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Sustained viral control after Nucleos(t)ide Analogue treatment withdrawal following Hepatitis B e Antigen seroconversion is associated with high Hepatitis B surface Antigen loss rates

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Summary: In a multi-ethnic cohort of patients with Nucleos(t)ide Analogue induced HBeAg seroconversion, sustained viral control despite treatment withdrawal led to higher HBsAg loss rates compared to viral relapse or treatment continuation. This suggests a predominant off-treatment non-cytolytic HBsAg clearance.

ABSTRACT

Background: Several studies have shown that Nucleos(t)ide treatment cessation in virally suppressed HBeAg-negative hepatitis patients may increase the chance of subsequent HBsAg loss. In the present study we examined predictive factors for HBsAg loss in HBeAg-positive patients with recent Nucleos(t)ide Analogue (NA) induced HBeAg seroconversion.

Methods: This is a retrospective, multi-ethnic, observational cohort study including mono-infected hepatitis B patients with NA-induced HBeAg seroconversion from 20 centres in Belgium, the Netherlands and Canada.

Results: The analysed cohort consisted of 178 predominantly male (75.3%) patients of mixed ethnicity (44.4% Asians, 48.9% Caucasians). Treatment was withdrawn in 105 patients and continued in 73, leading to HBsAg loss in respectively 14 patients off- and 16 patients on-treatment. A total of 62 patients relapsed of whom none showed HBsAg unless retreatment was initiated. Caucasian ethnicity was associated with a >5-fold higher chance ($p<0.001$) of HBsAg loss, but HBV genotype ($p=0.812$) was not. Overall, HBsAg loss rates were not affected by treatment cessation. Nevertheless, annual HBsAg seroclearance rates were higher in patients with sustained viral suppression after treatment cessation (8.4%) than in those that continued treatment (5.4%) or those that were retreated after relapse (1.5%) ($p<0.001$).

Conclusion: Sustained viral control after treatment withdrawal in patients with recent Nucleos(t)ide Analogue induced HBeAg seroconversion is associated with higher HBsAg loss rates, compared to treatment continuation or viral relapse. This points towards a predominant non-cytolytic clearance of HBsAg early after HBeAg seroconversion.

1. INTRODUCTION

Today, an estimated 240 million people worldwide are chronically infected with the hepatitis B Virus (HBV), putting them at high risk of liver cirrhosis, decompensation and hepatocellular carcinoma (HCC). HBV is a non-cytopathic hepatotropic DNA virus residing in the liver where it frequently causes a lifelong infection. The virus integrates in the host's genome, hampering sterilizing clearance from the liver. In contrast to exposed children, most adults clear the Hepatitis B surface Antigen (HBsAg) spontaneously from blood, within 6 months after infection.[1-3] Clearance of HBsAg is associated with an increase in transplant-free survival, a decrease in HCC incidence and a low risk of subsequent disease reactivation.[4, 5]

Extensive experimental evidence has demonstrated a role for both cytolytic and non-cytolytic clearance during acute HBV infection. Cytotoxic T Lymphocytes (CTL) can induce lysis of infected hepatocytes. Through release of interferon-gamma and tumour-necrosis factor alfa, they can also induce non-cytolytic intracellular HBV clearance, possibly via an APOBEC3-dependent mechanism.[6-16] The precise mechanism behind HBV clearance during chronic HBV infection remains subject of debate. From a clinical point of view, a predominant cytolytic mechanism has been put forward. Indeed, ALT flares during a chronic infection have repeatedly been associated with improved CTL-mediated HBV immune control.[17-22]

Nucleos(t)ide Analogues (NA) efficiently suppress viral replication by inhibiting the viral DNA-polymerase and improve survival of chronic HBV patients. However, NA-induced HBsAg loss is rare, requiring several years to often decades of treatment.[4, 5] Over the last years, the feasibility of stopping NA treatment prior to HBsAg loss in long-term virally suppressed HBeAg-negative hepatitis B patients has been thoroughly investigated. Although viral relapse was found to be frequent, subsequent HBsAg loss has been reported in up to 39% of the patients. This has been ascribed to beneficial ALT flares with concomitant restored HBV-specific T cell responses.[21, 23-26] However, in a recent, large cohort study of 629 HBeAg-negative hepatitis patients sustained viral suppression rather than any type of relapse following treatment withdrawal, was associated with HBsAg clearance, challenging the clinical concept of a predominant cytolytic viral clearance in chronic HBV.[23]

