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Stéphane Chevaliez,[1,2] Christophe Hézode,[2,3] Stéphane Bahrami,[4] Marion Grare,[1] and Jean-Michel Pawlotsky[1,2]

[1]National Reference Center for Viral Hepatitis B, C and Delta, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France; [2]INSERM U955, Créteil, France; [3]Department of Hepatology and Gastroenterology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France; [4]Paris Health Economics and Health Services Research Unit, LIC EA 4393, Hôpital Henri Mondor, Université Paris-Est, Créteil, France

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Corresponding author: Dr Stéphane Chevaliez, PharmD, PhD, Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France. Tel: +33-1-4981-2828; Fax: +33-1-4981-2839. E-mail: stephane.chevaliez@hmn.aphp.fr

Abbreviations: HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; cccDNA: covalently closed circular DNA; HBeAg: hepatitis B e antigen; IFN: interferon; IU: international unit; HDV: hepatitis D virus; PCR: polymerase chain reaction; IQR: interquartile range.

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ABSTRACT

Background & aims

Information regarding long-term HBsAg kinetics during treatment with nucleoside/nucleotide analogues is limited. The aim of the present study was to assess whether finite nucleoside/nucleotide analogue treatment duration could be envisaged during the patient's lifetime.

Methods

Patients with chronic hepatitis B receiving different schedules of nucleoside/nucleotide analogues were followed for a median duration of 102 months, i.e. 8.5 years (interquartile range: 88-119 months). Long-term HBV DNA and HBsAg level kinetics were modeled in order to estimate time to clear HBsAg during therapy in patients with undetectable HBV DNA.

Results

Antiviral therapy was associated with a slow but consistent reduction in the level of HBsAg in most of them. Three patterns of HBsAg level declines were identified: decline during both the detectable and undetectable HBV DNA phases; decline during the HBV DNA detectable period only; decline during the HBV DNA undetectable period only. The mean HBsAg titer at the time when HBV DNA became undetectable was 3.29±0.49 Log_{10} international units (IU)/mL, and the mean slope was -0.007±0.007 Log_{10} IU/month, i.e. an average decline of 0.084 Log_{10} IU per year. The corresponding calculated median number of years needed to clear HBsAg was 52.2 years (interquartile range: 30.8-142.7).

Conclusions

This study, based on the very long-term follow-up of patients with chronic hepatitis B treated with potent nucleoside/nucleotide analogues, shows that HBsAg clearance is unlikely to occur during the patient's lifetime, even if HBV replication is well controlled. Thus, lifetime therapy is required in the vast majority of HBV-infected patients.

INTRODUCTION

Recent developments in the antiviral treatment of chronic hepatitis B virus (HBV) infection have emphasized the need for biomarkers that are predictive of treatment outcomes and can be used to tailor therapy to the individual patient. In chronic HBV carriers, hepatitis B surface antigen (HBsAg) is produced as a result of translation of messenger RNAs generated from transcriptionally active cccDNA or integrated HBV DNA sequences in the host genome. HBsAg is present in the envelope of infectious HBV virions and in noninfectious spheres and tubules. The latter exceed infectious virions when replication is active; they remain produced in large amounts when replication is controlled, either spontaneously (inactive carriers) or by antiviral therapy [1-4].

A number of recent studies have demonstrated the clinical utility of HBsAg quantification in monitoring HBV therapy [1, 5-14]. Indeed, early on-treatment serum HBsAg levels predict the sustained post-treatment response to therapy and the eventual subsequent HBsAg clearance in patients with both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative chronic hepatitis B treated with a finite duration of pegylated interferon (IFN)-α [1, 5-9]. HBsAg clearance, followed or not by HBs seroconversion (appearance of anti-HBs antibodies) characterizes a sustained remission of HBV infection.

Standardized quantitative HBsAg level assays are available. Three commercial assays, the HBsAg assay on Architect® device (Abbott Diagnostics, Chicago, Illinois), the HBsAg II Quant assay on Elecsys® or Cobas® e devices (Roche Diagnostics GmbH, Mannheim, Germany), and the Liaison®XL HBsAg Quant assay on Liaison®XL device (DiaSorin, Saluggia, Italy) are approved in the European Union; they are available for

research use only in the United States. These tests are inexpensive and easy-to-use, and high throughput is possible with automated platforms [15, 16]. Thus, HBsAg quantification can be easily used to monitor antiviral therapy in patients with chronic HBV infection.

Treatment of chronic hepatitis B with nucleoside or nucleotide analogues is aimed at reducing virus production by inhibiting the reverse transcriptase function of the HBV DNA polymerase, the enzyme responsible for viral replication. Nucleoside/nucleotide analogues do not exert a direct effect on HBsAg transcription and translation. Recent liver society guidelines recommended the use of entecavir or tenofovir as first-line therapy in patients with chronic hepatitis B and an indication for nucleoside/nucleotide analogues, because both drugs potently inhibit the HBV reverse transcriptase and have a high barrier to resistance. Entecavir and tenofovir have been shown to efficiently maintain suppression of HBV DNA levels for prolonged periods of time in the vast majority of treated patients [17-19]. As a result, most patients who started therapy with other drugs currently receive entecavir and/or tenofovir as part of their treatment regimen in areas where these drugs are available and reimbursed.

