Original article

Six-year follow-up of hepatitis B surface antigen concentrations in tenofovir disoproxil fumarate treated HIV–HBV-coinfected patients

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Background: Quantitative measurement of hepatitis B surface antigen (HBsAg) has been proposed as a surrogate marker of treatment efficacy when HBV DNA load becomes undetectable. Our main objective was to study the kinetics of HBsAg level in HIV–HBV-coinfected patients with undetectable HBV DNA load under treatment containing tenofovir disoproxil fumarate (TDF).

Methods: A retrospective analysis was performed on frozen serum samples of 33 HIV–HBV-coinfected patients who were treated with TDF and had undetectable HBV DNA for ≥1 year. Baseline and serial follow-up samples were assayed for HBsAg levels.

Results: The characteristics of the patients at TDF initiation were median age 43.6 years, median HBV DNA load 2 $\log_{10}$ IU/ml and median HBsAg concentration 3.4 $\log_{10}$ IU/ml. Ten patients were positive for hepatitis B e antigen. Baseline median HBsAg concentration, defined 1 year after HBV DNA became undetectable, was 3.1 $\log_{10}$ IU/ml. Overall, from years 1 to 6 and a median duration of TDF treatment of 2.6 years, the median HBsAg concentration decreased slowly. Notably, only 13 (39%) patients presented a constant decrease of HBsAg concentration, whereas the remaining had fluctuating or increasing HBsAg concentrations. The slope was not influenced by HBeAg status, HIV infection duration and $CD4^+$ T-cell count at baseline or at nadir.

Conclusions: Despite control of HBV DNA replication under efficient TDF treatment, HBsAg levels persistently decreased in only 39% of HIV–HBV-coinfected patients. Larger follow-up studies are needed to determine whether HBsAg concentration monitoring under analogue treatment can be used as a reliable marker for HBV clearance.

Introduction

The prevalence of HBV chronic infection in HIV patients is estimated to be approximately 7% in France [1]. Medications licensed in Europe and the US for the treatment of HBV infection include interferon-α, pegylated interferon (PEG-IFN), lamivudine (3TC), adefovir dipivoxil, entecavir and telbivudine; tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) are approved for HIV treatment and are also active against HBV. Guidelines recommend treating HIV–HBV-coinfected patients with at least one molecule active on both viruses, especially TDF [2].

Monitoring of HBV infection in HIV-infected patients relies on HBV viral load (HBV-VL) with the desired goal to maintain HBV-VL to the lowest value under treatment [3]. HBV-VL monitoring is essential to document the control of viral replication and to detect, as soon as possible, any viral breakthrough, which might precede a potentially dangerous clinical flare-up, especially in patients with advanced liver disease. Using the newest antiviral molecules, entecavir or TDF, HBV-VL decreases to undetectable levels, below 12–20 IU/ml as measured by the most recent molecular assays, within 1 year in most patients [3]. Therefore, when the control of HBV replication is obtained, HBV-VL monitoring is no longer an appropriate parameter to follow the evolution of chronic hepatitis.

In a few studies, correlations were found between the concentration of hepatitis B surface antigen (HBsAg) and

the level of different replicative forms of intrahepatic viral DNA [4–6]. However, the most consistent data converge towards a link between the level of intrahepatic covalently closed circular DNA (cccDNA) and the concentration of circulating HBsAg. The level of cccDNA in patients depends on several parameters. As shown by several authors, intrahepatic cccDNA is higher in hepatitis B e antigen (HBeAg)-positive than in HBeAg-negative patients, and reaches a lower value in inactive carriers and is even less in patients who cleared HBsAg [4,7]. These data resemble those recently published on quantitative HBsAg during the different phases of chronic hepatitis B infection with lower HBsAg concentrations during the inactive periods of the disease [8,9]. Caution should, however, be applied because the correlation between HBsAg and cccDNA has not been found by all investigators, suggesting that other parameters might also influence the production of HBsAg [10]. Indeed, HBsAg production is the result of transcription from several messenger RNAs, generated either from the cccDNA or from any (full or partial) integrated HBV DNAs. HBsAg messenger RNA transcription is thus dependent on many transcription factors, but is also likely regulated by the host's immune response [11]. In addition to the regulation at the transcription level, the secretion step out of the hepatocytes might also be affected by amino acid change on HBsAg; some variants seem less efficiently secreted and could accumulate in the hepatocyte with potential deleterious consequences [12]. Therefore, regulation of HBsAg production is certainly a complex association of events involving both the host's immune response and the virus. Yet, because most treated patients have undetectable viral load and since one ultimate therapeutic goal is HBsAg loss, quantitative HBsAg has been proposed as a surrogate marker of treatment efficacy when HBV-VL becomes undetectable.