HBsAg loss following NA withdrawal in HBeAg positive hepatitis patients with NA-induced HBeAg seroconversion, has been less well studied.[27-29] These data are relatively difficult to obtain, given the low prevalence of these patients and the common practice of continuing treatment until HBsAg loss in many countries. We previously showed that treatment cessation in patients with recent NA treatment-induced HBeAg seroconversion leads to high relapse rates and potential fatal outcomes.[30]

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Met opmerkingen [ES1]: Check for abbreviations through the whole manuscript, several are not explained (ALT, AST, gamma-GT,...)

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In the current study, we set out to [analysestudy](#) the effect of treatment cessation after HBeAg seroconversion on HBsAg loss in a large, multi-ethnic cohort of chronic hepatitis B patients with NA-induced HBeAg seroconversion. We demonstrate that even though overall treatment cessation did not affect HBsAg loss rates, sustained viral control following treatment withdrawal was associated with higher HBsAg loss rates, compared to treatment continuation or viral relapse after treatment cessation. These clinical data substantiate the potential for off-treatment non-cytolytic viral clearance during chronic HBV infections and incite further studies on immune-mediated HBV clearance mechanisms aimed at a functional HBV cure.

Met opmerkingen [ES2]: Otherwise 2x study

2. METHODS

2.1. Study design

This retrospective, real-world, observational cohort study included chronic hepatitis B patients with NA-induced HBeAg seroconversion from the Outpatient Hepatology Department of 20 hospitals in Belgium, the Netherlands and Canada between 1998 and 2016. Exclusion criteria were concomitant infection with the Hepatitis C Virus (HCV), Hepatitis Delta Virus (HDV) or Human Immunodeficiency Virus (HIV), the use of long-term immunosuppressive medication (>2 weeks) and HBsAg loss at the time of HBeAg seroconversion. Clinical follow-up of the patients was performed according to the international guidelines applicable at that time.

2.2. Data collection

Patients were identified based on available medical lab records as described previously.[30] Following HBeAg seroconversion, treatment was continued or interrupted at the discretion of the treating physician, according to the prevailing guidelines or reimbursement criteria in the different countries. HBeAg and HBsAg loss/seroconversion was defined as the loss of respectively HBeAg and HBsAg and the appearance of respectively anti-HBeAg antibodies and anti-HBsAg antibodies (Anti-HBsAb) on two occasions at least one month apart. Sustained viral suppression was defined as continuous HBV DNA suppression <2000 IU/mL. Virologic relapse was defined as a single HBV DNA elevation >2000 IU/mL after treatment stop, clinical relapse as virologic relapse with ALT-rise >2xULN. Relapse was considered persistent if detected on two or more occasions at least three months apart.

Data on demographics (date of birth, ethnicity, gender), virology (HBeAg status, HBsAg status, HBV viral load and HBV genotype), biochemistry (ALT, AST, gamma-GT), treatment (type and duration) and the presence of cirrhosis were collected systematically in one case report form per patient. The diagnosis of cirrhosis was made according to the international guidelines, through the combination of clinical, elastographical and imaging studies, or histologically.[3, 31] The Upper Limit of Normal (ULN) for ALT was defined as 40 IU/mL.[3] The limit of quantification for HBV DNA varied between 12 IU/mL and 2000 IU/mL, depending on the time of determination (2009 vs 1998). HBV DNA levels below the limit of detection and below the limit of quantification were given the arbitrary values of respectively 0 and the lower limit of quantification. HBV genotyping was performed using the INNO-LiPA test (Fujirebio, Ghent, Belgium).

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2.3. Statistical analysis

Continuous data are shown as mean $\pm$ standard deviation or median (IQR). Variables were compared between groups using a chi-square or Kruskal-Wallis test for categorical variables and a Mann-Whitney U test, student t test or one-way ANOVA for continuous variables. Consolidation treatment duration was calculated as time from HBeAg seroconversion until treatment cessation.