Information regarding HBsAg kinetics during treatment with nucleoside/nucleotide analogues is limited [5, 10-14, 18, 20-25]. These studies indicate nucleoside/nucleotide analogue therapy results in lesser overall declines in serum HBsAg levels and HBsAg clearance appears to be less frequent than in patients receiving pegylated IFN-α-based therapy. However, this could be biased by the fact that pegylated IFN-α is generally used in subsets of patients with a greater likelihood to clear HBsAg, e.g. patients with low HBV DNA and high alanine aminotransferase levels.

The aim of the present study was to assess whether finite nucleoside/nucleotide analogue treatment duration could be envisaged during the patient's lifetime. For this,

we assessed the long-term kinetics of serum HBsAg levels in patients with chronic hepatitis B treated with various successive schedules of nucleoside/nucleotide analogues and who maintained undetectable HBV DNA in the long term, and evaluated the clinical interest of HBsAg level quantification to tailor treatment duration.

PATIENTS AND METHODS

Patients

The study was a longitudinal analysis of HBsAg levels in prospectively collected serum samples from 30 patients with histologically proven chronic hepatitis B, treated with different nucleoside/nucleotide analogue schedules according to drug approvals and outcomes on therapy between December 1996 and December 2010 in the Department of Hepatology and Gastroenterology of our institution. All patients were positive for HBsAg and negative for anti-HBs antibodies at the beginning of the study. The selection criteria were a follow-up on treatment of at least 4 years and availability of stored sera for quantification of HBsAg levels. All patients followed in our institution who met these criteria were included; they were representative of HBV-infected patients seen in the area, as shown by their genotype distribution (Table 1). A total of 604 serial samples (mean: 20.1±7.4 per patient; range: 10-42) collected from April 2000 to December 2010 were available (no samples available prior to 2000). The study followed the principles of Good Clinical Practice and was approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale). All patients gave written informed consent. The drugs were given once daily at doses of 100 mg for lamivudine, 10 mg for adefovir dipivoxil, 0.5 mg or 1.0 mg for entecavir and 300 mg for tenofovir disoproxil fumarate.

Laboratory measurements

Serum HBV DNA levels were measured in each available sample by means of a real-time PCR assay (COBAS Ampliprep®/COBAS TaqMan®, Roche Molecular Systems, Pleasanton, California) [26]. Results were expressed in international units per milliliter (IU/mL). The lower limit of detection of the assay is 20 IU/mL.

Serum HBsAg levels were quantified by means of the Architect® HBsAg assay after 1:100 dilution of the sera. The dynamic range of quantification of this assay is 0.05 to 250.0 IU/mL (-1.3 to 2.4 Log_{10} IU/mL). Samples with HBsAg levels <0.05 IU/mL at 1:100 dilution were retested undiluted, while those with HBsAg >250.0 IU/mL at 1:100 dilution were retested at a final dilution of 1:999, according to the manufacturer's instructions.

The HBe system was assessed by means of commercial enzyme immunoassays (VIDAS™ HBe and VIDAS™ Anti-HBe, Biomérieux, Marcy-l'Etoile, France). Anti-hepatitis D virus (HDV) antibodies were detected by means of an enzyme immunoassay (ETI-AB-DELTAK-2, Bio-Rad Laboratories, Hercules, California). Basal core promoter and precore mutations were sought by means of a line probe assay after nested PCR amplification of the corresponding genomic region (INNO-LiPA HBV PreCore, Innogenetics, Gent, Belgium), according to the manufacturer's instructions.

The HBV genotype was determined in all patients by directly sequencing a portion of the overlapping genes encoding HBsAg and the B and C subdomains of the HBV reverse transcriptase. Sequence analysis was followed by phylogenetic analysis. Briefly, after extraction of viral DNA from 200 µl of serum using the QIAamp MinElute Virus Vacuum Kit (Qiagen GmbH, Hilden, Germany), a hemi-nested polymerase chain reaction (PCR) was used to amplify a 492-bp fragment with primers POL-1, POL-2 and

HBPr-94, as previously described [27-29]. PCR products were sequenced by means of the Big-Dye Terminator v3.1 sequencing kit on the ABI 3100 sequencer (Applied Biosystems, Foster City, California), according to the manufacturer's protocol. Phylogenetic analyses were performed using different prototype HBV genotype A to H sequences by using software from the Phylogeny Inference Package (PHYLIP) version 3.65.

Statistical analysis

Statistical analysis was performed with Stata® 10.0 (StataCorp LP, College Station, Texas). We assumed that the HBsAg titer follows a Log-normal distribution. Patient-specific slopes of HBsAg decline were estimated using linear regression and used to predict time to undetectable HBsAg whenever the decline was statistically significant, using the first sample with undetectable HBV DNA as a starting point. The sample distribution of times to undetectable HBsAg was described using the median and dispersion has been characterized by the first and third quartiles (interquantile range [IQR]). In addition, the population-level mean and standard deviation were estimated using a mixed linear model with random coefficients and similarly used to estimate the time to undetectable HBsAg.

RESULTS

Characteristics of the study patients

Table 1 shows the individual demographic, virological and histological data of the patients. Twenty-four men and 6 women were enrolled in the long-term longitudinal study of HBsAg levels. The mean age of the patients was 55.6±10.1 years (range: 30.6-

88.1); 16 of them were Caucasian, 6 were Asian, and 8 were African. Eighteen patients (60.0%) were HBeAg-negative. Among the 12 patients who were HBeAg-positive before starting therapy, 6 were still HBeAg-positive at the beginning of follow-up in this study; 3 of them seroconverted during the study period and simultaneously became HBV DNA-negative.