Quantitative HBsAg measurement has appeared promising during PEG-IFN treatment. Brunetto *et al.* [13] have determined therapeutic predictive values for HBsAg clearance under treatment in HBeAg-negative patients, and Moucari *et al.* [14] have also shown in a few patients that HBsAg quantification might be predictive of a sustained virological response in HBeAg-negative patients.

HBV treatment with nucleotides could be as long as 15 years according to some authors, but the kinetics of HBsAg during treatment with nucleotide analogues have not been extensively studied. Some data have been presented recently but they were mostly issued from short-term follow-up studies [5,8,15,16]. It should be stressed that the detailed mechanism of HBV protein production, independently of viral replication, remains largely unknown.

The aim of the present study was to analyse the evolution of HBsAg concentration in treated patients who had persistent undetectable HBV-VL. We present the 6-year kinetics of HBsAg in HIV–HBV-coinfected patients under TDF treatment and perfectly controlled for HBV replication.

Methods

Patients with HIV–HBV coinfection (n=202), as determined by the presence of anti-HIV and HBsAg for ≥6 months were selected amongst HIV-infected patients (n=3,228) regularly attending the HIV clinical unit of the Infectious Diseases Department at Pitié-Salpêtrière Hospital (Paris, France) and registered after informed consent in the New AIDS Data Information System (NADIS) database.

Only 88 HIV–HBV-coinfected individuals receiving TDF as part of their highly active antiretroviral therapy (HAART) with undetectable HIV viral load (<40 copies/ml) were considered for enrolment in the study. This last criterion was used as a surrogate marker of adherence to antiviral treatment, with the purpose to preferentially include compliant patients. Among these 88 patients, 33 were enrolled because they had been receiving TDF for >2 years and their HBV-VL (HBV Roche TaqMan CAP/CTM; Roche Diagnostics, Meylan, France; lower limit of quantification 12 IU/ml) had been undetectable for ≥1 year under TDF treatment.

HBsAg quantification (Architect; Abbott Diagnostics, Rungis, France) was performed at enrolment corresponding to the time when TDF was introduced. Baseline, defined as the time 1 year after plasma HBV DNA had become undetectable, and yearly serial follow-up samples for each patient who remained HBV-DNA-negative were analysed for HBsAg quantification. The lower limit of detection was 0.05 IU/ml and the linear range covered 0.05–250 IU/ml. Samples above the upper limit of quantification were diluted in phosphate-buffered saline as recommended.