A cox regression model was used to investigate factors predictive of (1) HBsAg loss following HBeAg seroconversion (model 1) and (2) relapse after treatment cessation (model 2). Follow-up time was calculated as time from HBeAg seroconversion to HBsAg loss, retreatment initiation or lost to follow-up (model 1) or as time from treatment cessation to any type of relapse, HBsAg loss or lost to follow-up (model 2). Treatment cessation was included as a time-dependent covariate. Variables with $p < 0.100$ in univariate analysis were further investigated in multivariate analyses. A clock-reset approach was applied to plot Kaplan-Meier curves. Data were analysed in SPSS version 24.0. All statistical tests were two-sided and p -values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Cohort characteristics

The analysed cohort consisted of 178 predominantly male (75.3%) patients of mixed ethnicity (44.4% Asians, 48.9% Caucasians) with NA-induced HBeAg seroconversion after a median treatment duration of 15.5 (IQR 8.2-29.3) months. Following HBeAg seroconversion, 105 patients continued treatment for a median of 11.4 (6.1-18.0) months before withdrawal, whereas 73 patients continued treatment until the end of study inclusion or HBsAg loss (Figure 1). (Figure 1) Patient characteristics per treatment status after HBeAg seroconversion are depicted in Table 1. Overall, characteristics were similar between both groups. However, the continuous treatment group consisted of significantly more start-of-treatment cirrhotic patients (41.1% vs 22.8%; $p=0.008$), was more frequently treated with second generation NA (83.5% vs 41.0%; $p<0.001$) and had a significantly shorter follow-up after HBeAg seroconversion (median 22.2 versus 64.2 months; $p<0.001$) compared to the treatment stop group.

3.2. HBsAg loss and relapse rates after treatment stop (Figure 1)

Thirty patients presented with HBsAg loss at a median of 38.0 (12.9-59.5) months after HBeAg seroconversion: 16 on-treatment after a median consolidation therapy of 14.3 (8.2-41.4) months and 14 off-treatment at a median of 46.0 (15.6-162.4) months after treatment cessation. 7/105 patients were lost to follow-up immediately after treatment cessation. Viral relapse was noted in 62/98 patients at a median of 6.1 (3.5-16.7) months after treatment cessation, of whom 30 showed concomitant ALT-rise $>2\times\text{ULN}$. Relapse was transient in 18 patients, persistent in 28 and another 16 were retreated immediately after a first episode of relapse. Of the 28 patients with persistent relapse, 15 were subsequently retreated. A total of 31 patients were therefore retreated following viral relapse. This retreatment led to HBsAg loss in 2 patients respectively 5.1 and 7.4 years after retreatment start (Figure 1). Both patients showed an at onset severe clinical relapse for which immediate retreatment was deemed necessary. Remarkably, all patients with off-treatment HBsAg loss showed persistently low ALT ($<1.5\times\text{ULN}$) and HBV DNA ($<2000\text{ IU/mL}$) levels after HBeAg seroconversion. (Figure 2)

Met opmerkingen [ES3]: Is this significantly shorter on treatment compared to treatment cessation?

Met opmerkingen [ES4]: Percentages? Is easier to read and compare with published data. Also for the other data

3.3. Caucasian ethnicity, but not treatment cessation is associated with HBsAg loss

Factors assessed to predict HBsAg loss after HBeAg seroconversion in the entire cohort are displayed in Table 2. Caucasian ethnicity (HR 8.166; $p<0.001$), higher HBV DNA (HR 2.185 per log increment; $p<0.001$), higher Gamma-GT (HR 1.002 per unit increment; $p=0.003$) and older age at start of treatment (HR 1.034 per year increment; $p=0.004$) were associated with HBsAg loss in a univariate cox regression model. In multivariate analyses, only Caucasian ethnicity and higher HBV DNA levels at start of treatment remained significantly associated (Table 2). HBV genotype was not associated with HBsAg loss ($p=0.812$) in the sub-population of 48 patients in which HBV genotyping was available.

