance testing prior to starting anti-HBV therapy.

Our study had several limitations. First, the inclusion criteria of the parent antiretroviral treatment trials may have skewed our study population. Even though both parent studies were focused on participants with CD4 T-cell counts less than 300 cells/μl, we were still able to see an association between CD4 T-cell count and HBV infection. The exclusion of patients with very high AST and ALT may have underestimated the prevalence of CH-B, especially in those with HBeAg-positive disease. However, we believe that this exclusion did not substantially alter the HBV DNA findings or the association with lower $CD4^+$ T-cell counts, as discussed above. In addition, HIV–HBV coinfected patients are recognized to have lower ALT levels than are HBV monoinfected patients despite higher HBV DNA levels [6]. Second, we were not able to characterize liver disease using a liver biopsy because biopsies were not feasible and are not readily available in resource-limited countries. However, we did use a noninvasive marker of liver disease (AAL reactivity) that correlates with the level of fibrosis [11] and does not use platelet count, which can be affected by HIV. About 15% of the cohort had a level of AAL reactivity that was consistent with cirrhosis, which was almost exclusively found in individuals of African descent; however, the only sign of liver disease in these individuals was a trend toward lower albumin. This prevalence is similar to that described in HBV mono-infected cohorts with ALT cutoffs that were less than 2 × the ULN [21]. A Ugandan study found a 17% prevalence of significant fibrosis in HIV monoinfected patients [22]; thus, the cirrhosis prevalence in our study of HIV–HBV coinfected individuals is possible. Further studies are needed to characterize liver disease in HIV–HBV coinfected patients from resource-limited settings including those with high ALT/AST levels. Third, we did not find an association in the multivariable analysis with HBV genotype and HBV DNA, but these data are limited by the inability to genotype 16% of the individuals. This lack of association was also seen in a study from Denmark of 784 HBV monoinfected patients [23]. However, in another study, HBV DNA levels were the highest in HBeAg-positive patients with HBV genotype D [24].

In summary, we found a variable prevalence (2–11%) of hepatitis B in HIV-infected patients from diverse settings prior to starting HAART. Thus, it is important for providers to know the prevalence of HBV in their country. HBV DNA levels were relatively low in HBeAg-negative patients; thus, this serologic marker may be useful in prioritizing patients on their need for HBV treatment in settings in which HBV DNA is not available. HIV–HBV coinfection, especially those with high HBV DNA, was associated with lower $CD4^+$ T-cell counts prior to HAART initiation, which emphasizes the need for HBV testing in HIV-infected patients. Further work is needed to study HIV–HBV coinfection in patients with high ALT/AST levels, to understand the mechanism for $CD4^+$ T-cell loss in HIV–HBV coinfection, and to optimize treatment for HIV–HBV coinfection in resource-limited settings.

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Conflicts of interest

There are no conflicts of interest.

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