The HBV genotype could be identified in 29 of the 30 patients. Ten patients were infected with genotype A, 6 with genotype B, 1 with genotype C, 4 with genotype D, 5 with genotype E, 1 with genotype F and 2 with genotype G. In the remaining patient, no sample with an HBV DNA level >2 Log_{10} was available during follow-up for HBV genotype determination. Twenty-five of the 30 patients (83.3%) had significant fibrosis (METAVIR grade ≥F2) and 13 patients had compensated cirrhosis (F4). None of them was coinfected with HDV (Table 1).

Nucleoside/nucleotide analogue treatment received and virological response

The median follow-up in this study was 102 months, i.e. 8.5 years (IQR: 88-119 months). The 30 patients received different schedules of nucleoside/nucleotide analogues as part of their regular treatment. The successive treatments received by each patient are shown in the supplementary Figure; selected examples are shown in Figure 1. In general, the patients were successively switched to treatment schedules which were more potent and/or which had a higher barrier to resistance, e.g. lamivudine to lamivudine plus adefovir, then to entecavir or tenofovir, alone or in combination, as a reflection of the history of HBV therapy (supplementary Figure). At the end of follow-up, 5 patients were on entecavir monotherapy (median duration of entecavir therapy: 32 months; IQR: 19-33 months), one patient was on tenofovir monotherapy for 12 months,

11 patients were treated with lamivudine and tenofovir (median duration of this regimen: 15 months; IQR: 12-20), one with entecavir and adefovir for 18 months, 10 with entecavir and tenofovir (median duration of this regimen: 29 months; IQR: 13-42), and 2 with lamivudine and adefovir (72 and 102 months, respectively). Undetectable HBV DNA in serum (<20 IU/mL) was achieved in all treated patients. The median duration of undetectable HBV DNA was 47 months (IQR: 24-77) in the 30 patients.

Long-term HBsAg level kinetics during nucleoside/nucleotide analogue therapy

Median duration of monitoring of serum HBsAg levels on nucleoside/nucleotide analogue therapy was 102 months, i.e. 8.5 years (IQR: 88-119 months) in the 30 patients. For each patient, HBsAg kinetics were modeled by means of linear regression; the slope and intercept were evaluated during the HBV DNA-detectable and -undetectable periods, respectively. Individual patient data are shown in Table 2 (slope and intercept) and in the supplementary Figure (HBV DNA and HBsAg level kinetics according to the treatments received); selected examples are shown in Figure 1.

As shown in Figure 1 and in the supplementary Figure, the kinetics of serum HBV DNA and HBsAg levels were not parallel. Antiviral therapy was associated with a slow but consistent reduction in the level of HBsAg throughout the study period in 27 of the 30 treated patients (Patients 1 to 27; Table 2). In these patients, the slopes of HBsAg concentration ranged from -0.072 to -0.002 and the mean annual HBsAg decline was -0.138±0.171 Log_{10} IU/year (range -0.870 to -0.020). Only one out of the 27 patients (Pt-12) lost HBsAg during the follow-up period, after 29 months of undetectable HBV DNA. This patient had a more rapid slope of HBsAg decline during the undetectable HBV DNA phase (Table 2). None of the remaining 26 patients lost HBsAg during follow-up and

their annual HBsAg decline was slow (mean: -0.110±0.091 Log IU/year, range -0.419 to -0.020).

Influence of virological breakthroughs due to HBV resistance on HBsAg level kinetics

Among the 30 patients, 7 were treated *de novo* with the combination of lamivudine and adefovir (Patients 8, 9, 11, 15, 21, 23 and 25). The remaining 20 patients initially received lamivudine monotherapy and subsequently added-on adefovir as a result of virological failure. In 3 of them (Patients 5, 7 and 13), follow-up started when they were already on lamivudine plus adefovir, so no sequence data was available on lamivudine monotherapy (supplementary Figure). As shown in Table 1, at the time of virological breakthrough on lamivudine monotherapy, population sequencing identified a dominant lamivudine-resistant viral population in 13 patients (Patients 2, 3, 6, 10, 14, 16, 17, 18, 19, 20, 22, 28 and 29) and a dominant wild-type population in 5 patients (Patients 4, 12, 24, 26 and 30); no sequence data was available in the remaining 2 patients (Patients 1 and 27). In 12 of the 13 patients in whom selection of lamivudine-resistant HBV variants was documented as the cause of treatment failure, the HBsAg level modestly increased at the time of virological breakthrough. This elevation was transient and the pre-breakthrough slope was restored when adefovir was added in all cases (supplementary Figure). HBsAg data was missing at the time of breakthrough in the remaining patient.

Patterns of HBsAg level declines in patients with detectable and undetectable HBV DNA phases

Three distinct patterns of HBsAg level declines were identified in the 22 patients in whom detectable and undetectable HBV DNA phases were present (Patients 1 to 22): decline during both the detectable and undetectable HBV DNA phases (n=15, Patients 1 to 15); decline during the HBV DNA detectable period only (n=5, Patients 16 to 20); decline during the HBV DNA undetectable period only (n=2, Patients 21 and 22) (Table 2 and Figure 1). In 12 of the 15 patients in whom HBsAg levels declined during both the HBV DNA detectable and undetectable periods, the HBsAg level decline was less pronounced during the HBV DNA undetectable phase than during the HBV DNA detectable phase (Table 2). It was more pronounced in two cases (Patients 1 and 7) and identical in one case (patient 6). No comparison could be made between the detectable and undetectable periods in the remaining 5 patients, as no HBsAg level kinetics were available during the HBV DNA undetectable and detectable periods in 3 (Patients 23 to 25) and 2 of them (Patients 26 and 27), respectively (Table 2 and Figure 1). Three patients seroconverted in the e system during follow-up at the time they became HBV DNA undetectable, including 2 in whom HBsAg level decline subsequently slowed down (Patients 2 and 5) and one in whom it did not (Patient 20).

