Kinetics of Hepatitis B Core–Related Antigen and Anti–Hepatitis B Core Antibody and Their Association With Serological Response in Human Immunodeficiency Virus–Hepatitis B Coinfection

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Kinetics of hepatitis B core-related antigen and anti-hepatitis B core antibody and their association with serological response in HIV-hepatitis B co-infection

Running head: Novel biomarkers in HBV-HIV co-infection

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Main point:

Serum quantification of Hepatitis B core-related antigen and anti-Hepatitis B core antibodies could be useful in predicting HBeAg-seroclearance in HIV-HBV co-infected patients undergoing long-term

TDF-containing ART. Nevertheless, we emphasize that their performance is not better than other, currently available markers.

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Abstract

Background: To describe the kinetics of hepatitis B core-related antigen (qHBcrAg) and anti-hepatitis B core antibody (qAnti-HBc) during tenofovir (TDF)-treatment and assess their ability to predict HBeAg-seroclearance in patients co-infected with HIV and hepatitis B virus (HBV).

Methods: Serum qHBcrAg, qAnti-HBc and HBV-DNA were obtained at TDF-initiation and every 6-12 months. On-treatment kinetics of qHBcrAg (ΔqHBcrAg) and qAnti-HBc (ΔqAnti-HBc) were estimated using mixed-effect linear regression. Hazard ratios (HR) assessing the association between markers and HBeAg-seroclearance were calculated using proportional hazards regression and sensitivity (Se) and specificity (Sp) of marker levels in predicting HBeAg-seroclearance were assessed using time-dependent ROC curves.

Results: During a median 4.6 years, cumulative incidence of HBsAg-seroclearance and HBeAg-seroclearance were 3.2% (n=5/158) and 27.4% (n=26/95), respectively. ΔqHBcrAg was biphasic in HBeAg-positive (-0.051 and -0.011 $\log_{10}$U/mL/month during $\leq$18 months and >18 months, respectively) and monophasic in HBeAg-negative patients. ΔqAnti-HBc was monophasic regardless of HBeAg-status. In HBeAg-positive patients, baseline qHBcrAg and qAnti-HBc levels were associated with HBeAg-seroclearance (adjusted-HR=0.48/$\log_{10}$U/mL; 95%CI=0.33-0.70 and unadjusted-HR=1.49/$\log_{10}$PEIU/mL; 95%CI=1.08-2.07, respectively). Cutoffs with the highest accuracy in predicting HBeAg-seroclearance at 36 months were qHBcrAg<6.5 $\log_{10}$U/mL at month-24 (Se=1/Sp=0.58) and baseline qAnti-HBc$\geq$4.1 $\log_{10}$PEIU/mL (Se=0.42/Sp=0.81).

Conclusions: In co-infected patients undergoing TDF, qHBcrAg/qAnti-HBc could be of use in monitoring HBeAg-seroclearance.

Key words: hepatitis B; HIV; seroclearance; hepatitis B core-related antigen; anti-hepatitis B core antibody.

Background

In HIV-positive individuals, it was recently estimated that 7.4% worldwide were co-infected with chronic hepatitis B virus (HBV) [1]. HIV-HBV co-infection has been associated with increased risk of liver cirrhosis, hepatocellular carcinoma, and hepatic decompensation particularly when HBV replication is left uncontrolled [2,3]. Tenofovir (TDF)-containing antiretroviral therapy (ART) effectively provides dual activity against HIV and HBV in co-infected patients, allowing suppression of HBV replication [4]. Wide-spread TDF use in co-infected patients has led to substantial decreases in HBV-DNA detection, with almost 85% of patients able to achieve undetectable HBV-DNA [5].

In HBV mono-infected patients, the viral markers indicating improved prognosis are suppression of HBV-DNA, hepatitis B “e” antigen (HBeAg) seroclearance (for those with HBeAg-positive serology), and more importantly hepatitis B surface antigen (HBsAg) seroclearance [4]. During TDF use in HIV-HBV co-infected patients, almost half of those who are HBeAg-positive exhibit HBeAg-seroclearance and few overall have HBsAg-seroclearance [6,7]. There are, however, exceptions in which higher rates of seroclearance are observed in co-infected patients with more severe immunosuppression [8–10]. As antiviral therapy inhibits viral replication of HBV-DNA, its levels do not accurately reflect intrahepatic HBV activity in patients undergoing antiviral treatment [11]. Other markers of treatment efficacy, such as quantification of HBeAg (qHBeAg) and HBsAg (qHBsAg), have been useful in predicting seroclearance events, but their stability during long-term treatment makes their use in routine clinical practice debatable [6,12].

Recently, quantitative hepatitis B core-related antigen (qHBcrAg) and anti-hepatitis B core antibody (qAnti-HBc) have been gaining attention in HBV mono-infection. qHBcrAg consists of three proteins, hepatitis B core antigen (HBcAg), HBeAg and a 22KDa *precore* protein (p22cr), which are transcribed from the *precore*/*core* gene and share an identical 149 amino acid sequence [13]. This surrogate marker strongly correlates with covalently-closed circular (ccc)DNA and total intrahepatic HBV-DNA during antiviral-induced HBV suppression [13–18]. qAnti-HBc involves quantification of the standard marker anti-HBc antibodies and is thought to reflect HBV-specific adaptive immunity. Baseline levels of this marker have been shown to bear reliable prediction of HBeAg seroconversion in HBV mono-infected patients undergoing antiviral therapy [19].

Nevertheless, current research on these markers has mainly focused on HBV mono-infected patients, while no study to date has examined its relevance in HIV-HBV co-infection. This is particularly concerning as qAnti-HBc levels are linked to host immunity [20], which could be impaired during HIV infection. The aim of the present study was to describe qHBcrAg and qAnti-HBc kinetics during long-term TDF treatment in a large, prospective cohort of ART-experienced HIV-HBV co-infected patients. We further aimed to evaluate the association between these markers and HBeAg or HBsAg-seroclearance and their predictive capacity for these events.

Methods

Patients and data collection

Patients were selected from the French HIV-HBV Cohort Study [21]. Briefly, this prospective study recruited 308 HIV-infected patients with chronic HBV infection from seven clinical

centers located in Paris and Lyon, France during May 2002–May 2003. Patients were included if they had HIV-positive serology confirmed by western blot and HBsAg-positive serology for more than 6 months. Participants were followed prospectively every six to twelve months until 2010-2011.

For this analysis, we included patients who initiated TDF-containing ART, had a minimum of two consecutive visits undergoing TDF-containing ART (lasting more than 6 months), with an available sample at TDF-initiation and at least once during follow-up. Patients with detectable HCV-RNA or HDV-RNA or those undergoing intensification with standard or pegylated-interferon (peg-IFN) were excluded from analysis (Supplementary figure 1). All patients gave written informed consent to participate in the cohort and the study received ethical approval in accordance with the Helsinki declaration.

Baseline was defined as the study visit at or directly prior to TDF-initiation. Follow-up began at TDF-initiation and continued until last study visit, TDF discontinuation, meeting any one of the exclusion criteria, or death; whichever occurred first. Demographic information were collected at study inclusion. HIV- and HBV-related variables were collected before TDF initiation and at each follow-up visit.

Assessing markers of viral activity

Serum HBV-DNA was quantified at baseline and every 6 months by a real-time PCR assay (COBAS® AmpliPrep/COBAS TaqMan®, detection limit: 12 IU/mL; or COBAS® Amplicor HBV Monitor, detection limit: 60 IU/mL; Roche Diagnostics, Meylan, France). HIV-RNA viral load was measured at cohort inclusion and every six months using either a branched-DNA (b-DNA 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 yearly using commercial enzyme-linked immunoassays (EIAs) (Diasorin, Antony, France). All other markers were collected at baseline and every 6 months. Serum qHBsAg levels (IU/mL) were quantified using the ARCHITECT HBsAg assay (Abbott Laboratories, Rungis, France) [7,22]. Serum qHBeAg levels were quantified using either ARCHITECT or Elecsys HBeAg assay (with Modular E170 analyzer; Roche Diagnostics). qHBeAg levels were expressed in Paul Ehrlich Institute units (PEIU)/mL [23]. qHBcrAg (U/mL) was measured using a commercially-available, automated HBcrAg chemiluminescence EIA (Lumipulse® G System, FujiRebio Europe, Gent, Belgium) [24]. When initial qHBcrAg levels were above 7 $\log_{10}$ U/mL, a 1/100 dilution was performed to obtain results within the range of quantification. Finally, IgG and IgM anti-HBc antibodies were quantified using ARCHITECT Anti-HBc II assay (Abbott Laboratories, Rungis, France) with an automated ARCHITECT i4000 system. Anti-HBc antibody levels were reported in PEIU/mL [25].