Interestingly, treatment cessation was not associated with HBsAg loss (HR 1.794; $p=0.171$). Restricting the analysis to patients with a consolidation treatment of at least 6 months (HR 2.041; $p=0.166$) or 1 year (HR 1.756; $p=0.379$) did not change these results (Figure 3A, B and C). Overall mean annual HBsAg loss rates were 5.4% and 5.1% on- and off-treatment respectively ($p=0.561$) in our cohort. Anti-HBsAg antibody development was observed in 12 and 7 patients on- and off-treatment respectively. Patients with on-treatment HBsAg loss tended to develop anti-HBsAb more rapidly, but there was no difference in cumulative rate of anti-HBsAb development 2 years after HBsAg loss (68.9% vs 66.6%; $p=0.754$) (Figure 3D).

3.4. Sustained viral suppression after treatment cessation is associated with higher HBsAg loss rates

Next, we examined whether annual HBsAg loss rates differed according to the virological outcome and (re)treatment status after HBeAg seroconversion using a clock-reset approach. Patients were considered as continuously treated ($n=178$) until treatment was interrupted and as viral suppressed ($n=98$) until relapse occurred ($n=62$) with ($n=31$) or without ($n=31$) need for subsequent retreatment (Table 3). Baseline characteristics predicting HBsAg loss did not differ significantly between these 4 groups, apart from a lower proportion of Caucasians in the relapsers that were not retreated (Table 3). Of the 36 patients with continuous sustained viral control after treatment cessation, 14 (38.9%) lost HBsAg at a median of 1.5 (0.5-7.3) years after treatment cessation. The rate of HBsAg loss per year was 5.4% if treatment was continued after HBeAg seroconversion, but 8.4% if treatment was stopped with subsequent sustained viral control ($p<0.001$; Table 3). In case of retreatment after relapse, the HBsAg loss rate was only 1.5% per year ($p<0.001$). In addition, no HBsAg loss was observed in patients ($n=31$) that showed a mild relapse (median HBV DNA 4.02 log IU/mL; median ALT 0.9 ULN), but were not retreated. None of them developed a biochemical flare $>2\times$ ULN during a median follow-up time of 1.9 (0.8-3.8) years after relapse detection. As the proportion of Caucasian patients in all 3 groups of

patients with HBsAg loss was similar (49.0% vs 48.9% vs 54.8%), the significant difference in annual HBsAg loss rates (5.4% vs 8.4% vs 1.5%) cannot be ascribed to ethnic background differences. These data therefore suggest that sustained viral control following treatment withdrawal is associated with higher HBsAg loss rates, irrespective of ethnicity.

4. DISCUSSION

In the present study we report the effect of NA treatment cessation after HBeAg seroconversion on HBsAg loss. We demonstrate for the first time that annual HBsAg loss rates are higher in patients with sustained viral suppression after treatment cessation compared to patients in whom treatment is continued or patients with viral relapse following treatment cessation.

Overall, HBsAg loss rates were not affected by treatment cessation. In addition, there was no effect of prolonging consolidation treatment before treatment cessation. This points towards a predominant effect of an improved viral immune control on subsequent HBsAg loss. In start-of-treatment HBeAg negative HBV patients, however, HBsAg loss rates are substantially higher after treatment cessation.[19, 21, 23] HBeAg negative hepatitis patients are less susceptible to CTL-induced viral clearance and show a lower abundance of NK and B-cell related transcripts.[27-29] Thus, in these patients viral rebound after treatment withdrawal may be needed to elicit immune responses strong enough to clear HBsAg.[19, 21, 23, 26, 28, 29]

Importantly, we observed higher annual HBsAg loss rates among patients with sustained viral control after treatment cessation (8.4%) compared to patients that continued treatment (5.4%) or patients with relapse after treatment cessation with (1.5%) or without (0.0%) subsequent retreatment. In our cohort, a total of 14 patients showed off-treatment HBsAg loss. All of them had persistent low ALT (<1.5 ULN) and HBV DNA (<2000 IU/mL) levels following HBeAg seroconversion. No HBsAg loss was noticed in patients with viral relapse unless retreatment was initiated with subsequent complete viral suppression. Overall, this substantiates a predominant non-cytolytic HBV clearance early after HBeAg seroconversion.

