Long-term Clinical Outcome of Antiviral Therapy for Chronic Hepatitis B

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CONTENTS

General introduction

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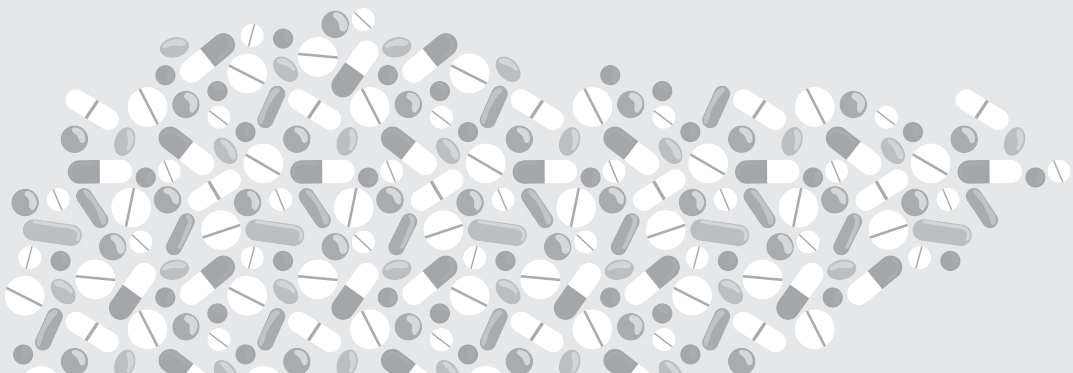

The hepatitis B virus (HBV) was discovered by Dr. Baruch Samuel Blumberg in the 1960s when he identified the 'Australia antigen', which is now known as the hepatitis B surface antigen (HBsAg). For his discovery he received the Nobel Prize in Medicine in 1976.

The HBV is a small DNA virus (40-42 nm) belonging to the family of the Hepadnaviridae, who have a strong preference for infecting hepatocytes.[1-2] HBV virions consist of an outer lipoprotein envelope containing HBsAg. The core is located within this outer layer and contains the viral genome and the viral polymerase, the latter being necessary for viral synthesis. Furthermore, according to their differences in sequence, HBV is classified into eight genotypes (A-H). Besides producing virions, infected cells also produce two types of non-infectious HBsAg particles: 20 nm spheres and filamentous forms with the same diameter; these particles are produced in excess of the amount of complete virions.

CHRONIC HEPATITIS B

Infection with HBV can lead to acute hepatitis, chronic hepatitis, (decompensated) cirrhosis and hepatocellular carcinoma (HCC). Chronic infection with HBV affects an estimated 350-400 million people worldwide and 500.000 CHB patients die annually because of (decompensated) cirrhosis or HCC.[4] An estimated 45% of the infected population worldwide lives in high endemic areas (hepatitis B prevalence >8%) including Asia and sub-Saharan Africa. The different HBV genotypes have a typical geographic distribution with A and D as Caucasian and B and C as South-East Asian genotypes.[3] The risk of developing chronic hepatitis B (CHB) depends on the age of infection. Perinatal infection from the infected mother to the child in high endemic countries results in CHB in an estimated 90%. In contrast, infection with HBV in adults with a mature immune system, mainly through sexual contacts or by (sharing) needles, results only in 5% in chronic disease. [3, 5]

CHB is characterized by HBsAg-positivity for at least 6 months and is classified into four phases distinguished by the presence of hepatitis B e antigen (HBeAg) or its antibody anti-HBe and serum levels of HBV DNA and alanine aminotransferase (ALT) (Table).[3-4, 6] The first phase is the immune tolerance phase, characterized by HBeAg-positivity, high levels of HBV DNA, normal ALT levels and low rates of fibrosis progression. In this phase, the virus is not recognized by the host immune system, resulting in a high viral load and no or limited liver inflammation. The second phase, known as the immune clearance phase, is characterized by HBeAg-positivity, high levels of HBV DNA and elevated ALT levels. In this phase, the activated host immune system tries to eradicate the virus within the infected hepatocytes, which can lead to progression

Table 1 Chronic hepatitis B is divided into four phases.

Phase	HBeAg	HBV DNA	ALT
Immune tolerance	Positive	>200.000	Normal
Immunce clearance	Positive	>20.000	Elevated
Inactive carrier	Negative	<2000	Normal
HBeAg-negative chronic hepatitis B	Negative	Fluctuating	Fluctuating

of fibrosis. Prolonged hepatic inflammation (as measured by ALT) in this phase is an indication for starting antiviral therapy to reduce the risk of disease progression.[7-8] The inactive carrier phase can develop after HBeAg seroconversion (HBeAg-negativity and anti-HBe positivity) and is further characterized by persistently low levels of HBV DNA and ALT. This phase is associated with a good long-term prognosis, a low rate of progression to cirrhosis and HCC and the highest rate of HBsAg seroconversion. HBeAg-negative CHB is also characterized by HBeAg- negativity and lower HBV DNA levels, but with a high risk of disease progression to advanced fibrosis, cirrhosis and HCC. However, both HBV DNA and ALT can fluctuate, and distinction from 'inactive carriers' can therefore be difficult, indicating the importance of serial measurements of HBV DNA and ALT. This distinction is of clinical importance as therapy is indicated in patients with HBeAg-negative CHB.

TREATMENT OF CHRONIC HEPATITIS B

First-line therapy currently consists of immune modulating peginterferon (PEG-IFN) and 5 nucleos(t)ide analogues (NA). A one year course of PEG-IFN injections aims at an off-treatment sustained response and is associated with considerable side-effects.[9] After 1 year of treatment with PEG-IFN 22-27% of HBeAg-positive patients achieved HBeAg seroconversion, increasing to 29-32% 6 months post-treatment.[10-11] HBeAg seroconversion was sustained in most patients during prolonged follow-up.[12] In HBeAg-negative CHB, 36% of patients treated with PEG-IFN for 1 year achieved a response (HBV DNA levels <20.000 IU/ml with normal ALT) after 24 weeks of off-treatment follow-up.[13] However, response to PEG-IFN in HBeAg-negative patients is durable in only a limited number of patients.[14]

The introduction of NA has recently changed the landscape of CHB management for they proved, at least in the short term, to be a safe, effective and well-tolerated treatment option.[15] Continued NA therapy can adequately suppress HBV DNA levels, normalize ALT and improve liver histology in the absence of genotypic resistance.[15] Suppression of HBV DNA to undetectable serum levels is the most commonly used surrogate endpoint of NA therapy as HBV DNA levels are associated with risk of

disease progression in untreated patients.[16-17] However, avoiding genotypic resistance to NA is also an important long-term issue as this could result in virological- and biochemical breakthrough and clinical detoriation.[18]

In HBeAg-positive patients, lamivudine (LAM) achieves suppression of HBV DNA to undetectable levels in 40% after one year of treatment,[11] compared to 21% in adefovir (ADV) treated patients,[19] and 60% in patients treated with telbivudine (LdT).[20] The newest NA entecavir (ETV) and tenofovir (TDF) are able to achieve undetectable HBV DNA levels in 67% and 76% of HBeAg-positive patients after 1 year of therapy.[19, 21] Virological response rates are considerably higher in HBeAg-negative patients because of lower baseline HBV DNA levels.[19, 22] Resistance rates are also lowest with ETV and TDF, and therefore these NA are currently first choice as monotherapy to initiate oral antiviral therapy whenever available.[6, 8] Furthermore, the excellent virological data from ETV and TDF have already been linked to histological regression of fibrosis, but not yet to a better clinical outcome in successfully treated CHB patients.[23] However, the data has its limitations as they are derived from large registration trials, in which patients are treated and monitored according to protocols and included only when complying with strict inclusion- and exclusion criteria. Moreover, patients from clinical practice may have co-morbidities or been pre-treated with older NA, which could result in higher resistance rates and lower response rates.[24-28]

SEROLOGICAL RESPONSE DURING NA THERAPY

HBsAg is an early marker of infection with HBV, and is therefore often used as a screening tool.[5] HBsAg clearance is currently the most definite endpoint of therapy, as it is associated with a better clinical outcome and a very low probability of reactivation in the absence of immune suppression.[29-31]

HBeAg seroconversion is achieved in 20% of patients treated with NA during the first year of therapy, which could induce immune control over HBV and a better clinical outcome in CHB.[4, 19-21, 32] Despite the possibility of achieving inactive disease after HBeAg-seroconversion, it has been shown that NA treated patients who discontinue treatment have a high probability of virological relapse and thus developing active HBeAg-negative CHB.[33] It is therefore likely that, in the absence of useful predictors for sustained response, NA therapy should only be stopped when HBsAg seroconversion is achieved.

Quantification of HBsAg levels has recently gained attention because of its correlation with intrahepatic HBV covalently closed circular (ccc)DNA, the main replicative template of HBV. Through its association with cccDNA, HBsAg is hypothesized to be a marker for immunological response to hepatitis B therapy, independent of viral

replication as measured using HBV DNA levels in serum.[34] Serum HBsAg levels could therefore be a possible predictor of (sustained) serological response during antiviral therapy.[35-36] Despite achieving undetectable HBV DNA during prolonged treatment, HBsAg seroconversion rates are low and thus prolonged NA therapy remains necessary for the majority of patients.[30-31] NA therapy suppresses viral replication by inhibiting HBV polymerase, but HBsAg is produced through a different pathway, resulting in only a limited decline in HBsAg during short-term NA therapy.[37, 36, 38-39] Quantification of HBsAg could thus be a possible tool to monitor NA therapy after achieving undetectable HBV DNA and could help identifying patients likely to achieve HBsAg seroconversion.

The general aims of this thesis are (1) to explore the long-term virological and clinical efficacy of nucleos(t)ide analogue therapy in both NA-naïve and NA-experienced patients, (2) to explore baseline- and on-treatment predictors for serological response during CHB therapy, and (3) to investigate the effect of long-term oral antiviral therapy on HBsAg levels.

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1

Entecavir treatment for chronic hepatitis B: adaptation is not needed for the majority of naïve patients with a partial virological response

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ABSTRACT

Background

Entecavir (ETV) is a potent inhibitor of viral replication in nucleos(t)ide analogue (NA)-naïve chronic hepatitis B (CHB) patients. The aim of this study was to investigate the long term efficacy and safety of ETV in NA-naïve CHB patients, particularly in those with detectable HBV DNA after 48 weeks, in whom treatment adaptation is suggested by current guidelines.

Methods

In a multi-center cohort study we investigated 333 CHB patients treated with entecavir monotherapy.

Results

The NA-naïve population consisted of 243 patients, while 90 were NA-experienced. Virological response (VR, HBV DNA <80 IU/mL) was achieved in 48%, 76% and 90% of HBeAg-positive and in 89%, 98% and 99% of HBeAg-negative NA-naïve patients at week 48, 96 and 144, respectively. Thirty-six of 175 (21%) NA-naïve patients with at least 48 weeks follow-up had a detectable load at week 48 (partial virological response, PVR). Twenty-nine (81%) patients with PVR reached VR during prolonged ETV monotherapy and none of them developed ETV-resistance. Among 22 patients with HBV DNA <1000 IU/mL at week 48, VR was achieved in 21 (95%) patients, compared to eight (57%) of 14 patients with HBV DNA ≥1000 IU/mL. Continuous HBV DNA decline was observed in most patients without VR during follow-up and in three patients adherence was suboptimal according to the treating physician. ETV was safe and did not affect renal function or cause lactic acidosis.

Conclusion

ETV monotherapy can be continued in NA-naïve patients with a detectable HBV DNA at week 48, particularly in those with a low viral load at week 48, as long-term ETV leads to a virological response in the vast majority of patients.

INTRODUCTION

Current treatment guidelines consider nucleos(t)ide analogues (NA) and peginterferon (PEG-IFN) as first line treatment of chronic hepatitis B (CHB). The ultimate goal of treatment is prevention of cirrhosis, hepatic decompensation and hepatocellular carcinoma.[1] Entecavir (ETV) is a cyclopentyl guanosine analogue and showed superior biochemical, virological and histological efficacy compared to lamivudine (LAM) in large phase III trials.[2-3] Moreover, genotypic resistance to ETV is rare in NA-naïve patients through five years of continuous therapy.[4] However, the efficacy of ETV is seriously compromised in LAM-refractory chronic HBV patients with increasing rates of genotypic ETV resistance and patients experiencing a virological breakthrough.[5] Recently we translated these previous findings to clinical practice in a large European multicenter study, as ETV was very effective in NA-naïve patients during the first year of therapy, but less effective in patients with LAM resistance at baseline.[6]
Avoiding viral resistance is a cornerstone of CHB treatment, as resistance is associated with a worsened outcome.[7] Moreover, persistent viremia has been identified as a risk factor for a dismal outcome after two years of treatment with telbivudine (LdT).[8] Therefore, current European guidelines have focused on patients with a partial virological response (PVR), defined as >1 log IU/mL decline in HBV DNA from baseline but a detectable load at week 24 (LAM and LdT) or week 48 (adefovir (ADV), ETV and tenofovir (TDF)). It is suggested that these patients could be at risk for developing genotypic resistance and that treatment adaptation in patients with a persisting viral load after 48 weeks should thus be considered.[9] However, evidence supporting these guidelines is scarce and based on data from studies with less potent NA.[8, 10] It is thus unclear whether treatment adaptation is necessary for naïve patients treated with the more potent drug ETV. The aims of this cohort study were therefore (1) to investigate the efficacy of ETV in clinical practice beyond one year for NA-naïve and –experienced chronic hepatitis B patients, (2) to explore baseline factors associated with a PVR to ETV in NA-naïve patients and (3) to investigate whether a PVR compromises long-term ETV treatment success.

MATERIALS AND METHODS

Study population

In this investigator-initiated cohort study within the European network of excellence for Vigilance against Viral Resistance (VIRGIL), all consecutive adult CHB patients treated with ETV monotherapy between 2005 and May 2010 in 10 large European referral centers were included. Further eligibility criteria were: a viral load of at least

2000 IU/mL at the initiation of ETV monotherapy, and duration of ETV monotherapy for at least 3 months. Patients were excluded if they had viral co-infections (HIV, HCV, HDV) and if they had a liver transplantation before start of ETV therapy. All 220 patients from the previously published study cohort were included in this study and 198 new patients were enrolled.[6] In total, 418 chronic HBV patients treated with ETV monotherapy were identified. Eighty-five patients did not fulfill the entry criteria and were excluded from analysis: 33 subjects had been treated with ETV monotherapy for less than three months, 58 patients had a baseline HBV DNA of less than 2000 IU/mL, 7 patients were co-infected with HCV, 1 patient was co-infected with HDV and 1 patient had a liver transplantation before start of treatment. A total of 333 patients were thus eligible for this analysis. Twenty (6%) of these patients could not be traced anymore after several attempts and were therefore considered lost to follow up. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Patients gave written informed consent according to standards of the local ethics committees.

Follow-up of participants

All subjects were prospectively monitored every three months. At every visit routine examination with biochemical (ALT, bilirubin, albumin) and virological (HBV DNA level, HBeAg, anti-HBe) assessments took place. Genotypic analysis was done (a) at baseline in all NA-experienced HBV patients, (b) in case of virological breakthrough, defined as an increase in serum HBV DNA level > 1 log (10-fold) above nadir on at least two occasions after initial virological response, or (c) in case of serum HBV DNA > 200 IU/mL at the end of follow-up. If ETV-resistant mutations were detected during follow-up, genotypic analysis was performed at baseline in NA-naïve subjects. In NA-experienced patients, genotypic resistance was also assessed in stored serum samples obtained at the end of all previous NA-treatment regimes. HBV genotype was determined at start of ETV therapy. The diagnosis of cirrhosis was based on histology or ultrasound examinations.

Endpoints

The primary outcome was virological response (VR), defined as serum HBV DNA levels < 80 IU/mL (approximately 400 copies/mL) during the on-treatment follow-up period. Secondary endpoints were HBeAg loss and seroconversion (in HBeAg-positive patients), HBsAg loss and seroconversion, emergence of ETV-related mutations and ALT normalization. Renal function was assessed by calculation of the estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2 using the Modification of Diet in Renal Disease equation, based on the serum creatinine level, age, sex, and race.

Laboratory tests

Serum alanine aminotransferase (ALT), bilirubin, albumin levels and international ratio of prothrombin time were measured locally using automated techniques. Hepatitis B surface antigen (HBsAg), antibody against HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays in all centers. Serum HBV DNA levels were measured using a quantitative real-time polymerase chain reaction assay, the COBAS AmpliPrep-COBAS TaqMan HBV test (CAP-CTM; Roche Molecular Systems, Inc., Branchburg, NJ, USA), with a lower limit of detection of 12 IU/mL, in nine of ten centers. In one center serum HBV DNA was measured using Roche Amplicor (linear dynamic range, 400 to 200,000 copies/mL; Roche Diagnostic Systems, Branchburg, NJ, USA). A conversion factor of 5.26 copies/IU was used for conversion of copies/mL to IU/mL. HBV genotypes and detection of HBV polymerase gene mutations was determined by direct sequencing or using the INNO-LiPA assay (Innogenetics, Gent, Belgium).

Data analysis

HBV DNA levels were logarithmically transformed for analysis. ALT levels are expressed as values representing a ratio to the local upper limit of normal (xULN). Continuous variables were expressed as means ± SD or median (IQR) where appropriate. Follow-up times were calculated from the date of ETV treatment initiation to the date of event or censorship. The cumulative probability of achieving virological response was estimated by Kaplan-Meier analysis. Cox's regression analysis was used to study which of the following baseline factors were associated with virological response to ETV monotherapy: Age, gender, race, body mass index (BMI), HBV genotype, HBeAg status, viral load, ALT level, presence of cirrhosis, prior treatment with LAM, prior history of LAM resistance, presence of LAM resistance at baseline, duration of LAM therapy, prior treatment with ADV, prior history of ADV resistance, prior treatment with (peg)interferon, ETV dosage, and treatment center. Factors that correlated strongly (that is presence of collinearity), were compared in separate models with each collinear variable by using the Akaike information criterion method. A Cox model was used to estimate the influence of prior treatment with LAM and prior treatment with ADV on the virological response to ETV, adjusted for the confounding effects of HBeAg status, viral load, and prior treatment with LAM. The covariate ETV dosage was not included, as it was not associated with virological response in the univariate proportional hazards analysis and, when included in the model, did not improve model fit. All statistical tests were two-sided, and a p-value < 0.05 was considered to be statistically significant. SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of the study population are shown in table 1. One-hundred-forty-three (43%) patients were HBeAg-positive, median ALT was 1.7 (1.0-3.3) x ULN and mean HBV DNA was 6.2±1.7 log IU/ml at baseline. NA-experienced patients

Table 1 Baseline characteristics of the study population.

	All patients	NA-naïve	NA-experienced	
	N=333	N=243	N=90	P value
Age	43±14	43±14	41±13	0.12
Gender (male)	248 (75%)	177 (73%)	70 (79%)	0.26
Race				0.57
Caucasian	160 (48%)	114 (47%)	46 (51%)	
Asian	92 (28%)	70 (29%)	22 (24%)	
Other	81 (24%)	59 (24%)	22 (24%)	
BMI	25±4.1	25±3.5	25±5.0	0.21
ALT (xULN)	1.7 (1.0-3.3)	1.7 (1.0-3.5)	1.7 (1.0-3.2)	0.78
HBV DNA (Log IU/ml)	6.2±1.7	6.2±1.7	6.2±1.8	0.86
HBeAg-positive	143 (43%)	86 (36%)	57 (63%)	< 0.001
Genotype (N=265)				0.80
A	56 (21%)	40 (22%)	16 (20%)	
B	23 (9%)	14 (8%)	9 (11%)	
C	37 (14%)	25 (14%)	12 (15%)	
D	127 (48%)	91 (50%)	36 (44%)	
Other	22 (7%)	14 (8%)	8 (10%)	
Dosage entecavir (0.5 mg)	265 (80%)	238 (98%)	27 (30%)	< 0.001
Presence of cirrhosis	90 (27%)	57 (24%)	33 (37%)	0.04
MELD score	7.8±2.1	7.5±1.9	8.4±2.4	0.02
Previous treatment with (PEG-)IFN	74 (22%)	49 (20%)	25 (28%)	0.14
Previous treatment with LAM				
LAM-experienced	72 (22%)		72 (80%)	
Prior history of LAM resistance	35 (11%)		35 (39%)	
LAM-resistance at baseline	14 (4%)		14 (16%)	
Previous treatment with ADV				
ADV-experienced	51 (15%)		51 (57%)	
Prior history of ADV resistance	14 (4%)		14 (16%)	
ADV resistance at baseline	12 (4%)		12 (13%)	
Previous treatment with TDF	4 (1%)		4 (4%)	
Previous treatment with LdT	2 (1%)		2 (2%)	

Table 2 Virological and biochemical response to entecavir, number of observed events.