In 3 patients (Patients 28 to 30), serum HBsAg levels slightly increased during the study period, including in 2 patients who lost their HBV DNA and remained HBV DNA undetectable for 32 and 80 months, respectively (patients 29 and 30). In the third patient (patient 28), only one clinical specimen was available during the HBV DNA undetectable period (Table 2).

Estimation of time to clear HBsAg during therapy

Statistical modeling was used to estimate the theoretical amount of time required to clear HBsAg after HBV DNA became undetectable in the 18 patients in whom a consistent reduction of HBsAg levels was observed during the study period (we excluded from this analysis the 3 patients who experienced an increase in HBsAg level during the full follow-up period, the 5 patients who experienced an increase in HBsAg level during the HBV DNA undetectable period, the patient who rapidly cleared HBsAg during therapy, and the 3 patients in whom linear regression could not be applied because only one value was available). In these patients, including 4 who were HBeAg-positive and 14 who were HBeAg-negative, the mean HBsAg titer at the time when HBV DNA became undetectable was 3.29±0.49 Log_{10} IU/mL. The mean slope was -0.007±0.007 Log_{10} IU per month, i.e. an average decline of 0.084 Log_{10} IU per year. This slope inversely correlated with the initial HBsAg level (c=0.527).

The corresponding calculated median number of years needed to clear HBsAg was 52.2 years (IQR: 30.8-142.7), reflecting the wide dispersion of observed kinetics. There was no influence of the treatment received on this prediction.

DISCUSSION

Quantification of HBsAg levels is emerging as a contributive tool in monitoring HBV infection and antiviral therapy outcomes, in complement to HBV DNA levels [4, 9, 11, 30]. In the present study, we characterized very long-term HBsAg level kinetics during prolonged oral treatment with nucleoside/nucleotide analogues in HBV mono-infected patients with HBeAg-positive and –negative chronic hepatitis B, and derived a prediction, based on mathematical modeling, of the theoretical duration of therapy in order to achieve undetectable HBsAg levels.

Our results showed a progressive reduction of HBsAg levels in the majority of patients who achieved undetectable HBV DNA during therapy. However, the HBsAg level reductions were very slow and did not parallel that of HBV DNA levels. This result was not surprising, since HBsAg is not a marker of viral replication, but rather reflects a reduction in translation of mRNAs produced from transcriptionally active cccDNA and integrated HBV sequences [31]. HBsAg loss was a rare event, which occurred in only one patient in this study. In the remaining patients, HBsAg slowly decreased during the HBV DNA detectable and/or undetectable periods and was still detectable at the end of follow-up. In general, but not always, the decline was steeper during the HBV DNA detectable period and tended to slow when HBV DNA became undetectable. Different patterns of HBsAg level decline were observed (Figure 1 and supplementary Figure), but the subgroup numbers were small and it is difficult to know whether they were representative of the general population.

In this study, pretreatment samples were not available for HBsAg quantification. It was thus not possible to assess spontaneous variations prior to initiation of antiviral therapy and compare them with the on-treatment HBsAg level kinetics. However, a recent study showed median HBsAg level decreases of 0.041 and 0.043 Log_{10} IU per year in untreated HBeAg-negative patients with active and inactive disease, respectively [32]. These titer reductions were only twice slower than our observed average decline of 0.084 Log_{10} IU per year on treatment, which inversely correlated with the HBsAg level at the start of follow-up. This suggests that, except in the minority of patients who rapidly clear HBsAg on therapy, the sustained control of HBV replication does not dramatically accelerate natural HBsAg clearance. This observation is in keeping with the lack of strong relationship between HBsAg levels and HBV replication [1, 2, 4].

Interestingly, in 12 out of 13 patients who experienced a virological breakthrough due to lamivudine resistance during the study period, the HBsAg level slightly increased concomitantly to the peak of HBV DNA, and declined again as soon as viral replication was controlled by adefovir add-on. This probably reflects the small proportion of HBsAg produced from messenger RNAs generated during the active replication process, most of HBsAg being generated from cccDNA and HBsAg-coding sequences integrated in the genome of host cells without the need for infectious virion production.

The mechanisms underlying HBsAg clearance during antiviral therapy remain unknown. Our observation that the steepest decline in HBsAg levels occurred during the period when HBV DNA was detectable in the majority of cases is in keeping with two, non-mutually exclusive hypotheses. On the one hand, HBsAg clearance could be principally of immunological origin, specific immune responses triggered by the production of viral proteins being required to eliminate cells that contain cccDNA and produce HBsAg. In this respect, more fluctuations of HBsAg levels were observed during the HBV DNA-positive than during the HBV DNA-negative period in many patients (supplementary Figure). These minor fluctuations could be the result of immune elimination, but could also reflect the fluctuations of HBV replication. The alternative hypothesis is based on the fact that patients with controlled HBV replication continue to constitutively produce large amounts of HBsAg from replication-inactive, transcription-active cccDNA and coding sequences that are integrated in the host cell genome. Therefore, the very slow long-term decrease of HBsAg levels in the patients with controlled HBV replication may reflect the slow reduction of the infected cell pool size in the liver. Indeed, living infected hepatocytes transmit cccDNA to progeny cells. However, in the absence of infectious virion production, natural infected cell death is not

compensated by *de novo* infection of noninfected cells and could explain the very slow decline in HBsAg production.