On the other hand, several NA stop studies in relatively small cohorts of start-of-treatment HBeAg negative patients found that ALT flares after viral rebound may facilitate HBsAg loss via a T cell dependent mechanism, suggesting a cytolytic HBV clearance.[19, 21, 26] A recent study in a large cohort of Taiwanese start-of-treatment HBeAg-negative patients confirmed the existence of these beneficial flares, but found that they are responsible for only a minority of the observed HBsAg loss cases.[23] In our study cohort of recently HBeAg seroconverted patients, beneficial flares were fully absent. Therefore, based on the current findings, we hypothesize that overall, clearance of the Hepatitis B Virus during chronic infection occurs mainly through a non-cytopathic mechanism resulting from the improved immune control after HBeAg seroconversion. Further studies should aim at unravelling which immune responses contribute to a persistent off-treatment viral suppression in the search for a functional HBV cure.[8, 9, 11]

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Patients with viral relapse after treatment cessation showed lower HBsAg loss rates regardless of retreatment initiation, highlighting the need to carefully select patients eligible for treatment stop in order to maximize functional cure rates. As of today, no accurate marker to predict sustained viral suppression off-treatment is available in clinical practice.[32] Prospective studies assessing innovative biomarkers for safe treatment discontinuation are urgently needed. PD1 expression on HBV-specific T-lymphocytes might be an attractive candidate. In a recent prospective NA stop study, the fraction of anti-HBV core and polymerase specific T cells expressing PD1+ before treatment cessation could predict persistent viral control after withdrawal. In addition patients with sustained response after treatment cessation, had relatively high CD8 T cell expression levels of PD1.[33] On the other hand, another small scale prospective NA stop study showed that on-therapy PD1 expression was low in patients going on to clear HBsAg loss following relapse after treatment cessation.[26] However, as all of these studies included only start-of-treatment HBeAg-negative patients, it remains to be determined whether similar immunological markers are applicable after HBeAg seroconversion.

HBsAg loss rates were high in our cohort. Mean annual HBsAg loss rates of 5.4% and 5.1% were noted respectively on- and off-treatment. In start-of treatment HBeAg-negative patients, mean HBsAg seroclearance rates of only 0.15% on- and 1.78% off-treatment were reported.[23] In treatment-naïve patients annual HBsAg clearance rates are in the same order of magnitude ranging from 0.45% to 2.38%.[23, 34] As HBeAg seroconversion is achieved through an improved immune control, patients in our cohort are probably more prone to clear HBsAg, partially explaining the higher HBsAg loss rates observed ~~seen~~ here.

In addition, we observed a >5-fold increased chance of HBsAg loss in Caucasian patients compared to other ethnicities, which comprised almost half of the entire cohort. This might explain why lower mean off-treatment annual HBsAg loss rates of 2.45% were found by Chen *et al.* in 134 Asian patients in whom treatment was withdrawn after HBeAg seroconversion.[35] Previous studies corroborate an up to 2.8 fold lower chance of HBsAg loss in Asian patients.[36][37] In our cohort, this ethnic difference in HBsAg loss rates could not be ascribed to HBV genotype. Most probably, host factors such as differences in HLA polymorphisms between Caucasian and Asian patients, contribute to the ethnic differences in HBsAg loss rates observed here.[38]

This study has some limitations. Although the study was intrinsically retrospective, we estimate that the choice of hard biological endpoints, such as HBsAg loss and ALT levels, has -will have-avoided overcome a major selection bias. Nevertheless, a variation in the number of lab values available per patient in this setting cannot be overcome. In addition, the continuous treatment group contained significantly more cirrhotic patients and was more frequently treated with second generation

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Met opmerkingen [ES6]: could this composition of the cohort also not in part explain the higher rates compared to the literature and discussed in the previous paragraph? Could, if you agree, make this more clear, as in the way it is now written, you do not link the higher rates to the ethnic composition of the cohort

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Met opmerkingen [ES7]: right?

Met opmerkingen [ES8]: Perhaps also add a remark somewhere on the suspected reason for that (I suppose decision of the treating physician because of the potential risk of decompensation in case of flare after treatment stop, but just a hypothesis of course, as you have no data on the decision process)

Nucleos(t)ide Analogues. Nevertheless, as cirrhosis and the type of NA did not influence the chance of HBsAg loss in our cohort, this unbalance did not influence our results. Third, HBV genotyping and quantitative ~~HBsAg~~epatitis B surface Antigen values were mostly not measured during routine clinical practice. We were able to evaluate the contribution of HBV genotypes to HBsAg loss in a sub-population of 48 patients of whom 8 showed HBsAg loss. However, we were not able to evaluate the effect of quantitative ~~HBsAg~~epatitis B surface Antigen levels.