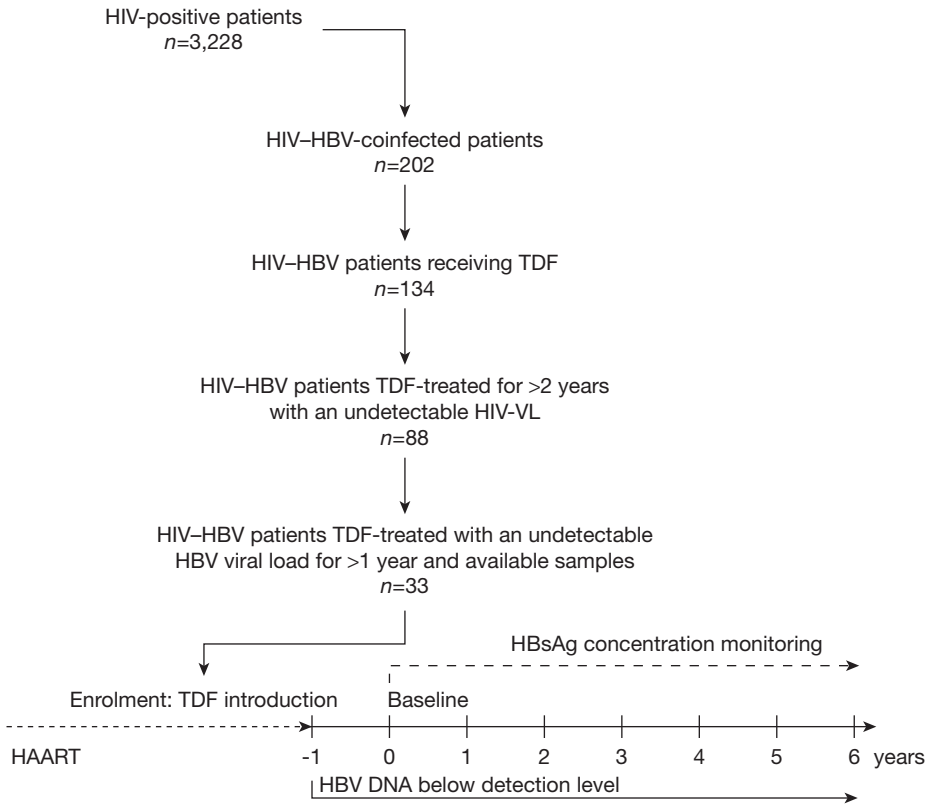
For each patient, the slope was calculated by subtracting the HBsAg concentration at year 'n' to the value at year 'n+1'. Patients were classified according to their yearly HBsAg slope either as persistently increasing or decreasing. Patients with positive and negative slopes over time were classified as 'fluctuating'. The mean slope was calculated for each patient during the follow-up period.

Means or medians were compared using non-parametric tests, the Mann–Whitney test for two groups or the Kruskal–Wallis test for three or more groups. A P-value ≤0.05 was considered significant.

Results

Among 202 HIV–HBV-coinfected patients followed in our centre, 88 were receiving a TDF-containing

Figure 1. General outline of the study



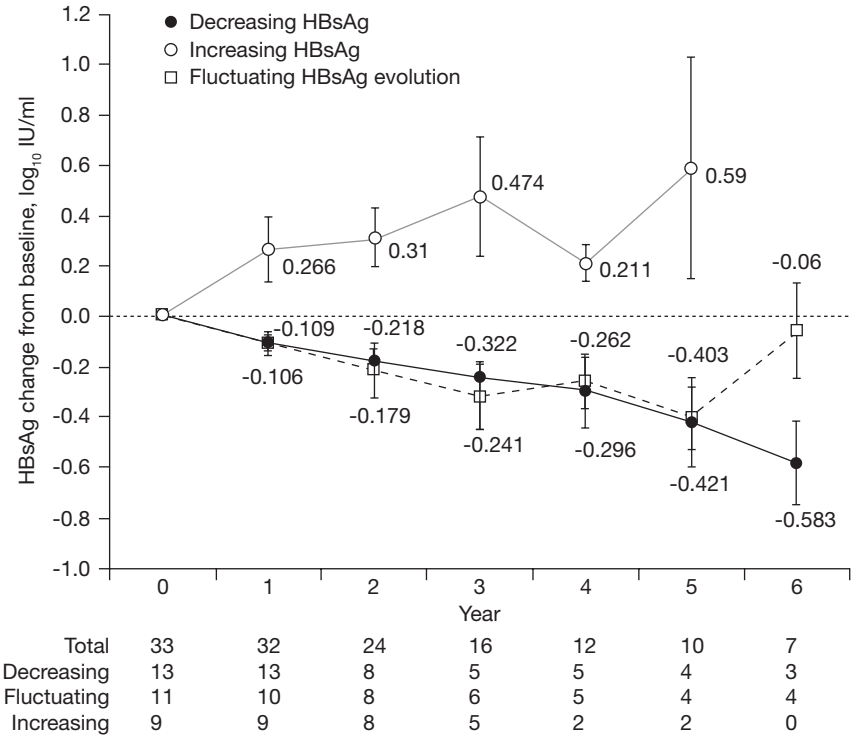
Patients were enrolled (time point -1 year) if they had been treated for >2 years with tenofovir disoproxil fumarate (TDF) and if their HBV viral load (HBV-VL) was below detection level for >1 year. Hepatitis B surface antigen (HBsAg) kinetics were assessed from baseline (time point 0), defined as 1 year after HBV-VL had become undetectable, and up to 6 years. HAART, highly active antiretroviral therapy.