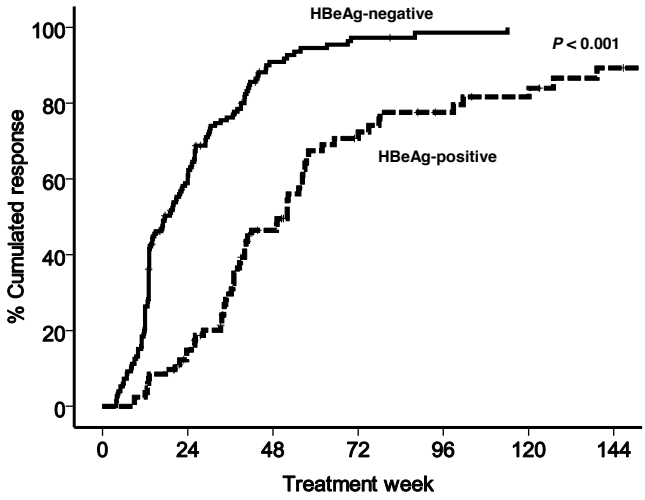
	NA-naïve patients (N=243)	LAM-experienced patients (N=72)			ADV-experienced patients[a] (N=43)	
		No LAM-resistance (N=37)	Prior history of LAM-resistance (N=21)	LAM-resistance at baseline (N=14)	LAM-resistance (N=18)	No LAM-resistance (N=25)
Baseline HBV DNA (Log IU/ml)	6.2±1.7	6.4±1.8	6.3±1.5	5.6±1.8	5.9±1.8	5.4±1.8
Median follow-up (month, IQR)	19 (11-32)	25 (13-32)	19 (9-31)	10 (6-15)	15 (9-28)	32 (23-39)
Virological response	208/243 (86%)	28/37 (76%)	11/21 (52%)	3/14 (21%)	6/18 (33%)	22/25 (88%)
Virological breakthrough	5/243 (2%)	0/37 (0%)	5/21 (23%)	7/14 (50%)	7/18 (39%)	1/25 (4%)
ETV resistance	0/243 (0%)	0/37 (0%)	3/21 (14%)	0/14 (0%)	3/18 (17%)	1/25 (4%)
ALT normalization	126/171 (74%)	14/24 (56%)	8/11 (62%)	4/7 (57%)	6/9 (67%)	12/16 (75%)
HBeAg loss	18/86 (21%)	6/26 (23%)	4/14 (29%)	0/7 (0%)	3/14 (21%)	4/12 (33%)
HBeAg seroconversion	13/86 (15%)	5/26 (19%)	2/14 (14%)	0/7 (0%)	2/14 (14%)	3/12 (19%)
HBsAg loss	3/243 (1%)	2/37 (5%)	0/21 (0%)	0/14 (0%)	0/18 (0%)	2/25 (8%)

[a]The antiviral effect of entecavir is described for 43 patients who were directly switched to entecavir

were more often HBeAg-positive (p=0.001), had more often cirrhosis (p=0.04), had a higher MELD score (p=0.02) and were more often treated with 1 mg ETV (p=0.001) as compared to NA-naïve patients. In addition, 87% of LAM-resistant patients and 86% of ADV-resistant patients were treated with 1 mg. Overall median follow-up was 20 (IQR 11-32; range 3-51) months.

Efficacy of entecavir in NA-naïve patients

In total, 243 (73%) patients were NA-naïve and treated for a median of 19 (IQR 11-32; range 3-45) months (Table 2). For HBeAg-positive patients (n = 86), the cumulative probability of achieving VR at week 48, 96 and 144 was 48% (95% CI 36-60), 76% (66-86) and 90% (81-99), respectively (Figure 1). HBeAg loss rates were 10% at week 48, 21% at week 96 and 34% at week 144. Corresponding rates for HBeAg seroconversion were 8%, 16% and 24%. ETV therapy was not stopped in any patient achieving HBeAg seroconversion. HBsAg loss occurred in one (1%) of the HBeAg-positive patients. For HBeAg-negative patients (n = 157), the cumulative probability of achieving VR at week 48, 96 and 144 was 89% (95% CI 84-93), 98% (95-100)



Number of patients without response							
HBeAg-positive	86	70	28	17	11	8	4
HBeAg-negative	157	63	8	3	1	0	0
Total number of patients in follow up	243	226	175	142	106	78	49

Figure 1 Kaplan-Meier curve for the probability of achieving virological response for 243 NA-naïve patients according to HBeAg-status at baseline. P-value by Log-Rank testing.

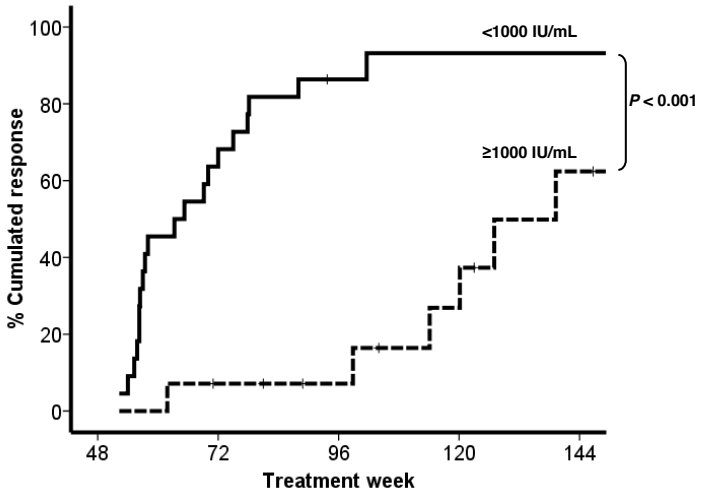
and 99% (97-100), respectively (Figure 1). HBsAg loss occurred in two (1%) HBeAg-negative patients. There were no significant differences in virological response rates per center. Five patients experienced a virological breakthrough, but no genotypic resistance to ETV was detected.

Partial virological response in NA-naïve patients

Partial virological response (PVR) at week 48 occurred in 36 (21%) of 175 NA-naïve patients with at least 48 weeks follow-up. High baseline HBV DNA (OR 0.67; 95% CI 0.50-0.89; p=0.005) and HBeAg positivity (OR 0.25; 95% CI 0.10-0.60; p=0.002) were the only independent risk factors for having a PVR. Overall follow-up of patients with a PVR lasted for 27 (19-35) months. Twenty-nine (81%) of 36 patients achieved a VR beyond week 48 (Table 3). Moreover, 10 patients needed more than 96 weeks of continuous ETV therapy to achieve a VR. Patients achieving a VR had a lower HBV DNA at week 48 than those who did not achieve a VR during prolonged ETV monotherapy. Patients with a PVR were stratified according to their viral load at week 48 (Figure 2). Twenty-one (95%) of 22 patients with HBV DNA <1000 IU/mL and 8 (57%) of 14 patients with HBV DNA ≥1000 IU/mL achieved a VR without treatment adaptation during prolonged treatment beyond week 48. Overall, a continuous HBV DNA decline was observed in six (86%) of seven patients without VR at the end

Table 3 NA-naïve patients with a partial virological response at week 48. Characteristics of 36 patients with a detectable HBV DNA after 48 weeks of continuous ETV monotherapy.

	All patients	Response	Without response	
	N=36	N=29	N=7	P value
Age	41±14	43±14	33±13	0.13
Gender (male %)	30 (83%)	26 (90%)	4 (57%)	0.07
Race				0.09
Caucasian	17 (47%)	15 (51%)	2 (29%)	
Asian	11 (31%)	8 (28%)	3 (43%)	
Other	8 (22%)	6 (21%)	2 (29%)	
HBeAg-positive	28 (78%)	22 (76%)	6 (86%)	0.58
Presence of cirrhosis	7 (19%)	6 (21%)	1 (14%)	0.70
Follow-up (Months, IQR)	27 (19-35)	28 (19-35)	22 (19-28)	0.23
Baseline HBV DNA (Log IU/ml)	7.7±1.4	7.5±1.5	8.2±1.1	0.31
HBV DNA week 48 (Log IU/ml)	2.7±1.3	2.3±1.2	3.8±0.8	0.006
HBV DNA decline at week 48 (Log IU/ml)	5.0±1.3	5.1±1.3	4.3±0.8	0.13
HBV DNA last visit (Log IU/ml)			3.0±0.9	
Load >1000 IU/mL at week 48	14 (39%)	8 (28%)	6 (86%)	< 0.001



Number of patients without response					
<1000 IU/mL at week 48	22	8	2	1	1
≥1000 IU/mL at week 48	14[a]	12	10	7	3
Total number of patients in follow up	36	31	23	16	9

Figure 2 Kaplan-Meier curve for the probability of achieving virological response for NA-naïve patients with a PVR according to HBV DNA at week 48. [a]Three patients were switched to TDF+emtricitabine and one patient received TDF add-on therapy. P-value by Log-Rank testing.

of follow-up. In three (43%) patients non-compliance was suspected by the local physician. Five patients were switched to a TDF containing regimen (in two because of virological breakthrough, one patient first achieved VR); all achieved a VR during follow-up. In two patients ETV dosage was changed from 0.5 mg to 1 mg, both achieved a VR during follow up. However, no ETV-resistance was detected during follow-up, at virological breakthrough or at time point of treatment adaptation.

Efficacy of ETV in NA-experienced patients

Fifty-one (57%) NA-experienced patients had received prior treatment with ADV. To investigate the efficacy of ETV as salvage therapy for ADV-treated patients, the antiviral effect of ETV is given in table 2 for 43 (84%) subjects, who were directly switched to ETV monotherapy. Twelve (28%) of these patients had a history of genotypic resistance to ADV (rtN236T/ rtA181V/T). Seventy-two (80%) NA-experienced patients had received prior treatment with LAM. Adjusted for baseline viral load and HBeAg status, antiviral response to ETV was neither influenced by prior ADV therapy (HR 0.92; 95% CI 0.57-1.51; p=0.75) nor by previous ADV-resistance (HR 1.23; 95% CI 0.56-2.70;

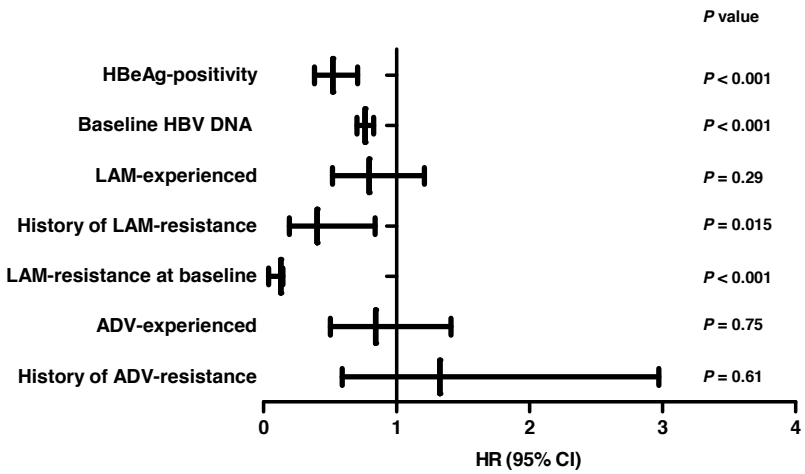


Figure 3 Adjusted hazard ratio (HR) of achieving virological response for both NA-naïve and NA-experienced patients. Based on the Cox's model adjusted for HBeAg status, mean baseline HBV DNA, LAM-experience, history of LAM-resistance, LAM-resistance at baseline, ADV-experience and history of ADV-resistance.

p=0.61) (Figure 3). In contrast, presence of LAM-resistant mutations at baseline (HR 0.13; 95% CI 0.04-0.42; p<0.001) and a previous history of LAM-resistance (HR 0.40; 95% CI 0.19-0.84; p=0.015) were significantly associated with a reduced probability of achieving VR.

Resistance surveillance

Eighteen patients experienced a virological breakthrough after a median follow-up of 20 (11-32) months. Five (2%) NA-naïve patients experienced a virological breakthrough, non-adherence was suspected in two of them and three were switched to a TDF containing regimen (TDF monotherapy in one, TDF add-on in one and TDF+emtricitabine in one). Six NA-experienced patients were switched to a TDF containing regimen (TDF monotherapy in two, TDF add-on in three and TDF+emtricitabine in one) and one patient stopped ETV without starting another NA. In four NA-experienced patients genotypic mutations to ETV were detected, one patient was previously only exposed to ADV and achieved a virological response before developing a virological breakthrough (Table 4). A TDF containing treatment regimen was initiated in all four patients, and a subsequent decline in HBV DNA was observed in three of them. No mutations associated with decreased sensitivity to ETV were observed in any of the NA-naïve patients, including those with a viral load > 200 IU/mL at the end of follow-up.

Table 4 Characteristics of four patients developing genotypic mutations to entecavir.

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	26	67	29	26
Gender	Male	male	male	Female
At start of entecavir				
HBeAg status	positive	positive	positive	Positive
HBV DNA (log10 IU/mL)	8.9	5.7	7.9	4.2
Prior LAM exposure	No	Yes	Yes	Yes
Prior LAM-resistance	No	Yes	Yes	Yes
Baseline LAM-resistance	No	No	No	No
HBV Genotype	A	D	A	C
Viral load at max. viral suppression (log10 IU/ml)	<1.9	5.3	2.9	3.8
Month of resistance	40	9	28	15
At time of resistance				
HBV DNA (log10 IU/ml)	5.4	5.3	6.3	5.5
Mutational pattern	M204V L180M I169T L217R	L180M M204V V173L A181T N236T	M204V L180M V173L S202 I169T	M204V L180M T184I
Adverse outcome	None	None	None	None
Non-compliance	No	No	No	No
Response to salvage therapy				
Salvage therapy	Addition of tenofovir	Tenofovir monotherapy	Addition of tenofovir	Tenofovir monotherapy
Follow-up (months)	44	18	54	18
HBV DNA at last F/U (log10IU/ml)	< 1.9	< 1.9	< 1.9	4.0

Safety surveillance

Adverse events included: dizziness, headaches and loss of appetite (in three different patients). None of the patients developed clinically evident lactic acidosis, but lactate was not routinely measured. One patient died because of a hepatocellular carcinoma, which was already present at start of ETV, one patient had a recurrence of his hepatocellular carcinoma, and one patient died because of a non-Hodgkin lymphoma. To assess renal safety we analyzed creatinine levels in a subset of 188 patients with an available baseline creatinine. Mean estimated glomerular filtration rate (eGFR) at baseline was 95.8 mL/min/1.73m^2. The mean decrease in eGFR during follow-up was 1.2±18.1 mL/min/1.73m^2 (p=0.38). None of the patients experienced an increase in serum creatinine > 0.5 mg/dL. In a subset of 9% of patients with an eGFR <70 mL/

min/1.73m^2 eGFR increased with 1.4±10.0 mL/min/1.73m^2 (p=0.60*).* Age was the only risk factor significantly associated with developing an eGFR <60 mL/min/1.73m^2.

DISCUSSION

The current multicenter study showed that ETV is effective up to three years in NA-naïve patients, irrespective of having a virological response at week 48. The vast majority of NA-naïve patients with a PVR achieved undetectable HBV DNA through prolonged therapy without treatment adaptation. Genotypic resistance to ETV was not detected in any of the patients with a PVR at week 48. We showed that ETV is a safe antiviral drug with a good renal tolerance and minimal side effects. Persistent viral replication and the development of resistance during treatment with NA for CHB have been associated with an adverse treatment outcome.[8, 11] Therefore, EASL guidelines suggest treatment adaptation in patients with a PVR to prevent treatment failure and the development of resistance.[9] PVR is defined as a decline of >1 log IU/mL in HBV DNA but failure to achieve undetectable HBV DNA levels at week 48 in patients treated with continuous ETV monotherapy.

In our cohort of NA-naïve patients treated with ETV, 36 patients failed to achieve a VR at week 48. Among these patients with a PVR, 81% achieved a VR without treatment adaptation through 15 additional months of therapy. Cumulated probability of achieving VR beyond week 48 was higher for patients with HBV DNA <1000 IU/mL at week 48. Importantly, despite two patients experiencing a virological breakthrough, no resistance was detected in these NA-naïve patients. This is in accordance with the ETV phase III trial in which, albeit with incomplete follow-up, a substantial number of patients achieved a response beyond the first year of treatment, whilst genotypic resistance remained rare through 5 years of continuous monotherapy.[4, 12-13] Our findings are in contrast with previous studies on LdT/LAM and ADV in which persistent viral replication at week 24 and week 48 of therapy was identified as a predictor of the emergence of subsequent viral resistance.[8, 10] This highlights that treatment paradigms based on data from studies investigating agents with a low barrier to resistance cannot be translated to newer and more potent drugs as ETV and TDF.

Nevertheless, not all ETV-treated patients with a PVR achieved VR through prolonged treatment. As we, after thorough examination, determined non-compliance in three (43%) of these seven patients, this explains in our opinion primarily the inability to achieve HBV DNA undetectability. The problem of non-adherence is supported by a previous study suggesting partial response to ADV is most likely due to non-compliance and host pharmacological factors.[14] One of seven patients without a VR experienced a virological breakthrough and treatment was adapted by the treating

physician. However, it is important to note that six (86%) of seven patients who failed to achieve a VR during follow-up still had a declining load at end of follow-up, which suggests that achieving a VR can probably be reached in the majority of cases. Patients with a PVR could therefore be considered 'slow responders' instead of partial responders. Taken together, our study shows that continuing ETV appears safe and effective in patients with detectable HBV DNA at week 48, especially in patients with a lower viral load at week 48, of whom 95% achieved VR.

Decreased sensitivity to ETV for LAM-refractory patients was soon known after introduction of this agent.[5, 15] Our study confirms these results as the antiviral efficacy of ETV is seriously diminished in these patients, even after correction for possible confounders as high baseline HBV DNA and HBeAg-positivity. Moreover, our study underlines that even after resistance testing at baseline; the absence of LAM-associated mutations does not guarantee a susceptible virus during ETV treatment. This suggests that if there is a suspected history of LAM-resistance, TDF containing regimens should be preferred instead of ETV monotherapy as LAM-resistance strains remain susceptible to TDF monotherapy.[16]

Consistent with in vitro data, our study showed that antiviral efficacy of ETV treatment was not influenced by prior exposure or resistance to ADV.[17-21] Until now only small studies or studies with a relatively short follow-up have confirmed the in vitro efficacy of ETV in ADV-experienced or ADV-resistant patients in real life practice.[6, 22-24] Our findings are of particular interest because both ETV and TDF can thus be used as salvage therapy for ADV-experienced patients.[9, 25]

Data from the large phase III trials with a selected population showed that entecavir has few side effects in patients with compensated liver disease.[2-3] However, a recent report indicates that patients with decompensated cirrhosis are at risk for developing lactic acidosis.[26] We showed that ETV is safe during prolonged therapy in this heterogeneous cohort, even in the presence of cirrhosis. Moreover, we proved that ETV does not affect renal function, which might be a concern during TDF therapy.[27]

Limitations of our study are the observational design and the heterogeneous group of patients, yet we used Cox's regression to correct for confounders as treatment duration, HBV DNA, HBeAg status and previous LAM-resistance. Nevertheless, this heterogeneous population is also representative for clinical practice, and makes it possible to compare different groups of (NA-experienced) patients within one study.

In conclusion, in contrast to what is suggested in recently published EASL guidelines on the management of chronic hepatitis B, adjustment of ETV monotherapy in NA-naïve patients with a PVR at week 48 is not necessary. We demonstrated that continuous therapy beyond week 48 is safe and effective, and results in VR in the vast majority of patients, particularly in those with HBV DNA <1000 IU/mL at week 48. Furthermore, genotypic resistance to ETV was not observed in this subset of

NA-naïve patients. For both NA-naïve and NA-experienced patients, ETV proved to have a favorable safety profile.

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2

Efficacy and tolerance of a combination of tenofovir disoproxil fumarate plus emtricitabine in patients with chronic hepatitis B: a European multicenter study

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ABSTRACT

Background

The combination of tenofovir disoproxil fumarate (TDF) plus emtricitabine (FTC) is used extensively to treat HIV and also has potent activity against hepatitis B virus (HBV). The aim of this study was to assess the efficacy and tolerance of TDF+FTC in patients with chronic hepatitis B (CHB).

Methods

Seventy-eight consecutive CHB patients from five European centers were included. All started a TDF+FTC combination between October 2005 and March 2010. Virological, biochemical, and clinical data were recorded during follow-up. Tolerance was also monitored. Patients were classified into either treatment simplification (TS), where efficacy of the previous treatment was obtained at TDF+FTC initiation, and treatment intensification (TI), where the previous line of therapy had failed.

Results

TDF+FTC was given as a TI to 54 patients (69%) and as a TS to 24 (31%). Among patients with TI, 83% were males. The median baseline HBV-DNA was 4.4log IU/mL, and median alanine-transaminase (ALT) was 1.1 xULN. Sixty percent was HBeAg positive, 47% had significant fibrosis (≥F3 Metavir equivalent), and 29% had confirmed cirrhosis. Median treatment duration was 76 weeks (interquartile range 60–116). Kaplan–Meier analysis showed that 48 weeks after TI, the probability of becoming HBV-DNA undetectable was 76%, and reached 94% at week 96. No viral breakthrough occurred. Patients with TS (87% males, median baseline HBV-DNA 1.1 log IU/mL, median ALT 0.79 x ULN, 33% HBeAg positive, 61% with significant fibrosis) were treated for a median duration of 76 weeks. In this subgroup, all patients but one remained HBV-DNA undetectable and no ALT flare-up occurred during follow-up. Creatinine levels did not show kidney-function deterioration in either group of patients.

Conclusions

After a median follow-up of >76 weeks, the TDF+FTC combination showed encouraging antiviral efficacy and a good safety profile in all patients. TDF+FTC may represent an interesting clinical option to simplify therapy and increase the barrier to resistance, which should be assessed on the long term.

