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MAJOR ARTICLE

Association of Hepatitis B Core-Related Antigen and Antihepatitis B Core Antibody With Liver Fibrosis Evolution in Human Immunodeficiency Virus-Hepatitis B Virus Coinfected Patients During Treatment With Tenofovir

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Background. Quantitative hepatitis B core-related antigen (qHBcrAg) or antihepatitis B core antibody (qAnti-HBc) could be useful in monitoring liver fibrosis evolution during chronic hepatitis B virus (HBV) infection, yet it has not been assessed in human immunodeficiency virus (HIV)-HBV-coinfected patients undergoing treatment with tenofovir (TDF).

Methods. One hundred fifty-four HIV-HBV-infected patients initiating a TDF-containing antiretroviral regimen were prospectively followed. The qHBcrAg and qAnti-HBc and liver fibrosis assessment were collected every 6–12 months during TDF. Hazard ratios (HRs) assessing the association between qHBcrAg/qAnti-HBc and transitions from none/mild/significant fibrosis to advanced fibrosis/cirrhosis (progression) and from advanced fibrosis/cirrhosis to none/mild/significant fibrosis (regression) were estimated using a time-homogeneous Markov model.

Results. At baseline, advanced liver fibrosis/cirrhosis was observed in 40 (26%) patients. During a median follow-up of 48 months (interquartile range, 31–90), 38 transitions of progression (IR = 7/100 person-years) and 34 transitions of regression (IR = 6/100 person-years) were observed. Baseline levels of qHBcrAg and qAnti-HBc were not associated with liver fibrosis progression (adjusted-HR per $\log_{10}$ U/mL = 1.07, 95% confidence interval [CI] = 0.93–1.24; adjusted-HR per $\log_{10}$ Paul-Ehrlich-Institute [PEI] U/mL = 0.85, 95% CI = 0.70–1.04, respectively) or regression (adjusted-HR per $\log_{10}$ U/mL = 1.17, 95% CI = 0.95–1.46; adjusted-HR per $\log_{10}$ PEI U/mL = 0.97, 95% CI = 0.78–1.22, respectively) after adjusting for age, gender, duration of antiretroviral therapy, protease inhibitor-containing antiretroviral therapy, and $CD4^+/CD8^+$ ratio. Nevertheless, changes from the previous visit of qAnti-HBc levels were associated with liver fibrosis regression (adjusted-HR per $\log_{10}$ PEIU/mL change = 5.46, 95% CI = 1.56–19.16).

Conclusions. Baseline qHBcrAg and qAnti-HBc levels are not associated with liver fibrosis evolution in TDF-treated HIV-HBV coinfected patients. The link between changes in qAnti-HBc levels during follow-up and liver fibrosis regression merits further study.

Keywords. antihepatitis B core antibody; cirrhosis; hepatitis B core-related antigen; human immunodeficiency virus; liver fibrosis.

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With an estimated 257 million infected individuals worldwide and risk of progression to liver cirrhosis and hepatocellular carcinoma, chronic hepatitis B virus (HBV) remains a major global public health issue [1, 2]. Moreover, because HBV shares the same modes of transmission as human immunodeficiency virus (HIV), the global prevalence of chronic coinfection with HBV in HIV-positive individuals is estimated at 7.4% [1]. Because highly effective antiretroviral therapy (ART) has become available for HIV-positive individuals, acquired immune deficiency syndrome (AIDS)-related morbidity and mortality

have substantially declined in the last decades, giving way to others causes of disease, such as liver-related comorbidities and death [3]. Because of interactions between both viruses and an accelerated evolution toward severe complications in untreated HIV-HBV coinfected individuals [4], initiating ART containing tenofovir (TDF), an agent bearing dual activity against both HIV and HBV, is necessary [5, 6].

Despite the anti-HBV potency of TDF, the proportion of TDF-treated, HIV-HBV coinfected patients developing severe liver fibrosis remains high [7, 8], but not any higher compared with treated HBV-monoinfected patients [9]. Monitoring liver fibrosis generally involves biomarker-based scores, transient elastography, or, in rare cases, liver biopsies [10]; however, these markers can be expensive, unavailable, or invasive to patients. Serum markers of viral activity have been correlated with liver fibrosis levels and are arguably simpler to use [11]. Because suppression of HBV deoxyribonucleic acid (DNA) replication occurs in the majority of TDF-treated coinfection patients [12], HBV DNA would not be useful to monitor liver fibrosis [13]. Other markers, such as quantification of hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg), are stable for many treated coinfected patients and with their varying correlation with liver fibrosis [14, 15], they would also be poor candidates.

