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On-therapy HBsAg kinetics can predict HBsAg loss after nucleos(t)ide analogues interruption in HBeAg-negative patients. The cup is half full and half empty

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ABSTRACT

Background: Nucleos(t)ide analogues withdrawal may improve HBsAg loss rates. However, conditions to select patients are not well established.
Aims: to evaluate the impact of HBsAg kinetics before treatment interruption on post-treatment response.
Methods: Longitudinal, ambispective study in non-cirrhotic chronic hepatitis B HBeAg-negative patients, analysing on-treatment and post-treatment HBsAg kinetics. On-treatment HBsAg kinetics diagnostic accuracy (AUROC) to identify HBsAg loss was evaluated.
Results: 52 HBeAg-negative patients stopped treatment after 8.2 years, and 6 (11.5%) achieved HBsAg loss one year after withdrawal. Multivariate analysis showed that on-treatment HBsAg kinetics was related to HBsAg loss (OR=0.10; 95%CI=0.016–0.632; $p = 0.014$) with a high diagnostic accuracy (AUROC=0.935). A significant HBsAg decline ≥1 log10 IU/mL showed a positive and negative predictive value of 50% and of 97.6%, respectively. After treatment interruption, HBsAg decline speed (log10 IU/mL/year) accelerated in patients treated >6 years (from -0.06 to -0.20, $p<0.05$) and remained stable in treated <6 years (from -0.12 to -0.12 p=ns).
Conclusions: On-treatment HBsAg kinetics can predict post-treatment HBsAg loss rate. Half of patients with a significant HBsAg decline can eliminate HBsAg the first year after withdrawal. Post-treatment HBsAg decline is faster not only in patients who lost the HBsAg but also in those who remain HBsAg-positive.

1. Introduction

The hepatitis B virus (HBV) infection remains a global public health problem. In chronic hepatitis B (CHB), long-term administration of nucleos(t)ide analogues (NA) with high barrier to resistance, i.e., entecavir (ETV) or tenofovir disoproxil fumarate (TDF), is the treatment of choice [1]. The optimal therapeutic endpoint is the hepatitis B surface antigen (HBsAg) loss, which indicates suppression of HBV replication and viral protein expression. However, in CHB e antigen (HBeAg)-negative patients the decline of HBsAg during NA therapy is very slow (−0.1 log10 IU/mL/year) and HBsAg loss very infrequent (0.6–4.6%) [2–5]. Therefore, European Association for the study of the Liver (EASL) and American Association

HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; NA, nucleos(t)ides analogues; HBV, hepatitis B virus; OR, odds ratio; CI, confidence interval; CHB, chronic hepatitis B; ETV, entecavir; TDF, tenofovir disoproxil fumarate; EoT, end of treatment; TE, transient elastography; VR, virological relapse; CR, clinical relapse; ALT, alanine aminotransferase; ULN, upper limit of normality; IL, interleukin; IQR, interquartile range; Peg-IFN, pegylated interferon; AUROC, area under receiver operating characteristic; S, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

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for the Study of Liver Diseases (AASLD) guidelines recommended during years to maintain NA until HBsAg loss [6,7] and to monitor on-treatment HBsAg kinetics [2,5,8].

The Asian-Pacific consensus suggested in 2008 to discontinue NA therapy in CHB HBeAg-negative patients with undetectable HBV-DNA in three consecutive determinations separated by 6 months [9]. Important studies, not only in Asian population [10] but also in Caucasian patients [11,12] have shown that NA withdrawal after years of viral suppression, can improve HBsAg loss rates. Thus, EASL guidelines accepted in 2017 that NA could be discontinued in HBeAg-negative patients after 3 years of DNA suppression [1]. Recent studies evaluating NA withdrawal have shown that low HBsAg levels, at baseline and at the end of treatment (EoT), are related to HBsAg loss [10,13,14]. However, the optimal duration of therapy before discontinuation remains unclear and there are not well-established conditions to select these patients [15].

The hypothesis of our study was that HBsAg kinetics during NA therapy could affect the HBsAg kinetics after NA withdrawal. Thus, the primary aim was to evaluate HBsAg decline before and after treatment withdrawal in non-cirrhotic CHB HBeAg-negative patients. Secondary aim was to evaluate the influence of on-treatment HBsAg kinetics on post-treatment responses.

2. Materials and methods

2.1. Patients and study design

This is a single centre, longitudinal, ambispective study analysing HBsAg levels in non-cirrhotic CHB HBeAg-negative patients during NA therapy and after withdrawal. Patients were eligible if they had received a stable NA dose during a minimum of 3 years and achieved virological response (HBV-DNA below the limit of quantification <13 IU/mL).

Recruitment period was from December 2017 to October 2019. Exclusion criteria were: CHB HBeAg-positive patients, human immunodeficiency virus or hepatitis D virus coinfection, immunosuppressive therapy, history of hepatocellular carcinoma, transient elastography (TE) >9.4 kPa [16], absence of HBsAg determination before NA treatment, or inability to perform a close follow-up. HBsAg levels were determined before NA treatment, at year 1 and 3 after initiation and 1 year before withdrawal. Protocol visits were at EoT, and at weeks 4, 12, 24 and 48 after interruption.

The study protocol was approved by the Ethical Committee of our Institution "Comitè Ètic d'Investigació Clínica - Parc de Salut Mar", study reference 2018/7939/I, in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Variables and clinical definitions

The HBV-genotype was collected from electronic data. Demographic data and TE were assessed at EoT. After NA cessation, liver function, HBV-DNA, HBsAg levels, HBeAg, anti-HBe and anti-HBs were assessed at every protocol visit.