HAART and were controlled with regard to HIV-VL; we selected 33 patients satisfying inclusion criteria for whom HBV DNA remained undetectable under treatment (Figure 1).

The characteristics of the 33 study patients at enrolment, that is, when TDF was introduced, were median (range) age 43.6 years (21–65), HIV infection duration 13 years (2–24), CD4$^+$ T-cell count 490 cells/mm^3 (209–903), HBsAg concentration 3.4 $\log_{10}$ IU/ml (1.2–5.0) and HBV-VL 2 $\log_{10}$ IU/ml (0–10.5). Overall, 12 patients presented with residual circulating HBV-VL before TDF introduction despite antiviral treatment. All patients received either 3TC (24%) or FTC (76%) in association with TDF and 76% had a protease inhibitor in their HAART regimen. Ten patients were HBeAg-positive. The HBsAg concentration at enrolment was significantly higher among HBeAg-positive patients (median [range] 4.28 $\log_{10}$ IU/ml [2.89–5.00]) than among HBeAg-negative patients (median [range] 3.13 $\log_{10}$ IU/ml [1.17–4.18]; P=0.0006).

Baseline values, obtained during the first year after HBV DNA became undetectable, for HBsAg concentration ranged from 0.92 to 4.18 $\log_{10}$ IU/ml (median 3.1 $\log_{10}$ IU/ml) and were no longer different according to the HBeAg status. The median (range) TDF treatment duration was 2.6 years (1–6) and the median (range) follow-up duration after HBV DNA became undetectable was 4 years (1–6). Overall, from years 1 to 6 after HBV DNA became undetectable, HBsAg concentrations decreased slowly with a mean slope of -0.033 $\log_{10}$ IU/ml/year; however, three distinct patterns could be identified based on HBsAg kinetics. Notably, 13 patients presented a constant and persistent decrease of HBsAg concentration (mean slope -0.065 $\log_{10}$ IU/ml/year), 9 had a constant and persistent increase of HBsAg concentration (mean slope 0.133 $\log_{10}$ IU/ml/year), whereas 11 had fluctuating levels over time (mean slope -0.033 $\log_{10}$ IU/ml/year; Figure 2). The mean slope was not significantly influenced by HBeAg status, baseline HBsAg level, HIV infection duration, or the CD4$^+$ T-cell count

Figure 2. Evolution of HBsAg concentrations over time



The differences in hepatitis B surface antigen (HBsAg) level were calculated by subtracting the HBsAg concentration at each year time point to the HBsAg value when HBV DNA became first undetectable (baseline). Patients with persistently decreasing HBsAg concentrations are represented by closed circles, those with increasing concentrations by open circles and those with a fluctuating evolution by open squares and a dotted line. Below the graph, the total number of patients considered at each time point and their distribution in each group is indicated.

at baseline or nadir (Table 1). The influence of other antiretroviral-associated treatments could not precisely be investigated because of small numbers in each group. None of the patients lost HBsAg during the observation period. The maximum observed drop was 3.12 $\log_{10}$ IU/ml in a patient with an initial concentration of 4.34 $\log_{10}$ IU/ml, but was not associated with HBsAg disappearance.

