CROI 2015
Seattle, WA, USA

EFFECT OF IMMUNOSUPPRESSION AND ANTIVIRALS ON INTRACELLULAR HBV REPLICATION IN HIV-HBV CO-INFECTION

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Introduction

The formation of covalently closed circular DNA (ccc-DNA) within the hepatocyte is an important step in the replication cycle of hepatitis B virus, allowing production of viral RNAs that encode for polymerase, core, and envelope proteins.[1]

With a long half-life, ccc-DNA persists in the hepatocyte for long periods of time and can be detected in patients with inactive infection or even in those who achieved HBsAg-seroclearance.[2]

Reductions in ccc-DNA and total intracellular HBV-DNA have been observed during treatment with potent nucleoside/nucleotide analogues, yet still remain detectable within a year of treatment.[3,4]

Poor immune control has also been associated with higher levels of HBV ccc-DNA and total intracellular HBV-DNA[5], which causes some concern for patients infected with the human immunodeficiency virus.

Only few studies to date have examined intracellular replication in HIV-HBV co-infected patients[6], but none have had the capacity to fully determine risk-factors associated with its levels, especially in patients undergoing long-periods of highly active antiretroviral therapy (ART).

Objective

To examine the determinants of intracellular levels of replication in HBV-human immunodeficiency virus (HIV) co-infected patients.

Methods and Patients

Study Design
Cross-sectional study nested in an observational, multi-center, prospective cohort aimed at studying the determinants of liver fibrosis evolution among HIV-HBV co-infected patients.

Patients
Recruited from the French 2002 – 2010 HIV-HBV Cohort and Bi-LIVER Studies and enrolled in the present study according to the following inclusion criteria:

- HIV enzyme linked immunoabsorbant assay (ELISA) positivity confirmed by a Western-Blot
- Hepatitis B surface antigen (HBsAg) seropositivity >6 months
- Had an available paraffin-embedded liver biopsy for at least one time-point (liver biopsies obtained based on concomitant guidelines from the European Association for the Study of the Liver[7])

Sixty patients *overall* were included (46 with one liver biopsy and 14 with two liver biopsies). Subgroup analysis looking at changes of intracellular replication were performed among patients *undergoing TDF-containing ART* (one biopsy before and one after TDF-initiation).

Quantification of intracellular HBV-parameters

- DNA was extracted from biopsy specimens using the MasterPure DNA purification kit (Epicentre) according to manufacturer's instructions. Briefly, 500µL of lysis buffer was added to liver tissue and then gently ground using a plastic grinder. After an overnight proteinase K digestion at 42°C and RNase digestion (30min at 37°C), 500µL of precipitation buffer was added to each sample. Samples were well homogenized prior to a 10min centrifugation (10000g). The adequate volume of isopropanol was added to the supernatants and DNA was precipitated. Pellets were rinsed with ethanol 70° and resuspended in 50µL of Tris-EDTA buffer.
- Intrahepatic ccc-DNA and total HBV-DNA were quantified by real-time PCR using a LightCycler instrument (Roche Diagnostics, Mannheim, Germany) as described previously.[2]

Statistical analysis
Intracellular HBV-DNA levels were $\log_{10}$-transformed. Determinants were examined using mixed-effect linear regression model, accounting for the within-individual correlation of patients with more than one biopsy.

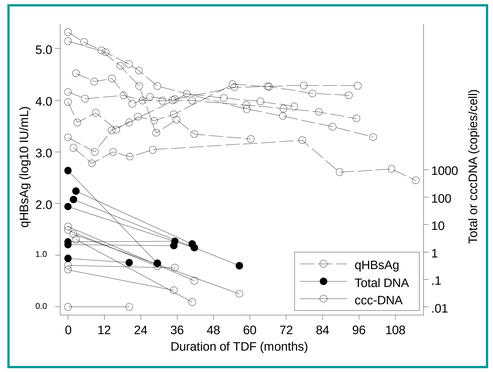
Results

Table 1. Description of study population at the time of first biopsy

	N=60
Demographic characteristics	
Male:Female ratio (% male)	53/7 (88.3)
Age, yrs†	42 (37-46)
From zone of high HBV-endemicity	8 (13.3)
HIV-related characteristics	
Known HIV-infection duration, yrs†	11.9 (7.1-15.6)
AIDS-defining illness	17 (28.3)
CD4+ cell count, /mm³†	460 (337-649)
Nadir CD4+ cell count, /mm³+	251 (108-321)
ART-naïve	3 (5.0)
Detectable HIV-RNA (>50 cps/mL)	19 (31.7)
HIV viral load, $\log_{10}$ cps/mL[1]†	3.64 (2.40-4.55)
Duration of ART, yrs†	7.3 (5.1-9.3)
HBV characteristics	
Known HBV-infection duration, yrs†	9.3 (6.1-13.2)
Detectable serum HBV-DNA (>60 IU/mL)	23 (46.9)
HBeAg-positive	35 (61.4)
F3-F4 fibrosis [N=59]	19 (32.2)
Cumulative LAM-duration, mos† [N=41][3]	69.3 (41.0-85.6)
Cumulative ADV-duration, mos† [N=6][3]	21.0 (3.9-32.3)
Cumulative TDF-duration, mos† [N=25][3]	17.8 (5.7-31.0)

All statistics are *n* (%), except for (†) where median (IQR) is given.[1]With detectable HIV-RNA. [2]With detectable HBV-DNA. [3]Among patients undergoing treatment.