Statistical analysis

All qHBcrAg and qAnti-HBc units were $\log_{10}$ transformed. Linear regression was used to estimate univariable differences in baseline qHBcrAg or qAnti-HBc, and their 95% confidence intervals (CI), across levels of determinants. A multivariable model was constructed by adding all covariables with a $p<0.2$ in univariable analysis and removing non-significant variables in backwards-stepwise fashion.

In longitudinal analysis, on-treatment kinetics of the changes of qHBcrAg (ΔqHBcrAg) and qAnti-HBc (ΔqAnti-HBc) were estimated using mixed-effect linear regression models with a

random-intercept to account for between-patient variability at baseline [26]. Models were stratified by HBeAg-status. Given the bi-phasic nature of ΔqHBcrAg kinetics among HBeAg-positive patients in preliminary analysis, we modeled qHBcrAg kinetics according to ≤18 and >18 months of TDF treatment. All of the models were adjusted *a priori* for factors influencing liver fibrosis or HBV replication: body mass index (BMI), age, concomitant lamivudine (LAM) treatment, cumulative treatment duration with LAM, undetectable HBV-DNA, CD4+ cell count >350 cells/mm^3 (if not already stratified), and baseline qHBcrAg or qAnti-HBc levels. For specific genetic and immunological determinants, we also included a cross-product term between TDF duration and presence of determinant along with its individual components (separately for each determinant), from which stratum-specific estimates could be calculated and differences in ΔHBcrAg and ΔqAnti-HBc could be tested.

We then evaluated the relationship between qHBcrAg or qAnti-HBc and HBeAg-seroclearance. First, univariable hazards ratios (HR) and 95%CI comparing hazards of HBeAg-seroclearance between continuous baseline levels of markers (qHBcrAg and qAnti-HBc separately) were estimated using Cox proportional hazards models. A multivariable model was constructed using the backwards-selection approach as described above. Second, we performed several analyses to explore the most adequate thresholds for both markers, individually or combined. We selected the following criteria to assess the predictive capacity of HBeAg-seroclearance: qHBcrAg log_{10} U/mL <7.5 at baseline and <6.5 at months 12, 24 and 36; qAnti-HBc ≥4.1 log_{10} PEIU/mL at baseline; qHBcrAg <7.5 log_{10} U/mL and qAnti-HBc ≥4.1 log_{10} PEIU/mL at baseline; qHBcrAg <7.5 log_{10} U/mL or qAnti-HBc ≥4.1 log_{10} PEIU/mL at baseline; and HBV-DNA <60 IU/mL at months 12, 24 and 36. Time-dependent receiver operating characteristic (ROC) curves were used to evaluate the sensitivity (Se) and specificity (Sp) of each criterion as predictor of HBeAg-seroclearance until specific time-points of TDF duration [27].

All statistical analysis was performed using STATA (v15.1, College Station, TX, USA) or RStudio (v1.1.453, Vienna, Austria) and significance was defined as a *p*-value <0.05.

Results

Baseline characteristics of the study population

Of the 158 patients, 95 (60%) were HBeAg-positive and 63 (40%) HBeAg-negative. At TDF-initiation, median age was 41.1 years (IQR=35.5-48.0) and the majority was male (84.2%). All patients were ART-experienced with median CD4+ cell count at 404/mm^3 (IQR=295-552) and 57.3% (*n*=90) having undetectable HIV-RNA. A total of 123 (78.3%) had detectable HBV-DNA with a median viral load of 5.2 $\log_{10}$ IU/mL (IQR=3.0-7.2). For those with available data on HBV genotypes, most patients harbored genotype A (68%), followed by E (12%), G (12%) and D (8%). The G to A nucleotide substitution at position 1896 of the *precore* region was identified in 21.8% of patients (*n*=22/101).

As shown in Table 1, HBeAg-positive patients were more often male (*p*<0.001), born in zone of low/moderate HBV-prevalence (*p*<0.001), diagnosed with an AIDS-defining event (*p*=0.04), had a longer duration of known HIV-infection (*p*=0.007), higher levels of HIV-RNA (*p*=0.03) and qHBsAg (*p*<0.001) than HBeAg-negative patients (Table 1). As expected, HBV-DNA and ALT/AST levels were higher among HBeAg-positive versus HBeAg-negative patients (both *p*<0.001).

Baseline determinants of qHBcrAg and qAnti-HBc levels

At TDF-initiation, median levels of unadjusted qHBcrAg were significantly higher in HBeAg-positive (7.8 $\log_{10}$ U/mL; IQR=7.0-8.2; *p*<0.001) versus HBeAg-negative patients (3.0 $\log_{10}$ U/mL; IQR=2.5-4.0), while median levels of unadjusted qAnti-HBc were greater in HBeAg-

218 negative (3.4 log_{10} PEIU/mL; IQR=3.0–3.9; *p*=0.008) versus HBeAg-positive patients (2.8 log_{10}
219 PEIU/mL; IQR= 1.1–4.0) (Table 1). In multivariable analysis (Table 2), baseline qHBcrAg levels
220 (adjusted mean log_{10} U/mL) were higher in those with an AIDS-defining event (6.3 versus
221 without advanced HIV disease, 5.7; *p*=0.06), ALT >70 IU/L (6.7 versus ALT ≤70 IU/L, 5.5;
222 *p*<0.001) and higher serum HBV-DNA (*p*<0.001). Baseline qAnti-HBc levels (adjusted mean log_{10}
223 PEIU/mL) were higher in older individuals (*p*=0.09), those without an AIDS-defining illness (3.0
224 versus with AIDS-defining illness, 2.3; *p*=0.047), with higher CD4+ cell count (*p*<0.001) and with
225 HBeAg-negative status at baseline (3.4 versus HBeAg-negative, 2.5; *p*<0.001).
226
227 ***On-treatment kinetics of qHBcrAg and qAnti-HBc levels***
228 Median follow-up of TDF treatment was 4.6 years (IQR=2.9-7.6). Adjusted ΔHBcrAg was faster
229 in HBeAg-positive versus HBeAg-negative patients, overall and during the first 18-months of
230 treatment (Table 3; figure 1A). In HBeAg-positive patients, adjusted ΔqHBcrAg during TDF
231 treatment was biphasic, with a significantly more rapid decline during the first 18 months (-
232 0.051 log_{10} U/mL/month) compared to thereafter (-0.011 log_{10} U/mL/month; p=0.007). In
233 HBeAg-negative patients, adjusted ΔqHBcrAg was linear during TDF treatment (-0.003 log_{10}
234 U/mL/month). ΔqHBcrAg was faster in HBeAg-negative patients with HBV genotype D infection
235 patients (*p*<0.001) and slower with genotype A infection (*p*=0.01) compared to other
236 genotypes, while faster ΔqHBcrAg was observed in HBeAg-negative patients with *precore*
237 mutations compared to without (*p*=0.009).
238
239 Both HBeAg-positive and HBeAg-negative patients had linear ΔqAnti-HBc during TDF treatment
240 (Figure 2A), while ΔqAnti-HBc was not different between HBeAg-positive versus HBeAg-
241 negative patients (-0.011 log_{10} PEIU/mL/month for both; p=0.7) (Table 3). In HBeAg-positive
242 patients, adjusted ΔqAnti-HBc was significantly faster in HBV genotype A versus non-A

infection (*p*=0.02). In both HBeAg-positive and HBeAg-negative patients, those with CD4+ cell count >350/mm^3 versus ≤350/mm^3 and those with a nadir CD4+ cell count >200/mm^3 versus ≤200/mm^3 exhibited a significantly faster ΔqAnti-HBc during TDF treatment (Table 3).