In conclusion, sustained viral suppression after treatment cessation as well as Caucasian ethnicity were associated with higher HBsAg loss rates following HBeAg seroconversion. Our data strongly suggest an important role for non-cytolytic HBV clearance in chronic HBV infections. Future studies should aim at unravelling the immune mechanisms involved in this process.

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CONFLICT OF INTEREST

TV is recipient of a 2014 mandate from the Belgian Foundation Against Cancer (mandate number: 2014-087) and received research funding from Gilead Sciences, Bristol-Myers-Squib and Roche Diagnostics.

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Met opmerkingen [ES9]: I think this was abbreviated previously

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		NA stop n=105	Continuous NA n=73	p-value
Baseline characteristics	Male gender	78 (74%)	56 (77%)	0.713
	Age at treatment start*	40.2±15.2	41.4±15.0	0.484
	Ethnicity			0.874
	Asian(n)	46	33	
	Black(n)	7	5	
	Caucasian(n)	52	34	
	Other(n)		1	
	Cirrhosis(n)	24 (22.8%)	30 (41.1%)	0.008
	Previous interferon treatment(n)	24 (22.6%)	19 (26.4%)	0.628
	HBV-genotype*	7A, 1B, 7C, 3D	10A, 4B, 10C, 5D, 1G	0.836
Treatment start	HBV DNA (¹⁰ log)*	6.86±1.62	7.13±1.85	0.296
	ALT (ULN)**	2.9 (2.0-5.4)	3.7 (1.7-7.0)	0.728
	Year of treatment start**	2006 (2003-2010)	2010 (2007-2012)	<0.001
HBeAg seroconversion	HBV DNA (¹⁰ log)*	1.75±0.73	1.85±1.08	0.588
	ALT (ULN)**	0.8 (0.5-1.0)	0.8 (0.6-1.2)	0.230
	Type NA			<0.001
	First generation (Lamivudine-Adefovir)	62	12	
	Second generation (Entecavir-Tenofovir)	43	61	
	Time to HBeAg seroconversion (months)**	17.8 (8.4-31.9)	14.2 (6.0-26.4)	0.611
	FU-time after HBeAg seroconversion (months)**	64.9 (34.0-97.2)	22.2 (12.0-42.1)	<0.001
	Consolidation treatment (months)**	11.4 (6.1-18.0)	NA	NA
	FU-time after treatment stop (months)**	39.8 (13.7-81.4)	NA	NA

Table 1: patient characteristics per treatment status after HBeAg seroconversion. Data were represented as mean±SD or median (IQR). *: HBV Genotyping was available in 48 patients. NA: Nucleos(t)ide Analogue. ULN: Upper Limit of Normal. ALT: Alanine Amino Transferase. *:mean±SD; **:median (IQR)

		Univariate		Multivariate model 1		Multivariate model 2		Multivariate model 3		Multivariate model 4	
		<i>p</i>	HR (95% C.I.)	<i>p</i>	HR (95% C.I.)	<i>p</i>	HR (95% C.I.)	<i>p</i>	HR (95% C.I.)	<i>p</i>	HR (95% C.I.)
Baseline characteristics	Caucasian ethnicity	<0.001	8.166 (2.847-23.425)	0.002	5.812 (1.950-17.324)	0.029	9.822 (1.271-75.911)	0.004	6.377 (1.748-22.797)	0.001	6.652 (2.285-19.364)
	Male gender	0.161	2.132 (0.740-6.138)								
	Prior interferon treatment	0.946	1.028 (0.457-2.313)								
	Cirrhosis	0.274	0.661 (0.316-1.386)								
Treatment start	Age at start of treatment (years)	0.004	1.034 (1.011-1.058)	0.119	1.020 (0.995-1.047)	0.164	1.021 (0.991-1.052)			0.080	1.020 (0.998-1.044)
	ALT (per unit increment)	0.474	1.000 (0.999-1.001)								
	AST (per unit increment)	0.145	1.001 (1.000-1.002)								
	Gamma-GT (per unit increment)	0.003	1.002 (1.001-1.004)			0.066	1.002 (1.000-1.003)				
	HBV DNA (10log)	<0.001	2.185 (1.520-3.139)	<0.001	1.770 (1.290-2.430)						
HBeAg seroconversion	Platelets (per unit increment)	0.826	0.999 (0.993-1.006)								
	ALT (per unit increment)	0.005	1.004 (1.001-1.006)					0.317	1.002 (0.998-1.007)	0.060	1.003 (1.000-1.005)
	AST (per unit increment)	0.164	1.011 (0.995-1.028)								
	Gamma-GT (per unit increment)	0.100	1.004 (0.999-1.009)								
	HBV DNA (10log)	0.023	1.696 (1.077-2.672)					0.664	1.148 (0.615-2.145)		
	Platelets (per unit increment)	0.478	1.002 (0.996-1.009)								
	Time to HBV DNA <2000 IU/mL (years)	0.898	1.018 (0.774-1.339)								
	Time to HBeAg seroconversion (years)	0.464	1.000 (0.999-1.000)								
Treatment cessation	First/second generation NA	0.607	0.824 (0.395-1.721)								
	Overall (Yes/No)	0.171	1.794 (0.778-4.137)								
	At least 6 month consolidation	0.166	2.041 (0.744-5.603)								
	At least 12 months consolidation	0.379	1.756 (0.501-6.159)								