Several studies suggested that quantification of serum HBsAg is useful to predict the virological response to antiviral therapy. A high negative predictive value has been reported in both HBeAg-negative and HBeAg-positive patients treated with pegylated IFN-α [1, 6-9, 33-35]. HBsAg appeared to be useful to identify non-responders as early as 12 to 24 weeks after the start of this therapy and tailor treatment duration in responders. Early stopping rules based on HBsAg level measurements on treatment have been proposed for patients not responding to pegylated IFN-α [1, 6-8, 33, 35].

On a different standpoint, the establishment of stopping rules for patients treated with nucleoside/nucleotide analogues would reduce the burden of a need for lifetime therapy. Such therapy can be withdrawn only in patients who lose HBsAg, probably after a consolidation period of yet unknown duration in the absence of published data. Therefore, serial HBsAg measurements could prove useful to inform patients about the expected treatment duration. A study in patients treated with lamivudine monotherapy showed that HBsAg levels decrease only in long-term on-treatment responders, while no changes are observed in patients who develop lamivudine resistance [36]. In a recent three-year study with tenofovir, a small number of patients who lost HBsAg had a rapid HBsAg level decline. In the remaining patients, HBsAg levels remained high [18, 30]. Another study suggested that a median 10.6 years of effective lamivudine therapy is needed to achieve HBsAg loss in patients with a virological response [20].

Our long-term follow-up study in patients receiving the most potent available HBV drugs revisits this prediction. Indeed, we projected the median number of years required to clear HBsAg (excluding patients without an HBsAg level decline) at 52.2 years (IQR: 30.8-142.7). This discrepancy with the latter study could be explained by the

patients' characteristics and the use of a "homebrew" quantitative HBsAg assay in the other study compared to a commercial, standardized assay in ours. Our patients were representative of patients currently followed in most specialized centers. Indeed, they were on a variety of antiviral agents that reflected the best available drugs at the time, and several were switched to more potent drugs with a better barrier to resistance because of failure to respond or development of HBV resistance. Interestingly, almost all of our patients were finally switched to entecavir and/or tenofovir, and this always resulted in undetectable HBV DNA without any inflexion of the HBsAg level slope. Overall, our data suggest that, in most patients with chronic hepatitis B treated with nucleoside/nucleotide analogues who achieve undetectable HBV DNA, finite treatment duration cannot be envisaged. The relatively small number of patients in our study does not call into question this conclusion, because a very large number of serial blood samples were available over many years of therapy and all of the statistical analyses were highly significant.

In contrast with previous studies including carefully selected patients receiving one single type of treatment in clinical trials, the population of our study reflects patients seen in real-life clinical practice, i.e. individuals who have received sequential anti-HBV therapies with increasing efficacy and barrier to resistance and have now achieved long-term control of HBV replication for several years. Their average follow-up of 8.5 years (up to 10 years) was substantially longer than in previous studies, which generally assessed HBsAg kinetics for periods of 2 to 3 years on treatment [5, 10-14, 18, 20-25]. We used mathematical modeling to calculate the average number of years required to clear HBsAg. This original approach allowed us to demonstrate that anti-HBV therapy with nucleoside/nucleotide analogues must be administered lifelong, a generally accepted but never formally demonstrated hypothesis.

In conclusion, our study, based on the very long-term follow-up of patients with chronic hepatitis B treated with potent nucleoside/nucleotide analogues, shows that HBsAg clearance is unlikely to occur during the patient's lifetime if it does not happen within a few months after HBV replication is well controlled. Thus, lifetime therapy is required in the vast majority of HBV-infected patients. Nevertheless, HBsAg level monitoring may help identify the few patients who may clear HBsAg on long-term therapy. How long treatment should be continued in these patients after HBsAg loss in order to prevent a recurrence of infection is unknown.

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FIGURE LEGEND

Figure 1. Selected examples of individual HBV DNA and HBsAg level kinetics. LAM denotes lamivudine, ADV adefovir, ETV entecavir and TDF tenofovir. The solid line with filled circles represents the kinetics of HBV DNA levels, in Log_{10} IU/mL. The dotted line is the best-fitted curve of HBsAg values, in Log_{10} IU/mL, during the HBV DNA detectable period (empty circles), then the HBV DNA undetectable period (crosses). The top and bottom shaded areas correspond to the lower limits of detection of the real-time PCR assay for HBV DNA and Architect® assay for HBsAg, respectively. The black arrow indicates HBe seroconversion. Examples are shown for different patterns of HBsAg level kinetics described in Table 2: decline during both the HBV DNA detectable and undetectable periods (Pts 3, 5, 6 and 13); decline during the HBV DNA detectable period only (Pt 18); decline during the HBV DNA undetectable period only (Pt 22); no possible assessment of decline during the undetectable period; no possible assessment of decline during the detectable period (Pt 25); increase in HBsAg levels (Pt 26). The patterns observed in the 30 patients are shown in the supplementary figure.

Table 1: Individual demographic, virological and pathological characteristics of the study population (Pt-1 to Pt-30). ND: not determined; NA: not applicable; Rx: treatment; FU: follow-up.