A flurry of research on new surrogate markers of hepatitis B, including quantification of hepatitis B core-related antigen (qHBcrAg) and quantification of antihepatitis B core antibody (qAnti-HBc), has suggested a role for their use in monitoring HBV-related liver fibrosis [16, 17]. Quantification of hepatitis B core-related antigen, an antigen consisting of proteins sharing an identical 149 amino acid sequence (including hepatitis B core antigen, HBeAg, and a truncated 22-kDa "precore" protein), is particularly interesting because of its strong correlation with liver fibrosis, as assessed via liver biopsies [18]. Antihepatitis B core antibody, being more closely linked with host immune responses, has been found to be a useful predictor of serological response [19] or clinical relapse [20] and could parallel liver fibrosis regression. Nevertheless, no study to date has examined qAnti-HBc as it relates to liver fibrosis evolution. More concerning, no assessment of the association between any of these markers with liver fibrosis has been conducted in treated HIV-HBV coinfected patients.

The objective of the present study is to determine in what respect qHBcrAg and qAnti-HBc levels are associated with liver fibrosis progression and regression in HIV-HBV coinfected patients undergoing TDF-containing ART. We also aimed to describe the kinetics of qHBcrAg and qAnti-HBc according to liver fibrosis stage at treatment initiation.

METHODS

Patients and Study Design

Patients were selected from the French HIV-HBV cohort study [21]. In brief, this cohort included 308 HIV-infected patients with chronic HBV infection from 7 clinical centers located in Paris and Lyon, France. Patients were recruited in 2002–2003 and followed prospectively every 6–12 months until 2010–2011. Inclusion criteria for this cohort were being at least 18 years old, HIV-positive serology confirmed by Western blot, and HBsAg seropositive for more than 6 months. Patients' demographic characteristics were collected at study inclusion, and clinical stage of HIV infection was classified according to the Centers of Disease Control and Prevention criteria. All patients gave their written informed consent to be included in the cohort, and the study protocol was approved in accordance with the Helsinki declaration.

For this analysis, we included patients who (1) initiated TDF-containing ART, (2) had a minimum of 2 consecutive visits undergoing treatment (lasting more than 6 months), and (3) had available quantification of qHBcrAg, qAnti-HBc, and assessment of a noninvasive measure of liver fibrosis before TDF initiation and at least once during follow-up. Patients with detectable HCV ribonucleic acid (RNA) or hepatitis delta virus (HDV) RNA and those undergoing intensification with standard or pegylated-interferon were excluded from analysis.

Baseline was defined as the study visit at or directly before TDF initiation. Follow-up was defined as all study visits thereafter (ie, every 6–12 months after TDF-initiation). Follow-up continued until TDF discontinuation, meeting any one of the exclusion criteria, loss to follow-up or death, whichever occurred first.

Assessing Markers of Viral Replication

Serum HBV-DNA viral load was quantified at baseline and every 6–12 months by a commercial polymerase chain reaction (PCR)-based assay (COBAS AmpliPrep/COBAS TaqMan, detection limit 12 IU/mL; or COBAS Amplicor HBV Monitor, detection limit 60 IU/mL [Roche Diagnostics Systems, Meylan, France]). Serum HIV-1 RNA viral load was measured at baseline and every 6–12 months using either a branched DNA technique (bDNA Quantiplex 3.0, detection limit 50 copies/mL [Bayer Diagnostics, Cergy-Pontoise, France]) or real-time PCR technique (COBAS AmpliPrep/COBAS TaqMan HIV-1 test, detection limit 40 copies/mL [Roche Molecular Systems, Meylan, France]). Qualitative HBsAg, HBeAg, and antibodies were detected at cohort inclusion and every yearly visit using commercial enzyme immunoassays (EIA).

Serum qHBcrAg (U/mL) was measured at baseline and every 6 months using commercially available, automated HBcrAg chemiluminescence EIA (Lumipulse G System; FujiRebio Europe, Gent, Belgium) [22]. For levels of qHBcrAg >7 log, a dilution 1/100 was performed as recommended by the manufacturer. In addition, anti-HBc antibodies (both immunoglobulin [Ig]G and IgM) were quantified at baseline and every 6–12 months using ARCHITECT Anti-HBc II assay (Abbott

Laboratories, Rungis, France), with an automated ARCHITECT i4000 system.