HBV-DNA was measured by polymerase chain reaction with a limit of quantification of 13 IU/mL (Versant HBV DNA 1.0®, Siemens Medical Solutions Diagnostics, New York, USA). Serum HBsAg quantification was introduced in our laboratory in July 2014 and was evaluated by Electro-chemiluminescence immunoassay Elecsys® HBsAgII (Roche Diagnostic, Rotkreuz, Switzerland). The assay ranged from 0.05 to 52,000 IU/mL. In highly concentrated samples above the upper limit, the value of manual dilution was multiplied by the dilution factor [17]. In patients who started NA treatment before July 2014, the HBsAg was analysed in cryopreserved serum samples part of the private collection (C.0000956) of the IMIM (Hospital del Mar Medical Research Institute) and were extracted in fasting conditions and centrifuged at 3000 rpm before preservation at −30 °C [5].

The on-treatment HBsAg kinetics was evaluated at different time points, calculated as delta of HBsAg levels from NA initiation to year 1 (Delta_1), year 3 (Delta_3) and to EoT (Delta_EoT) and the off-treatment HBsAg kinetics as delta of HBsAg from EoT to one year after interruption. Virological relapse (VR) was defined as positive HBV-DNA at any time point. Significant virological relapse (SVR) as HBV-DNA above 2000 IU/mL. Clinical relapse (CR) as an elevation of alanine aminotransferase (ALT) above 2 times the upper limit of normality (ULN) and HBV-DNA >2000IU/mL at any time point [18]. Sustained off-treatment response was defined as persistent ALT<2xULN and HBV-DNA<2000IU/mL and patients in "grey-zone" as ALT>2xULN or DNA>2000IU/mL at week 48 after withdrawal. Retreatment with NA was indicated if patient fulfilled any of the following criteria: severe flare (ALT>10xULN in two consecutive blood test for 2 weeks), moderate flare (ALT 5–10xULN in two consecutive blood test for 4 weeks) or mild persistent flare (ALT 2–5xULN and DNA>2.000 IU/ml persisting for more than 6 months).

2.3. Statistical analysis

Quantitative variables were expressed as medians and interquartile ranges (IQR, Q1-Q3). Categorical variables were expressed as proportions. Continuous variables were compared by the Mann–Whitney *U* test, Wilcoxon or Kruskall-Wallis when appropriate and categorical by the Pearson chi-square test or Fisher test. Differences between patients who achieved HBsAg loss and those who did not, were analysed by univariate analysis. Variables showing a P value<0.05 were included in a multivariate forward stepwise logistic regression analysis to determine independent predictors of HBsAg loss and expressed as odds ratio (OR) and 95% confidence interval (95%CI). The diagnostic accuracy of HBsAg decline to identify patients at risk of losing HBsAg was assessed using the area under the receiver operator characteristic (AUROC) curve (95%CI). The optimal HBsAg decline cut-off value to identify HBsAg loss was selected on the basis of sensitivity (S), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR) and negative likelihood ratio (-LR). Bootstrapping was used to perform an internal validation of the HBsAg kinetic diagnostic accuracy by generating 1000 resampling sets with random replacement. The results of the internal bootstrap validation gave estimation for the AUROC with the median (Percentile 5-Percentile 95). The correlation between treatment duration (years) and HBsAg decline (log 10 IU/mL) was evaluated by Pearson's coefficient (r). The cumulative HBsAg loss rate was evaluated by the Kaplan-Meier method (Breslow and Log-rank tests).

All statistical tests were two-sided and a P value<0.05 was considered significant. Analyses were performed with the SPSS® 25.0 (SPSS Inc., Chicago, IL, USA) and the AUROC of HBsAg decline and Bootstrapping were calculated with MedCalc® v19.1.3 (MedCalc Software).

3. Results

3.1. Study population and baseline characteristics

From January 1999 to December 2017, 148 CHB HBeAg-negative patiens started NA treatment in our hospital. Twenty-seven (18.2%) were lost during follow-up and 9 (6.2%) lost the HBsAg during therapy (6 under NA treatment [5] and 3 under pegylated interferon [peg-IFN] add-on strategy [17]). Therefore, 112 patients were evaluated, and sixty were excluded: 20 (17.8%) with cirrhosis, 6

Table 1
HBsAg kinetics and NA treatment duration.

