Hepatitis B core-related antigen quantification is an accurate predictor 12-months prior to hepatitis B "e" antigen-seroclearance in HIV-HBV coinfected patients treated with tenofovir

Lorenza Dezanet[1], Audrey Gabassi[2], Sarah Maylin[2], Hayette Rougier[3], Patrick Miailhes[4], Caroline Lascoux-Combe[5], Julie Chas[6], Pierre-Marie Girard[3], Constance Delaugerre[2], Karine Lacombe[1,3], Anders Boyd[1,3]

[1] INSERM, Sorbonne Université, Institut Pierre Louis d'Épidémiologie et de Santé Publique, Paris, France; [2]Laboratoire de Virologie, Hôpital Saint-Louis, AP-HP, Paris, France; [3]Service de maladies infectieuses, Hôpital Saint-Antoine, Paris, France; [4]Service de maladies infectieuses, Hôpital de la Croix-Rousse, Hospices Civils, Lyon, France, [5]Service de maladies infectieuses, Hôpital Saint-Louis, AP-HP, Paris, France; [6]Service de maladies infectieuses, Hôpital Tenon, AP-HP, Paris, France.

Introduction

For hepatitis B "e" antigen (HBeAg)-positive patients, HBeAg seroconversion is an important endpoint indicating long-lasting therapeutic response and clinical improvement.[1]

Despite prolonged periods of undetectable serum HBV-DNA, tenofovir (TDF)-treated patients co-infected with HIV-hepatitis B virus (HBV) do not immediately clear HBeAg, thereby highlighting the need for new markers of treatment efficacy.[2]

In the past years, new markers, such as quantitative hepatitis B core-related antigen (HBcrAg), have been gaining attention in patients chronically mono-infected with HBV. HBcrAg consists of three species of related proteins sharing an identical 149 amino acid sequence: HBcAg, HBeAg and p22cr.[3]

This surrogate marker strongly correlates with covalently-closed circular (ccc)DNA (correlation coefficient = 0.70) and serum HBV DNA, even when HBV DNA is undetectable in HBV mono-infected patients.[3,4]

Nevertheless, no previous study to date has examined its relevance during tenofovir (TDF) treatment in HIV-HBV co-infected patients.

Objectives

To describe the kinetics of HBcrAg and determine its accuracy as a predictor of HBeAg-seroclearance during the course of TDF-containing ART in HBeAg-positive HIV-HBV co-infected patients.

Methods and Patients

Study Design and population

This analysis is part of the French HIV-HBV cohort (2002-2011), a prospective, observational, multi-center study comprising 308 participants in 7 clinical centers in France.

Quantification of HBV-parameters

All HBV-parameters were obtained at baseline and every 6-12 months.

- **HBV-DNA detection:** PCR-Amplicor (Roche Diagnostic Systems, Meylan, France; detection limit 60 IU/mL).
- **HBcrAg level:** HBcrAg assay (Lumipulse system, FujiRebio, Inc.) with automated CLEIA system; reported in U/mL.
- **HBeAg quantification:** Architect i2000 analyzer assay (Abbott Laboratories, Rungis, France) and reported in Paul Erlich Institute units (PEI U)/mL.

Statistical analysis

- ✓ **Linear regression** was used to determine baseline determinants of HBcrAg level.
- ✓ **Mixed-effect linear models** was performed to estimate the change of HBcrAg levels over time. Values were adjusted *a priori* on baseline levels, body mass index, age, concomitant lamivudine (LAM) treatment, cumulative treatment duration with LAM, HBV-DNA level, and CD4+ cells/mm^3.

Inclusion criteria:

- HBsAg seropositivity >6mo
- HIV ELISA (+) confirmed by WB
- age over 18 years old
- minimum of 2 follow-up visits (>6 months)
- available sample at baseline and at least once during follow-up
- initiated TDF-containing antiretroviral treatment (ART)
- HBeAg-positive at baseline

Exclusion criteria:

- baseline or incident infection with HCV or HDV
- undergoing intensification with peg-IFN / IFN

95 participants included in this analysis

- ✓ **Cox proportional hazards regression** was used to assess the association between HBcrAg level and HBeAg-loss.
- ✓ **Time-dependent ROC curves** was carried out to evaluate prediction of HBeAg-loss using HBV-infection markers with sensitivity (Se) and specificity (Sp) at every yearly interval.
- ✓ Statistical significance was determined at a **p-value <0.05**