Assessing Liver Fibrosis Levels

Liver fibrosis was assessed at each yearly visit by the FibroTest calculated from a standard battery of biochemical levels [23]. Scores ranged from 0 to 1. METAVIR equivalents of these measures, established in the HIV-HBV coinfected population, were used to grade liver fibrosis: F0–F1, <0.48; F2, 0.48–0.58; F3, 0.59–0.73; F4, >0.73) [24].

Statistical Analysis

We defined 2 groups of patients based on their levels of liver fibrosis at baseline: no, mild, or moderate liver fibrosis (F0-F1-F2) and advanced liver fibrosis or cirrhosis (F3-F4). Baseline characteristics were compared between liver fibrosis groups using a Kruskal-Wallis test for continuous variables and Pearson's χ^2-test or Fisher's exact test for categorical variables.

All qHBcrAg and qAnti-HBc units were $\log_{10}$ transformed. We compared the on-treatment kinetics of qHBcrAg and qAnti-HBc, estimated using mixed-effect linear regression models with a random-intercept, between baseline liver fibrosis groups. Models assumed a linear change during follow-up. We included a cross-product term between TDF duration and liver fibrosis group along with its individual components, from which stratum-specific estimates were calculated and differences in slopes of HBcrAg and qAnti-HBc change were tested.

To examine liver fibrosis evolution, we modeled transitions between liver fibrosis groups during follow-up due to fluctuations generally observed in fibrosis during treatment. Time-homogeneous multistate Markov models were used to jointly model the transition intensity (TI) from F0-F1-F2 to F3-F4 and from F3-F4 to F0-F1-F2 between each follow-up visit via maximum likelihood methods. The hazard ratios (HRs) comparing TI per unit increase in marker levels at baseline, during follow-up and change between visits, were estimated with proportional hazards of the TI. Multivariable adjustment was made using predefined covariates from a previous study in TDF-treated coinfected patients: age, gender, duration of ART, and protease inhibitor (PI)-containing ART [7], as well as $CD4^+$/$CD8^+$ ratio during follow-up [25, 26]. Model fit was assessed by plotting the observed and expected prevalence of belonging to a given fibrosis group [27].

Statistical analysis was performed using STATA (v13.0; College Station, TX) or using the "msm" package in R (v3.5.2; Vienna, Austria) [28]. Significance was defined as a $P < .05$.

RESULTS

Baseline Characteristics of Study Population

Of the 308 patients enrolled in the French HIV-HBV cohort study, we excluded 97 patients with less than 2 consecutive visits undergoing treatment (lasting more than 6 months); 18 and 16 patients with detectable HCV RNA or HDV RNA, respectively; 7 patients undergoing intensification with standard or pegylated-interferon; 12 patients with insufficient data on qHBcrAg and qAnti-HBc; and 4 patients who did not have sufficient data on liver fibrosis.

Of the 154 patients included in analysis, 114 (74%) had no, mild, or moderate liver fibrosis (F0-F1-F2) and 40 (26%) had advanced liver fibrosis or cirrhosis (F3-F4) at baseline (Table 1). Overall, the study population was predominately male (84%) and had a median age of 41 years (interquartile range [IQR], 35–48). Almost all patients were ART-experienced (99%), with 57% having an undetectable HIV viral load and a median $CD4^+$ cell count of 404/mm^3 (IQR, 292–555). A total of 120 (79%) patients had detectable HBV viral load with a median 5.2 $\log_{10}$ IU/mL (IQR, 3.0–7.3) before TDF-containing ART initiation.

Patients with advanced liver fibrosis or cirrhosis (F3-F4) were more likely to be male ($P < .001$), older ($P < .001$), born in zone of low or moderate HBV-prevalence ($P < .001$), have cardiovascular disease ($P = .02$), longer estimated duration of HIV infection ($P < .01$) and HBV infection ($P < .01$), and a previous AIDS-defining event ($P = .01$) when compared with patients with none to moderate liver fibrosis. In addition, duration of previous ART exposure ($P < .01$) was longer in patients with advanced liver fibrosis or cirrhosis, as well as cumulative duration of lamivudine exposure ($P < .001$). Among the antiretrovirals previously used by patients, past exposure to zidovudine ($P < .01$) or zalcitabine ($P < .01$) was more common in patients with advanced liver fibrosis or cirrhosis.