	N = 52	NA treatment duration 3–6 years (*n* = 11, 21.2%)	NA treatment duration 6–9 years (*n* = 22, 42.3%)	NA treatment duration >9 years (*n* = 19, 36.5%)	P value
Before antiviral treatment					
Males, n (%)	39 (75)	7 (63.6)	17 (77.3)	15 (78.9)	ns
Caucasian, n (%)	30 (57.7)	3 (27.3)	10 (45.5)	17 (89.5)	<0.001
HBV Genotype, n (%)					ns
A	13 (25)	3 (27.3)	6 (27.3)	4 (21.1)	
B	2 (3.8)	0 (0)	1 (4.5)	1 (5.3)	
C	2 (3.8)	0 (0)	1 (4.5)	1 (5.3)	
D	32 (61.5)	8 (72.7)	12 (54.5)	12 (63.2)	
E	3 (5.8)	0 (0)	2 (9.1)	1 (5.3)	
HBsAg (IU/mL)	3821 (1587–7144)	5493 (1698–7005)	3206 (1558–8092)	4084 (1700–7007)	ns
HBsAg (IU/mL), n (%)					ns
<1000	10 (19.2)	2 (18.2)	4 (18.2)	4 (21.1)	
1000–10,000	34 (65)	8 (72.7)	15 (68.2)	11 (57.9)	
>10,000	8 (15.4)	1 (9.1)	3 (13.6)	4 (21.1)	
During NA treatment					
NA treatment, n (%)					ns
Tenofovir	34 (65)	8 (72.7)	15 (68.2)	11 (57.9)	
Entecavir	17 (33)	3 (27.3)	7 (31.8)	7 (36.8)	
Lamivudine	1 (2)	0 (0)	0 (0)	1 (5.3)	
Treatment duration (years)	8.17 (6.5–10.3)	3.98 (3.5–5.1)	7.95 (6.8–8.4)	11.25 (10.2–13.6)	<0.001
Delta_1 HBsAg (log10 IU/mL)	−0.01 (0.03-(−0.09))	−0.03 (−0.19-(−0.02))	0.01 (−0.05–0.07)	−0.02 (−0.17–0.01)	ns
Delta_3 HBsAg (log10 IU/mL)	−0.12 (−0.09(−0.24))	−0.15 (−0.44-(−0.07))	−0.07 (−0.17–0.03)	−0.15 (−0.28-(−0.04))	ns
Delta_1pre-EoT HBsAg (log10/mL)	−0.09 (−0.15-(−0.01))	−0.12 (−0.17-(−0.01))	−0.09 (−0.14-(−0.07))	−0.07 (−0.16-(−0.02))	ns
Delta of HBsAg per year (log10IU/mL/year)	−0.06 (−0.11-(−0.03))	−0.06 (−0.14- (−0.02))	−0.06 (−0.11-(−0.02))	−0.06 (−0.10 -(−0.04))	ns
End of treatment (EoT)					
Age (years)	52 (43–59)	44 (42–53)	53 (43–61)	55 (50–59)	ns
HBsAg (IU/mL)	817 (197–2486)	1350 (332–2639)	817 (221–3721)	558 (57–1628)	ns
HBsAg (IU/mL)					0.053
<100	8 (15.4)	0 (0)	2 (9.1)	6 (31.6)	
100–1000	19 (36.5)	4 (36.4)	10 (45.5)	5 (26.3)	
>1000	25 (48.1)	7 (63.6)	10 (45.5)	8 (42.1)	
Delta_EoT HBsAg (log 10 IU/mL)	−0.51 (−0.93–0.21)	−0.29 (−0.88-(−0.07))	−0.45 (−0.74-(−0.15))	−0.82 (−1.3-(−0.51))	0.017
Delta_EoT HBsAg, n (%)					0.105
<−1 log10 (IU/mL)	42 (80.8)	10 (90.9)	19 (86.4)	13 (68.4)	
≥−1 log10 (IU/mL)	10 (19.2)	1 (9.1)	3 (13.6)	6 (31.6)	
48 Weeks after NA interruption					
HBsAg (IU/mL)	364 (30–1973)	1022 (339–2394)	590 (104–2584)	130 (0–602)	0.097
HBsAg (IU/mL), n (%)					0.017
<100	13 (26.5)	1 (10)	4 (19)	8 (44.4)	
100–1000	18 (36.7)	3 (30)	9 (42.9)	6 (33.3)	
>1000	18 (36.7)	6 (60)	8 (38.1)	4 (22.2)	
Delta_1post-EoT HBsAg (log10 UI/mL)	−0.19 (−0.57-(−0.08))	−0.12 (−0.18-(−0.02))	−0.19 (−0.41-(−0.09))	−0.41 (−1.30-(−0.06))	ns
NA reintroduction, n (%)	3 (5.8)	1 (9.1)	1 (4.5)	1 (5.3)	ns
Sustained off-treatment response, n (%)	21 (40.4)	4 (36.4)	13 (49.1)	4 (21.1)	ns
HBsAg loss, n (%)	6 (11.5)	0 (0)	0 (0)	6 (31.6)	0.003

Quantitative variables are expressed as median (IQR). Qualitative variables are expressed as n (%). HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; NA: nucleos(t)id analogue; EoT: end of treatment; Sustained off-treatment response (ALT<2xULN and HBV-DNA<2000IU/mL).

(4.1%) without baseline serum sample, and 34 (30.4%) declined their participation. Fifty-two patients were included, 75% were males, the median (IQR) age was 52 (43–59), 65% received TDF, and treatment duration was 8.17 (6.51–10.29) years. HBV-genotype was evaluated in all patients and 32 (61.5%) were infected by genotype D. Main characteristics of included patients are depicted in Table 1.

3.2. HBsAg kinetics during therapy and after withdrawal

The HBsAg level (IU/mL) was 3821 (1587–7144) before antiviral treatment, 817 (197–2486) at EoT, and 364 (30–1973) 48 weeks after withdrawal. The HBsAg decline during NA therapy (log10 IU/mL) was −0.01 (0.03-(−0.09)) at year 1, −0.12 (−0.09(−0.24)) at year 3 and −0.51 (−0.93-(−0.21)) at EoT. The speed of HBsAg decline during NA therapy (log10 IU/mL/year) was −0.06 (−0.11-(−0.03)). We observed a correlation between treatment duration (years) and HBsAg decline during treatment (log 10 IU/mL)(r= −0.51; p<0.001). Therefore, the Delta_EoT (log 10 IU/mL) was higher as longer the treatment was: −0.29 in patients treated from 3 to 6 years (n = 11), −0.45 in treated from 6 to 9 (n = 22), and −0.82 in those treated >9 years (n = 19)(p = 0.017) (Table 1 and Fig. 1). However, the speed of HBsAg decline was the same in the three treatment periods −0.06 vs. −0.06 and −0.06 log10 IU/mL/year; p=ns).