Fig 1. HBV parameters among seven co-infected patients undergoing tenofovir-containing antiretroviral therapy with biopsy pairs



Median duration of treatment (between biopsies) was 35.3 months (range: 20.2-56.6). From before TDF-initiation to sequential biopsy, most exhibited strong declines in both median ccc-DNA (2.9 to 0.04 copies/cell, respectively, *p*=0.04) and intracellular HBV-DNA (45.1 to 1.4 copies/cell, respectively, *p*=0.03). Of note, intracellular replication was detectable at the end of follow-up for all patients, yet at very low levels.

Conclusions

- **In coinfected patients, severe immuno-suppression is associated with intracellular HBV replication.**
- **Treatment with TDF is linked to large declines in ccc-DNA, yet replication within the hepatocyte still persists after long periods of treatment.**

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Acknowledgements

Sponsored by IMEA (Institut de Médecine et d'Epimiologie Appliquée). A unrestricted grant for the French HIV-HBV Cohort was provided from Gilead Sciences; Inc.

A. Boyd was awarded a post-doctoral fellowship with the Agence nationale de recherches sur le sida et les hépatites virales and is currently under a post-doctoral fellowship funded by SIDACTION.

Table 2. Determinants of circular covalently-closed (ccc) DNA and total intracellular HBV-DNA

	HBV ccc-DNA				Total intracellular HBV-DNA			
	Univariable		Multivariable[1]		Univariable		Multivariable[1]	
	Δ (95%CI)	*p*	Δ (95%CI)	p	Δ (95%CI)	p	Δ (95%CI)	p
Female vs male	-0.70 (-1.09, -0.32)	<0.001	-0.48 (-0.84, -0.12)	0.01	-0.56 (-1.28, 0.16)	0.13		
Age (per year)	0.00 (-0.02, 0.03)	0.8			-0.01 (-0.05, 0.02)	0.4		
AIDS-defining illness	0.62 (0.11, 1.14)	0.02			0.82 (0.22, 1.43)	0.008		
CD4+ T cells >500/mm³	-0.48 (-0.91, -0.06)	0.03			-0.36 (-0.98, 0.25)	0.2		
Nadir CD4+ >250/mm³	-0.70 (-1.12, -0.27)	0.001	-0.57 (-0.95, -0.19)	0.004	-0.90 (-1.46, -0.33)	0.002	-0.78 (-1.27, -0.29)	0.002
Detectable HIV-RNA	0.28 (-0.18, 0.73)	0.2			0.15 (-0.57, 0.86)	0.7		
Duration of ART (/yr)	0.01 (-0.04, 0.06)	0.7			-0.02 (-0.08, 0.04)	0.5		
HBV-DNA (/$\log_{10}$ IU/mL)	0.22 (0.14, 0.29)	<0.001			0.40 (0.31, 0.49)	<0.001		
HBeAg-positive serology	0.83 (0.43, 1.22)	<0.001	0.76 (0.39, 1.13)	<0.001	1.36 (0.85, 1.88)	<0.001	1.35 (0.88, 1.82)	<0.001
ALT >ULN	0.03 (-0.43, 0.49)	0.9			0.50 (-0.11, 1.12)	0.11		
Cumulative LAM-duration								
[0, 36) months	0				0			
[36, 76) months	0.32 (-0.30, 0.93)	0.3			0.92 (0.16, 1.67)	0.02		
≥76 months	-0.27 (-0.82, 0.28)	0.3			-0.13 (-0.88, 0.62)	0.7		
Cumulative TDF-duration								
0 months	0		0		0		0	
[0, 36) months	-0.39 (-0.79, 0.01)	0.06	-0.43 (-0.83, -0.04)	0.03	-0.83 (-1.46, -0.21)	0.009	-0.84 (-1.36, -0.33)	0.001
≥36 months	-1.07 (-1.60, -0.54)	<0.001	-0.88 (-1.40, -0.35)	0.001	-1.62 (-2.18, -1.06)	<0.001	-1.58 (-2.25, -0.91)	<0.001

[1] In the model for HBV ccc-DNA, the following determinants were not included in the multivariable analysis because they did not meet the pre-specified *p*-value threshold: AIDS-defining illness (*p*=0.198) and CD4+ cell count >500/mm³ (*p*=0.286). In the model for total intracellular HBV-DNA, this was the case for the following determinants: AIDS-defining illness (*p*=0.141), CD4+ cell count >250/mm³ (*p*=0.120), and cumulative LAM-duration (*p*=0.357). For both models, serum HBV-DNA variables were not included due to the high collinearity with both treatment duration and HBeAg-status.