Of note, baseline qHBsAg levels were not significantly correlated with individual ΔqHBcrAg in HBeAg-positive individuals (Bland-Altman rho=-0.06, *p*=0.11) and HBeAg-negative individuals (Bland-Altman rho=0.0004, *p*=0.9). Baseline qHBsAg levels were also not significantly correlated with individual ΔqAnti-HBc in HBeAg-positive individuals (Bland-Altman rho=0.06, *p*=0.12) and HBeAg-negative individuals (Bland-Altman rho=-0.08, *p*=0.12).

Association of qHBcrAg and qAnti-HBc levels with seroclearance

During TDF treatment, 26 HBeAg-positive patients achieved HBeAg-seroclearance (cumulative incidence=27.4%, 95%CI=18.7%-37.5%). Of the patients with HBeAg-seroclearance, HBeAg seroconversion occurred in 8 (cumulative incidence=8.4%, 95%CI=3.7%-15.9%). Patients with lower baseline qHBcrAg level had a higher rate of HBeAg-seroclearance (Figure 1B) after adjusting for change in CD4+ cell count from previous visit (adjusted-HR=0.48, 95%CI=0.33–0.70) (Table 4). Patients with higher baseline qAnti-HBc level had higher rates of HBeAg-seroclearance (HR=1.49, 95%CI=1.08-2.07) (Figure 2B), while no other variable was below the *p*-value threshold considered for multivariable analysis (Table 4). In addition, ΔqAnti-HBc was also faster among patients achieving HBeAg-seroclearance during follow-up (-0.017 $\log_{10}$ PEIU/mL/month) compared to those without HBeAg-loss (-0.010 PEIU/mL/month; *p*<0.001).

HBsAg-seroclearance occurred in only 5 patients (cumulative incidence=3.2%, 95%CI=1.0%-7.2%). There were no statistically significant differences in their baseline characteristics compared to those without HBsAg-seroclearance (Supplementary table 1). Among those with

HBsAg-seroclearance, median baseline qHBcrAg was 6.7 log_{10} U/mL (range HBeAg-positive=6.7-8.0, HBeAg-negative=3.7-4.0) and median baseline qAnti-HBc was 3.2 log_{10} PEIU/mL (range HBeAg-positive=1.2-4.6, HBeAg-negative=1.8-3.6). ΔqHBcrAg appeared steeper during the first 12 months of TDF treatment in these individuals (Figure 1C), while the change in qAnti-HBc was flat during follow-up (Figure 2C). Given the few numbers of patients with HBsAg-seroclearance, this endpoint was not considered further in analysis.

Predictive capacity of HBV serological markers on HBeAg-seroclearance

Table 5 gives the Se and Sp of various criteria in predicting HBeAg-seroclearance at specific time-points during TDF treatment. As expected, undetectable HBV-DNA for most time-points provided optimal Se, but consistently low Sp in predicting long-term HBeAg-seroclearance. qHBcrAg <6.5 log_{10} U/mL at months 12, 24 or 36 showed comparable Se to undetectable HBV-DNA, but higher Sp especially for predicting HBeAg-seroclearance in the 12 months following qHBcrAg measurement. Baseline qAnti-HBc $\geq$4.1 log_{10} PEIU/mL showed high Sp, but very low Se in predicting HBeAg-seroclearance. When combining qHBcrAg <7.5 log_{10} U/mL and qAnti-HBc $\geq$4.1 log_{10} PEIU/mL, both assessed at baseline, we found the highest levels of Sp in predicting HBeAg-seroclearance of the studied markers, but low Se.

Discussion

Despite the extensive research on novel biomarkers of HBV viral activity, none to date have examined their kinetics or association with seroclearance in HIV-HBV co-infected patients undergoing TDF treatment. We have demonstrated a gradual decrease of on-treatment qHBcrAg and qAnti-HBc levels, compatible with a cumulative therapeutic benefit of long-term nucleos(t)ide analogue (NA) therapy. Our findings indicated that lower baseline levels of qHBcrAg and higher qAnti-HBc antibodies were strong, independent predictors of HBeAg

seroclearance, suggesting some clinical applicability in TDF-treated HIV-HBV co-infected patients. To our knowledge, this study is the first to report the clinical utility of qHBcrAg or qAnti-HBc levels as a predictor of HBeAg-seroclearance in the treated HIV-HBV co-infected population.

Despite the fact that all previous studies examining qHBcrAg and qAnti-HBc antibodies have been performed in HBV mono-infected patients, we did observe several similarities to our co-infected population [19,28]. A decrease in qHBcrAg during TDF treatment was indeed observed for both HBeAg-positive or HBeAg-negative patients [28,29]. In addition, we demonstrated a bi-phasic decline in HBeAg-positive patients, which probably reflected the initial, effective inhibition of circulating virus in the blood associated with TDF followed by reductions of the cccDNA pool [30]. For qAnti-HBc antibodies, its kinetics in TDF-treated HIV-HBV coinfected patients showed a steady decline, for both HBeAg-positive or HBeAg-negative patients, similar to HBV mono-infected individuals treated with peg-IFN or NAs [19,31,32]. Interestingly, the observation of higher median baseline qAnti-HBc levels in HBeAg-negative versus HBeAg-positive patients could be the result of increased immune activity in patients previously able to clear HBeAg [33,34].

We were able to identify other viral characteristics associated with qHBcrAg and qAnti-HBc kinetics. The presence of a *precore* mutation in HBeAg-negative patients appeared to accelerate declines in qHBcrAg. Indeed, as *precore* mutations can block the synthesis of HBeAg without adversely affecting HBV replication [35], the more rapid decrease in qHBcrAg might partly reflect viral suppression linked to TDF treatment. Whether this is due to reduction of the cccDNA pool in hepatocytes or from immunological clearance of virus in patients harboring *precore* mutant variants is unknown and cannot be confirmed with the data collected in our

study. Nevertheless, it should be noted that the rates of declines observed in these patients were much slower than most of the average rates of declines in HBeAg-positive patients.

On-treatment qAnti-HBc kinetics were faster in HBV genotype A versus non-A genotypes, specifically among HBeAg-positive patients. In studies on the natural history of HBV mono-infection, HBeAg-positive patients with HBV genotype A have demonstrated higher rates of sustained transaminase normalization, HBV-DNA clearance, transition to inactive HBV carrier state after HBeAg-seroconversion, and HBsAg-seroclearance [36,37]. These data would suggest that a more robust antiviral immune response could be elicited against genotype A infection, yet the exact mechanisms remain unknown ([38]). The faster decrease in qAnti-HBc levels builds on these findings, suggesting that anti-HBV-specific immunity could be more active in HBeAg-positive genotype A infected patients, even during TDF treatment.

Interestingly, given that HIV-related immunity also exerts an effect on anti-HBV-specific immunity [39], qAnti-HBc levels would assumedly be influenced by the degree of HIV-induced immunosuppression. This effect was already apparent at baseline, at which point qAnti-HBc levels were lower in patients with a previous AIDS-defining illness and lower CD4+ T cell counts. Additionally, more rapid declines of qAnti-HBc were observed in patients with higher baseline (>350 cells/mm^3) and nadir (>200 cells/mm^3) CD4+ cell counts, regardless of baseline HBeAg-status, supporting the concept that more pronounced immunosuppression lends to slower declines of qAnti-HBc [40]. How this translates to HBeAg-seroclearance is unclear. Faster slopes of qAnti-HBc were associated with HBeAg-seroclearance, suggesting that longer time spent at higher levels of qAnti-HBc levels (i.e. with more active anti-HBV immunity) does not lead to seroclearance. In addition, we observed no significant association with CD4+ cell counts and HBeAg-seroclearance.