Patient	Age (years)	Gender	Race	HBe Status			Genotype	Basal core promoter and precore mutations		Dominant lamivudine-resistant variants at virological breakthrough on lamivudine monotherapy	METAVIR Score	
				Prior to Rx	Start of FU	End of FU		Basal core promoter ^{1762}AG**G**^{1764}	Precore G^{1896}		Necroinflammatory score	Fibrosis grade
Pt-1	66.7	F	Caucasian	-	-	-	A	ND‡	G	ND†	A2	F1
Pt-2	60.9	M	Caucasian	+	+	-	A	AGG	G	V173L; M204I	A2	F1
Pt-3	59.0	M	African	-	-	-	A	TGA	G	M204I	A2	F2
Pt-4	60.4	M	Caucasian	-	-	-	A	TGA	G	Wild-type	A2	F2
Pt-5	39.8	M	Caucasian	+	+	-	A	AGG	G	NA*	A2	F3
Pt-6	60.0	M	Asian	-	-	-	B	TGA	G	L80I; M204I	A1	F3
Pt-7	72.4	F	Asian	-	-	-	B	ND‡	A	NA*	A1	F4
Pt-8	47.8	M	Asian	-	-	-	B	AGG; TGA	G; A	NA**	A1	F3
Pt-9	46.8	M	Asian	-	-	-	B	TGA	G	NA**	A1	F4
Pt-10	88.1	F	Caucasian	+	-	-	C	TGA	G	G173L; L180M; M204I	A1	F4
Pt-11	30.6	M	Caucasian	-	-	-	D	AGG	A	NA**	A2	F1
Pt-12	61.4	M	Caucasian	-	-	-	D	ND‡	G	Wild-type	A2	F4
Pt-13	74.4	M	Caucasian	-	-	-	D	TGA	G	NA*	A2	F4
Pt-14	61.8	M	African	-	-	-	E	TGA	G	M204I/V	A1	F4
Pt-15	66.3	F	Caucasian	-	-	-	F	AGG	G	NA**	A3	F3
Pt-16	57.3	M	Caucasian	+	-	-	A	TGA	G	L180M;	A3	F4

										M204V		
Pt-17	53.3	M	African	-	-	-	ND§	TGA	A	M204I	A3	F4
Pt-18	37.8	M	African	+	-	-	E	TGA	A	V173L; L180M; M204V	A1	F2
Pt-19	57.7	M	Caucasian	+	-	-	G	TGA	G; A	M204V; L180M	A2	F4
Pt-20	59.8	M	Caucasian	+	+	-	G	AGG	G; A	M204I	A2	F4
Pt-21	55,3	M	Asian	-	-	-	B	TGA	G	NA**	A1	F2
Pt-22	43.3	M	Caucasian	-	-	-	D	ND‡	ND‡	L180M	A2	F2
Pt-23	45.2	M	African	+	+	+	A	Negative	G	NA**	A2	F2
Pt-24	65.8	M	Caucasian	+	-	-	A	TGA	G	Wild-type	A3	F4
Pt-25	42.9	M	African	+	+	+	E	AGG	G	NA**	A2	F1
Pt-26	46.7	M	Asian	+	-	-	B	TGA	G	Wild-type	A3	F4
Pt-27	42.1	F	Caucasian	-	-	-	ND‡	ND‡	ND‡	ND†	A2	F2
Pt-28	69.5	M	Caucasian	-	-	-	A	TGA	G	L180M; M204V	A2	F4
Pt-29	48.3	M	African	+	+	+	E	Negative	G	L180M; M204V	A3	F3
Pt-30	46.8	F	African	-	-	-	E	Negative	A	Wild-type	A1	F1

§Undetermined genotype

‡PCR amplification failure or lack of hybridization to the specific probes in spite of efficient PCR amplification

†HBV DNA level <2 Log_{10} IU/mL or insufficient amount of serum for resistance testing

*Patients initially treated with lamivudine alone who were already on lamivudine plus adefovir at the start of follow-up

**Patients treated *de novo* with a combination of lamivudine and adefovir

Table 2: Slopes and intercepts for Patients 1 to 30 during the full follow-up period, and split between the HBV DNA detectable and the following HBV DNA undetectable periods.