INTRODUCTION

Hepatitis B virus (HBV) is the leading cause of liver cancer and frequently leads to cirrhosis and liver failure.[1] The goal of nucleos(t)ide analog treatment is to suppress viral replication, to halt liver-disease progression, and to prevent the onset of complications. Management of antiviral therapy should be based on precise virological monitoring that enables early diagnosis of a partial response and also treatment failure.[2-4]

Virological response is defined by a decline in HBV-DNA levels during therapy[5] and different profiles of response may be distinguished. The initial response is characterized by a decrease of at least 1 log IU/mL in viral load by week 12. This definition was chosen as it exceeds variability in HBV-DNA assays and spontaneous variations of viral load during the course of infection. Primary non-response is defined as the result of either poor-treatment compliance or inadequate antiviral potency of the drug.[6] Current guidelines have focused on patients with a partial virological response, defined as a decline in HBV-DNA greater than 1 log IU/mL from baseline, but a detectable viral load at week 24 (for lamivudine- or telbivudine-based therapy) or week 48 (for adefovir, entecavir, tenofovir).[2] In these cases it is recommended to switch or to add a more potent drug and that has a complementary cross-resistance profile.

Virological breakthrough was defined by an increase of at least 1 log IU/mL compared to the lowest value during treatment, and was confirmed by a second test in a treatment-compliant patient.[7-8] Persistent viremia has been identified as a risk factor for a worse outcome and is associated with a greater risk of resistance.[9-11] Early adaptation of treatment is recommended, at least at the time of a virological breakthrough or in cases of insufficient viral suppression in compliant patients. The addition of a complementary drug is the preferred strategy.

With the availability of drugs that exhibit potent antiviral activity and have a high barrier to resistance, antiviral drug resistance is becoming a more manageable issue. Therefore, all the current guidelines have identified the persistence of viral replication, even at low levels, as a major target to prevent disease progression and to prevent the emergence of resistance.[12]

Tenofovir disoproxil fumarate (TDF) plus emtricitabine (FTC) is used extensively to treat HIV. Both drugs also exhibit potent activity against HBV.[13-16] Their combination[17] may be clinically relevant in increasing the barrier to resistance of TDF and alleviate the risk of antiviral drug resistance.[12] The objective of this study was to assess the efficacy and tolerance of the TDF+FTC combination for chronic hepatitis B (CHB) in a cohort of patients followed in five European clinical centers.

MATERIALS AND METHODS

Patients

Patients with chronic hepatitis B, treated with a combined TDF+FTC therapy, were consecutively recruited from five European Centers, which were all members of the European Network of Excellence VIRGIL (vigilance against viral resistance): Hospices Civils de Lyon, France; Erasmus MC, Rotterdam, Netherlands; Hospital Vall d'Hebron, Barcelona, Spain; Hôpital Cochin, Paris, France; Hannover Medical School, Hannover, Germany. All patients started the TDF+FTC combination between October 2005 and March 2010. Patients were classified into two groups: treatment simplification, i.e., two drugs in one pill per day, when efficacy of the previous line of therapy has been obtained before initiation of TDF+FTC, and treatment intensification (TI), when the previous line of therapy had failed. Patients were included in the analysis if they received the TDF+FTC combination for at least 12 weeks and they had no co-infection with HIV or HCV. Previous treatment history was recorded and coded as simple (≤1 molecule for ≤1 year) or complicated (>1 molecule and/or treatment duration >1 year), in accordance with the European Association for the Study of the Liver (EASL) guidelines.[2]

Patient follow-up

All patients were regularly monitored within their routine clinical follow-ups. Virological, biochemical, clinical, and tolerance data were assessed locally during the follow-up. The primary endpoint of interest was virological response, defined as HBV-DNA being undetectable (assessed by real-time PCR), according to the technique used. Secondary endpoints were time when HBV-DNA became undetectable, clinical improvement, alanine-transaminase (ALT) normalization, tolerance assessed by creatinine level during follow-up, and HBsAg and HBeAg loss and/or seroconversion. Renal-function impairment was defined as an increase in creatinine level >1.5 times the baseline value.

Statistical analyses

Normally distributed variables were presented as the mean +/- standard deviation, whereas skewed variables were presented as the median and interquartile (25–75%) range. Categorical variables were studied using the two-sided chi-square test or Fisher's exact test when necessary, whereas quantitative variables were analyzed using analysis of variance (ANOVA) or the non-parametric Kruskal–Wallis test as appropriate. A Kaplan–Meier analysis was performed to assess the probability of HBV-DNA being undetectable and for ALT normalization over time after TDF+FTC initiation. Follow-up times were calculated from the date of TDF+FTC initiation to

the date of event or censorship. Cumulative probabilities were compared between subgroups using the log-rank test. HBV-DNA values were dichotomized according to the 4 $\log_{10}$ IU/mL cut-off based on published data that showed clinical relevance.[6, 18] Age was analyzed by comparing patients below or above 40 years, a cut-off age above which HBV complications has been reported to increase.[4] Statistical analysis was performed using SPSS v.17.0 for Windows. All statistical tests were two-sided and a *p*-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

Seventy-eight consecutive CHB patients were included. All started on combined TDF+FTC therapy between October 2005 and March 2010. This TDF + FTC combination was given as treatment-intensification to 54 patients (69%) and as treatment simplification to 24 (31%) patients. Within the whole study population, 85% of patients were male, and the mean age was 49 years ±15 (Table 1). Median duration of follow-up was 76 weeks in both groups. Two patients had a co-infection with the hepatitis delta virus. The proportion of patients with significant fibrosis at baseline (Metavir score ≥F3) was slightly higher in the treatment-simplification group (61.1%) than in the treatment-intensification group (46.7%), although this difference was not statistically significant (*p*=0.30). Sixty percent of patients within the treatment-

Table 1 Patients' characteristics at the start of tenofovir (TDF) plus emtricitabine (FTC) therapy (n=78).

	Treatment intensification (n=54)	Treatment simplification (n=24)	p
Demographics			
Male (n (%))	45 (83.3)	21 (87.5)	0.75
Mean age (years) ± SD	47.8 ± 15.8	51.8 ± 14.0	0.29
Median BMI (kg.m-2) [IQ range]	23.7 [21.5-25.4]	23.9 [21.8-28.5]	0.48
BMI >25 (%)	45.0	26.7	0.15
Previous treatment history (complicated, n (%))	48 (88.9)	23 (95.8)	
Median TDF+FTC duration (weeks) [IQ range]	76 [60-116]	76 [52-120]	0.58
Median ALT (x ULN) [IQ range]	1.10 [0.64-1.95]	0.79 [0.53-1.11]	0.04
Significant fibrosis (Metavir ≥F3, %)	46.7	61.1	0.30
Proven cirrhosis (%)	28.9	38.9	0.44

Table 1 (continued) Patients' characteristics at the start of tenofovir (TDF) plus emtricitabine (FTC) therapy (n=78).

	Treatment intensification (n=54)	Treatment simplification (n=24)	p
Virological			
Median HBV-DNA (log10 IU/mL) [IQ range]	4.4 [3.0-5.8]	1.1 [1.1-1.1]	<0.001
HBeAg positive (%)	60.4	33.3	0.028
Co-infections (n)			
Delta	1	1	0.53
Genotype, n (%)			
A	11 (28.9)	4 (26.7)	1
B	6 (15.8)	1 (6.7)	0.66
C	5 (13.2)	4 (26.7)	0.25
D	11 (28.9)	5 (33.3)	0.75
E	4 (10.5)	1 (6.7)	1
F	1 (2.6)	0 (0)	1
Non available	16	9	

IQ, interquartile (25–75%); SD, standard deviation.

intensification group were HBeAg positive at baseline versus 33% of patients within the treatment-simplification group (p=0.028).

Analysis of changes in HBV-DNA levels during therapy

In the treatment-intensification group, median HBV-DNA level at initiation of TDF+FTC was 4.4 log IU/mL and all patients were HBV-DNA positive. Among patients with available HBV-DNA information after 48 weeks of combination therapy, 80% (33/41) had undetectable HBV-DNA and this proportion increased to 94% (15/16) at 96 weeks. Kaplan–Meier analysis indicated that the probability of being HBV-DNA undetectable at 24 weeks was 47%, reaching 76% at 48 weeks, and 94% at 96 weeks (Figure 1). Time to undetectable HBV-DNA was shorter in patients with baseline HBV-DNA below 4 log IU/mL compared to those with a viral load above 4 log IU/mL (log-rank p<0.001; see figure 2). However, no significant difference was observed according to HBeAg status at baseline, previous treatment history (simple vs. complicated), age, gender, or ethnicity.

In the group with treatment simplification, all patients but one remained HBV-DNA undetectable during follow-up. In one patient, relapse occurred between 72 and 96 weeks of follow-up, shortly after the patient deliberately stopped TDF+FTC therapy.

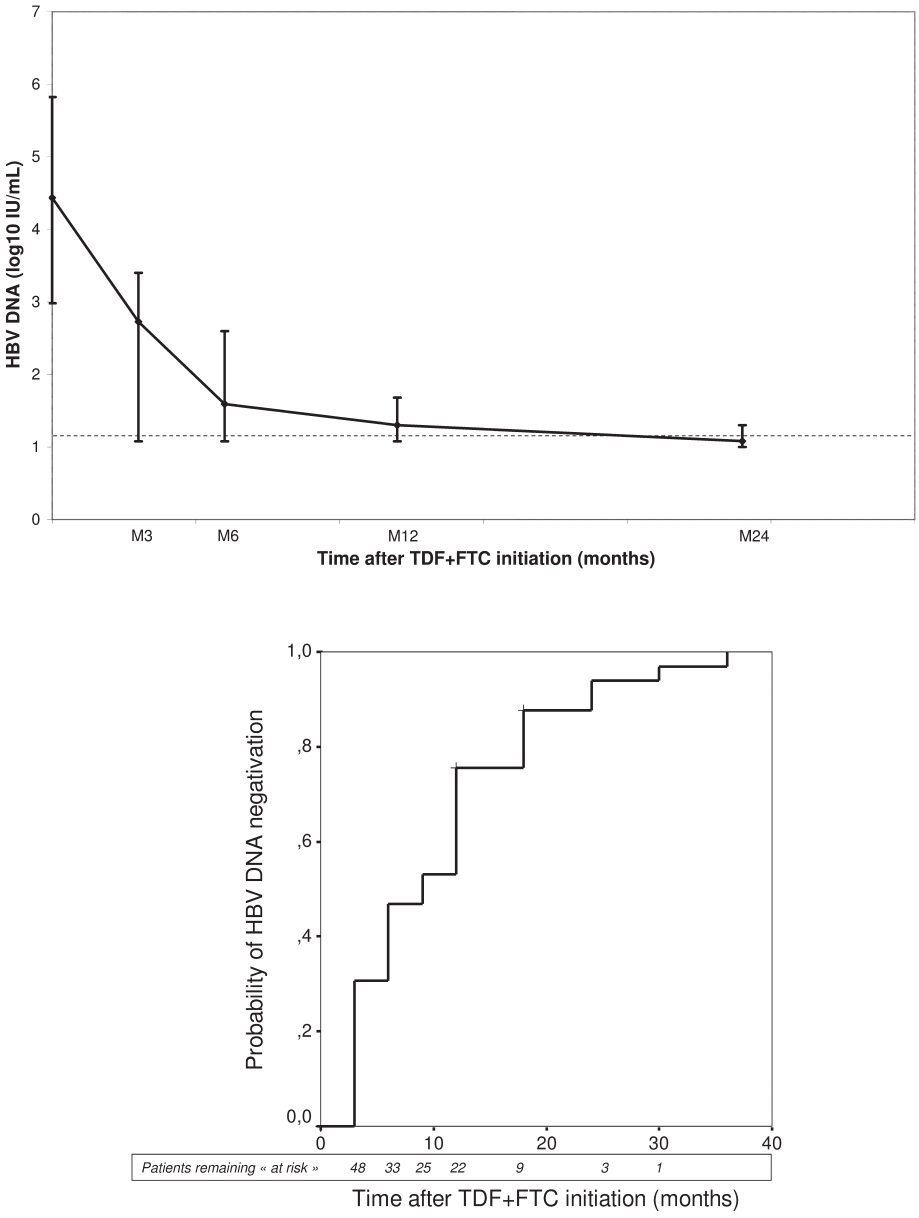

Figure 1 (A) HBV-DNA kinetics (median with interquartile range) after TDF+FTC initiation in patients with treatment intensification (*n*=54). (B) Kaplan–Meier analysis in patients with treatment intensification (*n*=54).

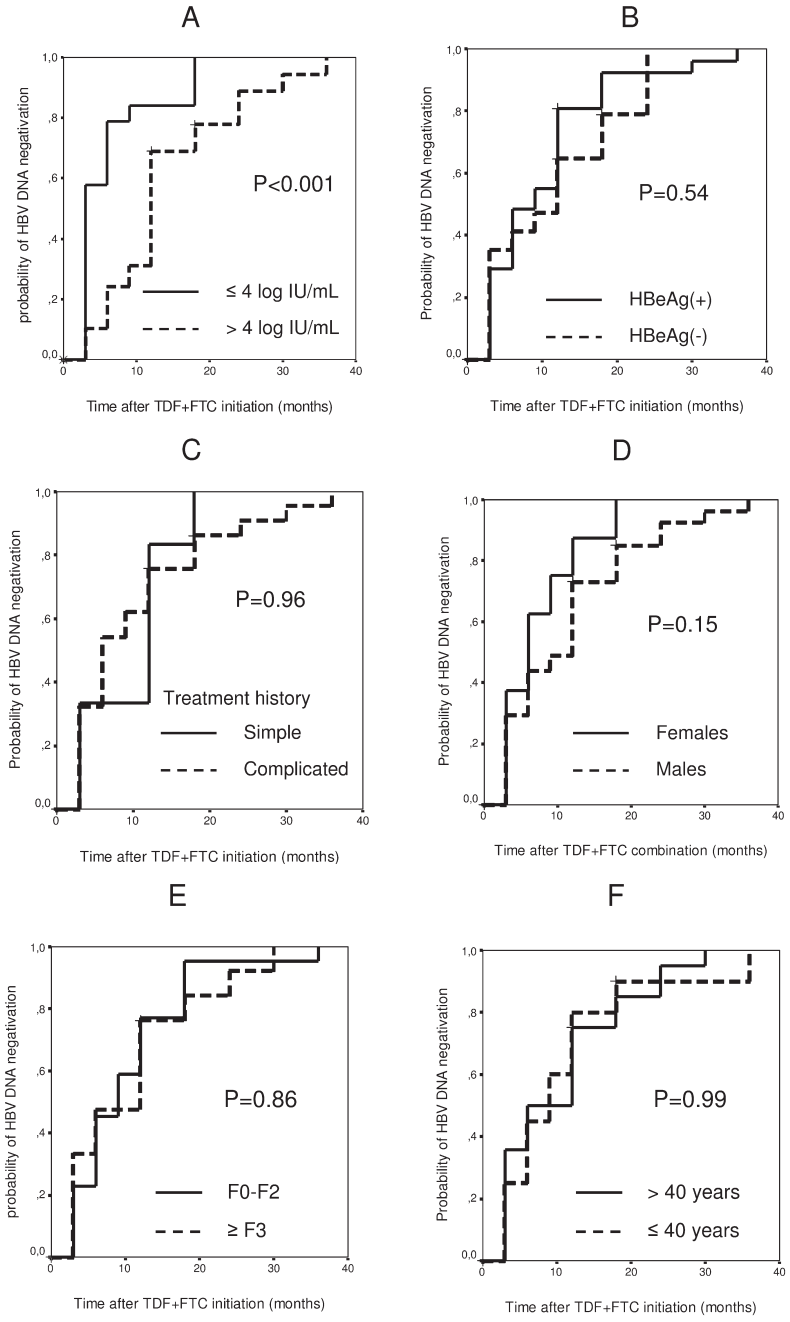

Figure 2 Kaplan–Meier analysis giving the probability of HBV-DNA negativity according to HBV-DNA level at baseline (A), HBeAg status at baseline (B), previous treatment history (C), gender (D), fibrosis at baseline (E), and age (F).

Analysis of clinical endpoints

No ALT flare occurred during follow-up for all patients. Two patients with decompensated cirrhosis at inclusion improved during the treatment-intensification follow-up. Four patients with treatment-intensification and cirrhosis decompensated during follow-up. Two of them already had an HCC at TDF+FTC initiation (one died), one developed HCC during follow-up and one had a cholestatic hepatitis associated with denutrition. HBV-DNA was undetectable at decompensation in three of these four patients.

Interestingly, HBe seroconversion was observed in 3 patients, who were HBeAg positive and received TDF+FTC intensification, at week 4, 16, and 112, respectively. HBs seroconversion was observed in only one patient in the treatment intensification group after 28 weeks of combination therapy.

Safety

Creatinine levels did not show kidney-function deterioration in either group of patients (Figure 3). Only one of the 78 patients had an increased creatinine level that was greater than 1.5 times the baseline value. Creatinine level in this patient increased from 92 µmol/L at baseline to 149 µmol/L at 24 weeks, but decreased back to normal value (76 µmol/L) at 48 weeks.

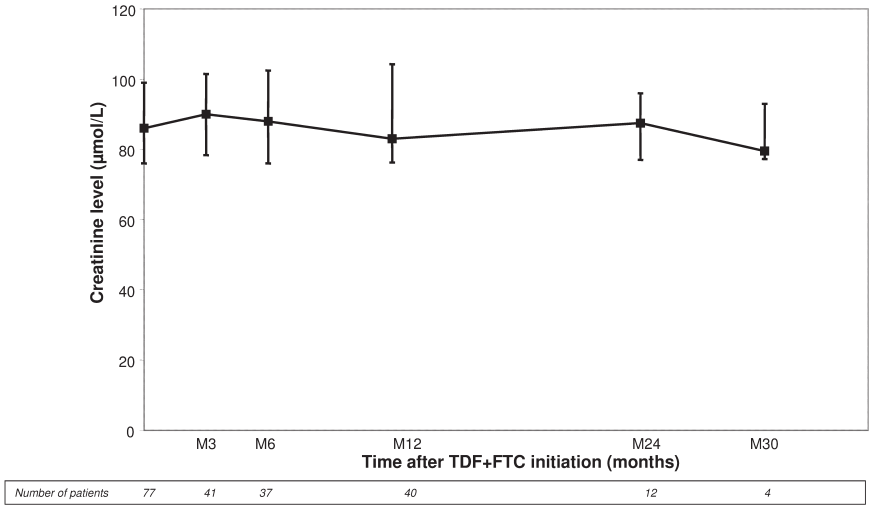

Figure 3 Median creatinine level, over time, in all patients (*n*=77). Error bars indicate the interquartile range.

DISCUSSION

To our knowledge, we have reported one of the largest clinical experience on the combination of TDF+FTC for the treatment of CHB besides clinical trials. Based on unselected patients, this longitudinal multicenter cohort study could be considered as representative of what is usually seen in routine clinical practice. Patients' baseline characteristics are indeed similar to previously reported data from field-practice experience. Age, gender, and significant fibrosis distributions were no different from what has been reported by Levrero et al. in a study that included 85 consecutive TDF-treated patients with a suboptimal response to adefovir (ADV) after 48 weeks of ADV±lamivudine.[19] These baseline characteristics are also similar to those of nucleos(t)ides-naive patients from an Italian cohort treated with entecavir (ETV) [20] and are similar to naïve or previously treated patients undergoing TDF therapy.[21]

The majority of our patients had a previous complicated treatment history. Only seven patients (belonging to the treatment-intensification group) had a simple history defined as no previous treatment (*n*=4) or treatment with a single molecule (*n*=3) during a maximum of one year. Our study, conducted in CHB patients from five European centers, shows that the probability of viral suppression increased over time to reach 76% after 48 weeks of treatment with the TDF+FTC combination and 94% after 96 weeks, thereby suggesting that TDF+FTC should be maintained beyond 48 weeks of therapy in patients with persistent viral replication.

In a recent randomized study that included 105 treatment-experienced patients with ADV failure and that compared a TDF monotherapy with a TDF+FTC combination, viral suppression was equivalent in both treatment arms and was achieved in 81% of subjects by week 48 [22] and in 82% of patients by week 168.[23] During a large multicenter long-term-resistance surveillance study in TDF-treated patients,[24] 51of 641 patients (8%) were viremic (>400 copies/mL) after week 72 and were eligible for an add-on comparison strategy.[25] Of these 51 patients, 13 (25%) remained on the TDF monotherapy whereas 38 (75%) also received FTC. Remaining on TDF monotherapy appeared to be as effective as adding FTC to ongoing TDF with, respectively, 69% and 61% of patients achieving viral suppression by week 192. Moreover, no viral resistance occurred during this period.[26]

These studies suggest that both strategies (TDF monotherapy or TDF+FTC) give similar results in terms of viral suppression. Of particular note, a recently published randomized study conducted at 39 sites worldwide, in patients with decompensated CHB liver disease with no history of previous treatment with ADV, TDF, or ETV, reported that viral suppression was obtained by week 48 in 70% of patients treated with TDF, 73% of patients treated with ETV, and 88% of patients treated with a TDF+FTC combination therapy.[27] However, this phase II study was designed to assess safety,

and was not powered to assess virological efficacy. It was, therefore, not possible to determine whether the TDF+FTC combination was superior or not in terms of viral suppression in this patient population.

In our study, time to viral suppression was shorter in patients with a low baseline viral load, which underlines the importance of early adaptation of treatment in cases of treatment failure, even in the absence of detectable mutation or biochemical breakthrough.

Our study failed to correlate viral response with known co-morbidity factors (Figure 2b, c, d), probably because our study population was mainly composed of difficult-to-treat patients. Our descriptive data on clinical endpoints are limited. Although it will take longer before the clinical benefits can be assessed, we have observed only four cases of decompensated cirrhosis with clinical impairment (one patient died from HCC), which is less than what was observed with a placebo or lamivudine,[28] or in a cohort of decompensated cirrhosis patients undergoing lamivudine treatment.[29] On the other hand, two patients with decompensated cirrhosis at baseline showed significant clinical improvement. Interestingly, Liaw et al. reported that TDF, ETV, and TDF+FTC were well tolerated in patients with decompensated cirrhosis, with improvement in biochemical and clinical parameters.[27] Six patients died (all deaths not being considered related to study drugs) and six underwent a liver transplantation.