Discussion

In contrast to PEG-IFN, nucleoside/nucleotide analogues are usually administered for prolonged periods of time to maintain viral suppression. The ultimate goals are to reach at least HBe seroconversion in HBeAg-positive patients and, better still, HBsAg clearance. TDF induces a strong inhibition of HBV replication in most patients, but the total duration of treatment required to clear HBsAg still remains undefined. Once HBV replication becomes undetectable under treatment, viral markers are lacking to further predict the infection evolution. Recent data suggest, however, that quantitative measurement of HBsAg might be a useful parameter to assess the probability for HBsAg seroconversion [17]. Moreover, the concept of reaching a sustained lower level of HBsAg under treatment to reduce liver disease progression is also supported by several reports indicating that lower HBsAg concentrations are found in the so-called 'low replicative carriers', characterized by no or mild liver disease [8,9]. The fact that the decrease of HBsAg concentration could be correlated with the decrease of cccDNA reinforces this idea [4]. Although this marker seems promising in predicting the chance to respond to IFN-based therapy, very few data are available on the long-term evolution of HBsAg concentration in nucleoside/nucleotide-analogue-treated patients [13,14]. Brunetto *et al.* [13] observed that the level of HBsAg after 48 weeks of treatment in HBeAg-negative patients, treated with PEG-IFN, 3TC or a combination of both, was significantly associated with sustained HBsAg clearance

Table 1. Main patient characteristics and evolution over time

Factor	Entire cohort (*n*=33)	Persistently decreasing HBsAg slope (*n*=13)	Fluctuating HBsAg slope (*n*=11)	Persistently increasing HBsAg slope (*n*=9)
Median age, years (range)	43.7 (20–64)	43.6 (27–53)	44.4 (20–60)	39.9 (28–64)
Median duration of HIV infection, years (range)	13 (1.8–24.1)	14 (2.6–24.1)	14.5 (4.9–23.1)	8.7 (1.8–16.8)
CDC classification				
A2, *n* (%)	11 (33)	2 (15)	5 (46)	4 (44)
A3, *n* (%)	11 (33)	6 (46)	2 (18)	3 (33)
B2, *n* (%)	2 (6)	–	–	2 (22)
C2, *n* (%)	1 (3)	1 (8)	–	–
C3, *n* (%)	8 (24)	4 (31)	4 (36)	–
3TC- or FTC-associated treatment				
3TC, *n* (%)	8 (24)	5 (39)	2 (18)	1 (11)
FTC, *n* (%)	25 (76)	8 (61)	9 (82)	8 (89)
PI-associated treatment, *n* (%)	25 (76)	10 (77)	7 (64)	8 (89)
HBeAg-positive, *n* (%)	10 (30)	5 (39)	3 (27)	2 (22.2)
Median $CD4^+$ T-cell count, cells/mm^3 (range)	319 (13–951)	269 (13–681)	511 (71–951)	255 (111–622)
Median $CD4^+$ T-cell nadir, cells/mm^3 (range)	153 (2–363)	100 (6–307)	191 (2–363)	244 (63–350)
Median baseline HBsAg level, log_{10} IU/ml (range)	3.4 (1.16–5.0)	3.45 (2.67–4.82)	3.08 (1.17–5.0)	3.12 (2.43–4.65)
Median HBsAg level kinetics, log_{10} IU/ml/year (range)	-0.033 (-0.475–0.413)	-0.065 (-0.440–-0.022)	-0.033 (-0.475–0.015)	0.133 (0.010–0.413)[a]

[a]Statistically significant values between the persistently increasing group and the fluctuating (P=0.002) or persistently decreasing (P<0.0001) groups. FTC, emtricitabine; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PI, protease inhibitor; 3TC, lamivudine.