More importantly, we found that higher levels of qHBcrAg at the time of initiating TDF-containing ART were independently associated with lower rates of HBeAg-seroclearance in HBeAg-positive patients. Previous research linking baseline qHBcrAg levels with HBeAg-seroclearance have mostly focused on treatment with peg-IFN. In 46 patients treated with peg-IFN, Chuaypen et al [18] reported that those with baseline qHBcrAg levels >8 $\log_{10}$ U/mL had a low risk of HBeAg seroclearance and suppression of HBV-DNA at 12 weeks post-treatment. Others have found that qHBcrAg levels at week 12 of peg-IFN treatment were predictive of post-treatment HBeAg-seroclearance [29], while another study investigating entecavir (ETV) with or without peg-IFN add-on therapy observed that higher qHBcrAg levels at both week 24 and week 36 were independently associated with a lower risk of HBeAg-seroclearance and HBV-DNA suppression 24-weeks post-treatment in both treatment arms [37]. With regards to NA-based therapy, baseline HBcrAg levels >5.7 $\log_{10}$ U/mL have been also associated with lack of HBeAg-seroconversion at 6 and 12 months of treatment [28] and faster declines of qHBcrAg were observed in patients with HBeAg-seroclearance as compared to those without [33]. Our findings in TDF-treated HIV-HBV co-infected patients fall in line with these studies.

Likewise, we found an association between anti-HBc antibody levels and HBeAg-seroclearance, indicating that patients with higher baseline total qAnti-HBc levels exhibited higher rates of HBeAg-seroclearance. Previous research has suggested that baseline qAnti-HBc strongly predicts HBeAg-seroconversion in populations of HBV mono-infected patients receiving either NA or peg-IFN therapy [19,25,32]. The fact that an association between qAnti-HBc levels and HBeAg-seroclearance was observed in HIV-HBV co-infected patients, given the effect of HIV-induced immunosuppression on its quantification and sometimes higher rates of HBeAg-seroclearance observed after anti-HBV containing ART [9,10], is noteworthy.

In terms of predictive capacity, we confirm the high sensitivity of qHBcrAg. We add to previous studies by showing that qHBcrAg levels have higher specificity in predicting HBeAg-seroclearance within 12-months of being measured, with waning specificity in predicting HBeAg-seroclearance thereafter. This finding implies that repeated measurements of qHBcrAg would be needed in order to continuously and more accurately gauge the risk of HBeAg-seroclearance. Indeed, other studies focusing on time-specific measurements of serum qHBcrAg levels have involved peg-IFN treatment in HBV mono-infected patients, with qHBcrAg measured at 12 weeks representing an important milestone for treatment response and stopping rules [18,29,41]. These studies have also demonstrated higher Se, but modest Sp when using qHBcrAg to predict HBeAg-seroconversion. On the other hand, serum anti-HBc antibodies had higher Sp at baseline than at any point during follow-up. Even when used as a complementary marker with qHBcrAg, predictive capacity did not improve. Nevertheless, the performance of these markers appears to be similar to qHBsAg and qHBeAg from previous studies in TDF-treated co-infected individuals [42]. Coupled with the marginally higher Sp when compared to using undetectable HBV-DNA to predict HBeAg-seroclearance, it is debatable to what extent these markers should be used in routine care (38).

Certain limitations of our study must be acknowledged. We only included analysis on HBeAg seroclearance and not HBeAg seroconversion, which could be considered a more desired endpoint of treatment response [4]. Considering that few patients achieved HBsAg seroclearance, our analysis on this endpoint was wholly descriptive. Second, there was substantial heterogeneity of HBcrAg levels observed during follow-up, making interpretability of this marker somewhat difficult. Finally, data on HBV genetic variability were available for patients with HBV-DNA replication, representing a group with more active HBV infection.

In conclusion, we provide a thorough understanding of qHBcrAg and qAnti-HBc kinetics, and their clinical applicability, in the context of HIV-HBV co-infection. Serum levels of qHBcrAg and qAnti-HBc could be useful in predicting HBeAg-seroclearance in HIV-HBV co-infected patients undergoing long-term TDF-containing ART. This is based on the high sensitivity of qHBcrAg and high specificity of qAnti-HBc, when compared to undetectable HBV-DNA, in predicting HBeAg-seroclearance. Nevertheless, whether they provide further clinical utility compared to qHBsAg and qHBeAg remains debatable.

Notes

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Table 1. Baseline characteristics of patients treated with tenofovir

	HBeAg status		
	HBeAg-positive (*n = 95*)	**HBeAg-negative** (*n = 63*)	***p***[a]
Demographics			
Male sex[b]	89 (93.7)	44 (69.8)	<0.001
Age (years)[c]	40.3 (35.0-46.7)	41.6 (37.2-49.6)	0.26
From zone of high HBV-prevalence[b]	8 (8.4)	32 (50.8)	<0.001
BMI (Kg/m^2)[c] [*N*=153]	22.4 (20.9-23.9)	22.8 (21.0-25.8)	0.27
HIV infection			
Estimated duration of HIV infection, years[c] [*N*=157]	12.0 (7.7-15.3)	8.3 (5.0-14.4)	0.007
AIDS-defining event[b]	29 (30.5)	10 (15.9)	0.04
HIV-RNA (log_{10} copies/mL)[c] [*N*=157]	1.7 (1.7-4.0)	1.7 (1.7-3.2)	0.03
HIV-RNA > 50 copies/mL[b] [*N*=157]	45 (47.8)	22 (34.9)	0.11
$CD4^+$ cell count (cells/µL)[c] [*N*=157]	423 (312-580)	388 (277-543)	0.24
Nadir $CD4^+$ cell count (cells/µL)[c] [*N*=143]	230 (78-321)	212 (118-324)	0.9
Duration of ART (years)[c]	7.0 (4.8-9.2)	6.1 (3.2-9.0)	0.17
Viral hepatitis			
Estimated duration of HBV infection (years)[c] [*N*=157]	7.9 (3.8-11.8)	7.7 (3.6-13.1)	0.8
HBV-genotype[b] [*N*=104]			
A	55 (67.1)	13 (59.1)	0.5
D	7 (8.5)	1 (4.6)	0.5
E	4 (4.9)	8 (36.4)	<0.001
G	12 (14.6)	0 (0)	0.05

Precore mutation[b] [*N*=101]	13 (15.9)	9 (47.4)	0.003
Concomitant LAM treatment[b]	67 (70.5)	39 (61.9)	0.26
HBV-DNA (log_{10} IU/mL)[c] [*N*=157]	6.6 (4.2-7.6)	1.9 (1.6-3.0)	<0.001
HBV-DNA <60 IU/mL[b] [*N*=157]	3 (3.2)	31 (49.2)	<0.001
ALT (IU/L)[c] [*N*=155]	63 (39-97)	28 (21-40)	<0.001
AST (IU/L)[c] [*N*=155]	44 (31-78)	27 (24-37)	<0.001
HBV serological markers			
qHBsAg (log_{10} IU/mL)[c] [*N*=157]	4.7 (4.3-5.1)	3.5 (3.3-3.9)	<0.001
qHBeAg level (PEIU/mL)[c] [*N*=70]	862 (328-1099)		
qHBcrAg level (log_{10} U/mL)[c]	7.8 (7.0-8.2)	3.0 (2.5-4.0)	<0.001
qAnti-HBc level (log_{10} PEIU/mL)[c]	2.8 (1.1-4.0)	3.4 (3.0-3.9)	0.008

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ART, antiretroviral treatment; BMI, body mass index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LAM, lamivudine; PEIU, Paul Ehrlich Institute units; qAnti-HBc, quantified anti-hepatitis B core antibody; qHBcrAg, quantified hepatitis B core-related antigen; qHBeAg, quantified hepatitis B e antigen; qHBsAg, quantified hepatitis B surface antigen.

[a]Comparing HBeAg-positive versus HBeAg-negative patients; significance determined using Kruskal–Wallis test for continuous variables and Pearson's X^2-test or Fisher's exact test for categorical variables.

[b]Number (%).

[c]Median (25–75th percentile).