The decline of HBsAg one year after NA interruption was −0.19 log10 IU/mL. Therefore, the speed of HBsAg decline after stopping treatment was faster than during therapy (−0.19 vs. −0.06 log10 IU/mL/year; p<0.001). The HBsAg decline after NA withdrawal compared to one year before, was similar in patients treated from 3 to 6 years (−0.12 vs. −0.12; p=ns) but accelerated in those treated from 6 to 9 (−0.19 vs. −0.09; p = 0.015), or >9 years (−0.41 vs. −0.07; p = 0.01)(Table 1 and Fig. 1). After NA interruption, the speed of HBsAg decline was faster not only in patients with HBsAg loss (−1.33 vs. −0.14 log10 IU/mL/year; p = 0.046) but also in those with persistence of HBsAg (−0.18 vs. −0.05 log10 IU/mL/year; p<0.001).

3.3. Predictors of HBsAg loss

Differences between patients who achieved HBsAg loss (n = 6) and those who did not (n = 46) were evaluated by univariate

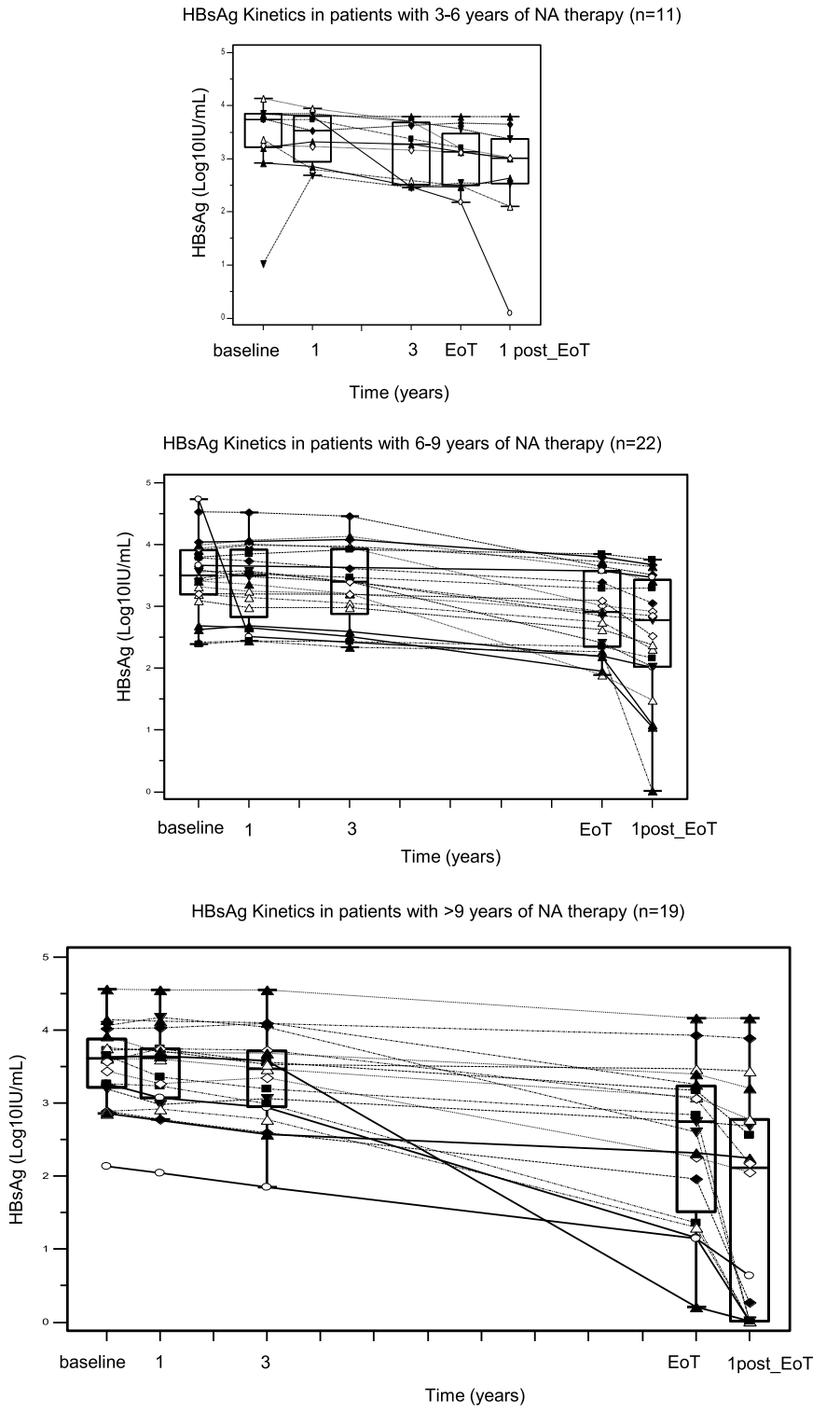


Fig. 1. HBsAg kinetics and treatment duration.
The kinetics of HBsAg (Delta_EoT HBsAg)(log 10 IU/mL) are depicted according to treatment duration in Fig. 1a (from 3 to 6 years; $n = 11$), Fig. 1b (from 6 to 9; $n = 22$) and Fig. 1c (longer than 9 years; $n = 19$). Each line represents one patient, and distribution of HBsAg values in each time point is represented by Box and whisker plots. The decline of HBsAg was higher as longer the treatment was, but the speed of HBsAg decline was the same independently of treatment duration. After stopping treatment, the speed of HBsAg decline was faster than during therapy, especially in those receiving NA longer than 6 years.