3 years post-treatment. Takkenberg *et al.* [16] also reported that in patients treated with PEG-IFN and adefovir dipivoxil combination for 48 weeks, baseline HBsAg levels predicted HBsAg clearance. However, no study has been performed in patients after they reached an undetectable HBV DNA level under oral treatment. Indeed, circulating HBsAg during HBV infection in a replicative patient is the result of surface antigen from Dane particles, but also and mainly from subviral particles in excess by a factor of 10^3 to 10^5 over the complete virus [18]. The first phase of HBsAg decrease in nucleoside/nucleotide-analogue-treated patients is therefore largely due to the efficient control of HBV replication, that is, Dane particle disappearance, and the true rate of HBsAg decrease should be studied once viral replication is controlled. In the present study, we extended up to 6 years the assessment of HBsAg kinetics in HIV–HBV-coinfected patients treated by TDF-containing HAART. By selecting only patients with both HBV and HIV persistently undetectable viral loads, it was assumed that compliance was high. Overall, there was a very slow decrease of HBsAg concentration during the follow-up with a mean slope of -0.033 log_{10} IU/ml/year; however, the decrease of HBsAg concentration was not constant and for almost one-third of patients the HBsAg concentrations increased with time. Moreover, unexplained fluctuations were frequently observed during the kinetics. Even when a decrease of HBsAg concentrations is observed, the very slow slope is worrying with regard to treatment duration. A simple mathematical approach based on the slope to calculate the delay for HBsAg loss, even in patients with a constant drop, is certainly irrelevant and would give a prediction extending over a life-time. One might also speculate that by reaching a lower concentration, potential immunosuppressive effects of HBsAg would abrogate and clearance would be triggered [19]. One might also wonder if reaching a value of 3 log_{10} IU/ml, a concentration classically found in the low replicative phase of the disease, would be enough to control any liver injuries [8,9].

Comparison of our data to an HBV-monoinfected cohort of patients was not possible locally in our centre. However, some data presented recently by Gane *et al.* [20] and Wursthorn *et al.* [21] in HBeAg-positive patients treated for 3 years with adefovir dipivoxil/TDF or telbivudine, respectively, found an estimated HBsAg slope of -0.25 log_{10} IU/ml per year, a value much higher than what is observed even for patients with persistently decreasing HBsAg concentration in our population [20,21]. However, this comparison is rather inappropriate because these studies were conducted in patients with high initial viral

loads; the HBsAg drop observed during the first year is thus likely due to the rapid drop of HBV-VL, that is, the production of Dane particles. When considering the HBsAg decrease in these two studies between year 2 and 3 after treatment initiation, when HBV replication is usually well-controlled in most patients, the slope seems then to reach a steady-state around -0.2 $\log_{10}$ IU/ml/year. Of note, none of the baseline parameters studied (HBeAg status, baseline HBsAg level, HIV infection duration and $CD4^+$ T-cell count at baseline or at nadir) could be identified as a predictor of the different HBsAg kinetics in our study. Surprisingly, although our observation relies on very few patients, individuals with persistently increasing HBsAg concentrations were not those who had gone through the most severe HIV-related disease, most of them being A2 or A3 according to the CDC classification (Table 1). Owing to the small sample size in our study, it is difficult to conclude if the difference in HBsAg decrease observed in our cases when compared with the literature is truly attributable to HIV coinfection or to other factors. Studies on larger populations, designed to understand the mechanisms of HBsAg production regulation once HBV replication is controlled, are needed.

Although our results clearly indicate divergent evolution of HBsAg concentration in patients with controlled viral replication, these data based on a retrospective analysis of few patients, have obvious limitations. Adherence to treatment was solely appraised by the efficacy to maintain HIV and HBV-VL below a detection level, but imperfect compliance could not be formally ruled out. It was also not possible to accurately assess the role of coadministered molecules on HBsAg kinetics because of the limited size of our retrospective study, but no obvious role of protease inhibitor, FTC or 3TC treatment was identified. Heterogeneity of our population in terms of length of HBV and HIV infection duration and immunological status should also be taken into consideration for unbiased interpretation of our observations.

In conclusion, if the desired goal to limit liver-related HBV injury is to reduce HBsAg and subsequently cccDNA to levels found in inactive carriers or in patients who cleared HBsAg, these results suggest that long-term treatment will be required. These data also demonstrate that all patients do not respond equally in terms of HBsAg decrease. Thus, longer follow-up studies are needed to determine whether HBsAg concentration monitoring under nucleoside/nucleotide analogue treatment, particularly when HBV-VL becomes undetectable, can be used as a predictive marker for HBV clearance in HIV–HBV-coinfected patients. While quantitative HBsAg might be relevant to follow treatment with interferon that acts through antiviral and immunological effects, the situation seems very different with analogue-based therapies acting specifically on viral replication.

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Disclosure statement

The authors declare no competing interests.

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