The main safety issue of nucleotide analogs is kidney dysfunction, especially with proximal tubulopathy.[30-31] At the time of the study's design, we focused on late markers estimated by the stability of serum creatinine. Except for one patient, our data did not suggest any kidney function impairment. Future studies assessing safety should also measure early markers of tubular dysfunction as well as comorbidities, which may also play a major role.

In conclusion, our results indicate that, in difficult-to-treat patients with a high exposure to antiviral drugs, a combination of TDF+FTC can reach a very high rate of viral suppression after 48 weeks of therapy, which further increased after 96 weeks. Several international guidelines still recommend treatment adaptation if viral suppression is not reached at one year. However, in the present study, HBV-DNA kinetics beyond 48 weeks of therapy strongly suggests maintaining treatment since no breakthrough has occurred. Several other studies confirm that, in cases of partial virological response, maintaining treatment allowed HBV-DNA to become undetectable over time.[32]

Thus, in our study, performed in the clinical practice with patients heavily exposed to antivirals, the TDF+FTC combination showed antiviral efficacy and a good safety profile in all patients, and may represent an interesting clinical option to increase barriers to resistance. Long-term follow-up will determine whether the objective of viral suppression can be reached in all these difficult-to-treat patients.

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Virological response to entecavir is associated with a better clinical outcome in chronic hepatitis B patients with cirrhosis

3

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ABSTRACT

Background

Entecavir (ETV) is a potent inhibitor of viral replication in chronic hepatitis B (CHB) and prolonged therapy may result in regression of fibrosis. The aim of this study was to investigate the effect of ETV on disease progression.

Methods

In a multicenter cohort study, we investigated 372 ETV treated patients. Clinical events were defined as developing hepatocellular carcinoma (HCC), hepatic decompensation or death. Virological response (VR) was defined as HBV DNA <80 IU/mL.

Results

Patients were classified as CHB without cirrhosis (n=274), compensated cirrhosis (n=89) and decompensated cirrhosis (n=9). Probability of VR was not influenced by severity of liver disease (p=0.62). During a median follow up of 20 (interquartile range 11-32) months, probability of developing clinical events was higher for cirrhotic patients (HR 15.41 (95% CI 3.42-69.54), p<0.001). VR was associated with a lower probability of disease progression (HR 0.29 (0.08-1.00), p=0.05), also after correction for established risk factors such as age. Benefit of VR was only significant in patients with cirrhosis (HR 0.22 (0.05-0.99), p=0.04), and remained after excluding decompensated patients (HR 0.15 (0.03-0.81), p=0.03). A higher HBV DNA threshold of 2000 IU/mL was not associated with probability of disease progression (HR 0.20 (CI 0.03-1.10), p=0.10).

Conclusions

VR to ETV is associated with a lower probability of disease progression in cirrhotic patients, even after correction for possible baseline confounders. When using a threshold of 2000 IU/mL, the association between viral replication and disease progression was reduced, suggesting that complete viral suppression is essential for NA therapy, especially in cirrhotic patients.

INTRODUCTION

An estimated 300-400 million people are chronically infected with hepatitis B virus (HBV), and over 500,000 chronic hepatitis B (CHB) patients die annually from complications of (decompensated) cirrhosis and hepatocellular carcinoma (HCC).[1] The ultimate goal of CHB therapy is thus prevention of cirrhosis, hepatic decompensation and/or HCC.[2] A pivotal study from Asia showed that the risk of progression to cirrhosis, HCC and liver-related mortality strongly correlates with circulating HBV DNA levels, and a reduction of HBV DNA levels to low or undetectable has been adopted as an important endpoint for measuring antiviral efficacy in CHB patients.[3-4]

Entecavir (ETV) showed superior biochemical and virological efficacy compared to lamivudine (LAM) in large phase III trials including CHB patients with compensated liver disease.[5-6] In addition, Shim et al recently showed that 1 year of ETV monotherapy is similarly effective in decompensated as in compensated patients.[7] Importantly, after a median of 6 years of continuous ETV therapy, 88% of patients treated with ETV showed improvement in fibrosis score, including 10 patients with advanced fibrosis or cirrhosis at baseline.[8] In addition, Liaw et al showed that LAM therapy was able to reduce the incidence of disease progression and the risk of HCC in patients with advanced fibrosis or cirrhosis compared with placebo.[9] However, besides histological improvement, ETV monotherapy has not yet clearly proved its value in prevention of clinical events such as HCC, decompensation and death.

The aims of this cohort study were therefore (1) to compare the antiviral efficacy of ETV between patients with different severity of liver disease at baseline, (2) to explore baseline factors associated with the occurrence of clinical progression and (3) to investigate whether virological response during ETV therapy results in lower probability of clinical progression in CHB patients.

MATERIALS AND METHODS

Study population

In this investigator-initiated cohort study within the European network of excellence for Vigilance against Viral Resistance (VIRGIL), all consecutive adult CHB patients (HBsAg positive for at least 6 months) treated with ETV for at least 3 months between 2005 and May 2010 in 10 large European referral centers were included. Patients were excluded if they had viral co-infections (HIV, HCV, HDV), had an HCC at baseline and if they had undergone a liver transplantation before start of ETV therapy. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and

the principles of Good Clinical Practice. Patients gave informed consent according to standards of the local ethics committees.

Follow-up of participants

All subjects were treated and prospectively monitored at the discretion of the local treating physician. Every 3-6 months routine examination with biochemical (serum ALT, bilirubin, albumin, INR, creatinine) and virological (serum HBV DNA level, HBeAg, anti-HBe, HBsAg, anti-HBs) assessments took place. Screening for HCC was performed at least yearly by alpha-fetoprotein and/or ultrasound in cirrhotic patients and in non-cirrhotic patients when other risk factors were present.[10] Diagnosis of compensated cirrhosis at baseline was based on the following criteria: histological and/or ultrasound signs associated with cirrhosis (spleen size >12 cm, portal vein>16 mm, or nodules within the hepatic parenchyma). Diagnosis of decompensated cirrhosis at baseline was based on the presence of: ascites confirmed by ultrasound, jaundice with a bilirubin level >2.0 mg/dL, bleeding esophageal varices, or hepatic encephalopathy in cirrhotic patients.

Endpoints

The primary outcome was the occurrence of a clinical event defined as a composite of development of hepatic decompensation, HCC, or death. Hepatic decompensation was defined according to previously listed criteria in patients previously compensated. HCC was confirmed by either histo-cytological examination or was diagnosed if 2 coincident imaging techniques (ultrasound, computed tomography, or magnetic resonance imaging) showed a focal lesion larger than 2 cm with arterial hypervascularization, or if one imaging technique showed a focal lesion larger than 2 cm with arterial hypervascularization in the presence of an alpha fetoprotein level greater than 400 ng/mL. Secondary endpoints were virological response (VR, serum HBV DNA levels <80 IU/mL), HBeAg seroconversion (in HBeAg-positive patients), HBsAg seroclearance and biochemical response (ALT normalization in patients with abnormal ALT at baseline).

Laboratory tests

Serum ALT, bilirubin, albumin levels and INR of prothrombin time were measured locally using standardized automated techniques. Hepatitis B surface antigen (HBsAg), antibody against HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays. Serum HBV DNA levels were measured using a quantitative real-time polymerase chain reaction assay, the COBAS AmpliPrep-COBAS TaqMan HBV test (CAP-CTM; Roche Molecular Systems, Inc., Branchburg, NJ, USA), with a

lower limit of detection of 12 IU/mL, in nine of ten centers. In one center serum HBV DNA was measured using Roche Amplicor (linear dynamic range, 400 to 200,000 copies/mL; Roche Diagnostic Systems, Branchburg, NJ, USA). A conversion factor of 5.26 copies/IU was used for conversion of copies/mL to IU/mL. HBV genotypes and detection of HBV polymerase gene mutations was determined by direct sequencing or by line-probe assay (Innogenetics, Gent, Belgium).

Data acquisition and analysis

Data acquisition from the patients' chart was performed by one experienced investigator (RZ). Data was systematically collected through a standardized clinical research form and entered for analysis within a database. HBV DNA levels were logarithmically transformed for analysis. ALT levels are expressed as values representing a ratio to the local upper limit of normal (xULN). Continuous variables are expressed as means ± SD or median (interquartile range, IQR) where appropriate. Follow up time was calculated as the time interval between start of ETV therapy and diagnosis of a clinical event or the end of follow-up. Patients were censored when antiviral therapy regimen was adapted. The cumulative probability of achieving primary or secondary endpoints was estimated by Kaplan-Meier analysis. The influence of VR was analyzed by a time-dependent analysis. Therefore VR was entered within a model as a time-dependent co-variable: all patients started (and thus were at risk) within the group without VR and were switched to the group with VR after achieving this endpoint. All baseline variables with a p-value <0.10 within the univariate analysis were entered within a multivariate model. All statistical tests were two-sided, and a *P* value <0.05 was considered to be statistically significant. SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics

In total, 437 chronic HBV patients treated with ETV were identified. Sixty-five patients did not fulfill the entry criteria and were excluded. Forty patients were treated for less than 3 months, 1 patient was less than 6 months HBsAg positive, 9 patients were co-infected with HCV or HDV, 5 patients had an HCC at baseline, 2 patients had undergone liver transplantation and 18 patients received concomitant antiviral therapy. A total of 372 patients were thus eligible for this analysis. The study population consisted of 274 patients with CHB without cirrhosis, 89 patients with compensated cirrhosis and 9 patients with decompensated cirrhosis. Baseline characteristics of the study population are shown in table 1 according to their initial baseline liver

Table 1 Baseline characteristics of the study population according to the severity of liver disease at baseline.

	No cirrhosis	Cirrhosis	Decompensated cirrhosis	
	N=274	N=89	N=9	P value
Age	41±14	51±14	51±10	<0.001
Gender (male %)	200 (73%)	71 (80%)	6 (67%)	0.38
Race				0.28
Caucasian	137 (50%)	41 (46%)	3 (33%)	
Asian	78 (29%)	19 (21%)	4 (44%)	
Other	59 (22%)	29 (33%)	2 (22%)	
ALT (xULN)	1.6 (1.0-3.0)	1.4 (1.0-3.1)	2.8 (1.9-18.2)	0.008
HBV DNA (Log10 IU/ml)	5.9±2.1	5.3±2.2	6.7±1.5	0.05
HBeAg-positive	116 (42%)	56 (63%)	4 (44%)	0.62
Genotype (N=277)				0.71
A	41(19%)	14 (24%)	2 (29%)	
B	21 (10%)	4 (7%)	-	
C	28 (13%)	7 (12%)	3 (43%)	
D	104 (49%)	29 (50%)	2 (29%)	
Other	18 (9%)	4 (7%)	-	
Bilirubin (mg/dl)	0.7±0.6	0.9±0.7	8.7±10.8	<0.001
Albumin, g/dl	4.4±0.4	4.1±0.5	3.1±0.2	<0.001
INR	1.0±0.1	1.2±0.2	1.4±0.2	<0.001
Platelet count (103/µl)	213±57	140±63	118±100	<0.001
Dosage entecavir (0.5 mg %)	229 (84%)	61 (69%)	5 (56%)	0.004
Previous treatment with (PEG-)IFN	64 (23%)	18 (20%)	-	0.18
Previous treatment with LAM	56 (20%)	29 (33%)	4 (44%)	0.02
Previous treatment with ADV	36 (13%)	28 (32%)	2 (22%)	0.001

disease severity. Overall median follow-up of the study population was 20 (IQR 11-32) months, and did not differ between the 3 groups (p=0.24).

Virological, serological and biochemical endpoints

The cumulative probability of achieving VR was 68% at week 48, 87% at week 96 and 93% at week 144 of ETV therapy. VR rates were not significantly influenced by severity of liver disease at baseline (p=0.62; figure 1). Also after correction for important baseline variables (HBeAg status, HBV DNA, previous LAM and ADV exposure), probability of achieving VR was not different between the 3 groups (p=0.50). HBeAg seroconversion was achieved in 26 (17%) of 154 HBeAg-positive patients and tended to be higher in patients with decompensated cirrhosis (p=0.06). HBsAg seroclearance

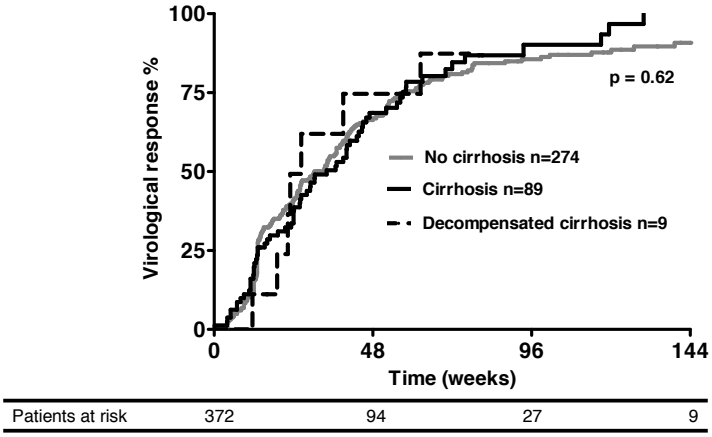
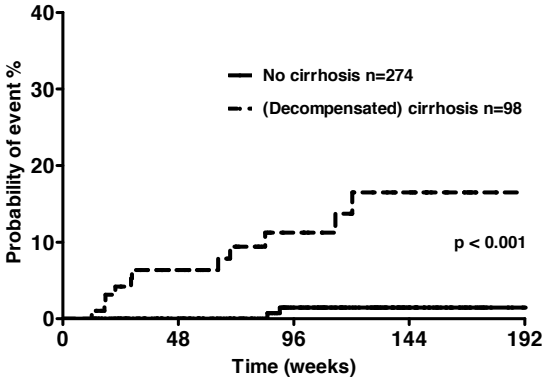


Figure 1 Kaplan-Meier for the cumulative probability of achieving virological response for entecavir treated patients. Stratified according to severity of liver disease at baseline.

was achieved in 3 HBeAg-positive and 3 HBeAg-negative patients and did not differ between the 3 groups (p=0.81). Genotypic resistance to ETV was detected in 5/111 (5%) NA-experienced patients. Biochemical response (normalization of ALT) was achieved in 200 (78%) of the 255 patients with a baseline ALT above the upper limit of normal. The biochemical response rates were also comparable between the 3 groups (p=0.35). MELD score did not change significantly for the 2 groups with cirrhosis at baseline during the study period (+0.0±2.9 for compensated cirrhosis, p=0.95 and -1.4±6.9 for decompensated cirrhosis, p=0.67).

Factors associated with clinical events in patients treated with entecavir

Within the study population 13 patients developed a clinical event: 6 patients developed an episode of hepatic decompensation (3 of these patients died), 3 patients developed an HCC and 7 patients died. The cumulative probability of an event was 0%, 2%, 2% and 2% at years 1, 2, 3 and 4 for non-cirrhotics versus 6%, 11%, 17% and 17% for cirrhotic patients (Figure 2, p<0.001). The higher cumulative probability of an event in cirrhotic patients remained when excluding decompensated patients (p<0.001). Two patients, both without cirrhosis, developed an ALT flare (ALT >10xULN) on therapy, but both patients did not experience clinical disease progression subsequently. Three of 9 decompensated patients had an ALT flare at baseline, of whom all achieved ALT normalization. In univariate analysis, patients with an event tended to be older (p=0.12, table 2) and more often had cirrhosis (p<0.001). Virological



Patients at risk					
No cirrhosis	274	205	123	50	6
Cirrhosis	98	76	42	23	2

Figure 2 Kaplan-Meier for the cumulative probability of developing a clinical event for entecavir treated patients. Stratified according to the presence of (decompensated) cirrhosis at baseline. A clinical event was defined as developing hepatocellular carcinoma, hepatic decompensation or death.

Table 2 Univariate Cox analysis of potential risk factors for developing clinical events within 372 patients treated with entecavir.

Risk factor	HR (95% CI)	P value
Age (per year)	1.03 (0.99-1.07)	0.12
Female gender	0.52 (0.12-2.35)	0.52
Asian ethnicity	0.88 (0.24-3.21)	0.85
ALT (xULN)	1.00 (0.92-1.08)	0.90
HBV DNA (Log10 IU/ml)	1.07 (0.82-1.39)	0.62
HBeAg-negativity	1.60 (0.54-4.75)	0.40
Genotype A	0.99 (0.21-4.67)	0.99
Bilirubin (mg/dl)	1.00 (0.99-1.01)	0.54
Albumin, (g/dl)	0.74 (0.67-0.83)	<0.001
INR	38.53 (6.12-242.62)	<0.001
Platelet count	0.99 (0.98-1.00)	0.02
Cirrhosis	15.41 (3.42-69.54)	<0.001
Decompensated cirrhosis	16.76 (4.58-61.29)	<0.001
Previous treatment with (PEG-)IFN	0.23 (0.03-1.80)	0.16
Previous treatment with LAM	2.85 (0.96-8.49)	0.07
Previous treatment with ADV	2.52 (0.83-7.71)	0.11
Virological response*	0.29 (0.08-1.00)	0.05

*According to a Cox model with this variable as a time dependent covariate.

breakthrough was observed in 18 patients of whom 2 patients developed a clinical event. In 8 (44%) of these patients non-compliance was suspected to be the cause of the virological breakthrough, as no resistant mutants were detected. However, only in 1 patient did the occurrence of virological breakthrough coincide with decompensation and death. The occurrence of a virological breakthrough was not associated with a higher probability of disease progression (p=0.14).

Virological response and clinical events

Five patients (1 non-cirrhotic patient, 3 patients with compensated cirrhosis and 1 patient with decompensated cirrhosis) developed a clinical event after achieving VR (Figure 3), with a median time to VR of 32 (15-53) weeks and a median time to event of 65 (28-88) weeks. To investigate the clinical effect of response to ETV, we studied the influence of VR on developing clinical events, with VR as time-dependent covariate within a Cox model (Table 2, figure 4A). Patients with a VR during ETV therapy had a lower probability of developing a clinical event (HR 0.29, 95% CI 0.08-1.00, p=0.05). This effect was both significant among all patients with cirrhosis (HR 0.22, 95% CI 0.05-0.99, p=0.04, figure 4B) and among those with compensated cirrhosis alone (HR 0.15, 95% CI 0.03-0.81, p=0.03). For the 9 patients with decompensated cirrhosis, VR was not significantly associated with a lower probability of disease progression (95% CI 0.06-13.06, p=0.86), which is probably influenced by the small number of patients within this group. VR was also not significantly associated with a lower probability of disease progression in the non-cirrhotic population (HR 0.24, 95% CI 0.02-3.76, p=0.27)Importantly, when separately including age or other significant baseline variables within a multivariate model, VR remained significantly associated with a lower

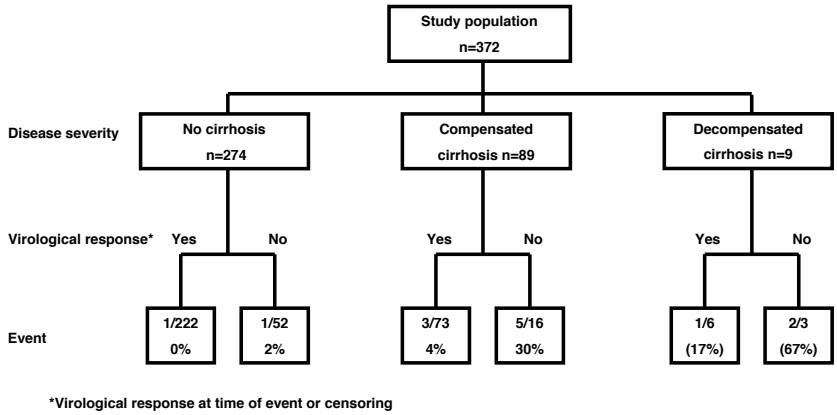


Figure 3 Distribution of clinical events within the study population according to the severity of liver disease at baseline and achievement of VR (HBV DNA <80 IU/mL).

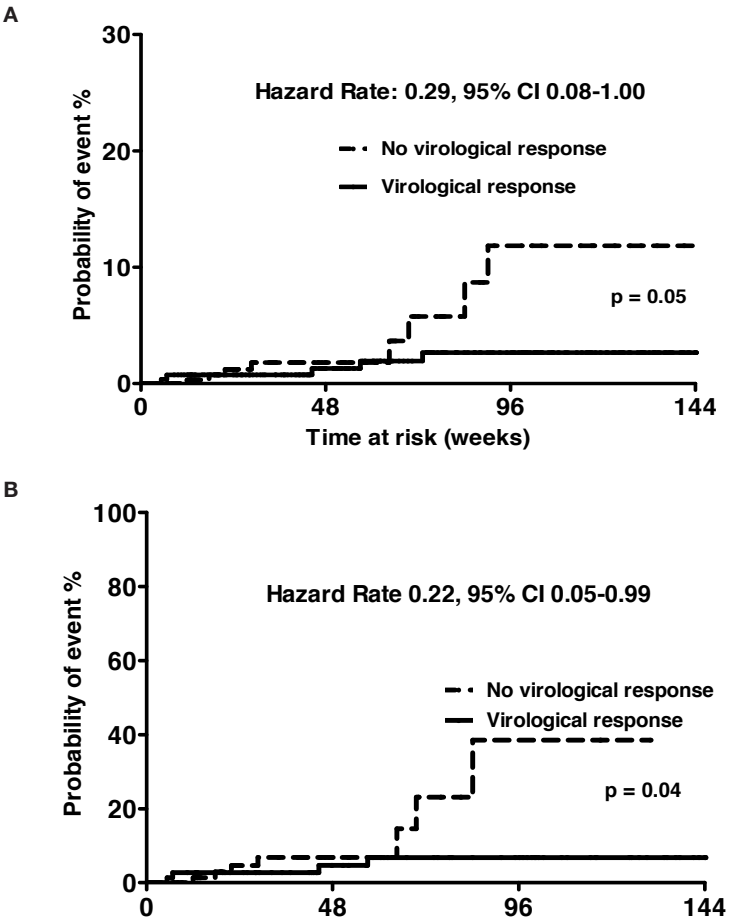


Figure 4 The cumulative probability of developing a clinical event during entecavir therapy for (A) all patients and (B) patients with cirrhosis, according to virological response. For this analysis, all patients started within the group without virological response and were censored and switched to the virological response group when HBV DNA <80 IU/mL was achieved and followed until occurrence of event or censoring. Time in the virological response group was thus calculated minus time to achieve virological response.

probability of events for the cirrhotic population (Table 3). Due to the limited number of events within the study population we could only include one baseline variable besides VR within the models. The influence of VR within the cirrhotic population became even stronger when early clinical events were excluded. When events during the first 6 or 12 months were excluded the association between VR and clinical events remained significant (HR 0.14, 95% CI 0.03-0.77, p=0.02 and HR 0.07, 95% CI 0.01-0.48, p=0.006).