Table 2. Baseline determinants of hepatitis B core-related antigen and anti-hepatitis B core antibody

	qHBcrAg (log_{10} U/mL)						qAnti-HBc (log_{10} PEIU/mL)					
	Univariable			Multivariable			Univariable			Multivariable		
	Coef.	(95% CI)	*p*	Coef.	(95% CI)	*p*	Coef.	(95% CI)	*p*	Coef.	(95% CI)	*p*
Age (per year)	-0.047	(-0.092, -0.002)	0.04				0.031	(0.002, 0.061)	0.04	0.023	(-0.004, 0.051)	0.09
Sex (male)	2.084	(1.115, 3.052)	<0.001				-0.242	(-0.908, 0.424)	0.5			
From zone of high HBV-prevalence[a]	-2.545	(-3.303, -1.786)	<0.001				0.605	(0.053, 1.157)	0.03			
BMI (per kg/m²) [*N*=153]	-0.099	(-0.225, 0.027)	0.12				-0.018	(-0.103, 0.066)	0.7			
AIDS-defining illness	0.915	(0.061, 1.768)	0.04	0.555	(-0.025, 1.135)	0.06	-0.743	(-1.295, -0.191)	0.009	-0.523	(-1.039, -0.007)	0.047
HIV-infection duration (per year) [*N*=157]	0.064	(-0.006, 0.134)	0.07				-0.005	(-0.052, 0.041)	0.8			
HBV-infection duration (per year) [*N*=157][a]	-0.062	(-0.124, -0.0001)	0.05				0.031	(-0.010, 0.072)	0.14			
HIV-RNA (per log_{10} copies/mL) [*N*=157]	0.232	(-0.069, 0.533)	0.13				-0.226	(-0.421, 0.032)	0.02			
CD4 cell count [*N*=157]												
per 100 /mm³	0.016	(-0.143, 0.175)	0.8				0.193	(0.094, 0.292)	<0.001	0.198	0.105, 0.292)	<0.001
> 350 /mm³	0.279	(-0.504, 1.061)	0.5				0.432	(-0.074, 0.938)	0.09			
CD4 nadir (> 250 cells/mm³) [*N*=143]	0.203	(-0.602, 1.009)	0.6				0.520	(0.022, 1.018)	0.04			
Duration of ART (per year)	-0.040	(-0.143, 0.062)	0.4				0.015	(-0.052, 0.082)	0.7			
Concomitant LAM treatment	0.477	(-0.314, 1.268)	0.24				-0.344	(-0.859, 0.171)	0.19			

Cumulative LAM duration (per year)	0.074	(-0.050, 0.197)	0.24				0.005	(-0.075, 0.086)	0.9			
HBeAg-positive[a]	4.153	(3.765, 4.540)	<0.001				-0.949	(-1.423, -0.475)	<0.001	-0.943	(-1.399, -0.487)	<0.001
Precore mutation [*N*=101]	-0.724	(-1.643, 0.196)	0.12				-0.591	(-1.424, 0.241)	0.16			
HBV-DNA (per $\log_{10}$ IU/mL) [*N*=157]	0.726	(0.621, 0.831)	<0.001	0.622	(0.507, 0.737)	<0.001	-0.029	(-0.130, 0.073)	0.6			
ALT (per IU/mL) [*N*=155]	0.010	(0.006, 0.014)	<0.001				0.001	(-0.001, 0.004)	0.4			
AST (per IU/mL) [*N*=155]	0.024	(0.015, 0.032)	<0.001				0.001	(-0.005, 0.007)	0.8			
ALT $\leq$ 70 IU/mL [*N*=155]	-2.680	(-3.423, -1.937)	<0.001	-1.145	(-1.776,-0.513)	<0.001	0.277	(-0.283, 0.837)	0.3			
AST $\leq$ 70 IU/mL [*N*=155]	-2.639	(-3.527, -1.751)	<0.001				0.201	(-0.443, 0.844)	0.5			
qHBsAg level ($\log_{10}$ IU/mL) [*N*=157]	1.535	(1.246, 1.824)	<0.001				-0.160	(-0.406, 0.087)	0.20			
qHBeAg level ($\log_{10}$ PEIU/mL) [*N*=70]	0.571	(0.269, 0.873)	<0.001				0.0001	(-0.001, 0.001)	0.8			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ART, antiretroviral treatment; BMI, body mass index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LAM, lamivudine; PEIU, Paul Ehrlich Institute units; qAnti-HBc, quantified anti-hepatitis B core antibody; qHBcrAg, quantified hepatitis B core-related antigen; qHBeAg, quantified hepatitis B e antigen; qHBsAg, quantified hepatitis B surface antigen.

[a]The following variables were not considered in multivariable analysis: *both models* – HBV and HIV-infection duration (unable to assess true infection duration), zone of HBV-prevalence, sex (collinear with other variables), AST $\leq$ 70 IU/mL (preferred over other transaminase variables) and HIV RNA (no longer below *p*-value threshold); qHBcrAg – HBeAg status, *precore* mutation and qHBsAg (collinearity) ; qAnti-HBc –CD4+ nadir >250/mm^3, concomitant LAM treatment, and *precore* mutation (no longer below *p*-value threshold).

Table 3. Kinetics of hepatitis core-related antigen and anti-hepatitis B core antibody during follow-up

	Decline in qHBcrAg log_{10} U/mL per month [a]						Decline in qAnti-HBc log_{10} PEIU/mL per month [a]			
	HBeAg+				HBeAg-		HBeAg+		HBeAg-	
	≤18 months Δ (95% CI)	*p* for intx[a]	>18 months Δ (95% CI)	*p* for intx	Δ (95% CI)	*p* for intx	Δ (95% CI)	*p* for intx	Δ (95% CI)	*p* for intx
Overall	*N = 95*				*N = 63*		*N=95*		*N=63*	
	-0.051 (-0.062, -0.040)		-0.011 (-0.024, 0.003)		-0.003 (-0.006, -0.001)		-0.011 (-0.013, -0.009)		-0.011 (-0.013, -0.009)	
HBV genotype	*N = 82*				*N = 22*		*N=82*		*N=22*	
A	-0.054 (-0.068, -0.040)	0.9	-0.068 (-0.090, -0.045)	0.29	0.001 (-0.006, 0.008)	0.01	-0.014 (-0.016, -0.012)	0.02	-0.005 (-0.011, 0.001)	0.6
D	-0.024 (-0.064, 0.017)	0.12	-0.038 (-0.054, -0.021)	0.4	-0.018 (-0.029, -0.008)	<0.001	-0.009 (-0.014, -0.003)	0.08	-0.002 (-0.012, 0.008)	0.4
E	-0.041 (-0.092, 0.011)	0.7	-0.041 (-0.060, -0.019)	0.9	-0.003 (-0.015, 0.008)	0.9	-0.013 (-0.020, -0.005)	0.9	-0.011 (-0.021, -0.002)	0.17
G	-0.075 (-0.117, -0.034)	0.27	-0.049 (-0.065, -0.033)	0.24	-		-0.013 (-0.018, -0.008)	0.9	-	-
YMDD mutation[b]	*N = 82*	0.17		0.07	*N = 21*		*N=82*	0.6	*N=21*	
Yes	-0.070 (-0.094, -0.046)		-0.044 (-0.060, -0.028)		-		-0.014 (-0.018, -0.011)		-	-
No	-0.051 (-0.065, -0.036)		-0.011 (-0.015, -0.007)		-0.004 (-0.010, 0.002)		-0.014 (-0.016, -0.011)		-0.070 (-0.132, -0.008)	
Precore mutation[c]	*N = 82*	0.3		0.9	*N = 19*	0.009	*N=82*	0.5	*N=19*	0.3
Yes	-0.048 (-0.083, -0.014)		-0.049 (-0.064, -0.033)		-0.007 (-0.014, -0.001)		-0.012 (-0.016, -0.007)		-0.004 (-0.010, 0.002)	
No	-0.053 (-0.067, -0.040)		-0.011 (-0.014, -0.007)		0.005 (-0.003, 0.014)		-0.013 (-0.016, -0.011)		-0.009 (-0.017, -0.001)	