At TDF-initiation, median levels of unadjusted qHBcrAg and qAnti-HBc were not significantly different in patients with F0-F1-F2 liver fibrosis (6.9 $\log_{10}$ U/mL and 3.3 $\log_{10}$ Paul-Ehrlich-Institute [PEI] U/mL, respectively) when compared with F3-F4 (5.9 $\log_{10}$ U/mL and 3.3 $\log_{10}$ PEI U/mL, respectively) (Table 1).

Evolution of Liver Fibrosis and Levels of Markers

Follow-up was a median 48 months (IQR, 31–90). The qHBcrAg decreased an average −0.209 $\log_{10}$ U/mL per year (95% confidence interval [CI], −0.245 to −0.173) for individuals with F3-F4 fibrosis and −0.220 $\log_{10}$ U/mL per year (95% CI, −0.248 to −0.192) for those with F0-F1-F2 fibrosis (Figure 1A). Likewise, qAnti-HBc decreased an average −0.161 $\log_{10}$ PEI U/mL per year (95% CI, −0.187 to −0.136) for individuals with F3-F4 fibrosis and −0.142 $\log_{10}$ PEI U/mL per year (95% CI, −0.161 to −0.122) for those with F0-F1-F2 fibrosis (Figure 1B). No significant differences in qHBcrAg and qAnti-HBc kinetics were observed between F0-F1-F2 and F3-F4 fibrosis levels (P for interaction = .6 and .23, respectively). Fibrosis levels during follow-up regarding baseline liver fibrosis status are shown in Figure 2.

Table 1. Baseline Characteristics of Patients Treated With Tenofovir Stratified on Liver Fibrosis Status

Characteristics	Total (*n* =154)	Liver Fibrosis Levels at TDF Initiation		*P*[a]
		F0-F1-F2 (*n* = 114)	F3-F4 (*n* = 40)	
Demographics				
Male gender[b]	129/25 (84/16)	89/25 (78/22)	40/0 (100/0)	<.001
Age (years)[c]	41 (35–48)	40 (34–44)	48 (41–52)	<.001
BMI (per kg/m^2) [N = 149]	22.5 (20.9–24.5)	22.8 (21.3–24.8)	21.5 (20.4–23.1)	.009
From high HBV-prevalence zone	39 (25)	37 (32)	2 (5)	<.001
Alcohol consumption (glasses/day) [N = 140]	0 (0–2)	1 (0–2)	0 (0–2)	.70
Cardiovascular disease	25 (16)	14 (12)	11 (27)	.02
Diabetes	7 (4)	2 (2)	2 (5)	.27
HIV Infection				
Estimated duration of HIV infection, years[c] [N = 153]	10.9 (6.0–15.0)	10.0 (5.3–13.8)	13.0 (9.9–16.0)	<.01
AIDS-defining illness[b]	39 (25)	23 (20)	16 (40)	.01
CD4$^+$ cell count (cells/μL)[c] [N = 153]	404 (292–555)	399 (292–552)	406 (269–565)	.87