Since an HBV DNA <2000 IU/mL is associated with immune control in natural history and peginterferon studies, we investigated the influence of achieving this endpoint for patients with cirrhosis, instead of the previously used HBV DNA <80 IU/mL, on occurrence of clinical events. When HBV DNA <2000 IU/mL was applied as a time dependent covariate within a Cox model, achievement of this endpoint was not significantly associated with a lower probability of developing clinical events for patients with cirrhosis (HR 0.20, 95% CI 0.03-1.10, p=0.10). In contrast, for a threshold of 200 IU/mL (HR 0.12, 95% CI 0.03-0.58, p=0.007) the association with probability of disease progression remained significant.

Table 3 Multivariate Cox models including virological response and different baseline variables. For 98 patients with (decompensated) cirrhosis at baseline treated with entecavir. Virological response was included in all models as time dependent covariate.

	HR (95% CI) Virological response	P-value
Model 1		
Virological response & age	0.22 (0.05-0.99)	0.04
Model 2		
Virological response & albumin	0.02 (0.00-0.44)	0.01
Model 3		
Virological response & INR	0.15 (0.02-1.00)	0.04
Model 4		
Virological response & platelets	0.11 (0.02-0.75)	0.01

DISCUSSION

This is the first study showing that VR to ETV reduces the probability of developing clinical events in CHB patients with cirrhosis. Importantly, this relationship remained significant when adjusted for different baseline variables. The probability of achieving VR within our study population was not influenced by liver disease severity at baseline.

Current antiviral therapy focuses on established surrogate endpoints such as HBV DNA suppression, as well as HBeAg and HBsAg seroconversion to assess and compare antiviral therapy in the short term as these endpoints are easy to measure and occur relatively frequently.[2] Treatment with the most potent antivirals, such as ETV and tenofovir (TDF), led to VR in the vast majority of patients, both within the registration trials and within large academic cohort studies.[11-14] Moreover, Chang et al. showed that continuous long-term ETV was able to reduce fibrosis, even in the presence of cirrhosis at baseline.[8] In addition, adefovir has shown to be effective and safe in patients

before and after liver transplantation, resulting in an improved clinical status.[15] Recent studies by Liaw et al. showed in decompensated patients that ETV was superior to adefovir (ADV) on virological endpoints and that ETV, TDF and the fixed combination of TDF+emtricitabine had a similar safety profile and a comparable efficacy.[16-17]

A survival benefit has only been shown for LAM treated patients with advanced liver disease. LAM therapy reduced the risk of disease progression and development of HCC in the absence of genotypic resistance, compared to patients treated with placebo.[9] A second observation within this study was that patients developing LAM resistance were at increased risk for developing liver related complications.[9] This underlines that, especially in patients with advanced liver disease, first line therapy should comprise a potent NA to avert viral resistance and suppress HBV DNA to the lowest level possible and to avoid viral resistance. Our findings are thus in accordance with this previous study in LAM treated patients and underline the preventive effect of potent NA therapy for cirrhotic patients in reducing the risk of developing clinical events such as hepatic decompensation, HCC and death. This lower probability of disease progression in patients with (decompensated) cirrhosis who achieved VR was present even after correction for different baseline variables within a multivariate model. In contrast, for the non-cirrhotic population there was no significant effect of VR on clinical disease progression. This discrepancy is probably caused by the relatively low incidence of complications within this population.[4] A longer follow-up and more patients would probably be required to find a preventive effect of treatment. A recent study from Greece could not show the prevention of HCC development in LAM treated patients, but a trend towards this effect was found after VR (HBV DNA <200 IU/mL) in patients without cirrhosis.[18] However, when a clinical threshold is passed, potent NA therapy is not always lifesaving in patients with decompensated cirrhosis. An initial high rate of disease progression was also seen in our 9 decompensated patients, but this small group does not allow us to draw conclusions on this topic.

Interestingly, when investigating a higher HBV DNA threshold of 2000 IU/mL, which in the natural history is generally used to distinguish inactive carriers from active disease (based on a higher risk of developing HCC and progression of disease),[3-4] we could not find a significant association between achieving this virological threshold and developing clinical events within our study. This suggests on one hand that a possible difference between a host-induced and NA-induced inactive carrier state (HBV DNA <2000 IU/mL) and on the other hand that NA induced viral suppression should probably be quite vigorous to minimize the risk of developing complications.

In conclusion, our study showed the beneficial effects of VR to ETV in patients with cirrhosis, in preventing liver disease progression for CHB patients. VR during potent NA therapy is achieved in the majority of patients and could thus minimize risk of developing complications.

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Peginterferon results in higher serological, but not virological, response rates when compared to continuous entecavir

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ABSTRACT

Background

HBeAg and HBsAg clearance are associated with an improved prognosis in chronic hepatitis B patients (CHB). These endpoints are more often achieved with a one year course of peginterferon (PEG-IFN) compared with one year of nucleos(t)ide analogue (NA) therapy. However, prolonged NA therapy may result in comparable serological response rates as with PEG-IFN.

Methods

We compared serological and virological response rates among HBeAg-positive CHB patients treated with long-term continuous entecavir (ETV) (n=91) for a median of 92 (IQR 50-132) weeks or one year of PEG-IFN (n=266) with comparable follow up.

Results

Median follow-up was 92 weeks (IQR 78–198) for patients treated with PEG-IFN, and 92 (IQR 50-132) weeks for patients treated with ETV. Finite PEG-IFN therapy resulted in significantly higher rates of HBeAg seroconversion (adjusted hazard ratio (HR): 3.16, $p<0.001$) and HBsAg clearance (HR 5.66,$p=0.027$) when compared to prolonged ETV treatment, whereas ETV resulted in higher rates of HBV DNA undetectability (OR 31.14, $p<0.001$) also after adjustment for HBV genotype and other relevant baseline factors.

Conclusions

Our study shows that finite PEG-IFN is associated with a higher probability of serological, but not virological, response for HBeAg-positive CHB patients when compared to prolonged ETV, even after correction for baseline differences.

BACKGROUND

Prolonged infection with the hepatitis B virus (HBV) may ultimately result in severe liver-related morbidity and mortality, and treatment of CHB is therefore indicated in patients with persistent liver inflammation.[1-3] HBeAg seroconversion (HBeAg clearance with positive anti-HBe) and Hepatitis B surface Antigen (HBsAg) seroclearance are important treatment end-points in HBeAg-positive CHB,[1] since they are associated with disease remission, a reduced risk of hepatocellular carcinoma and an improved prognosis.[4-6] These serological endpoints are more often achieved with a one year course of peginterferon (PEG-IFN) when compared to one year of nucleo(s)tide analogues (NA), but prolonged NA therapy may result in serological response rates approximating those achieved with PEG-IFN.[7-8] However, head-to-head comparisons of finite PEG-IFN versus long-term NA therapy have not been performed, and differences in baseline characteristics prohibit direct comparison of previously published study results. We therefore aimed to compare rates of HBeAg seroconversion and HBsAg clearance, as well as HBV DNA undetectability, in HBeAg-positive CHB patients treated with continuous entecavir (ETV) monotherapy or one year of PEG-IFN with subsequent off-treatment follow-up.

METHODS

Patients and follow-up

A total of 266 HBeAg-positive CHB patients were treated with PEG-IFN alfa-2b (PegIntron, Schering-Plough, Kenilworth, USA) for 52 weeks ± lamivudine (LAM, Zeffix, GlaxoSmithKline, Greenford, UK).[8] Patients were subsequently followed-up for another 6 months off-treatment and were enrolled in a long-term follow-up study.[9] Key inclusion criteria for this study were: HBsAg positive for at least 6 months before randomization, HBeAg positivity, elevated serum alanine aminotransferase (ALT) levels <10 times the upper limit of normal (ULN), and serum HBV DNA of more than 1.0×10^5 copies/mL. Another 91 consecutive NA-naïve patients were treated with ETV 0.5 mg daily as recommended by current treatment guidelines and were followed-up at the outpatient clinic at least every 3-6 months. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice.

Laboratory assays

(Anti-)HBeAg and HBsAg tests were performed using commercially available ELISA kits. HBV DNA was measured at baseline using real-time Taqman based methods.[8] ALT was measured locally and was expressed as multiples of the upper limit of normal (ULN). HBV genotype was assessed by line-probe assay (Innogenetics, Ghent, Belgium).

Statistical analysis

HBeAg seroconversion (HBeAg negativity with anti-HBe) and HBsAg clearance rates were compared by Kaplan-Meier and Cox-proportional hazard analyses. Rates of HBV DNA undetectability were evaluated at week 78 (i.e. 6 months post-treatment for patients treated with PEG-IFN) and at last follow-up evaluation. The analysis at last follow-up was limited to patients treated with ETV for ≥78 weeks. Follow-up time was calculated from start of treatment, and is expressed as median with the interquartile range (IQR). Follow-up was terminated in patients retreated after PEG-IFN and serological status before retreatment used as outcome parameter. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used to perform statistical analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

Study Cohort

Treatment outcomes did not differ between patients treated with PEG-IFN ± LAM, and patients were therefore pooled for this analysis.[8-9] Median follow-up was 92 weeks (IQR 78–198) for patients treated with PEG-IFN, and 92 (IQR 50-132) weeks for patients treated with ETV ($p<0.001$). A total of 323, 271, 166, and 109 patients were still in follow-up at weeks 48, 78, 96 and 144, respectively. HBV genotype distributions among patients treated with PEG-IFN were A/B/C/D/other in 34%, 9%, 15%, 39% and 4%, compared to 27%, 8%, 21%, 33% and 11% in the ETV group ($p=0.11$). The PEG-IFN and ETV groups were also well-balanced with regard to age (34.95 versus 36.93 years, $p=0.21$), sex (78% male versus 75%, $p=0.55$) and previous IFN therapy (both 21%, $p=0.97$). Baseline HBV DNA levels were higher in patients treated with PEG-IFN (9.06 versus 7.98 log copies/mL, $p<0.001$) as were ALT levels (4.30 versus 3.05 times the upper limit of normal, $p=0.004$).

HBeAg seroconversion

A total of 114 (32%) patients achieved HBeAg seroconversion in a median of 78 weeks (IQR 52–120). By Kaplan-Meier analysis, the cumulative probability of HBeAg seroconversion was higher in patients treated with PEG-IFN for one year versus those treated with long-term ETV (p=0.007). PEG-IFN therapy remained an independent determinant of HBeAg seroconversion in a Cox proportional hazard model; the hazard rate (HR) for PEG-IFN versus ETV was 3.16 (95% CI: 1.64 – 6.75, p<0.001), after adjustment for HBV genotype, baseline ALT and baseline HBV DNA (Figure 1A).

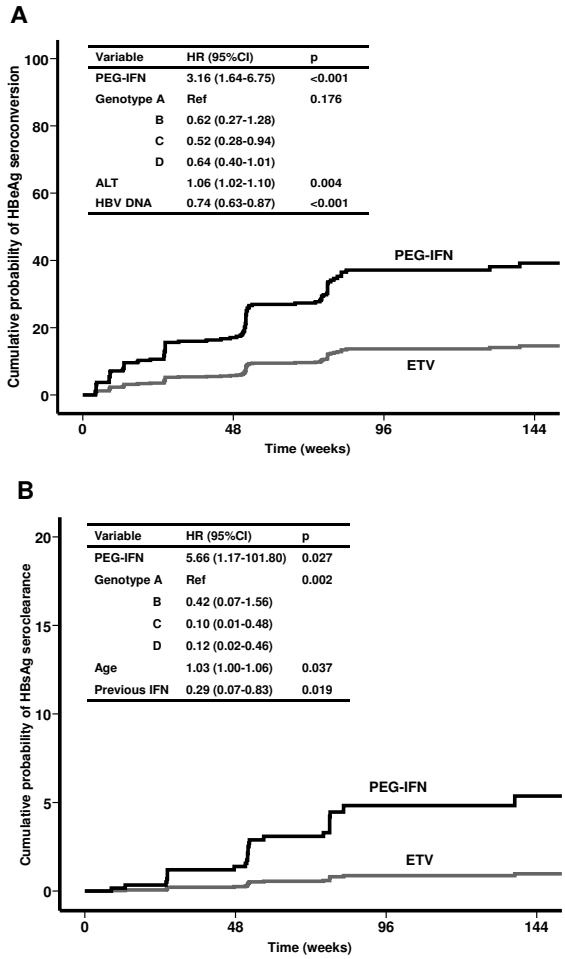

Figure 1 Cox-proportional hazard plots of the probability of HBeAg seroconversion (A) and HBsAg clearance (B) in patients treated with PEG-IFN (± LAM) or ETV.

HBsAg loss

A total of 30 (8%) patients cleared HBsAg in 92 (IQR 78–170) weeks. By Kaplan-Meier analysis, cumulative HBsAg clearance rates were higher in patients receiving PEG-IFN therapy compared to patients treated with ETV (p=0.032). In a Cox proportional hazards model, PEG-IFN therapy was independently associated with HBsAg clearance, with a HR of 5.66 (95% CI: 1.17 – 101.80, p=0.027) after adjustment for HBV genotype, age and previous IFN exposure (Figure 1B).

HBV DNA undetectability

At week 78, a total of 69 (21% of total cohort) patients had undetectable HBV DNA. HBV DNA undetectability was achieved in 77% of ETV treated patients, compared to only 8% of patients treated with PEG-IFN (p<0.001). At last follow-up evaluation, 90 (39%) of patients achieved undetectable HBV DNA, comprising 92% of patients on ETV, and 19% of PEG-IFN treated patients (p<0.001). In a logistic regression model, ETV therapy (OR 31.14, 95% CI: 13.30 – 72.90, p<0.001), HBV genotype A (OR 3.54, 95% CI: 1.58 – 7.95, p=0.002) and log HBV DNA (OR 0.65, 95% CI: 0.48 – 0.89, p=0.007) but not ALT (p=0.74) were independently associated with HBV DNA undetectability at week 78. An interaction term of HBV genotype and therapy was non-significant (p=0.26).

DISCUSSION

This retrospective cohort study compared a finite course of PEG-IFN to prolonged potent NA therapy for serological and virological response rates. We found that PEG-IFN results in higher rates of HBeAg seroconversion and HBsAg seroclearance than continuous ETV therapy, and this difference remained after adjustment for important baseline factors. Nevertheless, HBV DNA undetectability was achieved in only a minority of PEG-IFN treated patients, whereas most patients on ETV achieved this endpoint.

PEG-IFN and the NA have distinctly different modes of action in CHB.[10] PEG-IFN is an immunomodulator with limited direct antiviral efficacy that is able to induce a host immune response in a subset of patients.[11] NA competitively inhibit HBV polymerases and thus HBV DNA production. Several studies have shown that currently approved potent NA ETV and TDF can induce and maintain undetectable HBV DNA levels for prolonged therapy duration with a low risk of viral resistance or complications.[7, 12] However, relapse is common after discontinuation.[13] HBeAg seroconversion has previously been shown to be associated with an improved prognosis,[4-6] and is considered a first step towards immune control over HBV. The current study shows

that this endpoint is more often achieved with a finite course of PEG-IFN than with prolonged ETV therapy, suggesting that immune control can more often be achieved with PEG-IFN. However, long-term follow-up studies of patients treated with PEG-IFN have revealed that some patients maintain elevated HBV DNA levels after HBeAg seroconversion.[9, 14] Similarly, in patients treated with NA, viral rebound is frequently observed when therapy is discontinued after HBeAg seroconversion.[15]

Our study also shows that a finite course of PEG-IFN results in superior rates of HBsAg seroclearance, the closest outcome to clinical cure one can hope to achieve in CHB.[16-17] HBsAg clearance is durable, confers an excellent long-tem prognosis and is associated with a low probability of HBV reactivation in immune competent patients.[4, 18] Although HBsAg clearance is only rarely achieved with currently available agents, particularly in patients treated with NA, we showed that a reasonable proportion of patients treated with PEG-IFN may achieve this end-point during treatment and long-term off-treatment follow-up. This is in line with recent studies showing increased decline of HBsAg levels in patients treated with PEG-IFN, when compared to patients treated with ETV.[19-21]

Despite the serological responses, persistence of HBV DNA after PEG-IFN is a reality in the majority of patients.[9, 14] In contrast, nearly all patients on ETV achieved HBV DNA undetectability, and thus disease remission, while on-treatment. Taking into consideration the considerable side-effects of PEG-IFN and the limited response rates, PEG-IFN therapy should be limited to those patients with the highest chances of achieving both serological and virological response, whereas ETV is a powerful treatment option for the vast majority of patients.

Although the current study was not randomized, we conducted a thorough multivariate analysis including all major determinants of serological response.[22] Of note, the HBV genotype distribution was comparable in the two cohorts, and we adjusted for baseline ALT and HBV DNA levels, age, sex, presence of cirrhosis and previous IFN exposure when necessary. A second limitation is that we pooled patients treated with PEG-IFN ± LAM to increase power. Importantly, several independent randomized studies and a meta-analysis have revealed no benefits of combination therapy.[8, 13-14, 22]

In conclusion, our study shows that a finite course of one year PEG-IFN results in superior rates of HBeAg seroconversion and HBsAg seroclearance, but not HBV DNA undetectability, when compared to prolonged ETV monotherapy.

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5

Precore and core promoter mutants are associated with higher HBeAg seroconversion but low disease remission rates in HBV patients treated with nucleos(t)ide analogues

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ABSTRACT

Background

HBeAg seroconversion in chronic hepatitis B (CHB) patients is considered an important event as it is associated with better clinical outcome. However, a proportion might not achieve inactive disease after HBeAg seroconversion.

Methods

We determined presence of precore (PC) and base core promoter (BCP) mutations in 137 consecutive HBeAg-positive patients treated with nucleos(t)ide analogues (NA) at baseline by INNO-LiPA HBV PreCore assay (Innogenetics). Baseline HBeAg and HBsAg levels were measured using the ELECSYS platform.

Results

The majority (>60%) of patients with genotype B, C and D had PC/BCP mutants present at baseline, compared to only 40% of genotype A (p=0.02). Patients with mutants had lower HBV DNA (p=0.006), HBsAg (p=0.04) and HBeAg (p<0.001) levels compared to patients with wildtype. During a median treatment of 29 (IQR 18-56) months, 45 patients achieved HBeAg seroconversion. Probability of HBeAg seroconversion during therapy was higher in patients with PC/BCP mutants (HR 2.2, 95% CI: 1.13 - 4.44, p=0.01). After HBeAg seroconversion, patients with BCP mutants tended to have a higher probability of HBeAg relapse (p=0.07), and PC mutants tended to be associated with a lower probability of achieving HBV DNA <2000 IU/mL 1 year after seroconversion (p=0.07).

Conclusions

The presence of PC and/or BCP mutants in NA-treated HBeAg-positive patients appears an independent predictor of HBeAg seroconversion. However, BCP mutants were associated with a possible risk of serological relapse, while PC mutants tended to have a lower probability of achieving virological remission the first year after HBeAg seroconversion.

INTRODUCTION

Hepatitis B e Antigen (HBeAg)-positive chronic hepatitis B (CHB) is an early stage of the disease continuum.[1-3] HBeAg does not appear to be required for infection with hepatitis B virus (HBV), nor for viral replication, but the presence of HBeAg in serum is associated with higher levels of HBV DNA and was shown to be an risk factor for the development of hepatocellular carcinoma.[4-6] HBeAg clearance has therefore been adopted as a treatment endpoint for HBeAg-positive CHB in all major HBV guidelines.[3, 7]

About 20% of patients treated with nucleos(t)ide analogues achieve HBeAg seroconversion after one year of treatment.[7-8] However, emerging data show that mere loss of HBeAg from serum may be insufficient to induce disease remission. Indeed, reversal to HBeAg positivity is frequently observed after NA-induced HBeAg seroconversion, as is persistence of detectable HBV DNA levels after discontinuation of therapy.[9] Similarly, while HBeAg negativity induced by peginterferon (PEG-IFN) therapy seems to be more durable, a proportion of patients fail to achieve low levels of HBV DNA and HBsAg clearance.[10-12] A possible explanation for these observations is the presence of viral strains with mutations in the precore (PC) and basal core promoter (BCP) regions that prohibit the synthesis of HBeAg.[13] In patients treated with PEG-IFN, presence of these mutants predisposes to persistent replication after HBeAg clearance, possibly through positive selection during antiviral therapy,[14] and these mutants may therefore also predict persistence of substantial HBV DNA replication after HBeAg clearance in patients treated with NA.[14-15]

The aim of the current study was therefore to investigate the relationship between presence of PC or BCP mutants and the probability of NA-induced HBeAg seroconversion. Secondly, we assessed the relation between these mutants and true disease remission after HBeAg seroconversion in patients treated with NA.