Baseline $CD4^+$ cell count	*N = 95*	0.6		0.9	*N = 62*	0.06	*N=95*	0.001	*N=62*	0.006
>350 cells/mm^3	-0.052(-0.066, -0.038)		-0.050 (-0.069, -0.031)		-0.002 (-0.006, 0.001)		-0.015 (-0.017, -0.012)		-0.010 (-0.013, -0.007)	
≤350 cells/mm^3	-0.046 (-0.064, -0.027)		-0.008 (-0.012, -0.003)		-0.006 (-0.010, -0.003)		-0.010 (-0.013, -0.007)		-0.062 (-0.095, -0.030)	
Nadir $CD4^+$ cell count	*N=87*	0.60		0.10	*N=56*	0.3	*N=87*	0.004	*N=56*	0.007
>200 cells/mm^3	-0.047 (-0.063, -0.031)		-0.066 (-0.082, -0.049)		-0.004 (-0.008, -0.001)		-0.015 (-0.018, -0.013)		-0.009 (-0.012, -0.007)	
≤200 cells/mm^3	-0.053 (-0.069, -0.037)		-0.003 (-0.007, 0.001)		-0.006 (-0.009, -0.003)		-0.011 (-0.014, -0.009)		-0.006 (-0.008, -0.004)	

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; PEIU, Paul Ehrlich Institute units; qAnti-HBc, quantified anti-hepatitis B core antibody; qHBcrAg, quantified hepatitis B core-related antigen.

Decline in markers were determined using a mixed-effect linear model adjusted for baseline levels, body mass index, age, concomitant lamivudine treatment, cumulative treatment duration with lamivudine, HBV-DNA level and $CD4^+$ cells count (if not stratified).

[a] Slopes were compared between determinant groups, *p* values derived from a Wald X^2- test of an interaction term (calculated as the cross-product between duration of tenofovir treatment and presence of determinant), which is included in a mixed-effect linear model with its individual components.

[b] Mutation at position rtM204 of the *pol* gene.

[c] Mutation in the nucleotide at position 1896 (G versus A) of the *precore* region.

Table 4 – Factors associated to HBeAg-seroclearance during follow-up

Risk-Factor	Univariable		Multivariable Model 1[a]		Multivariable Model 2[b]	
	HR (95%CI)	*p*	HR (95%CI)[c]	*p*	HR (95%CI)	*p*
Age at baseline (per year)	1.04 (0.99-1.09)	0.10				
Male versus female sex	1.77 (0.24-12.92)	0.6				
From zone of high HBV-prevalence	0.89 (0.17-4.78)	0.9				
BMI at baseline (per kg/m²)	0.94 (0.82-1.06)	0.3				
AIDS-defining illness at baseline	1.35 (0.60-3.01)	0.5				
HIV-infection duration at baseline (per year)	0.99 (0.91-1.09)	0.9				
HBV infection duration at baseline (per year)	0.97 (0.91-1.04)	0.4				
HIV-RNA (per $\log_{10}$ copies/mL)[d]	1.00 (0.95-1.06)	0.9				
CD4 cell count[d]						
per 100mm^3	1.00 (0.99-1.01)	0.8				
>350/mm^3	1.06 (0.98-1.14)	0.16				
per 250/mm^3 change from prior visit	0.98 (0.96-1.01)	0.17	0.98 (0.96 – 1.00)	0.08		

Duration of ART (per year)[d]	1.00 (0.99-1.01)	0.3				
Concomitant LAM treatment[d]	1.01 (0.98-1.05)	0.4				
Cumulative LAM duration (per year)[d]	1.00 (0.99-1.01)	0.3				
HBV-DNA at baseline (per $\log_{10}$ IU/mL)	0.89 (0.73-1.08)	0.24				
HBV-DNA (per $\log_{10}$ IU/mL)[d]	1.01 (0.99-1.03)	0.32				
ALT (per 10 IU/mL)[d]	1.00 (0.99-1.01)	0.26				
Baseline qHBsAg level ($\log_{10}$ IU/mL)	0.46 (0.26-0.83)	0.009				
Baseline qHBeAg level <100 PEIU/mL	2.64 (0.99-7.05)	0.05				
Baseline qHBeAg level <10 PEIU/mL	6.64 (2.40-18.36)	<0.001				
Baseline qHBeAg level (per PEIU/mL)	0.47 (0.30-0.72)	0.001				
Baseline qHBcrAg level (per $\log_{10}$ U/mL)	0.49 (0.34-0.71)	<0.001	0.48 (0.33 – 0.70)	<0.001		
Baseline qAnti-HBc level (per $\log_{10}$ PEIU/mL)	1.49 (1.08-2.07)	0.02			1.49 (1.08-2.07)	0.02

ALT, alanine aminotransferase; ART, antiretroviral treatment; BMI, body mass index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HR, hazard ratio; LAM, lamivudine; PEIU, Paul Ehrlich Institute units; qAnti-HBc, quantified anti-hepatitis B core antibody; qHBcrAg, hepatitis B core-related antigen; qHBeAg, quantified hepatitis B e antigen; qHBsAg, quantified hepatitis B surface antigen. Analysis on the 95 HBeAg-positive patients, among whom 26 had HBeAg-loss.

In multivariable analysis, qHBeAg and qHBsAg were not further considered as the intent was to study qHBcrAg and qAnti-HBc antibodies. Univariable HR are provided for comparison.

[a] Model 1: the following variables were no longer below the *p*-value threshold – age at baseline and CD4 cell count >350/mm^3

[b] Model 2: the following variables were no longer below the *p*-value threshold – age at baseline, CD4 cell count >350/mm^3 and per 250/mm^3 change from prior visit.

[c] All HR are adjusted for the variables listed in the column.

[d] Time-varying covariate

Table 5. Quantifiable HBV markers in predicting HBeAg seroclearance

		Classification Probabilities [a]											
		M24		**M36**		**M48**		**M60**		**M72**		**M96**	
		***n*=78**		***n*=63**		***n*=40**		***n*=34**		***n*=27**		***n*=13**	
Criteria	***N***	**Se**	**Sp**	**Se**	**Sp**	**Se**	**Sp**	**Se**	**Sp**	**Se**	**Sp**	**Se**	**Sp**
qHBcrAg (log_{10} U/mL)													
<7.5 at baseline	95	0.86	0.61	0.66	0.61	0.73	0.65	0.72	0.67	0.69	0.70	0.58	0.79
<6.5 at M12	95	0.82	0.67	0.77	0.68	0.71	0.70	0.69	0.72	0.70	0.76	0.60	0.87
<6.5 at M24	84	††	††	1	0.58	0.97	0.61	0.94	0.64	0.96	0.69	0.80	0.82
<6.5 at M36	76	††	††	††	††	1	0.42	1	0.44	1	0.48	0.85	0.58
qAnti-HBc (log_{10} PEIU/mL)													
≥4.1 at baseline	95	0.51	0.80	0.42	0.81	0.44	0.83	0.43	0.84	0.41	0.86	0.33	0.95
qHBcrAg <7.5 and qAnti-HBc ≥4.1	95	0.37	0.89	0.31	0.90	0.32	0.91	0.30	0.92	0.27	0.94	0.21	0.98
qHBcrAg <7.5 or qAnti-HBc ≥4.1	95	1	0.52	0.78	0.52	0.85	0.56	0.87	0.59	0.84	0.63	0.69	0.73
HBV-DNA criteria [b]													

HBV- at M12	95	0.92	0.60	0.76	0.61	0.77	0.64	0.68	0.64	0.63	0.66	0.53	0.72
HBV- at M24	84	††	††	1	0.33	1	0.35	1	0.37	1	0.41	0.92	0.52
HBV- at M36	76	††	††	††	††	1	0.17	1	0.18	1	0.20	0.91	0.22

HBV, hepatitis B virus; qAnti-HBc, quantified anti-hepatitis B core antibody; qHBcrAg, quantified hepatitis B core-related antigen

Analysis on 95 HBeAg-positive patients, among whom 26 had HBeAg seroclearance.