CD4$^+$ cell count (cells/μL)[c] [N = 153]				.71
≥500	57 (37)	41 (36)	16 (40)	
≥350 and <500	43 (28)	34 (30)	9 (22)	
<350	53(35)	39 (34)	14 (35)	
Nadir CD4$^+$ cell count (cells/μL)[c] [N = 111]	223 (103–321)	224 (108–321)	197 (71–314)	.57
HIV-RNA <50 copies/mL [N = 153]	87 (57)	62 (54)	25 (62)	.40
HIV-RNA ($\log_{10}$ copies/mL)[c] [N = 153]	1.70 (1.70–3.50)	1.70 (1.70–3.71)	1.70 (1.70–2.63)	.54
Antiretroviral Therapy				
ART-naive	2 (1)	2 (2)	0 (0)	.99
Duration of prior c-ART therapy (years)[c]	6.9 (4.1–9.2)	6.0 (3.5–8.8)	7.7 (6.7–9.2)	<.01
Previous antiretroviral exposure [N = 152]				
Zidovudine	126 (83)	87 (76)	39 (97)	<.01
Stavudine	94 (62)	65 (57)	29 (72)	.11
Didanosine	92 (60)	65 (57)	27 (67)	.29
Zalcitabine	38 (25)	21 (18)	17 (42)	<.01
Nevirapine	29 (19)	22 (19)	7 (17)	.77
Efavirenz	64 (42)	47 (41)	17 (42)	.95
Indinavir/r	69 (45)	44 (39)	25 (62)	.01
Saquinavir/r	28 (18)	18 (16)	10 (25)	.21
ART backbone				.70
NRTI only	27 (17)	20 (17)	7 (17)	
NRTI + NNRTI	45 (29)	36 (32)	9 (22)	
NRTI + PI	58 (38)	42 (37)	16 (40)	
NRTI + NNRTI + PI	19 (12)	12 (10)	7 (17)	
Other	5 (3)	4 (3)	1 (2)	
Viral Hepatitis				
Estimated duration of HBV infection (years)[c] [N = 119]	7.9 (3.7–12.3)	6.9 (3.0–10.8)	11.5 (6.9–15.3)	<.01
HBV-DNA <60 IU/mL [N =152]	32 (21)	25 (22)	7 (17)	.58
HBV-DNA ($\log_{10}$ IU/mL)[c] [N =153]	3.9 (2.3–6.6)	4.3 (2.3–6.9)	3.3 (2.2–5.4)	.33
HBV Genotype [N = 101]				.55
A	67 (66)	51 (66)	16 (67)	
G	15 (15)	10 (13)	5 (21)	
D	8 (8)	6 (8)	2 (8)	
E	11 (11)	10 (13)	1 (4)	
qHBsAg $\log_{10}$ IU/mL	4.2 (3.5–4.9)	4.4 (3.6–4.9)	3.6 (3.0–4.6)	<.01
HBeAg positive	92 (60)	69 (60)	23 (57)	.74
qHBcrAg $\log_{10}$ U/mL	6.8 (3.5–7.9)	6.9 (3.1–8)	5.9 (3.6–7.5)	.64
anti-HBc antibodies, $\log_{10}$ PEI U/mL	3.3 (2.2–4.0)	3.3 (1.7–3.9)	3.3 (2.5–4.1)	.49
Concomitantly treated with LAM[b]	104 (67)	75 (66)	29 (72)	.43
Previous LAM-exposure [N = 152]	135 (89)	97 (85)	38 (95)	.24
Cumulative LAM duration (months)	51.2 (23.5–75.9)	45.4 (17.2–69.9)	72.5 (47.9–84.0)	<.001
ALT (IU/L)[c] [N = 117]	42 (28–73)	41 (26–76)	45 (30–72)	.74
AST (IU/L)[c] [N = 117]	34 (25–57)	32 (25–52)	41 (31–69)	.04