analysis (Table 2). HBsAg level before NA therapy was similar between groups (3062 and 3822 IU/mL; p=ns) as the Delta HBsAg at first and third year of treatment (−0.01 vs. −0.01 and −0.12 vs. −0.19 log 10 IU/mL, respectively, p=ns). However, treatment duration was longer in patients with HBsAg loss compared who did not (12.8 vs. 7.9 years; $p = 0.001$). All patients with HBsAg loss (100%) were treated longer than 9 years, compared with 28.3% of those without HBsAg loss ($p = 0.004$). Moreover, the speed of HBsAg decline was faster in patients with HBsAg loss compared with those who did not (0.14 vs. 0.05 log10 IU/mL/year; $p = 0.006$). As

Table 2
HBsAg kinetics and variables associated with HBsAg loss.

	N = 52	HBsAg+ (n = 46, 88.5%)	HBsAg- (n = 6, 11.5%)	P value	OR (95% CI), p value
Before antiviral treatment					
Males, n (%)	39 (75)	34 (73,9)	5 (83.3)	ns	
Caucasian ethnicity, n (%)	30 (57.7)	24 (52.2)	6 (100)	0.026	
HBV Genotype D, n (%)	32 (61.5)	28 (60.9)	4 (66)	ns	
HBsAg (IU/mL)	3821 (1587–7144)	3822 (1578–7159)	3062 (1822–4495)	ns	
HBsAg (IU/mL)				ns	
<1000	10 (19.2)	9 (19.6)	1 (16.7)		
1000–10,000	34 (65)	30 (65.2)	4 (66.7)		
>10,000	8 (15.4)	7 (15.2)	1 (16.7)		
During NA treatment					
Antiviral treatment, n (%)				ns	
Tenofovir	34 (65)	30 (65.2)	4 (66.7)		
Entecavir	17 (33)	15 (32.6)	2 (33.3)		
Lamivudine	1 (2)	1 (2.2)	0 (0)		
Treatment duration (years)	8.17 (6.5–10.3)	7.9 (5.9–10.1)	12.8 (10.3–16.2)	0.001	
Treatment duration, n (%)				0.004	
3–6 years	11 (21.1)	11 (23.9)	0 (0)		
6–9 years	22 (42.3)	22 (47.8)	0 (0)		
>9 years	19 (36.6)	13 (28.3)	6 (100)		
Add-on Peg-IFN, n (%)	19 (36.6)	18 (39)	1 (16.7)	ns	
Delta_1 HBsAg (log 10 IU/mL)	−0.01 (0.03-(−0.09))	−0.01 (−0.08–0.03)	−0.01 (−0.19–0.03)	ns	
Delta_3 HBsAg (log 10 IU/mL)	−0.12 (−0.09(−0.24))	−0.12 (−0.20- (−0.01))	−0.19 (−0.33-(−0.04))	ns	
Delta_1pre-EoT HBsAg (log10 IU/mL)	−0.09 (−0.15-(−0.01))	−0.09 (−0.14-(−0.02))	−0.16 (−0.29-(−0.01))	ns	
Delta_EoT HBsAg/year (log10IU/mL/year)	−0.06 (−0.11-(−0.03))	−0.05 (−0.10-(−0.02))	−0.14 (−0.16-(−0.11))	0.006	
End of treatment (EoT)					
Age (years)	52 (43–59)	51 (43–60)	54 (53–56)	ns	
HBsAg_EoT (IU/mL)	817 (197–2486)	1176 (258–2957)	21.4 (14.3–401)	0.002	
HBsAg (IU/mL)				0.001	
<100	8 (15.4)	4 (8.7)	4 (66.7)		
100–1000	19 (36.5)	17 (37.0)	2 (33.3)		
>1000	25 (48.1)	25 (54.3)	0 (0)		
Delta_EoT HBsAg (log 10 IU/mL)	−0.51 (−0.93–0.21)	−0.47 (−0.87- (−0.15))	−1.75 (−2.11- (−1.45))	<0.001*	**0.10 (0–016–0.632), p = 0.014**
Delta_EoT HBsAg, n (%)				0.001	
<−1 log10 (IU/mL)	42 (80.8)	41 (89.1)	1 (16.7)		
≥−1 log10 (IU/mL)	10 (19.2)	5 (10.9)	5 (83.3)		
48 Weeks after NA interruption					
HBsAg (IU/mL)	364 (30–1973)	590 (135–2489)	<0.05 (BLD)	0.001	
Delta_1post-EoT HBsAg/year (log10IU/mL/year)	−0.19 (−0.57-(−0.08))	−0.18 (−0.40-(−0.07))	−1.33 (−2.60-(−1.16))	0.02	

Quantitative variables are expressed as median (IQR). Qualitative variables are expressed as n (%). HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; NA: nucleos(t)id analogue; Peg-IFN: pegylated interferon; EoT: end of treatment; BLD: below limit of detection.

consequence, patients with HBsAg loss showed greater Delta_EoT HBsAg (−1.75 vs. −0.47 log10 IU/mL; p <0.001) and lower HBsAg levels before interruption (21.4 vs. 1176 IU/mL; p = 0.002).

Multivariate analysis including HBsAg_EoT, treatment duration and Delta_EoT, showed that only the Delta_EoT was associated with HBsAg loss after NA interruption (OR=0.10; 95%CI=0.016–0.632; p = 0.014).