[a] As sensitivity (Se) and specificity (Sp) are calculated from survival probabilities, all patients (*N*) are included in analysis.

[b] HBV- was defined as <60 IU/mL

For information, the number of patients (*n*) considered (i.e. those not lost to follow-up or having HBeAg seroclearance) during each time-interval has been included.

†† Not evaluated

Figure legends

Figure 1.

Hepatitis B core-related antigen quantification (qHBcrAg) according to (A) hepatitis B e antigen (HBeAg) status at tenofovir (TDF)-initiation, (B) cumulative probability of HBeAg seroclearance, (C) hepatitis B surface antigen (HBsAg) seroclearance during follow-up. Individual levels are expressed as grey lines.

Figure 2.

Anti-hepatitis B core antibodies quantification (qAnti-HBc) according to (A) hepatitis B e antigen (HBeAg) status at tenofovir (TDF)-initiation, (B) cumulative probability of HBeAg seroclearance, and (C) hepatitis B surface antigen (HBsAg) seroclearance during follow-up. Individual levels are expressed as grey lines.

Figure 1

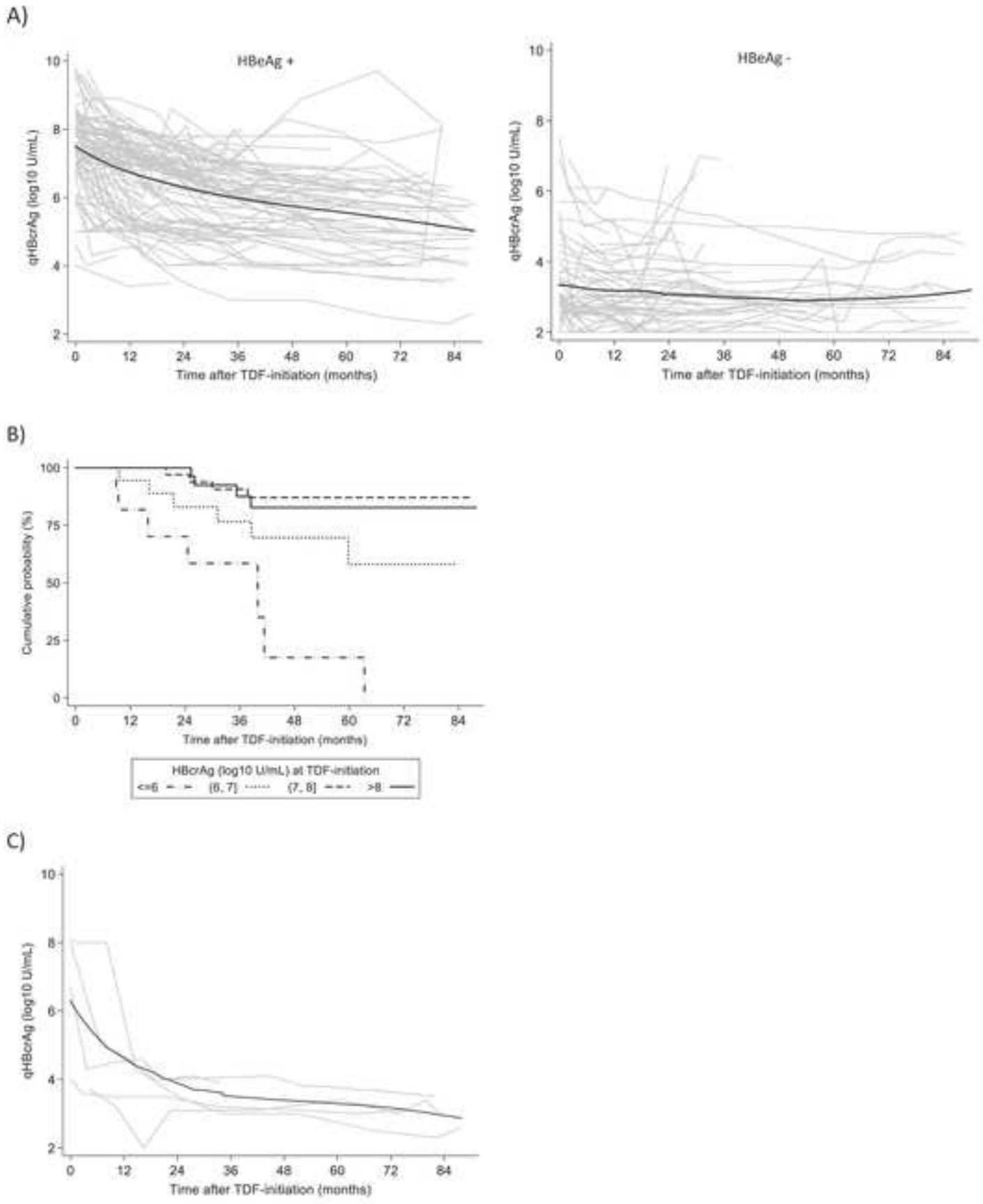
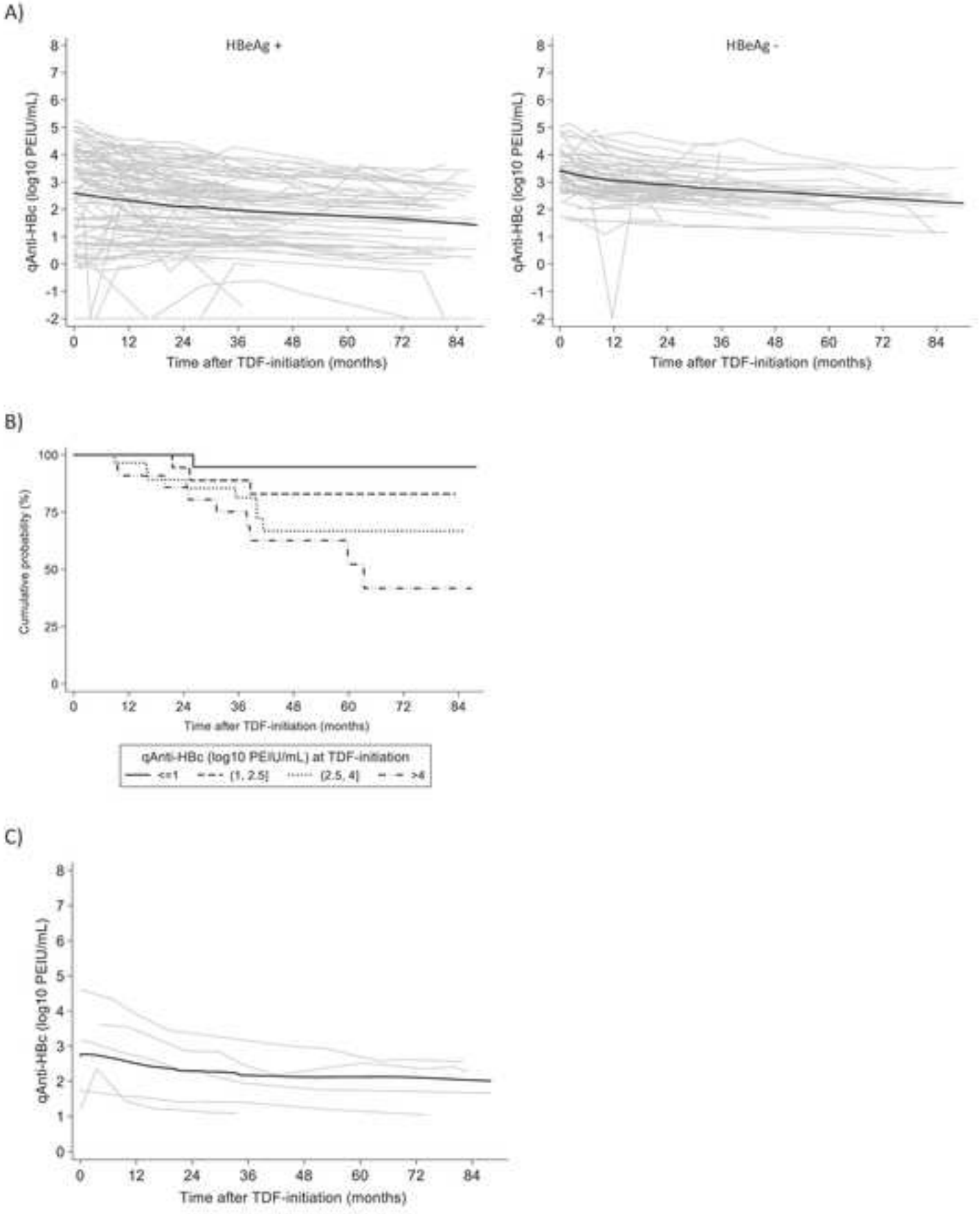
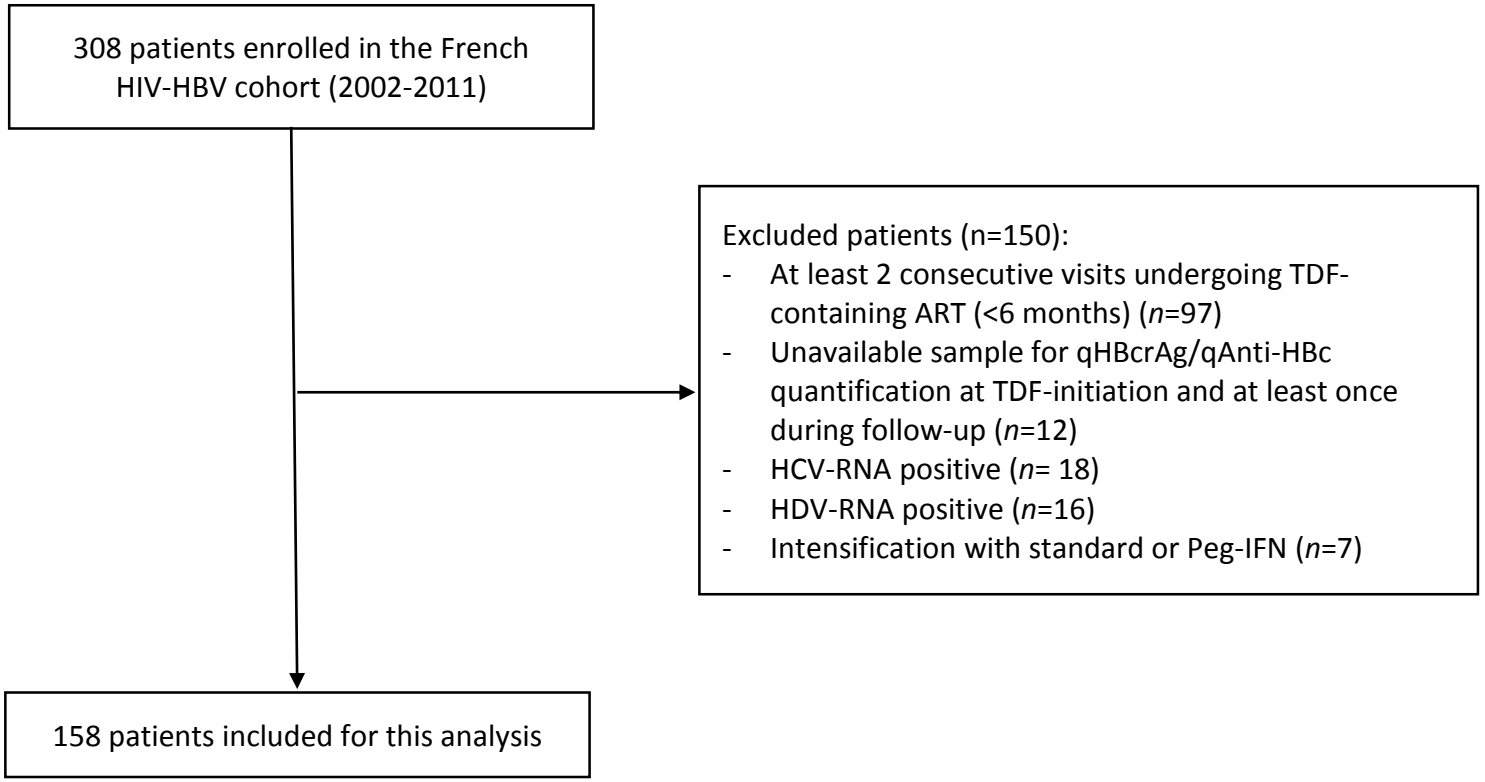