PATIENTS AND METHODS

Study population

All consecutive patients who were serum positive for HBeAg and negative anti-HBe and treated with NA therapy for at least 6 months from January 1st 1996 onwards at the Erasmus MC University Medical Center Rotterdam were enrolled. Patients were excluded when no stored baseline sample was available for retesting, when co-infected with hepatitis C virus, hepatitis D virus or human immunodeficiency virus, when treated with peginterferon within 6 months before start of NA therapy or when peginterferon was (temporarily) added to the NA regimen.

Follow up evaluation

ALT and virological parameters were assessed at least every 3-6 months. HBeAg seroconversion was defined as loss of HBeAg with concurrent appearance of anti-HBe. HBsAg seroconversion was defined as loss of HBsAg with appearance of anti-HBs. Serological recurrence was defined as a confirmed reappearance of HBeAg in at least 2 consecutive samples after HBeAg seroconversion. Virological remission was defined as achieving HBV DNA <80 IU/ml within one year after achieving HBeAg seroconversion. In patients in whom treatment was stopped after HBeAg seroconversion accompanied by an HBV DNA <80 IU/mL, relapse was defined as confirmed HBV DNA level above 2000 IU/mL and/or restart of antiviral therapy.

Laboratory testing

ALT level was measured using automated techniques. The presence of PC and/or BCP mutants was assessed at baseline by Innolipa HBV Precore assay (Innogenetics, Ghent, Belgium). This sensitive probe assay allows for detection of PC mutants (at nucleotide position G1896) and BCP mutants (at nucleotide positions A1762 and G1764), even when a minority species is present.[16] Patients were thus classified as having wildtype virus (WT, no PC or BCP mutants were detected), PC (PC mutants detected with or without WT), BCP (BCP mutants detected with or without WT), or as both (both PC and BCP mutants were found). HBV DNA quantification was performed using a TaqMan polymerase chain reaction (PCR) assay (lower limit of quantification 373 copies/mL) based on the EuroHep standard[17] or by using the AmpliPrep-COBAS TaqMan HBV test (CAP-CTM v 2.0; Roche Molecular Systems, Inc., Branchburg, NJ, USA, lower limit of detection 20 IU/mL). HBeAg and HBsAg were quantified at baseline using the Roche ELECSYS assay (Roche Diagnostics, Indianapolis, IN, USA) using a standardised protocol.

Statistical analysis

Data are presented as either mean (SD) or median (interquartile range, IQR) where appropriate. Associations between variables were tested using Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents when appropriate. Time-dependent analyses (HBeAg seroconversion, HBeAg seroreversion and virological relapse) were performed by Kaplan-Meier survival analysis and Cox' regression analysis. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

Role of the funding source

The sponsor of this study was Foundation for Liver and Gastrointestinal Research (SLO) in Rotterdam, the Netherlands. Financial support and test kits were provided by Innogenetics, Ghent, Belgium. The funding source did not have influence on study design, data collection, analysis and interpretation of the data, writing of the report nor the decision to submit for publication.

RESULTS

Patient characteristics

The study population consisted of 137 HBeAg-positive, anti-HBe-negative CHB patients (table). Median follow up of the study population was 29 (IQR 18-56) months from start of NA therapy.

Table 1 Characteristics of the study cohort.

Characteristics	Study population (n=137)
Demography	
Mean (SD) age, years	36.5 (14.5)
Male	105 (77%)
Race	
Caucasian	84 (61%)
Asian	40 (29%)
Other	13 (9%)
Laboratory results	
Median (IQR) ALT*	2.3 (1.3-4.5)
Mean (SD) HBV DNA, log IU/mL	7.4 (1.6)
Mean (SD) HBsAg, log IU/mL	4.1 (1.1)
Mean (SD) HBeAg, log IU/mL	2.1 (1.0)
HBV Genotype	
A	45 (33%)
B	24 (18%)
C	22 (16%)
D	38 (28%)
Other/mixed	8 (6%)

Table 1 (continued) Characteristics of the study cohort.

Characteristics	Study population (n=137)
INNO-LiPA result	
Wildtype	53 (39%)
Precore	24 (18%)
Basal core promoter	33 (24%)
Precore and basal core	27 (20%)
Nucleos(t)ide analogue regimen	
Lamivudine	52 (38%)
Entecavir	41 (30%)
Adefovir**	32 (23%)
Tenofovir**	12 (9%)

*Multiples of upper limit of the normal range. **Adefovir+lamivudine in 2 and tenofovir+lamivudine in 3.

Prevalence of PC and BCP mutants at baseline

Within the total cohort 39% of patients had only WT detectable. PC and/or BCP mutants were thus detected in 84 (61%) of patients (table).After stratification by HBV genotype, the distribution of PC and/or BCP mutants differed widely (p=0.02, figure 1). The majority of patients with genotype A had only WT virus (60%) present at start of NA therapy. In contrast, patients infected with HBV genotypes B, C and D had

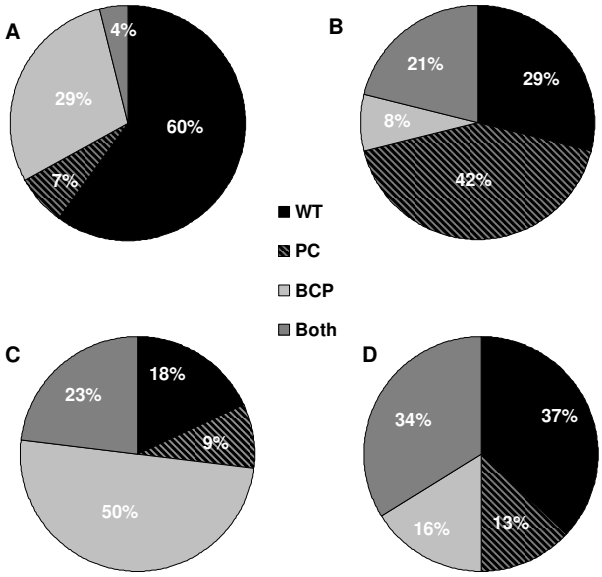


Figure 1 Frequency of PC and BCP mutants at baseline in the study cohort by HBV genotype.

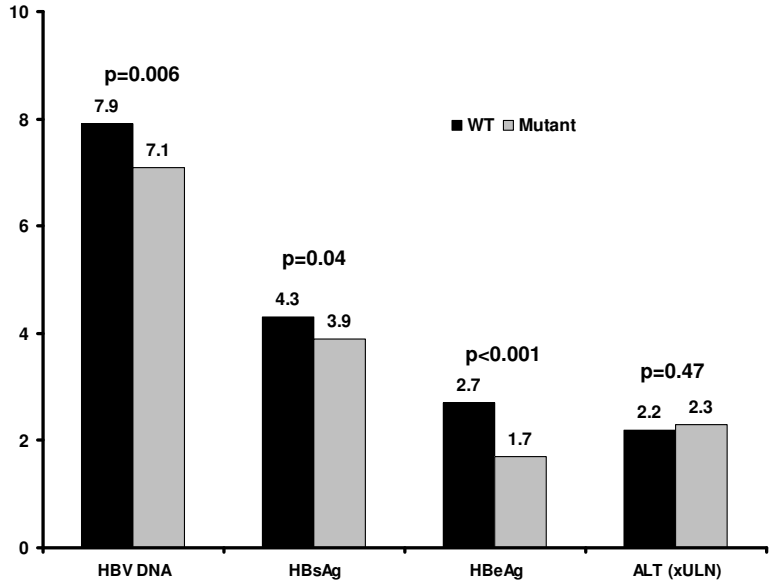


Figure 2 Relationship between presence of PC and/ or BCP mutants at baseline and virological and biochemical parameters of CHB infection. The y-axis represents mean values for: HBV DNA log IU/mL, HBsAg log IU/mL, HBeAg log IU/mL, and median for ALT xULN.

mutants present in 63 to 82% of patients. Patients with only WT virus at baseline had higher levels of HBV DNA, HBsAg and HBeAg compared to patients with PC and/ or BCP mutants (Figure 2). In contrast, ALT levels were comparable between both groups.

Relationship between PC and BCP mutants and serological response

A total of 45 (33%) patients achieved HBeAg seroconversion after a median of 76 (28-137) weeks (Figure 3). Patients with PC/BCP mutants present at baseline had a higher probability of achieving HBeAg seroconversion compared with WT patients (HR 2.20, 95% CI: 1.13 - 4.44, p=0.01, figure 4). In univariate analysis, higher baseline ALT was also associated with a higher probability of achieving HBeAg seroconversion (HR 1.05, 95% CI 1.02 – 1.07, p=0.002). In contrast, higher levels of HBV DNA (HR 0.87, 95% CI: 0.74 – 1.01, p=0.08) and HBsAg (HR 0.84, 95% CI 0.71 - 1.00, p=0.05) were associated with a lower probability of HBeAg seroconversion. Baseline HBeAg (HR 0.93, 95% CI 0.68 – 1.26, p=0.62) levels were not associated with probability of achieving HBeAg seroconversion. After correction for baseline HBV DNA, HBsAg, HBeAg, HBV genotype and ALT within a Cox regression model, the presence of PC/ BCP mutants at baseline remained independently associated with a higher probability of achieving HBeAg seroconversion (95% CI 1.11-6.15, p=0.03). Only 7 (16%) of

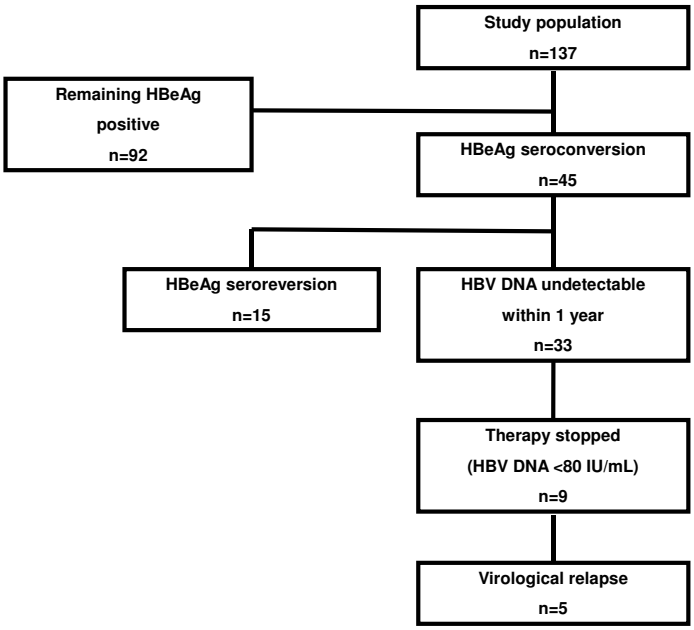
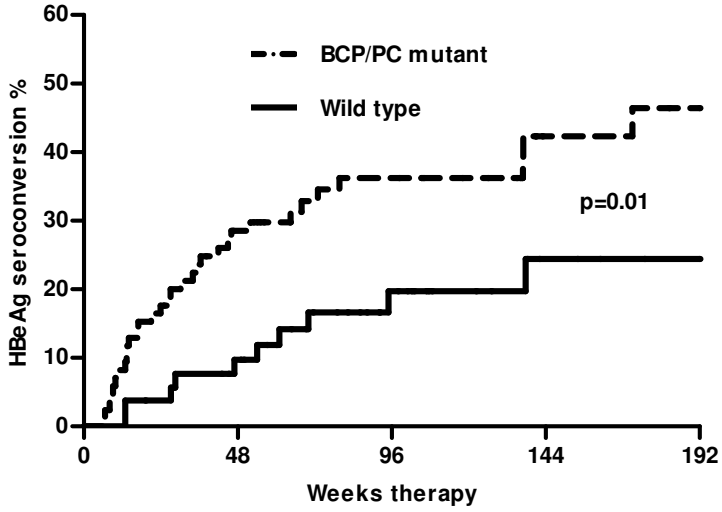


Figure 3 Study flow chart of 137 HBeAg-positive patients.



At risk					
BCP/PC mutant	84	56	36	16	12
Wild type	53	44	26	14	11

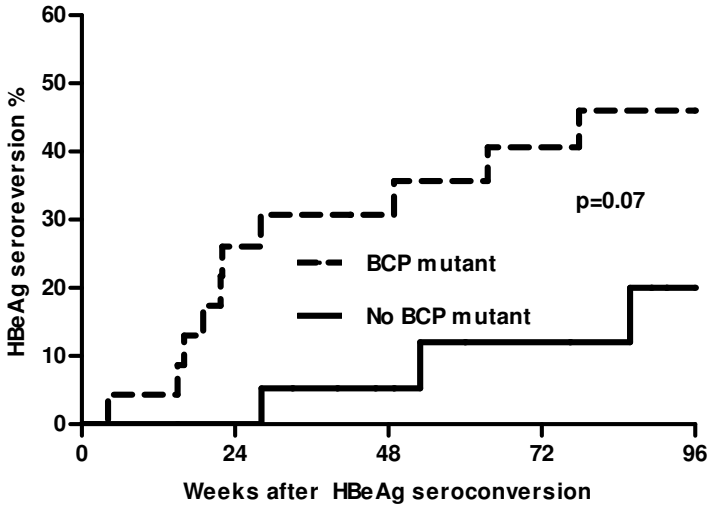
Figure 4 Cumulated probability of achieving HBeAg seroconversion during NA therapy up to 192 weeks of continuous therapy. Comparison between patients with WT virus and patients with detectable PC/BCP mutants at baseline.

patients achieved HBV DNA negativity before achieving HBeAg seroconversion. Probability of achieving HBV DNA negativity did not differ between patients with or without HBeAg seroconversion (p=0.29 by log rank). HBsAg seroconversion was achieved in only 6 (4%) patients and did not differ between patients with WT and PC/BCP mutants (p=0.99).

Follow-up after HBeAg seroconversion

After achieving HBeAg seroconversion, 15 of 45 (33%) patients had HBeAg-seroreversion (HBeAg reappearance confirmed in a consecutive sample). In 11 of these 15 (73%) patients HBeAg relapse occurred before cessation of therapy. HBeAg seroreversion tended to occur more frequently in patients with BCP mutants (HR 2.69, 95% CI 0.86-8.49, p=0.07, figure 5). Eight (73%) of these 11 patients remained HBeAg-positive during prolonged follow up. Fifteen (33%) patients experienced confirmed anti-HBe relapse after achieving HBeAg seroconversion, but this was not related to the presence of mutants.

A total of 33 (73%) patients (17 LAM, 6 ADV, 9 ETV and 1 TDF) achieved virological remission (HBV DNA <80 IU) within one year after HBeAg seroconversion. After exclusion of 7 patients with documented genotypic resistance to their current NA regimen



At risk					
BCP mutant	24	17	14	11	10
No BCP mutant	21	18	15	12	8

Figure 5 Comparison between patients with BCP mutants and patients without BCP mutants at baseline for confirmed reappearance of HBeAg after achieving HBeAg seroconversion (n=45).

(6 LAM and 1 ADV), the presence of PC mutants at baseline remained associated with a lower probability of achieving virological remission within one year after HBeAg seroconversion compared with patients without PC mutants (73% versus 96%, p=0.07). Importantly, type of therapy was not a significant predictor for achieving this endpoint (p=0.39).

NA therapy was stopped after at least 6 (median 18, IQR 10-48) months of consolidation therapy in 9 patients (7 LAM, 1 ETV and 1 ADV) who achieved HBeAg-seroconversion and HBV DNA <80 IU/mL. Two of these 9 (22%) patients had WT virus at baseline. However, after cessation of therapy within this group, 5 (56%) patients had virological relapse (confirmed HBV DNA >2000 IU/mL or restart of NA therapy) after a median follow up of 25 (15-157) weeks. Probability of virological relapse was not significantly influenced by the presence of PC and/or BCP mutants (p=0.63 by log rank), by type of therapy (p=0.55 by log-rank) or by length of consolidation therapy (p=0.43 by log-rank).

DISCUSSION

This study shows that the presence of PC/BCP mutants in HBeAg-positive patients is HBV genotype dependent and associated with a higher rate of HBeAg-seroconversion during NA therapy. However, after HBeAg seroconversion there was a trend towards a higher rate of HBeAg relapse in patients with BCP mutants and a lower rate of virological remission in patients with PC mutants.

HBeAg seroconversion during the natural course of CHB used to be associated with inactive disease. However, with rising knowledge and possibilities to quantify lower levels of viral replication, it is becoming increasingly clear that a substantial proportion of patients develops active HBeAg-negative CHB after HBeAg seroconversion and thus remains at risk for clinical progression of liver disease.[5, 18-19] Current data indicate a possible role for mutations within the PC or BCP region for developing active HBeAg-negative CHB. Mutations in both of these regions are associated with higher levels of viral replication after achieving HBeAg seroconversion.[14, 20-21] In this study we showed that these mutants are already detectable in a considerable number of HBeAg-positive patients at initiation of therapy, particularly in non-genotype A patients.

In addition, we showed that the presence of these mutants is predictive for achieving HBeAg seroconversion during NA-therapy. This is supported by previous studies showing that a PC mutant at position 1896 results in a stopcodon and thus cannot produce HBeAg and that BCP mutants show a diminished precore mRNA and HBeAg production.[22-23] Despite this higher rate of serological response, patients with BCP

mutants have a higher likelihood of serological reversion of HBeAg and those with PC mutants of maintaining higher serum HBV DNA levels after HBeAg seroconversion. BCP mutants are still able to produce HBeAg at a lower level and it was suggested that an accumulation of additional mutations could cause relapse of HBV DNA.[15, 21] Further evidence that achieving HBeAg seroconversion in these patients does not confer real immune control over the virus is supported by our finding that they have a low probability of achieving HBsAg seroconversion. These findings are important because stopping of NA therapy is still a major point of discussion in current HBV treatment guidelines.[7, 24]

Possible limitations of our study are the retrospective design and the heterogeneity of the cohort. However, the different regimens are not associated with different rates of HBeAg seroconversion and the current design enabled us to include a substantial number of HBeAg positive patients with long-term follow up.[25-26] Second, the INNO-LiPA assay detects mutations on position 1896 (PC), 1762 and 1764 (BCP), which are the most established mutated positions.[13] However, this assay is more sensitive than conventional sequencing, and can thus detect mutant virus at lower levels.[16]

In conclusion, the presence of PC and/or BCP mutants in HBeAg-positive patients was HBV genotype dependent, and independently associated with a higher probability of achieving HBeAg seroconversion during long-term NA therapy. However, after HBeAg-seroconversion patients with BCP mutants frequently have serological relapse while patients with PC mutants often do not achieve a complete virological response. HBsAg seroconversion was rare and not affected by the presence of PC and/or BCP mutants.

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Serum HBsAg decline during long-term potent nucleos(t)ide analogue therapy for chronic hepatitis B and prediction of HBsAg loss

6

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ABSTRACT

Background

Entecavir (ETV) and tenofovir (TDF) both potently inhibit viral replication in chronic hepatitis B (CHB) infection, but their long-term effect on serum HBsAg levels and HBsAg loss are lacking.

Methods

Seventy-five (32 HBeAg-positive) CHB patients with virological response (VR; HBV DNA <100 IU/mL) to either ETV or TDF for at least one year were included. HBsAg was quantified at baseline, at VR and yearly afterwards.

Results

Baseline HBsAg was higher in HBeAg-positive compared with HBeAg-negative patients (4.1 versus 3.5 log IU/mL, $p<0.001$). HBsAg decline two years after VR was more pronounced in HBeAg-positive patients (0.81 versus 0.15 log IU/mL, $p=0.03$). Older age and high baseline ALT were associated with HBsAg decline in HBeAg-positive patients. HBsAg decline was larger in patients achieving HBeAg loss. Predicted median time to HBsAg loss was 36 years for HBeAg-positive and 39 years for HBeAg-negative patients.

Conclusions

Long-term ETV and TDF therapy leads to significant HBsAg decline in HBeAg-positive patients. The majority of patients still need several decades of therapy to achieve HBsAg loss.

INTRODUCTION

Continuous therapy with entecavir (ETV) or tenofovir (TDF) results in durable suppression of viral replication in the majority of chronic hepatitis B (CHB) patients.[1-2] Current treatment guidelines emphasize HBV DNA suppression as a prerequisite for the prevention of complications of HBV-related liver disease.[3]

Serum hepatitis B surface antigen (HBsAg) loss is the preferred endpoint of HBV therapy and approximates clinical cure of the infection. However, HBsAg loss is rarely observed during therapy with ETV and TDF up to 5 years.[1-2]

Recent data indicate that HBsAg quantification during HBV treatment has additional value to HBV DNA quantification and on-treatment HBsAg was shown to predict a sustained off-treatment response to PEG-IFN therapy.[4-7] HBsAg levels reflect intrahepatic covalently closed circular DNA (cccDNA), the limiting factor in complete clearance of CHB infection, and could probably be used as a surrogate marker for the interaction between the immune system and the virus.[8-9]

However, data on the effect of nucleos(t)ide analogue (NA) therapy on HBsAg levels are unclear and predominantly described for less potent NA.[5, 8, 10-11]

The kinetics of serum HBsAg levels during long-term potent suppression of HBV DNA by TDF and ETV therapy are currently unknown. The aims of our study were (1) to investigate HBsAg kinetics in patients successfully treated with long-term ETV or TDF, (2) to identify factors associated with HBsAg decline and (3) to predict treatment duration required to achieve HBsAg loss.