3.4. Diagnostic accuracy of HBsAg decline to predict HBsAg loss

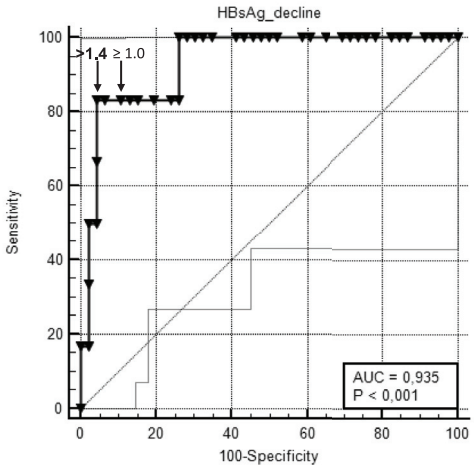
The diagnostic accuracy of HBsAg kinetics during antiviral treatment was excellent to identify patients at risk of losing HBsAg after treatment withdrawal. The AUROC (95% CI) of HBsAg decline was 0.935 (0.83–0.98) (Fig. 2). The bootstrap method showed a median AUROC (Percentile 5-Percentile 95) for the HBsAg kinetics of 0.75 - 0.99 to identify HBsAg loss.

The optimal Delta_EoT cut-off to identify patients at risk of losing HBsAg was > −1.4 log10 IU/mL (S = 83.3%, Sp= 95.7%, PPV=71.4% and NPV=97.8%). Other cut-offs were evaluated for an easier applicability in real clinical practice (Fig. 2). The Delta_EoT cut-off ≥ −1 log10 IU/mL showed good accuracy to identify HBsAg loss (S = 83.3%, Sp = 89.1%, PPV = 50% and NPV = 97.6%). Characteristics of the included patients according to the optimal (−1.4 log10 IU/mL) and useful (−1 log10 IU/mL) cut-offs to identify patients at risk of losing HBsAg are depicted in the Supplementary Tables 1 and 2, respectively.

3.5. Probability of HBsAg loss according to HBsAg kinetics during antiviral treatment

A Delta_EoT ≥ −1 log10 IU/mL was observed in 10 (19.2%) patients (Supplementary Table 2). Patients with a Delta_EoT ≥ −1 log10 IU/mL were usually Caucasian (90% vs. 50%, p = 0.02) but HBsAg levels before NA were similar between groups (3330 vs. 3891 IU/mL; p=ns). Patients with a Delta_EoT ≥ −1 log10 IU/mL had a longer treatment duration (10.2 vs. 7.9 years; p = 0.02) and faster HBsAg decline (−0.15 vs. −0.05 IU/mL/year; p = 0.001). Thus, HBsAg at EoT was lower in patients with a Delta_EoT ≥ −1 log10 IU/mL (114 vs. 1277 IU/mL; p = 0.01). Therefore, 5 (50%) out of 10 patients with a Delta_EoT ≥ −1 log10 IU/mL showed HBsAg <100 IU/mL before interruption and 5 achieved HBsAg loss during the first year after withdrawal (p<0.001 in both cases).

The cumulative rate of HBsAg loss at weeks 4, 12, 24, 36 and 48 after withdrawal was 1.9%, 1.9%, 5.8%, 9.6% and 11.5%. The probability of HBsAg loss one year after NA interruption was 50% in patients with a Delta_EoT ≥ −1 log10 IU/mL and 2.4% in those with Delta_EoT < −1log10 IU/mL (log-rank p<0.001; Breslow p<0.001) (Fig. 3). Moreover, patients with a Delta_EoT ≥ −1log10 IU/mL



HBsAg decline during treatment (Log10 IU/mL)	S (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)	+LR	-LR
≥ 1.0	83.3 (36-99)	89.1 (76-96)	50.0 (29-71)	97.6 (87-99)	7.67	0.19
> 1.2	83.3 (36-99)	89.3 (82-99)	62.5 (35-84)	97.7 (88-99)	12.78	0.18
> 1.4	83.3 (36-99)	95.6 (85.2-99)	71.4 (38-91)	97.8 (88-99)	19.17	0.17

Fig. 2. Diagnostic accuracy of HBsAg decline to identify patients at risk of losing HBsAg.
Diagnostic accuracy (AUROC, 95% CI) of HBsAg decline to identify patients at risk of losing HBsAg after treatment interruption. Evaluation of different cut-offs according to sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio. AUROC: area under receiver operating characteristic; CI: confidence interval; S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; +LR: positive likelihood ratio; -LR: negative likelihood ratio.