A)
HBeAg +
HBeAg -
qHBcrAg (log10 U/mL)
Time after TDF-initiation (months)
B)
Cumulative probability (%)
Time after TDF-initiation (months)
HBcrAg (log10 U/mL) at TDF-initiation
<=6
(6, 7]
(7, 8]
>8
C)
qHBcrAg (log10 U/mL)
Time after TDF-initiation (months)

Figure 2


A)
HBeAg +
HBeAg -
qAnti-HBc (log10 PEIU/mL)
Time after TDF-initiation (months)
B)
Cumulative probability (%)
Time after TDF-initiation (months)
qAnti-HBc (log10 PEIU/mL) at TDF-initiation
<=1
(1, 2.5]
(2.5, 4]
>4
C)
qAnti-HBc (log10 PEIU/mL)
Time after TDF-initiation (months)

Supplementary Figure 1. Selection of patients for analysis



Supplementary Table 1. Baseline characteristics of patients with and without HBsAg-seroclearance

	HBsAg seroclearance (*n = 5*)	No HBsAg seroclearance (*n* = 153)	*p*[a]
Male sex[b]	4 (80.0)	129 (84.3)	0.8
Age (years)[c]	43.2 (35.4-48.3)	41.1 (36.0-47.9)	0.6
From zone of high HBV-prevalence [b]	1 (20.0)	39 (25.5)	0.8
BMI (Kg/m^2)[c] [*N*=153]	22.1 (21.4-23.9)	22.6 (20.9-24.6)	0.9
AIDS-defining event[b]	1 (20.0)	38 (24.8)	0.8
CD4$^+$ cell count (cells/µL)[c] [*N*=157]	316 (303-405)	405 (294-559)	0.3
Nadir CD4$^+$ cell count (cells/µL)[c] [*N*=143]	194 (129-281)	227 (103-321)	0.6
Duration of ART (years)[c]	3.5 (1.1-7.5)	7.0 (4.4-9.0)	0.3
HBeAg-positive[b]	3 (60.0)	92 (60.1)	0.9
Estimated duration of HBV infection (years)[c] [*N*=157]	1.1 (0.8-7.4)	8.2 (3.7-12.4)	0.11
HBV-DNA (log_{10} IU/mL)[c] [*N*=157]	2.7 (2.3-6.1)	4.0 (2.2-6.6)	0.9
qHBsAg (log_{10} IU/mL)[c] [*N*=157]	2.9 (2.6-5.1)	4.3 (3.5-4.8)	0.4
qHBeAg level (PEIU/mL)[c] [*N*=70][d]	1036 (236-1094)	779 (328-1099)	0.9
qHBcrAg level (log_{10} U/mL)[c]	6.7 (4-8)	6.8 (3.4-7.9)	0.8
qAnti-HBc level (log_{10} PEI U/mL)[c]	3.2 (1.7-3.6)	3.3 (2.2-4.0)	0.9

ART, antiretroviral treatment; BMI, body mass index; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PEIU, Paul-Ehrlich Institut units; qAnti-HBc, quantified anti-hepatitis B core antibody; qHBcrAg,

quantified hepatitis B core-related antigen; qHBeAg, quantified hepatitis B e antigen; qHBsAg, quantified hepatitis B surface antigen.

[a]Comparing patients with and without HBsAg-seroclearance; significance determined using Kruskal–Wallis test for continuous variables and Pearson's X^2-test or Fisher's exact test for categorical variables.

[b]Number (%).

[c]Median (25–75th percentile).

[d]Only for HBeAg-positive patients