Patients	HBsAg kinetics profile	Full follow-up period			HBV DNA detectable period			HBV DNA undetectable period		
		Slope	p-value	Intercept	Slope	p-value	Intercept	Slope	p-value	Intercept
Pt-1	HBsAg level decline during the detectable and undetectable HBV DNA periods	-0.0017	0.05	3.4812	-0.0026	0.319	3.4311	-0.0043	0.39	3.5627
Pt-2		-0.0114	<0.001	3.3664	-0,0174	0.001	3.0415	-0,0121	0.008	4.4803
Pt-3		-0.0041	<0.001	4.1134	-0.0055	0.083	4.0878	-0.0004	0.82	3.9941
Pt-4		-0.0062	0.004	3.5898	-0.0205	0.631	3.2066	-0.0076	<0.001	3.6689
Pt-5		-0.0208	<0.001	2.5813	-0.0253	<0.001	2.4298	-0.0162	0.01	2.5174
Pt-6		-0.0142	<0.001	2.8725	-0.0110	0.068	2.9931	-0.0110	0.0	2.7023
Pt-7		-0.0161	<0.001	2.9536	-0.0099	0.011	3.1267	-0.0302	<0.001	3.2641
Pt-8		-0.0052	<0.001	3.3791	-0.1308	ND*	2.4700	-0.0054	<0.001	3.3932
Pt-9		-0.0035	0.009	3.1974	-0.0062	0.534	3.1783	-0.0029	0.19	3.1729
Pt-10[1]		-0.0071	<0.001	3.1170	-0.0116	0.072	3.0267	-0.0025	0.083	3.0069
Pt-11		-0.0027	0.046	3.5442	-0.0461	0.074	3.2670	-0.0006	0.642	3.4367
Pt-12		-0.0873	<0.001	3.2095	-0.0840	0.005	3.1661	-0.0742	ND*	2.5664
Pt-13		-0.0146	<0.001	3.0620	-0.0163	<0.001	3.0079	-0.0111	0.041	2.9893
Pt-14		-0.0115	<0.001	2.9346	-0.0082	<0.001	3.1445	-0.0017	0.62	2.6445
Pt-15		-0.0086	0.014	3.8911	-0.0807	0.343	3.7329	-0.0013	0.537	3.6429
Pt-16	HBsAg level decline during the detectable HBV DNA period only	-0.0029	0.036	3.8947	-0.0198	0.001	2.5211	+0.0042	0.791	3.8709
Pt-17		-0.0090	<0.001	3.0890	-0.0110	<0.001	2.9780	+0.0082	0642	2.9471
Pt-18		-0.0042	0.008	3.9348	-0.0038	0.118	3. 9620	+0.0115	0.18	3.7319
Pt-19		-0.0026	0.084	3.3819	-0.0026	0.234	3.4003	+0.0060	0.42	3.1220
Pt-20		-0.0162	<0.001	2.7145	-0.0331	<0.001	1.3415	+0.0265	0.306	2.6145

Pt-21	HBsAg level decline during the undetectable HBV DNA period only	-0.0159	<0,001	2.4068	+0.0246	0.275	2.7124	-0.0162	<0.001	2.4205
Pt-22		-0.0027	0.072	3.4962	+0.0092	0.447	3.7251	-0.0005	0.82	3.4033
Pt-23	HBsAg level decline during the HBV DNA-detectable period (only one undetectable HBV DNA value)	-0.0349	0.001	2.6885	-0.0401	0.001	2.4831		ND*	
Pt-24		-0.0023	0.35	3.6831	-0.0019	0.63	3.7044		ND*	
Pt-25		-0.0109	<0.001	3.8075	-0.0118	0.001	3.7728		ND*	
Pt-26	HBsAg level decline during the HBV DNA-undetectable period (not enough detectable HBV DNA value)	-0.0058	<0.001	3.7668		ND*		-0.0058	<0.001	3.7668
Pt-27		-0.0038	0.014	4.1357		ND*		-0.0038	0.01	4.1357
Pt-28	HBsAg level increase	+0.0086	0.348	3.4145	+0.0460	0.022	7.2227		ND*	
Pt-29		+0.0003	0.633	4.1820	+0.0007	0.442	4.2061	+0.0002	0.987	4.1713
Pt-30		+0.0018	0.027	4.5011	+0.0034	0.207	4.5384	+0.0022	0.149	4.4776

[1]An initial period with undetectable HBV DNA, observed during the first eighteen months of follow-up before the patient broke through on lamivudine therapy, was not taken into consideration in these calculations

*ND: not determined because not enough values were available

Figure

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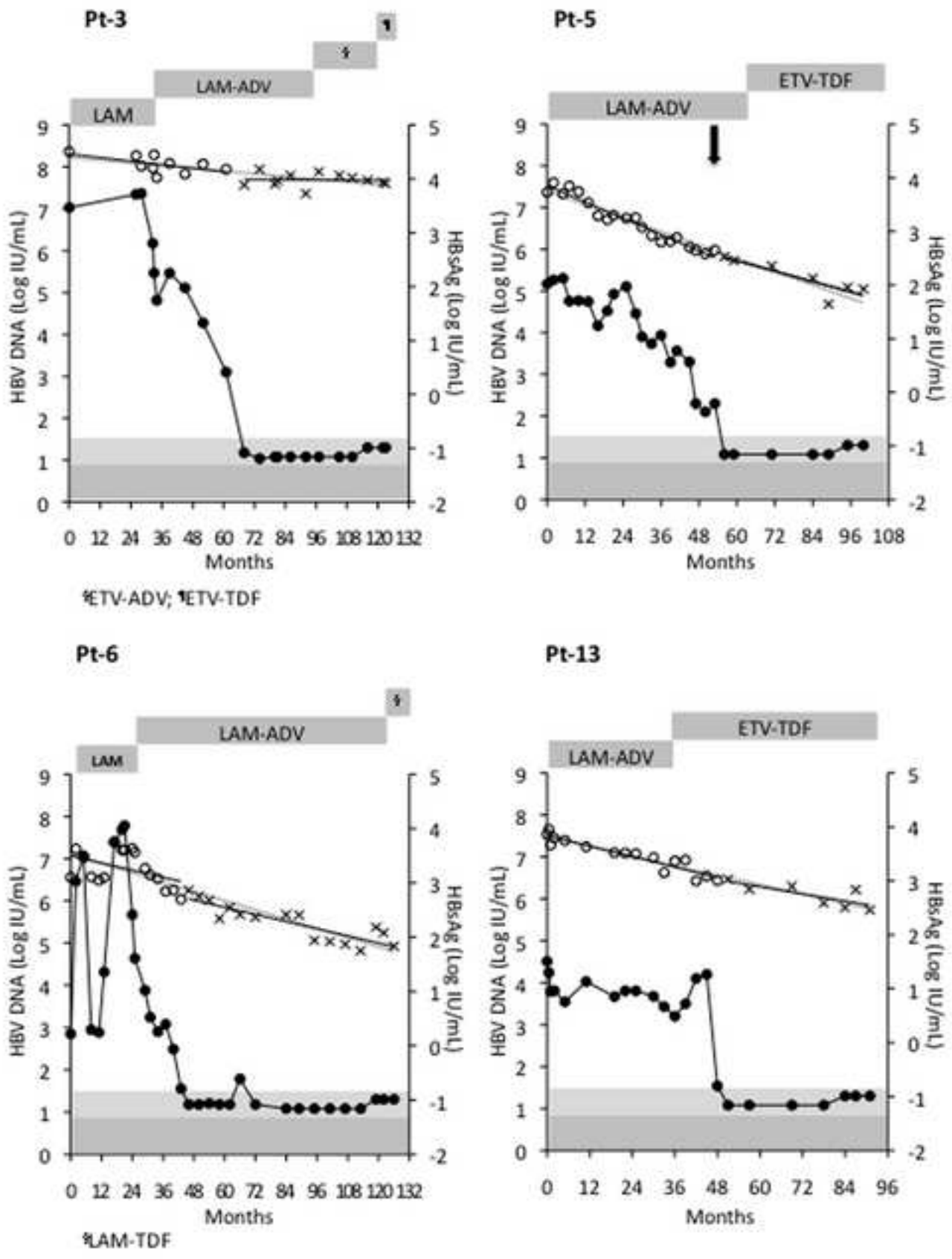