Results

Baseline characteristics of patients treated with tenofovir

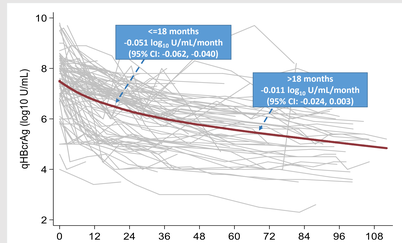
	HBeAg-positive (*N*=95)
Male gender *	89 (94)
Age (years) **	40 (35-47)
From zone of HBV-high prevalence *	8 (8)
AIDS-defining illness *	29 (31)
CD4+ cell count (cells/mm^3) **	423 (312-580)
Nadir CD4+ cell count (cells/mm^3) **	230 (78-321)
HIV-RNA > 50 copies/mL*	45 (48)
Duration of prior ARV therapy (years) **	7 (5-9)
HBV-infection duration (years) **	8 (4-12)
Concomitantly treated with LAM *	67 (71)
HBV-DNA (log_{10} IU/mL) **	6.6 (4.2-7.6)
ALT (IU/mL) **	63 (39-97)
HBeAg level (PEI U/mL) **	862 (328-1099)
HBcrAg level (log_{10} U/mL) **	7.8 (7.0-8.2)

*Number (%); **median (25-75th percentile); HIV- and HBV-infection duration were estimated from first positive serology.

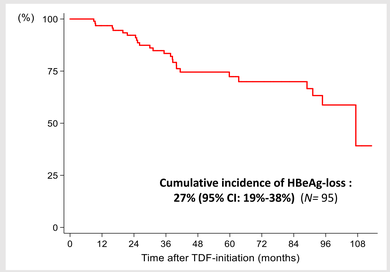
Baseline determinants of HBcrAg (log_{10} U/mL)

	Diff.	(95% CI)	p
From zone of HBV-high prevalence	-0.754	(-1.478, -0.031)	0.04
Duration of prior ARV therapy (yrs)	-0.086	(-0.146, -0.027)	0.004
ALT ≤ 70 IU/mL	-0.564	(-0.994, -0.135)	0.009
AST ≤ 70 IU/mL	-0.571	(-1.032, -0.110)	0.02
HBeAg level (PEI U/mL)	0.469	(0.211, 0.727)	<0.001

qHBcrAg during long-term TDF-treatment



Cumulative probability of HBeAg-loss



Association between HBcrAg levels and HBeAg-loss

HBeAg-loss occurred in 26 patients within a median 32 months (IQR=21-40). Median follow-up time was 4.6 years (IQR: 2.9 –7.6).

Baseline HBcrAg level <6.5 log_{10} U/mL was a predictor of HBeAg-loss during follow-up (HR = 5.46; 95% CI: 2.43–12.27; p <0.001), after adjustment for CD4+ cell count per 250/mm^3 change from prior visit (HR = 0.98; 95% CI: 0.96-1.00; p = 0.09).

HBcrAg level and prediction of HBeAg loss

		Classification Probabilities									
		M24 n=78		M36 n=63		M48 n=40		M72 n=27		M96 n=13	
Criteria	N	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp
HBcrAg level <6.5 log_{10} U/mL											
M12	95	**0.82**	**0.67**	0.77	0.68	0.71	0.70	0.70	0.76	0.60	0.87
M24	84	††	††	**1**	**0.58**	0.97	0.61	0.96	0.69	0.80	0.82
M36	76	††	††	††	††	**1**	**0.42**	1	0.48	0.85	0.58
HBV-DNA <60 IU/mL											
M12	95	0.92	0.60	0.76	0.61	0.77	0.64	0.63	0.66	0.53	0.72
M24	84	††	††	1	0.33	1	0.35	1	0.40	0.92	0.52
M36	76	††	††	††	††	1	0.17	1	0.20	0.91	0.22

Conclusions

- HBcrAg levels 12-months prior to loss appears to have high sensitivity in predicting HBeAg-seroclearance.
- Although specificity in predicting long-term HBeAg-seroclearance was low, it remained mostly higher than HBV-DNA detection.
- CD4 level does not influence the decrease of HBcrAg levels.
- HBV-DNA only provides optimal sensitivity for long-term prediction.

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Contact: anders.boyd@iplesp.upmc.fr