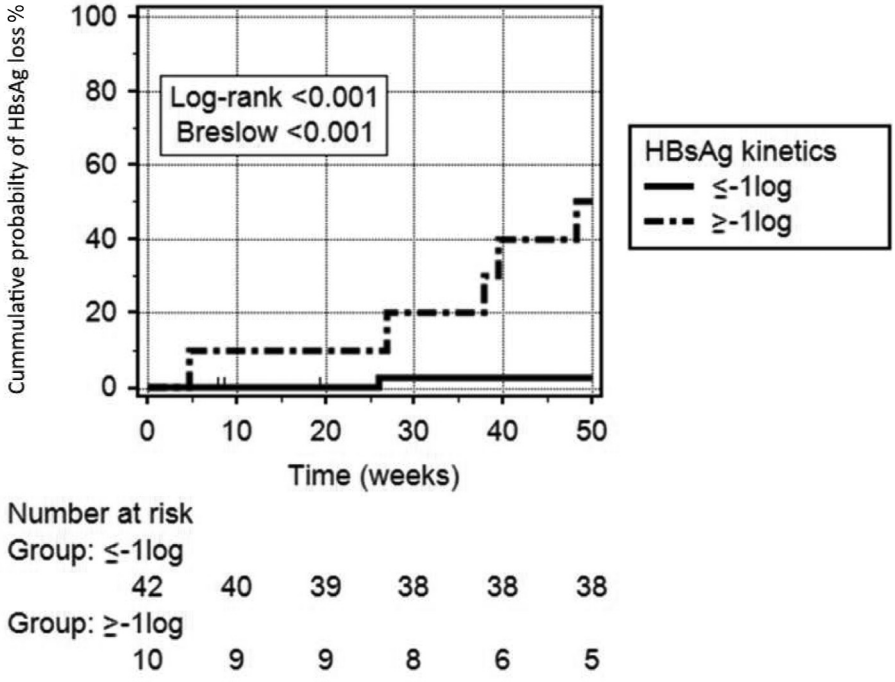


Fig. 3. Cumulative probability of HBsAg loss after NA interruption according to HBsAg kinetics during NA therapy.
Patients with a Delta_EoT HBsAg ≥ -1log10 IU/mL showed an HBsAg loss cumulative probability of 50% one year after NA interruption compared to 2.5% in those with a Delta_EoT HBsAg < -1log10 IU/mL (log-rank= $p<0.001$; Breslow $p=<0.001$).

that one year after treatment interruption persisted with HBsAg-positive showed lower HBsAg levels compared to those with a Delta_EoT < -1log10 IU/mL (103 vs. 766 IU/mL; $p = 0.002$).

3.6. Response after NA withdrawal

The HBsAg loss was observed in 6 (11.5%) patients and seroconversion (positive anti-HBs) in 4 (66.7%) of them. There were no differences in baseline characteristics or in on-treatment HBsAg kinetics between patients with and without seroconversion. However, the 4 patients who developed positive anti-HBs had been treated with TDF and the two others with ETV. Virological relapse was identified in 51 (98.1%), SVR in 24 (46.2%) and CR in 5 (9.6%) patients. Severe flare leading to NA reintroduction was observed in 3 (5.8%) but neither acute liver failure nor hepatic decompensation occurred. Patients with NA reintroduction were Caucasian ($n = 1$), Asian ($n = 1$) and Hispanic ($n = 1$), males ($n = 3$), 2 infected by genotype A and 1 genotype D, all of them treated with TDF during 4.7, 6 and 15 years. The three patients showed an HBsAg decline < 1 log10 IU/mL and HBsAg levels before interruption of 1781, 1632 and 807 IU/mL, respectively. Patients were retreated with the same NA and rapidly achieved virological response. Differences on response rates according the HBsAg kinetics during NA therapy are depicted in Supplementary Table 2 and Supplementary

Figure 1. Considering the HBsAg-positive patients without NA retreatment at week 48 ($n = 43$), 21 (48.8%) remained in sustained off-treatment response and 22 (51.2%) in grey zone. Among patients in grey zone, 20 (91%) showed a SVR and only 2 (9%) a CR. Four out of 5 (80%) patients with an HBsAg decline ≥ 1 log10 IU/mL that one year after NA withdrawal persisted with HBsAg-positive remained in sustained off-treatment response (persistent ALT<2xULN and HBV-DNA<2000IU/mL), compared to 48% (18 out of 38) of patients with HBsAg decline < 1 log10 IU/mL ($p = 0.17$).

3.7. HBsAg kinetics according to HBV genotype and NA therapy

The HBsAg kinetics was evaluated before and after treatment withdrawal according to HBV genotype D vs. other genotypes (Supplementary Table 3). Patients infected by genotype D ($n = 32$) showed lower HBsAg levels (IU/mL) before treatment initiation compared to those infected by other genotypes (2497 vs 5708; $p = 0.03$). However, there were no differences in the on-treatment HBsAg kinetics at year 1, at year 3, at EoT or in the speed of HBsAg decline. After treatment interruption, there were no differences between patients according to HBV genotype.

Regarding the type of NA therapy, we did not find differences between patients treated with TDF ($n = 34$) or ETV ($n = 17$) on treatment duration (8.0 vs. 8.5 years; p=ns), levels of HBsAg_EoT (697 vs. 1143 IU/mL; $p = 0.31$), Delta-EoT (−0.62 vs. −0.48 log10 UI/mL; p=ns), Delta_1post-EoT HBsAg (−0.18 vs. −0.19 log10 UI/mL; p=ns), rates of HBsAg loss (11.8 vs. 11.8%; p=ns), VR (97.1 vs. 100%; p=ns), SVR (65 vs. 35.3%; p=ns), CR (11.8 vs. 5.9%; p=ns), or retreatment (8.8 vs. 0%; p=ns). However, patients treated with TDF compared to those treated with ETV showed earlier VR (4 vs. 12 weeks; $p<0.001$), SVR (12 vs. 30 weeks; $p<0.001$) and CR (10 vs. 48 weeks; $p = 0.14$). In patients who had received Peg-IFN ($n = 19$) [17] the add-on therapy was finished a median of 2.3 years before EoT and no differences in HBsAg loss rate were found (5.3 vs. 15.2%; p=ns). However, patients with add-on Peg-IFN showed faster (−0.10 vs. −0.05 log10 IU/mL/year; $p = 0.02$) and greater HBsAg decline (−0.74 vs. −0.46 log10IU/mL; $p = 0.056$).