MATERIAL AND METHODS

Study population

All consecutive CHB patients treated with ETV or TDF therapy at the Erasmus MC University Medical Center Rotterdam were included. Patients were eligible if they had a virological response (VR; HBV DNA <100 IU/mL) for at least 48 weeks between April 2003 and December 2009. Patients were excluded if they had viral co-infections (HIV, HCV, HDV). A total of 160 patients were identified of whom 85 did not fulfill entry criteria and were excluded: 27 had not achieved VR and 58 had less than 48 weeks follow up after VR. Seventy-five patients were eligible for this analysis. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and principles of Good Clinical Practice. Patients gave written informed consent according to standards of the local ethics committees.

Laboratory tests

Alanine aminotransferase (ALT) levels were expressed as ratio to the upper limit of the normal range (ULN; 30 U/L for females and 40 U/L for males). HBeAg and antibody against HBeAg (anti-HBe) status was determined using enzyme immunoassays. Serum HBsAg was quantified at baseline, at VR and each year after VR using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL). Serum HBV DNA levels were measured using the Cobas TaqMan (Roche Diagnostics; lower limit of quantification 20 IU/mL).

Statistical analysis

Continuous variables are presented as mean (standard deviation) or median (inter-quartile range), and compared using (non-)parametrical tests where appropriate. Categorical variables were compared by Chi-square or Fisher's exact test. HBsAg decline was analyzed with a repeated mixed regression model with a heterogeneous compound symmetry structure of the covariance matrix. Patient specific duration of treatment required to achieve HBsAg loss or one log drop was estimated applying a linear mixed regression model with random slope and intercept to the observed HBsAg measurements by the exact visit times. Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL) and SAS 9.2 (SAS Institute Inc., Cary, NC). All tests were two-sided and evaluated at the 0.05 level of significance.

RESULTS

Baseline characteristics

A total of 75 (32 HBeAg-positive and 56 male) CHB patients were included. Fifty-six patients were treated with ETV and 19 with TDF (in five patients combined with emtricitabine). Eighteen patients were previously treated with (pegylated) interferon, 21 patients were NA-experienced and 17 patients were previously treated with both. HBeAg-positive patients were younger (32 versus 43 years, $p=0.04$), had higher mean baseline HBV DNA (5.4 versus 4.3, $p=0.03$) and HBsAg levels (4.1 versus 3.4 log IU/mL, $p<0.001$) compared with HBeAg-negative patients. HBV genotype distributions among HBeAg-positive patients were A/B/C/D/other in 11 (34%), 5 (16%), 3 (9%), 8 (25%) and 5 (16%) compared to 7 (16%), 4 (9%), 7 (16%), 19 (44%) and 6 (14%) among HBeAg-negative patients ($p=0.31$). Other baseline variables (sex, race, ALT, therapy and cirrhosis) were also comparable for HBeAg-positive and HBeAg-negative patients. Baseline HBsAg and HBV DNA levels were correlated in HBeAg-positive ($R=0.44$, $p=0.02$), but not in HBeAg-negative patients ($R=-0.01$, $p=0.95$).

Median follow up lasted 28 (22-35) months after start of therapy (20 (15-28) months after VR). HBeAg loss occurred in 11/32 (34%) HBeAg-positive patients and HBsAg loss (serum HBsAg <0.05 IU/mL) was observed in 3/75 (4%) patients. Median time to achieve VR was 6 (2-10) months for HBeAg-positive and 3 (2-4) months for HBeAg-negative patients.

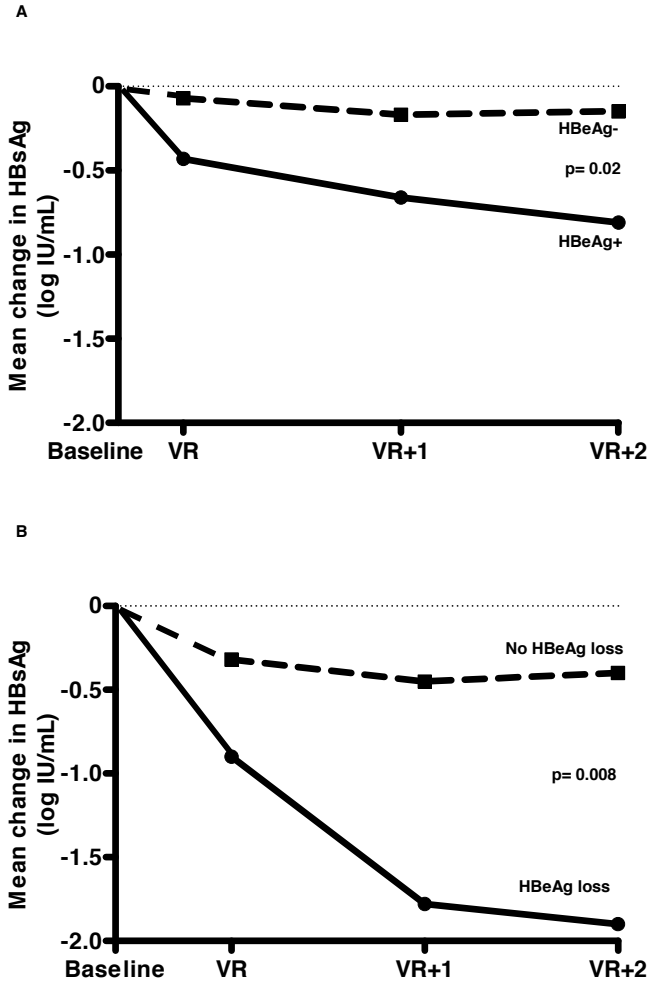

Figure 1 (A) Mean change compared to baseline for HBsAg in HBeAg-positive patients and HBeAg-negative patients treated with ETV or TDF. *Median time to VR was 3 (IQR 2-9) months. (B) Mean HBsAg decline compared to baseline for HBeAg-positive patients with or without HBeAg loss during ETV or TDF therapy. *Median time to VR was 6 (IQR 2-10) months.

HBsAg decline

In HBeAg-positive patients, the mean HBsAg decline at VR was 0.43 log IU/ml (p=0.06) compared to baseline (Figure 1). HBsAg decline progressed to 0.81 log IU/mL (p=0.003) two years after VR. In contrast, HBeAg-negative patients experienced a limited decline of 0.07 log IU/mL (p=0.27) at VR, and 0.15 log IU/mL (p=0.03) two years after VR (Figure 1). The decline in HBsAg two years after VR was more pronounced in HBeAg-positive patients (p=0.02).

HBsAg decline in relation to baseline factors

Baseline factors HBV genotype A (versus non-A), high HBV DNA, high ALT, high HBsAg and older age were significantly associated with more HBsAg decline in the HBeAg-positive population. Both current treatment regimen (ETV or TDF) and previous therapy were not significantly associated with HBsAg decline. In multivariate analysis only high ALT and older age remained significantly associated with HBsAg decline. None of the baseline factors were associated with HBsAg decline in HBeAg-negative patients.

HBsAg decline in relation to serological response

HBeAg-positive patients achieving HBeAg-loss had a more pronounced HBsAg decline compared to those who did not. Two years after VR HBsAg decline was 1.91 log/IU/mL in patients who lost HBeAg compared to only 0.41 log IU/mL for patients who did not (p=0.008).

We used linear mixed regression analysis of individual HBsAg decline curves to estimate the duration of therapy required to achieve (1) an HBsAg decline of 1 log IU/mL from baseline and (2) HBsAg clearance. The best fit was achieved with time as a linear effect. Median time to HBsAg loss for HBeAg-positive patients was 36 (9.6-98.3) years after start of therapy (Table 1). HBeAg-positive patients with a high baseline ALT had the strongest decline, but still needed a median time of 19 (7.3-99.9) years of therapy. HBeAg-negative patients had a limited HBsAg decline but, due to lower baseline HBsAg, needed a median interval of 39 (1.3-80.5) years to achieve HBsAg loss.

DISCUSSION

This is the first study on serum HBsAg levels in patients with long term suppression of HBV DNA by ETV or TDF treatment, the most potent antiviral agents currently available for the treatment of CHB. HBsAg levels decreased steadily in HBeAg-positive patients, especially in those with high baseline ALT, older age and HBeAg

Table 1 HBsAg declines and prediction of HBsAg loss in HBeAg-positive and negative patients.

	HBeAg-positive			HBeAg-negative		
	Overall (n=32)	High ALT (≥2xULN) (n=14)	Low ALT (<2xULN) (n=18)	Overall (n=43)	High ALT (≥2xULN) (n=18)	Low ALT (<2xULN) (n=25)
Baseline HBsAg (log IU)*	4.1 [0.6]	4.2 [0.7]	4.0 [0.6]	3.4 [0.7]	3.4 [0.9]	3.4 [0.9]
HBsAg decline (log/ year)**	0.11 [0.04; 0.34]	0.30 [0.06;0.82]#	0.07 [0.00; 0.13]#	0.07 [0.01; 0.18]	0.07 [0.04; 0.18]	0.07 [0.0; 0.18]
Years to 1log decline**	6.6 [1.7; 17.5]	3.6 [1.3; 16.7]	8.1 [0.0; 18.9]	8.0 [0.5; 14.9]	8.4 [2.1; 15.8]	5.7 [0.0; 14.9]
Years to HBsAg loss**	36.4 [9.6; 98.3]	19.5 [7.3; 99.9]	44.8 [1.2; 100.0]	38.9 [1.3; 80.5]	43.2 [10.3; 85.1]	29.7 [0.0; 75.1]

*Mean [SD] **Median [IQR] # p=0.003.

loss, while only a marginal HBsAg drop was observed in HBeAg-negative patients. The estimated duration required to achieve HBsAg loss was very long, but shortest for HBeAg-positive patients.

Previous studies suggest that HBsAg levels decrease slowly during treatment with NA monotherapy in HBeAg-positive patients.[8, 11-12] This study confirms these findings for the potent agents ETV and TDF. Our finding that the degree of HBsAg decline was most pronounced in HBeAg-positive patients with elevated baseline ALT and HBeAg loss indicates the need for an activated immune response against HBV before therapy is initiated. Nonetheless, we calculated that a median of 20 years will be needed to achieve HBsAg loss in these patients with high baseline ALT, whereas approximately 35 years of therapy would be needed for the overall HBeAg-positive population. The overall limited HBsAg decline in our NA treated patients and the absence of immune control also corroborate with the low chance of achieving sustained response after cessation of NA therapy.[13]

Remarkably, 10% of HBeAg-positive patients treated within the TDF phase III trial for up to 4 years lost HBsAg, which is the highest rate of HBsAg loss reported for potent NA therapy.[2] However, our study showed that the decline in HBsAg was similar in patients treated with ETV and TDF. It is thus unclear whether this high proportion of HBsAg loss should be attributed to TDF therapy itself, or is influenced by host and viral factors, such as HBV genotype.

HBsAg kinetics during NA therapy are conflicting for HBeAg-negative patients treated with NA therapy.[5, 10] Our study demonstrates that serum HBV DNA and HBsAg were not correlated at baseline and that potent NA therapy does not result in a clinically significant HBsAg decline in HBeAg-negative patients. In addition, Thompson et al. recently showed that the correlation between HBsAg and intrahepatic cccDNA is limited to HBeAg-positive patients in an untreated population, suggestion a more limited use of serum HBsAg in HBeAg-negative patients.[14] Recent natural history data indicate that HBsAg decline is limited in inactive carriers with only 0.04 log decline per year, which is comparable to our NA treated HBeAg-negative patients.[15] The estimated time to achieve HBsAg loss in our HBeAg-negative population was 39 years, which is longer than predicted for LAM-treated patients, suggesting indefinite NA therapy for the majority of these patients.[10] In our study, due to lower baseline HBsAg levels for HBeAg-negative patients compared to HBeAg-positives (3.4 vs. 4.1 log IU/mL, respectively), the difference in years to HBsAg loss is limited.

A possible limitation of our study is the relatively small number op patients included. This may have influenced our estimates on duration of HBsAg loss, as these estimates have a large variability. Five patients in our study were treated with a fixed combination of TDF and emtricitabine. However, HBsAg kinetics of these patients did not differ from patients treated with TDF monotherapy.

In summary, decline of serum HBsAg in HBeAg-positive patients treated with potent NA was significant but largely confined to patients with high baseline ALT, to older patients and to those achieving HBeAg-loss. In HBeAg-negative patients NA treatment resulted in limited HBsAg decline and no baseline predictors of HBsAg decline could be identified. We estimated that even in the most favorable group it will take a median of two decades to achieve HBsAg loss, while for the overall population it will take three to four decades. Our study thus suggests that HBsAg clearance, which is the preferred endpoint of HBV treatment, will probably remain a rare event during continued potent NA therapy.

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Hepatitis B surface antigen decline and clearance during long-term tenofovir therapy in patients co-infected with HBV and HIV

7

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ABSTRACT

Background

Kinetics of hepatitis B surface antigen (HBsAg) are predictive in hepatitis B virus (HBV) patients treated with peginterferon. Knowledge of their value in patients co-infected with HBV and human immunodeficiency virus (HIV) is lacking.

Methods

We quantified serum HBsAg in a Dutch multicenter cohort including 104 HIV/HBV patients treated with tenofovir (TDF) as part of HAART. Median duration of therapy was 57 (IQR 34-72) months.

Results

HBeAg-positive patients achieved an HBsAg decline of 2.2 log IU/mL, whereas HBeAg-negative patients only declined 0.6 log IU/mL log during 6 years of TDF. HBsAg decline at month 6 and 12 correlated with CD4 cell count for HBeAg-positive patients. Five (8%) HBeAg-positive and 3 (8%) HBeAg-negative patients cleared HBsAg. HBeAg-negative patients clearing HBsAg had lower baseline HBsAg compared to patients remaining HBsAg positive. The majority of patients clearing HBsAg achieved this endpoint within the first year. HBsAg decline at month 6 was predictive of achieving HBsAg seroclearance in HBeAg-positive patients.

Conclusions

TDF for HIV/HBV patients up to 6 years leads to significant HBsAg decline in the HBeAg-positive population. Early HBsAg kinetics were predictive for HBsAg seroclearance and correlated with increase of CD4 cell count, underlining the importance of immune restoration in HBV clearance.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is a potent inhibitor of both hepatitis B virus (HBV) and human immunodeficiency virus (HIV).[1-2] First data of its antiviral efficacy against hepatitis B virus (HBV) was derived from studies in HIV/HBV co-infected patients[3-5] and was subsequently demonstrated in a large randomized trial.[6] Unfortunately, serum hepatitis B surface antigen (HBsAg) seroclearance, the outcome closest to clinical cure of the disease, remains a rare event during long-term HBV therapy with potent nucleos(t)ide analogues (NA), especially among HBeAg-negative patients.[2, 7]
Quantification of HBsAg was recently correlated with intrahepatic markers of HBV, such as cccDNA and intrahepatic HBV DNA.[8-10] Moreover, HBsAg quantification has proven its value in predicting response for HBV mono-infected patients treated with pegylated interferon.[11-14] In contrast, data on HBsAg kinetics in NA-treated HBV patients is limited so far and its clinical use has not been proven yet.[15-18]
Despite effective suppression of viral replication by current highly active antiretroviral therapy (HAART) therapy, patients infected with both HIV and HBV have a higher risk of progression to cirrhosis and its complications compared with HIV mono-infected patients.[19-20] Clearance of HBV in these patients could therefore be of vital importance for the long-term prognosis of this co-infected population.
Knowledge of serum HBsAg kinetics and its predictive value during long-term potent suppression by TDF in HIV/HBV co-infected patients is limited, but may help to identify patients likely to clear HBsAg.[21] Recently we presented the long-term efficacy and safety during up to 5 years of TDF based therapy of HIV/HBV co-infection in a Dutch multicenter cohort study.[22] Using this cohort, we aimed in the current study (1) to investigate long term serum HBsAg kinetics in HIV/HBV co-infected patients treated with TDF, (2) to identify baseline- and on-treatment factors associated with HBsAg decline and HBsAg seroclearance and (3) to predict the duration of treatment required to achieve HBsAg loss for these patients using on treatment HBsAg declines.

PATIENTS AND METHODS

Study population

All consecutive HIV/HBV co-infected patients treated with TDF therapy at five Dutch centers specializing in viral hepatitis and HIV management were included. Patients were eligible for this study if they were HBsAg positive for at least 6 months, were anti-HIV positive at baseline and treated with TDF as a part of a HAART regimen for at least six months. Patients were excluded if they had hepatitis C or hepatitis delta co-infection, or concomitant pegylated interferon therapy. The study was conducted

in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Patients gave written informed consent according to standards of the local ethics committees. Diagnosis of cirrhosis at baseline was based on the following criteria: histological confirmation and/or clear signs on abdominal ultrasound associated with cirrhosis (spleen size >12 cm, portal vein>16 mm, or nodules within the hepatic parenchyma).

Laboratory tests

Serum alanine aminotransferase (ALT) levels were measured using automated techniques and are expressed as values representing a ratio to the upper limit of the normal range (ULN). Absolute numbers of CD4 T lymphocytes were assessed on whole blood by flow cytometry. Detection of HBeAg and antibody against HBeAg (anti-HBe) was performed using commercially available enzyme immunoassays. HBV genotype was determined at baseline by direct sequencing or using the INNO-LiPA assay (Innogenetics, Gent, Belgium). Serum HBsAg was quantified in stored samples at baseline, at 6 months and at yearly intervals thereafter using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL). Serum HBV DNA levels were measured using the Cobas TaqMan polymerase chain reaction (PCR) assays (Roche; lower limit of detection 20 IU/mL). HIV RNA was assessed quantitatively with the Cobas Ampliprep/Cobas Amplicor version 1.5 (lower limit of detection: 50 copies/mL; Roche Molecular Systems).

Statistical analysis

Serum HBsAg, HBV DNA and HIV RNA levels were logarithmically transformed for analysis. Continuous variables are presented as mean (standard deviation) or median (interquartile range), where appropriate. Continuous variables were compared using the t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square or Fisher's exact test. The decline of serum HBsAg from baseline was analyzed with a repeated mixed regression model with a heterogeneous compound symmetry structure of the covariance matrix. The patient specific duration of treatment required achieving a 1 log drop of HBsAg and HBsAg loss was estimated applying a linear mixed regression model with a random slope and random intercept to the observed HBsAg measurements by the exact visit times. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

A total of 104 patients were included in this analysis. Baseline characteristics of the study population are presented in table 1. Patients were predominantly male, of Caucasian origin and mostly harbored HBV genotype A. Baseline HBV DNA and HBsAg level were higher in HBeAg-positive patients compared with HBeAg-negative patients and strongly correlated within the HBeAg-positive population (r=0.62, p<0.001). Baseline HBsAg showed a moderate correlation with ALT (r=0.38, p=0.02)

Table 1 Baseline characteristics of the study population.

Characteristics	HBeAg positive (N=66)	HBeAg negative (N=38)	P
Age, (years)	43 ± 9	41 ± 9	0.40
Sex, (male %)	65 (99%)	27 (71%)	<0.001
Race (%)			<0.001
Caucasian	46 (70%)	12 (32%)	
Black	10 (15%)	23 (61%)	
Other	10 (15%)	3 (8%)	
ALT level, x upper limit of normal	1.7 (1.0 -2.9)	0.9 (0.6-1.1)	0.06
Mean HBV DNA level, $\log_{10}$ IU/mL	7.8 ± 1.5	3.0 (2.6)	< 0.001
Mean HBsAg level, $\log_{10}$ IU/mL	4.6 ± 1.1	2.8 (1.7)	< 0.001
HBV Genotype (N=83)			0.08
A	42 (66%)	9 (47%)	
B	1 (2%)	2 (11%)	
C	4 (6%)	0 (0%)	
D	5 (8%)	3 (16%)	
E	4 (6%)	0 (0%)	
G	8 (13%)	5 (26%)	
Cirrhosis	12 (18%)	2 (5%)	0.08
CD4 cell count (cells/mm^3)	288 (140-470)	260 (115-385)	0.29
HIV RNA, $\log_{10}$ copies/mL	3.1 ± 1.7	3.2 ± 1.7	0.83
Treatment regimen			0.33
2 NRTI + 1 NNRTI	47 (71%)	33 (87%)	
2 NRTI + PI/r	14 (21%)	4 (11%)	
Other	5 (8%)	1 (3%)	
Concomitant anti-HBV therapy			0.53
Lamivudine	60 (91%)	33 (87%)	
Emtricitabine	6 (9%)	5 (13%)	
Previous lamivudine-experience	37 (56%)	19 (50%)	0.54

in HBeAg-negative patients. Baseline HBsAg was higher for HBV genotype A versus genotype non-A (4.9 versus 4.2 log IU/mL, p=0.02) in HBeAg-positive patients; for HBeAg-negative patients this difference was not significant (3.2 versus 2.9 log IU/mL, p=0.65). Median follow-up of the study population was 57 (interquartile range 34-72) months.

HBsAg decline during treatment

HBsAg, HBV DNA and CD4 cell count kinetics up to 6 years of therapy are depicted in figure 1. HBsAg decline at year 1 was 1.2 log IU/mL (p<0.001 compared with baseline) for HBeAg-positive and only 0.2 log IU/mL (p= 0.58) for HBeAg-negative patients. HBsAg decline at 6 years of therapy was 2.2 log IU/mL for HBeAg-positive (p<0.001) and 0.6 log IU/mL for HBeAg-negative patients (p=0.07). Within the HBeAg-positive population, 68% of patients achieved at least a 1 log decline of HBsAg, compared to only 16% of HBeAg-negative patients. HBV DNA and HBsAg levels were significantly correlated during follow up (r>0.60).