Abbreviations: AIDS, acquired immunodeficiency syndrome; ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; c-ART, combined antiretroviral therapy; DNA, deoxyribonucleic acid; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B Virus; HIV, human immunodeficiency virus; LAM, lamivudine; N, total number of patients with data (if missing data were present); NNRTI, nonnucleoside/nucleotide reverse-transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse-transcriptase inhibitor; PEI, Paul-Ehrlich-Institute; PI, protease inhibitor; qAnti-HBc, quantitative antihepatitis B core antibody; qHBsAg, quantitative hepatitis B surface antigen; RNA, ribonucleic acid; TDF, tenofovir.

[a]F0-F1-F2 versus F3-F4 patients; Kruskal-Wallis test for continuous variables; Pearson χ^2 test or Fisher exact test for categorical variables.

[b]Number (%).

[c]Median (25th–75th percentile).

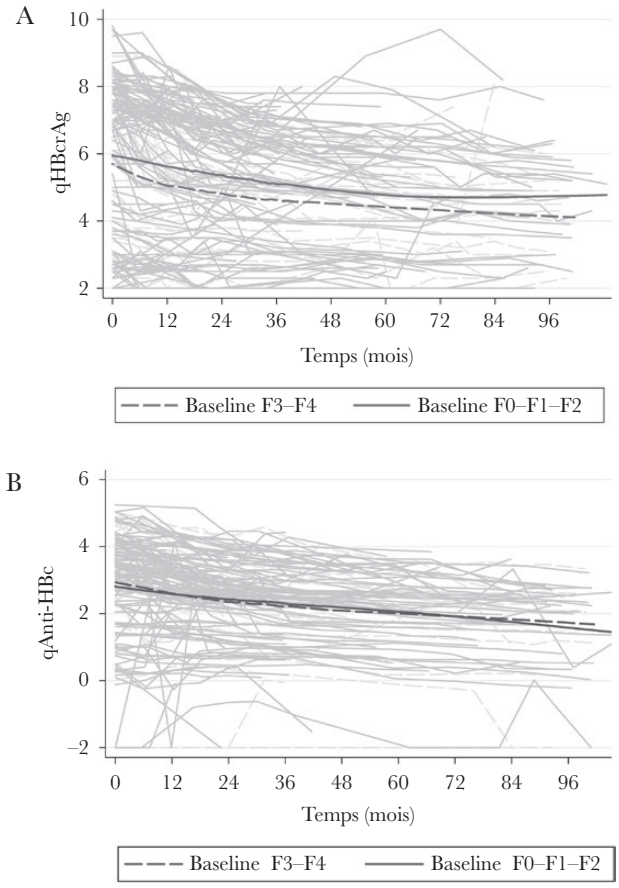

Figure 1. Kinetics of markers during follow-up depending on baseline liver fibrosis status. (A) hepatitis B core-related antigen quantification (qHBcrAg) according to baseline fibrosis status; (B) anti-hepatitis B core antibodies quantification (qAnti-HBc) according to baseline fibrosis status. Means are expressed as bold lines from a LOWESS curve and individual levels are expressed as gray lines.

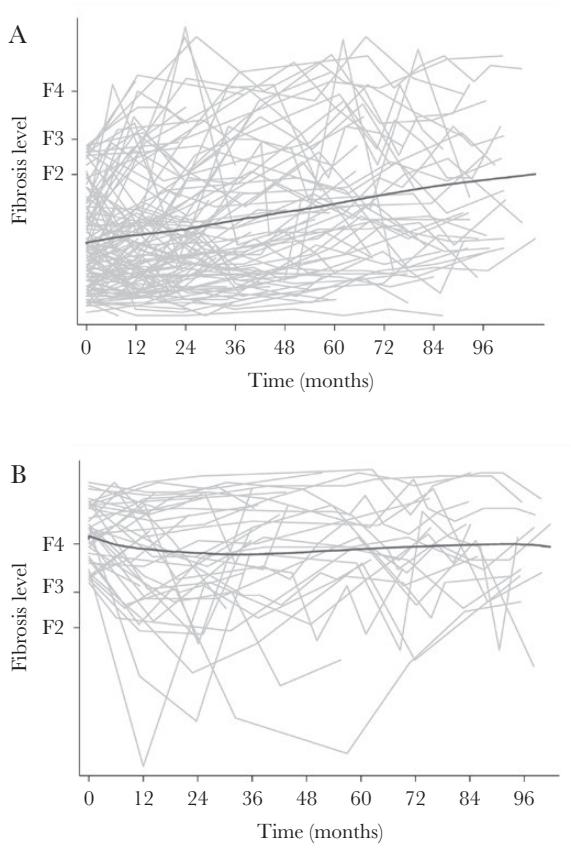

Figure 2. Fibrosis levels during follow-up in function of baseline liver fibrosis status. (A) Liver fibrosis quantification using FibroTest in patients with no, mild, or moderate liver fibrosis status at baseline (F0-F1-F2); (B) in patients with advanced liver fibrosis or cirrhosis at baseline (F3-F4). Means are expressed as bold lines from a LOWESS curve and individual levels are expressed as gray lines.

Marker Levels Associated With Transitions of Liver Fibrosis Over Time

In total, the median number of FibroTest assessments was 5 (IQR, 3–8) per patient, totaling 419 possible transitions between liver fibrosis status during follow-up. There were 38 transitions from F0-F1-F2 to F3-F4 fibrosis (TI = 7/100 person-years) and 34 transitions from F3-F4 to F0-F1-F2 (TI = 6/100 person-years).

In the univariable time-homogenous Markov models, qHBcrAg levels during follow-up were significantly and positively associated with transitions from F3-F4 to F0-F1-F2 liver fibrosis (Table 2). The qAnti-HBc levels at baseline and during follow-up were significantly and negatively associated with transitions from F0-F1-F2 to F3-F4, whereas change in qAnti-HBc levels from the previous visit was associated with transitions from F3-F4 to F0-F1-F2. Of note, no specific levels of qHBcrAg and qAnti-HBc were associated with transitions from F0-F1-F2 to F3-F4 (Supplementary Figure 1A and C, respectively) or with transitions from F3-F4 to F0-F1-F2 (Supplementary Figure 1B and D, respectively), except for qHBcrAg >6.5 log U/mL and qAnti-HBc >4.5 log PEI/mL with the latter transitions. In multivariable analysis, only change in qAnti-HBc levels from the previous visit was associated with transitions from F3-F4 to F0-F1-F2 (adjusted HR = 5.46; 95% CI, 1.56–19.16), after adjustment for age, gender, ART duration, PI-containing ART, and $CD4^+$/$CD8^+$ ratio during follow-up. Model fit was adequate with slight overestimation of being in the F3-F4 liver fibrosis state (Supplementary Figure 2).