4. Discussion

Our longitudinal study in non-cirrhotic HBeAg-negative patients with NA treatment withdrawal has shown that on-treatment HBsAg kinetics can predict theHBsAg decline after treatment interruption and the frequency of HBsAg loss .

Our study has shown a high incidence of HBsAg loss during the first year after treatment interruption (11.5%). Studies on Asian population have shown lower HBsAg loss rates (1.8%) [10,19] compared to European cohorts (from 9% to 22%) [11–14]. A recent systematic review of 25 studies showed a 2% HBsAg loss rate. However, only two studies included Caucasian patients, and most were Asian patients infected by HBV genotypes B or C [20]. In contrast, Kuhnhenn et al. have recently described low levels of HBsAg in HBeAg-negative patients infected by genotype B or D [21]. In our cohort, the 61.5% of patients were infected by genotype D. We have confirmed that patients with genotype D had lower levels of HBsAg at the initiation of NA treatment. However, we could not demonstrate differences between patients on treatment HBsAg kinetics or in the rate of HBsAg loss according to HBV genotypes.

Our study has shown a significant correlation between treatment duration and HBsAg decline. Therefore, the HBsAg decline was higher as longer the treatment was. The annual decline of HBsAg during NA treatment was very stable and slow (0.06 log10IU/mL/year) as we have previously reported [5]. The HBsAg decline during first 3 years of treatment was very low and HBsAg kinetics was not associated with HBsAg loss. However, patients with longer treatment duration had more probabilities of clearing the HBsAg during the first year after interruption. A recent meta-analysis has shown that antiviral therapy duration can be crucial to achieve a persistent viral remission after treatment interruption in HBeAg-negative patients [20]. Therefore, we consider that treatment longer than 3 years could be beneficial before interruption [1,9,15].

Patients with HBsAg loss, showed a greater on-treatment HBsAg decline and lower HBsAg values before interruption. The on-treatment HBsAg decline was independently associated with HBsAg loss and was a good predictor of HBsAg loss (AUROC=0.935). The optimal HBsAg decline cut-off was > 1.4 log10 IU/mL. However, to make the use of HBsAg kinetics easier in real clinical practice, we also evaluated the cut-off ≥1 log10 IU/mL that showed good PPV (50%) and excellent NPV (97.6%). Therefore, half of patients with an HBsAg decline ≥1log10 IU/mL achieved the HBsAg loss during the first year after NA withdrawal. Moreover 40% of them remained in sustained off-treatment response with low HBsAg levels (103 IU/mL) and no patients needed to be retreated. On the other hand, despite only 2.4% of patients with an HBsAg decline < 1 log10 IU/mL achieved the HBsAg loss, the 40% of them persisted in sustained off-treatment response.

t is important to note that after NA interruption the speed of HBsAg decline accelerated in patients treated longer than 6 years. It has been recently postulated that long-term HBV-DNA suppression can reinvigorate exhausted CD8+ T cells and restore the immune control against infected hepatocytes after withdrawal [14,22,23]. In consonance, our study has clearly shown that the speed of HBsAg decline after treatment interruption is 3 times faster than during therapy, not only in patients who lost the HBsAg but also in HBsAg-positive patients, being another argument in favour to stop treatment in these patients.

In terms of safety, only three patients developed a severe flare during the first 12 weeks after withdrawal and were retreated with the same NA showing an excellent response. In contrast, more than 90% of HBsAg-positive patients remained without antiviral treatment. Patients receiving ETV showed later virological relapse. Similarly, recent Asian studies [24,25] have reported that patients treated with ETV can develop the clinical or virological relapse later than those treated with TDF [26]. Thus, we consider that NA type should be considered for the monitoring after interruption. Another interesting point of our cohort was that 37% added-on Peg-IFN two years before EoT [17]. Patients with add-on Peg-IFN showed faster HBsAg decline despite no differences in HBsAg loss rate were found. Hence, an add-on strategy with Peg-IFN could be useful to accelerate HBsAg kinetics and to short NA duration before interruption [17,27].

Our study has some limitations. The limited number of included patients compared to Asian cohorts [10,19], a short time of follow-up, and the lack of an external validation. Nevertheless, it is important to note that these limitations have been compensated performing an internal validation of the HBsAg kinetics diagnostic accuracy and evaluating all included patients without any loss during follow-up. Moreover, the number of our included patients are similar to the previous European studies [11–14]. On the other hand, our study has several strengths. All included patients were HBeAg-negative at the NA initiation, who are the patients with less evidence on NA interruption. The long NA therapy has allowed to identify a significant correlation between treatment duration and HBsAg decline and to analyse the HBsAg kinetics before and after NA interruption in three different treatment time periodstime. Moreover, the evaluation of HBsAg kinetics before and after therapy has demonstrated an accelerated effect after withdrawal.

In conclusion, on-treatment HBsAg kinetics can predict the post-treatment HBsAg decline and the frequency of HBsAg with high accuracy. Half of patients with a significant HBsAg decline (≥ 1 log10 IU/mL) can eliminate HBsAg during the first year

after withdrawal compared to only few patient who did not show this kinetics. Importantly, after NA interruption HBsAg decline is faster not only in patients who lost the HBsAg but also in those who remain HBsAg-positive.

Conflict of interest

None declared.

Financial support statement

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Ethics approval statement

The study protocol was approved by the Ethical Committee of our Institution “Comitè Ètic d’Investigació Clínica - Parc de Salut Mar”, study reference 2018/7939/I.

Patients consent statement

All patients provided a written informed consent.

Guarantor of the article and final version of the manuscript

Jose A. Carrión Rodríguez certifies that all the authors have approved the final version of the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2021.12.017.

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