Table 2: Univariate and multivariate COX regression model to predict HBsAg loss following treatment-induced HBeAg seroconversion for the entire patient cohort. HR: Hazard Ratio, CI: Confidence Interval. ALT: Alanine aminotransferase, AST: Aspartate Aminotransferase, Gamma-GT: Gamma-Glutamyl transferase. Factors with p<0.100 in univariate analysis were further investigated in multivariate models.

Met opmerkingen [ES10]: Why some p values italic and others not?

	Treatment stop			Continuous treatment	p
	Sustained viral suppression: n=98	Relapse with retreatment: n=31	Relapse without retreatment: n=31	n=178	
HBsAg loss (n)	14 (14.3%)	2 (6.4%)	0	16 (9.0%)	<0.001
HBsAg loss rate per year (%)	8.4%	1.5%	0.0%	5.4%	<0.001
Caucasian ethnicity (n)	48 (49.0%)	17 (54.8%)	7 (22.6%)	87 (48.9%)	0.034
HBV DNA at treatment start (¹⁰ log) [°]	6.90±1.63	6.80±1.70	7.01±1.98	6.97±1.72	0.948
Gamma-GT at treatment start (IU/L) ^{**}	53 (23-106)	64 (23-129)	93 (35-163)	62 (29-132)	0.521
Age at treatment start (years) [°]	40.49±15.01	43.26±14.77	37.48±13.31	40.83±15.05	0.494
ALT at HBeAg seroconversion (ULN) ^{**}	0.8 (0.6-1.0)	0.8 (0.6-1.1)	0.7 (0.5-0.9)	0.8 (0.6-1.1)	0.616
HBV DNA at HBeAg seroconversion (¹⁰ log) [°]	1.74±0.70	1.73±0.67	1.67±0.54	1.79±0.88	0.856

Table 3: Overview of differences in HBsAg loss rate and factors predicting HBsAg loss according to (re)treatment status and virological outcome. Patients were considered as continuously treated until treatment was interrupted. Subsequently all patients were considered sustained responders until relapse occurred. °: mean±SD; **: median (IQR)

Met opmerkingen [ES11]: Explain the abbreviations in the legend

Figure 1: Flowchart of the study cohort according to treatment modality and virologic outcome.

Met opmerkingen [ES12]: Indicate percentages (as also discussed during presentation rehearsal)

Figure 2: Alanine Aminotransferase levels after HBeAg seroconversion in individual patients with off-treatment HBsAg loss occurring at the end of the curve (n=14).

Figure 3: Kaplan-Meier curves showing the cumulative probability of HBsAg loss and anti-HBsAg Antibody development after HBsAg loss. Analysis of (A) all patients; (B) all patients with at least 6 months consolidation treatment; (C) all patients with at least 12 months consolidation treatment and (D) all patients with HBsAg loss. *p-value=Log-Rank

Met opmerkingen [ES13]: Difficult to read the graphs and their legends: ensure quality of printed graphs and make sure they are "readable"