DISCUSSION

Despite the growing interest in novel surrogate markers of chronic HBV infection, such as qHBcrAg and qAnti-HBc, no study has examined their effect on liver fibrosis evolution in HIV-HBV coinfection. In our present work, we explored how these markers relate to liver fibrosis, as measured by a noninvasive biochemical marker, in a cohort of HIV-HBV coinfected patients with ART experience, both at the beginning of and during TDF treatment. Levels of qHBcrAg and qAnti-HBc at TDF-treatment initiation were not associated with progression or regression of liver fibrosis status. Nevertheless, there was a significant and independent association between the change in

Table 2. Levels of Markers as Determinants of Transitioning to and From None/Mild/Moderate Liver Fibrosis (F0-F1-F2) and Severe Fibrosis/Cirrhosis (F3-F4) During Tenofovir-Containing ART

	Univariable		Multivariable[a]	
Markers of HBV replication	F0-F1-F2 -> F3-F4	F3-F4 -> F0-F1-F2	F0-F1-F2 -> F3-F4	F3-F4 -> F0-F1-F2
qHBcrAg (log_{10} U/mL)				
At baseline	1.11 (0.99–1.25)	1.14 (0.98–1.33)	1.07 (0.93–1.24)	1.17 (0.95–1.46)
During follow-up	1.08 (0.95–1.23)	**1.23 (1.03–1.49)**	1.11 (0.92–1.33)	1.32 (1.02–1.71)
Change from previous visit	1.05 (0.58–1.88)	0.89 (0.53–1.51)	1.13 (0.55–2.31)	0.94 (0.44–2.00)
qAnti-HBc (log_{10} PEI U/mL)				
At baseline	**0.82 (0.69–0.97)**	0.86 (0.73–1.00)	0.85 (0.70–1.04)	0.97 (0.78–1.22)
During follow-up	**0.81 (0.68–0.98)**	0.93 (0.76–1.15)	0.92 (0.73–1.16)	1.09 (0.83–1.43)
Change from previous visit	1.48 (0.65–3.38)	**4.56 (1.91–10.91)**	2.27 (0.80–6.43)	**5.46 (1.56–19.16)**

Bold values refer to statistically significant results

Abbreviations: ART, antiretroviral therapy; PEI, Paul-Ehrlich-Institute; qAnti-HBc, quantitative antihepatitis B core antibody; qHBsAg, quantitative hepatitis B surface antigen.

[a]Adjusted for age, gender, duration of ART, protease inhibitor-containing ART and $CD4^+/CD8^+$ ratio during follow-up.

qAnti-HBc from the previous visit and transition from F3-F4 to F0-F1-F2.

We were unable to confirm previous studies in HBV-monoinfected patients in which positive associations between single-measured qHBcrAg or qAnti-HBc levels and liver fibrosis were observed [17, 18, 29–31]. This includes 1 study that found an association with qHBcrAg levels and development of liver fibrosis, as defined by the noninvasive marker of liver fibrosis FIB-4 [29]. In our study, we used a marker of liver fibrosis with high diagnostic accuracy in HIV-HBV coinfected individuals [24], suggesting that differences in fibrosis measurements did not result in discrepancies between studies.

The most evident difference in our study compared with others is that this evaluation included strictly HIV-HBV coinfected patients. Human immunodeficiency virus-induced immunosuppression has been shown to affect levels of qAnti-HBc, but not particularly qHBcrAg [32], and longer periods of HIV infection have been associated with higher degrees of biopsy-assessed liver fibrosis [33], both of which could have affected the association between these markers and fibrosis. Human immunodeficiency virus virological and immunological variables did not borne out as determinants for transitions between liver fibrosis states, with the exception of $CD4^+/CD8^+$ ratio during follow-up and liver fibrosis regression (HR = 2.90; 95% CI, 1.41–5.97), which has been associated with liver fibrosis levels in HIV infection [25, 26] and was adjusted for in our model. Other confounding variables include ART-related hepatotoxicity and metabolic hepatic manifestations. We adjusted our analysis for the use of PI-containing ART, a known risk factor of metabolic disorders and nonalcoholic steatohepatitis [34], and duration of ART. More generally, body mass index was stable and not associated with liver fibrosis progression. We did not collect data on all variables to calculate the homeostasis model assessment for insulin-resistance; nonetheless, only 4 patients were diabetic at baseline [35]. Taken together, any HIV-related bias would appear to be minimal, but without an HBV-monoinfected comparison group, it cannot be ruled out.