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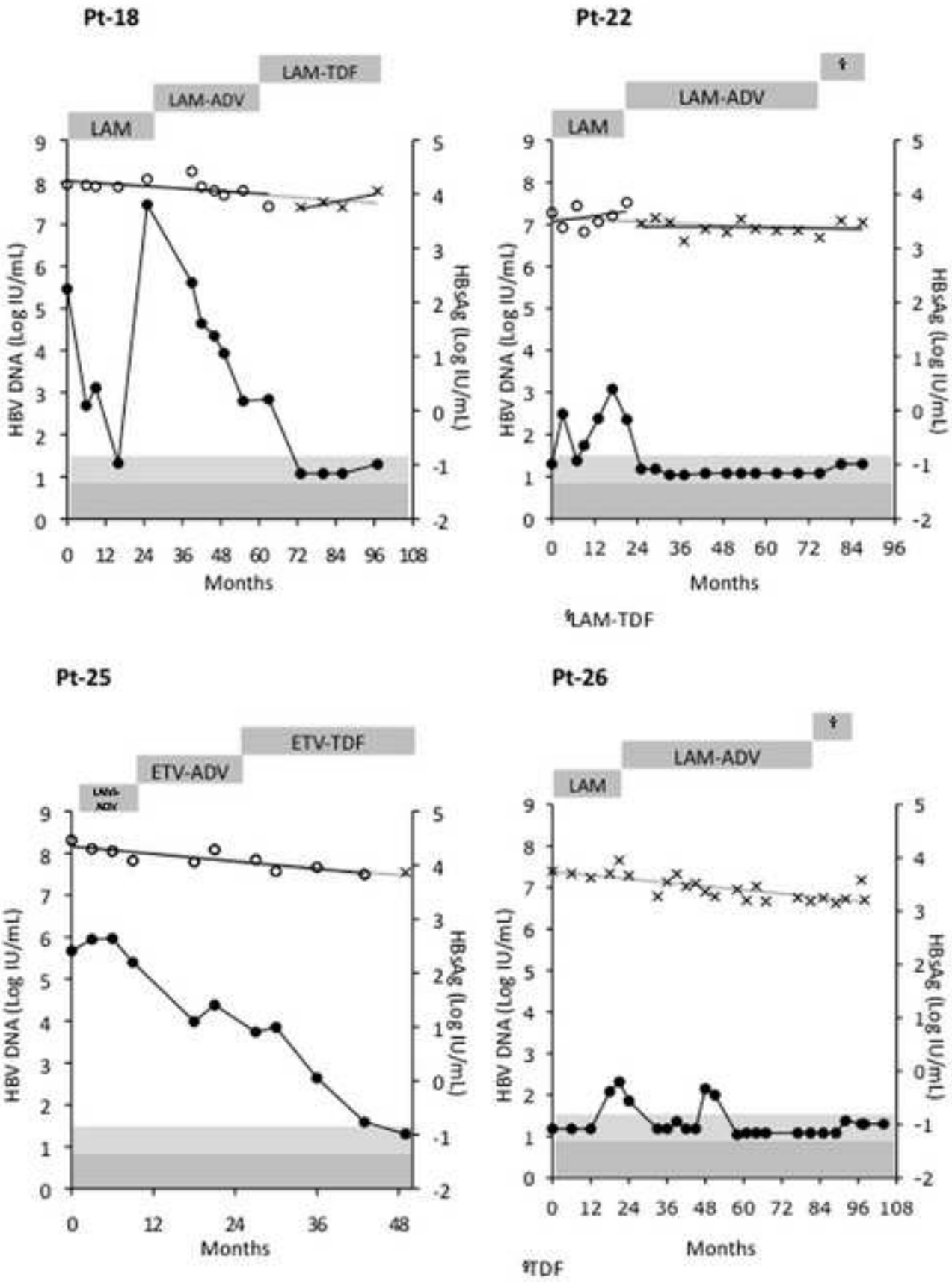

Supplementary material

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