Nevertheless, the finding on larger increases in qAnti-HBc antibodies between visits being associated with transition to lower fibrosis levels is intriguing. Given that the FibroTest is based on a battery of biochemical markers closely linked to liver inflammation [23], the transition towards lower liver fibrosis levels might in fact be more related to inflammatory responses than histopathological lessening of fibrosis [36]. Moreover, qAnti-HBc titers have been reported to be related to hepatic flares during the immune clearance phase of HBV infection [37, 38]. Because qAnti-HBc levels are considered a general marker of anti-HBV immunity [19], perhaps a sudden increase in antiviral immune response resulted in immediate intrahepatic control of the virus and then reduced liver inflammation [39, 40]. Nevertheless, transaminases levels (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and their changes between visits were not associated with transitions between states of liver fibrosis in our analysis, implying that if inflammation was causing this association, it would be independent of ALT/AST. Further research would be needed, to confirm this hypothesis and whether it also relates to biopsy-assessed liver fibrosis.

It should be noted that the kinetics of qHBcrAg and qAnti-HBc were no different for individuals with baseline F3-F4 versus F0-F1-F2 liver fibrosis, with decreases in markers observed for both groups. Previous research from our group among coinfected patients initiating TDF found no significant difference in time to undetectable HBV DNA between liver fibrosis groups [7]. However, HBeAg-seroclearance rates were higher in individuals with F3-F4 liver fibrosis, as found in other cohorts of HBV-monoinfected patients treated with potent nucleoside/nucleotide analogs [41, 42]. The findings on qHBcrAg and qAnti-HBc would more likely support the hypothesis that having liver fibrosis at baseline does not necessarily hinder suppression of viral activity during TDF-containing ART.

Certain limitations of our study need to be addressed. First, there were few events of liver fibrosis regression and progression

when evaluating transitions between states over time, so analyses were likely underpowered. Nevertheless, we did have sufficient statistical power to identify determinants of liver fibrosis evolution as from a previous analysis using the same model [7] and given the strength of association, adding more individuals to the analysis would likely not have rendered a significant association between markers and liver fibrosis change. Second, because noninvasive biochemical scores were used to assess liver fibrosis levels, our analysis was to some degree prone to measurement bias. Some of the marker levels used in the FibroTest could have been affected by metabolic dysfunction caused by HIV infection or certain antiretroviral agents, such as atazanavir [24, 36, 43, 44].

Finally, patients included in analysis were part of a hospital cohort beginning in the early 2000s and, as such, had extensive ART exposure and high prevalence of HIV-related immunosuppression before undergoing TDF-containing ART. These individuals might not be fully comparable to more contemporary cohorts of coinfected patients [9], and a marker such as qAnti-HBc, which is linked to overall immunity, merits further investigation in more immunocompetent coinfected populations.

CONCLUSIONS

Despite good performance of these markers in predicting serological responses [30], baseline qHBcrAg and qAnti-HBc were not associated with liver fibrosis evolution in our cohort of HIV-HBV coinfected patients undergoing TDF-containing ART. Sudden increases in qAnti-HBc were related to transitions towards lower fibrosis levels and hence could be interesting to use when monitoring decreases in liver inflammation and possibly liver fibrosis. However, further studies would be needed to clarify this association. Our findings support the need to assess other novel markers of viral activity, such as pregenomic HBV RNA, as predictors of liver fibrosis evolution in treated coinfected patients.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. R. C. and L. N. C. D. were responsible for statistical analysis, interpretation of the data, and drafting the manuscript. S. M. was responsible for hepatitis B core-related antigen (HBcrAg), hepatitis B surface antigen (HBsAg), and hepatitis B e antigen (HBeAg) quantification, interpretation of the data, and drafting the manuscript. A. G. and C. D. were responsible for HBcrAg, antihepatitis B core antibodies, HBsAg, and HBeAg quantification and drafted parts of the manuscript. H. R., P. M., C. L.-C., and J. C. acquired data for the cohort, assisted in interpreting data, and gave critical revisions of the manuscript. P.-M. G. and K. L. helped design, conceptualize, and obtain funding for the French HIV-HBV cohort study, coordinated data collection, and drafted the manuscript. A. B. coordinated data analysis, gave important comments on data interpretation, drafted the manuscript, and provided critical revisions of the manuscript. All authors approved the final version.

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