We investigated associations between baseline variables and HBsAg kinetics in HBeAg-positive and HBeAg-negative patients separately. Using univariate analysis, a more pronounced HBsAg decline was found in HBeAg-positive patients with either genotype A (p=0.08 versus genotype non-A), no previous lamivudine exposure (p=0.03), higher baseline HBsAg (p<0.001) and higher HBV DNA (p=0.03). In multivariate analysis, only higher HBsAg remained significant (p=0.02); in contrast HBV genotype A (p=0.58), previous lamivudine exposure (p=0.58) and higher HBV DNA (p=0.42) did not remain significantly associated with HBsAg decline. For HBeAg-negative patients, no significant baseline predictors of HBsAg kinetics could be identified.

CD4 cell count increased steadily during TDF therapy for both HBeAg-positive and HBeAg-negative patients, with the largest increase in the first six months (+129±155 cells/mm^3 for HBeAg-positive patients and +87±138 cells/mm^3 for HBeAg-negative patients; figure 1). Early HBsAg decline was correlated with an increase in CD4 cell count in HBeAg-positive patients at months 6 (r=0.34, p=0.04) and 12 (r=0.33, p=0.03). In 3 of 4 HBeAg-positive patients achieving clearance of HBsAg within the first year, this was accompanied by an increase in CD4 cell count of >150 cells/mm^3.

HBsAg kinetics in relation to HBeAg and HBsAg serological response

HBeAg-positive patients

The HBeAg-positive population comprised 66 patients, of whom 24 (36%) achieved HBeAg loss and 12 (18%) achieved HBeAg seroconversion. Kaplan-Meier estimates of HBeAg loss and HBeAg seroconversion were 17% and 8% at year 1 increasing to

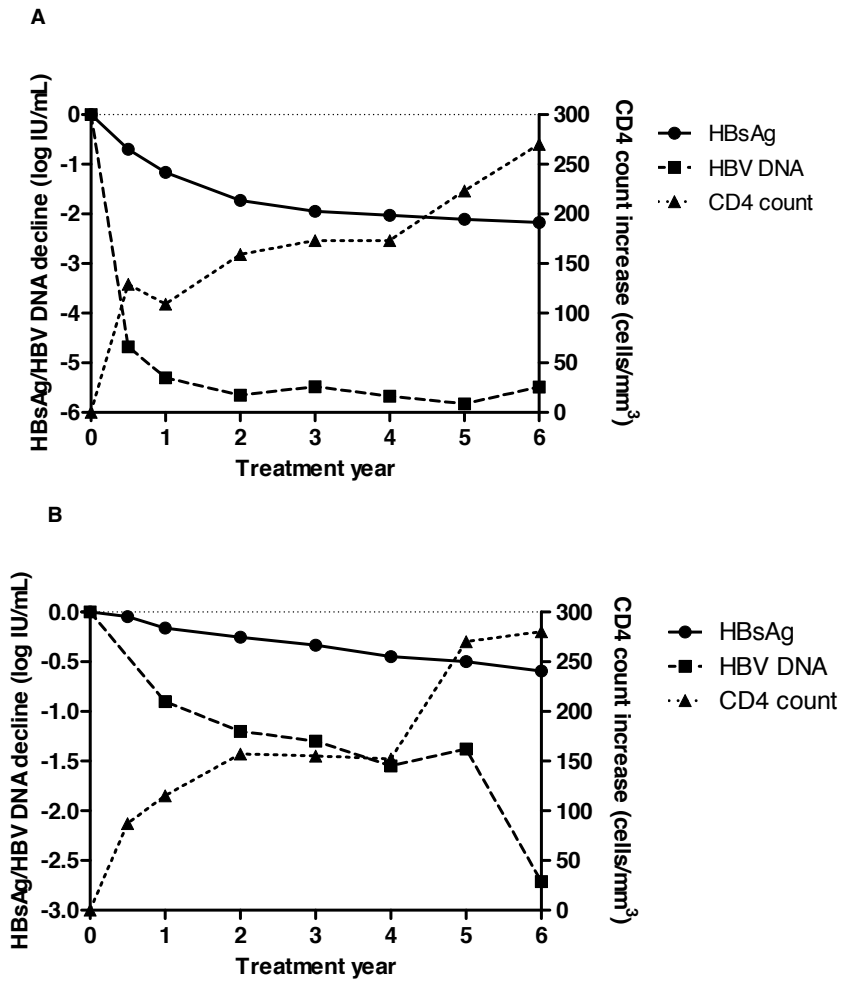
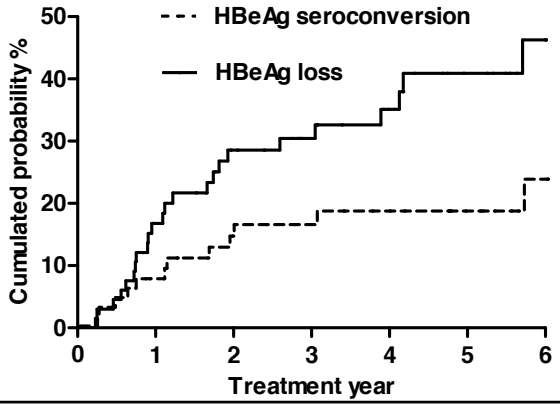


Figure 1 Kinetics of HBsAg, HBV DNA and CD4 cell count up to 6 years. Mean change compared to baseline for HBsAg, HBV DNA and CD4 cell count in (A) HBeAg-positive patients and (B) HBeAg-negative patients treated with TDF. The left Y-axis depicts the decline in HBsAg and HBV DNA, the right Y-axis depicts the increase in CD4 cell count.

47% and 24% at year 6 (Figure 2A). Baseline HBsAg tended to be lower in patients achieving HBeAg loss (4.3 vs. 4.8 log IU/mL, p=0.10). HBsAg decline up to 6 years for HBeAg-positive patients with or without HBeAg loss are depicted in figure 2B. Patients with HBeAg loss achieved a decline of 2.5 log IU/mL (p=0.006 compared with baseline) and patients remaining HBeAg-positive achieved a decline of 1.8 log IU/mL after 6 years of therapy (p<0.001).

A



At risk							
HBeAg loss	66	53	41	34	25	17	11
HBeAg seroconversion	66	58	47	41	32	24	16

B

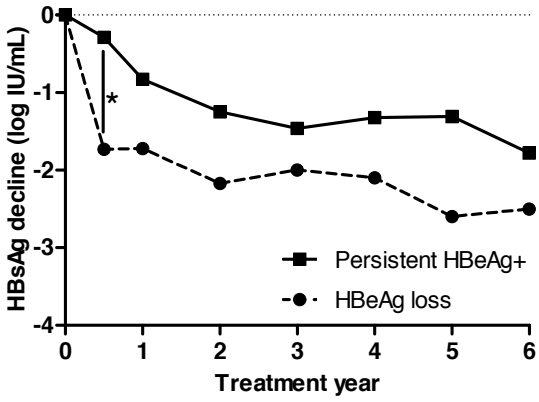


Figure 2 Serological response and HBsAg kinetics in HBeAg-positive patients. (A) Cumulative probability of achieving HBeAg loss or HBeAg seroconversion during TDF therapy. (B) Mean change compared to baseline in HBsAg for HBeAg-positive patients with or without HBeAg loss during six years of TDF therapy. *p=0.01 between both groups.

Five (8%) HBeAg-positive patients achieved HBsAg seroclearance within our study (Table 2). Three of those 5 (60%) also seroconverted, with detectable anti-HBs titers. Baseline HBsAg levels were comparable in HBeAg-positive patients achieving HBsAg seroclearance and those remaining HBsAg-positive (5.0 versus 4.6 log IU/mL, p=0.46). Individual HBsAg kinetics of patients achieving HBsAg seroclearance are depicted in figure 3a. An HBsAg decline of at least 2 log IU/mL at month 6 was

Table 2 Characteristics of patients with and without HBsAg clearance.

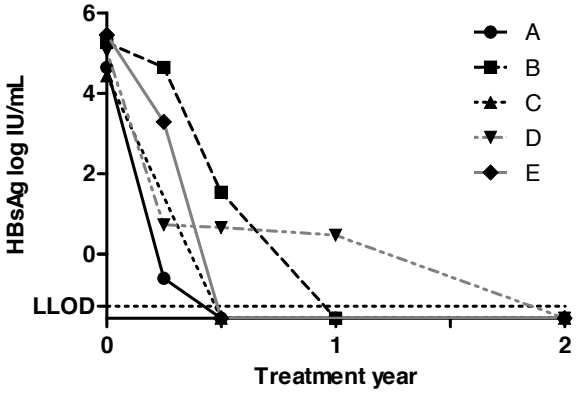
	HBeAg-positive			HBeAg-negative		
	HBsAg seroclearance			HBsAg seroclearance		
Baseline characteristics	No (N=61)	Yes (N=5)	P	No (N=35)	Yes (N=3)	P
Age, (years)	43 ± 9	38 ± 4	0.30	41 ± 9	42 ± 13	0.77
Sex, (male %)	60 (98%)	5 (100%)	0.77	25 (71%)	2 (67%)	0.86
Race (%)			0.85			0.96
Caucasian	42 (69%)	4 (80%)		11 (31%)	1 (33%)	
Black	9 (15%)	1 (20%)		21 (60%)	2 (67%)	
Other	15 (16%)	0		3 (9%)	0	
ALT level, x upper limit of normal	1.7 (1.0-2.9)	1.9 (1.8-2.8)	0.40	0.9 (0.5-1.2)	0.9 (0.8-1.0)	0.69
Mean HBV DNA level, log_{10} IU/mL	7.8 ± 1.5	7.9 ± 1.68	0.88	3.0 ± 2.6	3.6 ± 3.3	0.67
Mean HBsAg level, log_{10} IU/mL	4.6 ± 1.1	5.0 ± 0.42	0.46	2.8 ± 1.7	1.0 ± 2.6	0.05
HBV Genotype (N=83)			0.56			0.26
A	39 (66%)	3 (60%)		9 (53%)		
B	1 (2%)			2 (12%)		
C	4 (7%)			0 (0%)		
D	4 (7%)	1 (20%)		2 (12%)	1 (50%)	
Other/mixed	11 (19%)	1 20%)		4 (24%)	1 (50%)	
CD4 cell count (cells/mm^3)	296 (145-480)	250 (100-340)	0.25	270 (120-380)	210 (110-335)	0.64
HIV RNA, log_{10} copies/mL	3.0 ± 1.7	4.4 ± 1.1	0.04	3.2 ± 1.7	3.8 ± 2.1	0.54
Month 6						
Mean HBsAg level, log_{10} IU/mL	4.2 ± 1.1	-0.5 ± 1.5	<0.001	3.0 ± 1.7	0.2 ± 3.1	0.02

highly predictive of HBsAg seroclearance; 71% of these patients achieved HBsAg seroclearance and none of the patients with a decline of <2 log IU/mL. Predicted median time to HBsAg seroclearance for HBeAg-positive patients was 18 (IQR 10-28) years, while time needed to achieve an HBsAg decline of 1 log was 3 (2-5) years.

HBeAg-negative patients

Three (8%) HBeAg-negative patients achieved HBsAg seroclearance during TDF therapy up to six years, of whom only 1 patient (33%) seroconverted to anti-HBs. In contrast with HBeAg-positive patients, baseline HBsAg levels were considerably lower compared to patients remaining HBsAg positive (1.0 versus 3.0 log IU/mL,

A



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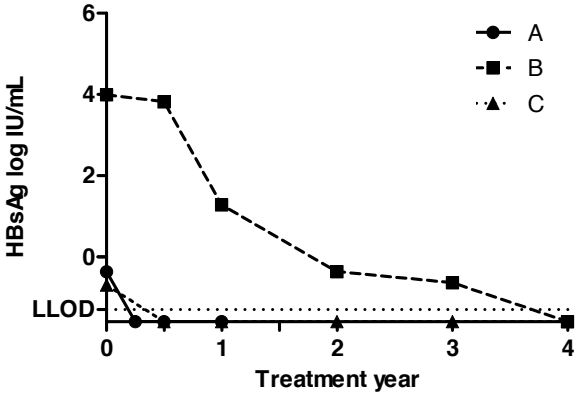

Figure 3 HBsAg kinetics in patients with HBsAg seroclearance. Individual HBsAg kinetics for (A) HBeAg-positive and (B) HBeAg-negative patients achieving HBsAg seroclearance during TDF therapy. LLOD: Lower limit of detection.

p=0.05, table 2). Two HBeAg-negative patients had already very low HBsAg levels at start of therapy and became HBsAg-negative within the first six months (Figure 3B). The third patient had a baseline HBsAg >4 log IU/mL, a fast decline within the first year and became HBsAg negative after 4 years. Predicted median time to HBsAg loss for HBeAg-negative patients was 42 (3-83) years and 10 (5-16) years were needed to achieve a decline of 1 log IU/mL.

DISCUSSION

This is the first large study investigating HBsAg kinetics in HIV/HBV co-infected patients treated with long-term TDF. A significant HBsAg decline of 2.2 log IU/mL at year 6 was noted within the HBeAg-positive population, while minimal HBsAg decline was observed in HBeAg-negative patients. An on-treatment correlation between CD4 count and early HBsAg decline was found. Early HBsAg decline was shown to be highly predictive of HBsAg seroclearance, which mainly occurred within the first year of TDF therapy.

HIV/HBV co-infection has a worse prognosis than mono-infection with either virus, and the presence of HBV is an important determinant of the prognosis in patients treated with HAART.[19-20] HBsAg positivity is the hallmark of HBV infection and HBsAg clearance signifies clinical cure. However, this endpoint is rarely achieved in HBV mono-infected patients, particularly not in HBeAg-negative patients.[2, 7, 22]

We showed that HBsAg decline in the first year was relatively fast, highly predictive for clearance of HBsAg and correlated with early on-treatment CD4 cell count in HBeAg-positive patients. These findings underline the importance of immune restoration by HAART therapy for HBV clearance in HIV/HBV co-infected patients. It could also be argued that HBsAg seroclearance in these patients is mainly a secondary effect of HAART therapy, caused by an increasing CD 4 cell count, instead of a direct effect of TDF on viral replication and HBsAg production or excretion.

In addition, our study showed that the vast majority of patients with HBsAg seroclearance achieved this endpoint within the first year of TDF based therapy. Five HBeAg-positive patients achieved HBsAg seroclearance, all of them showed a very fast decline of HBsAg, which was accompanied with a strong increase in CD 4 cell count. Early HBsAg kinetics were thus shown to be highly predictive for HBsAg seroclearance, which was also described by Wursthorn et al in telbivudine treated HBV mono-infected patients.[17] Three HBeAg-negative patients achieved HBsAg seroclearance, which is a rare event in NA-treated HBV mono-infected patients. Two of these HBeAg-negative patients had already very low baseline HBsAg levels at start of therapy and quickly achieved HBsAg seroclearance, indicating that this was

possibly already an ongoing event, which was probably accelerated by treatment and a consecutive increase in CD cell count. All patients achieving HBsAg seroclearance in our study were infected by non-Asian HBV genotypes, an important observation which was also noted in the pivotal entecavir and TDF trials.[2, 7] Genotypic and racial distribution thus seems important to take into account when comparing HBsAg seroclearance rates between studies with (different) NA as well as in peginterferon treated patients.[23]

Our study showed that HBsAg decline is mostly confined to HBeAg-positive HIV/HBV co-infected patients and tended to be steeper in HBeAg-positive patients achieving HBeAg loss. Both findings have also been noted in previous studies in HBV mono-infected patients, indicating that besides adequate viral suppression, baseline HBeAg status and achievement of HBeAg loss are both of significant importance and influence probability of HBsAg seroclearance.[2, 16-18, 24] Of the baseline variables studied, higher baseline HBsAg was associated with a stronger decline of HBsAg up to 6 years of continuous TDF therapy in HBeAg-positive patients. A smaller study in 33 HIV/HBV co-infected patients did not find this relation between HBsAg decline, HBeAg status and baseline HBsAg level.[21]

A limitation of our study is its retrospective design. However, due to the fact that TDF was first registered for HIV infected individuals, our cohort study has a long follow up with a median of more than four years. Moreover, to our knowledge this is the largest study investigating HBsAg kinetics in co-infected patients treated with TDF as part of antiretroviral therapy.

In conclusion, we showed that long-term TDF therapy leads to significant HBsAg decline in HBeAg-positive patients and that early HBsAg kinetics were highly predictive for HBsAg seroclearance. The on treatment correlation between CD4 cell count and HBsAg kinetics and the high rate of HBsAg seroclearance within the first year underlines the importance of immune restoration to clear HBsAg.

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Summary and discussion

TREATMENT OF CHRONIC HEPATITIS B

The landscape of treatment for chronic hepatitis B (CHB) has undergone great changes within the last years. Current therapeutic strategies focus on surrogate endpoints as virological response (HBV DNA suppression), biochemical response (ALT normalization), serological response (HBeAg and HBsAg loss or seroconversion) and histological response (histological improvement), to prevent the development of (decompensated) cirrhosis, hepatocellular carcinoma (HCC) and death.[1-2] First-line therapy of CHB comprises immune-modulating pegylated interferon (PEG-IFN) and 5 different nucleos(t)ide analogues (NA). PEG-IFN is registered for a finite duration of one year, after which medication is stopped and off-treatment durability of response is evaluated. PEG-IFN is administered once weekly subcutaneous by injection, has a considerable side effect profile and results in a sustained response in an estimated 30% of patients.[3] Alternatively, current guidelines suggest using daily oral NA therapy with successful suppression of HBV DNA in virtually all patients under continuous therapy and only limited side-effects. Five NA are currently approved for the treatment of CHB: lamivudine (LAM), entecavir (ETV), telbivudine (LdT), adefovir (ADV) and tenofovir (TDF).[4-6] All NA act by competitive inhibition of the hepatitis B virus (HBV) polymerase and thus suppress viral replication.[7]

VIROLOGICAL EFFICACY OF NA THERAPY IN NA-NAÏVE AND NA-EXPERIENCED PATIENTS

Large cohort studies with untreated Asian patients have shown that HBV DNA levels are associated with risk of liver disease progression and HCC development.[8-9] These levels of viral replication were subsequently used in current guidelines as a threshold for starting antiviral therapy. Complete suppression of viral replication to undetectable HBV DNA levels is thus currently considered the most important surrogate endpoint as it is also relatively frequently achieved during NA therapy. Current CHB guidelines advise to start TDF or ETV monotherapy as first line NA therapy (when available) because of their potency, high genetic barrier to resistance and negligible side effects.[1, 10] Different definitions of virological endpoints during NA therapy were also listed within these guidelines.[10] Virological response is defined as having an undetectable HBV DNA by sensitive PCR assay. Virological breakthrough is defined as a confirmed increase in HBV DNA of at least 1 log from the nadir (lowest level of HBV DNA achieved) and is generally associated with either non-compliance or by decreased susceptibility of the virus due the development of genotypic resistance. Based on their genetic barrier to resistance, patients with a detectable viral load at

week 24 (LAM and LdT) or week 48 (ADV, ETV and TDF) were defined as having a partial virological response. In case of partial virological response, the 2009 guidelines suggested to consider treatment adaption, despite lacking evidence for patients treated with ETV or TDF.[10-13] In addition, the majority of data on the long-term efficacy on ETV and TDF is currently from phase III registration studies including, treating and monitoring patients according to strict inclusion- and exclusion criteria, underlining the importance of evidence from real-life cohort studies.[7-8]

In **chapter 1** the influence of having a partial virological response on long-term virological outcome in NA-naïve patients treated with ETV was investigated within a European multicenter cohort. Partial virological response occurred in 21% of patients after 48 weeks of continuous ETV therapy. However, virological response was achieved with continued ETV in the vast majority of these patients without treatment adaptation, particularly in those with a low viral load (<1000 IU/mL) at week 48. Importantly, prolonged ETV monotherapy did not result in development of genotypic resistance to ETV. Further findings of this study were that previous ADV exposure or ADV resistance both not significantly lower virological response rates, but that patients with LAM resistance had a lower probability of achieving virological response. In addition, 4 NA-experienced (1 only ADV) patients developed mutations associated with genotypic resistance to ETV after which treatment was adapted. Persistent replication (detectable HBV DNA) at week 48 is thus not associated with an unfavorable virological outcome in ETV treated NA-naïve patients, which differs from previous data on older NA ADV, LAM and LdT. This discrepancy is probably caused by a higher genetic barrier to resistance (more mutations are necessary to develop clinical resistance) compared to older NA. Due to these low rates of resistance for ETV and TDF it is difficult to develop validated guidelines for treatment adaptation in clinical practice. Moreover, our findings underline that lessons from studies on NA with a different potency and different genetic barrier should be cautiously applied to patients treated with ETV or TDF.

According to cross resistance patterns of currently available NA, a TDF containing regimen should be prescribed as salvage therapy for patients with previous failure of LAM, LdT or ETV and an ETV containing regimen for those with ADV or TDF failure.[14] Current guidelines consider only *monotherapy* with NA as first-line in patients with CHB, as the benefit of *combination therapy* has not yet convincingly been proven in NA-naïve patients.[1, 10] According to the same guidelines, in case of genotypic resistance, switching to another drug without cross-resistance or add-on (combination) therapy is suggested. Therefore, the fixed combination of TDF and emtricitabine (FTC, structurally comparable to LAM), which is currently widely used for HIV-infected

patients, could be a simple and elegant tool in patients with previous treatment failure, because of a single pill combination regimen. However, currently there is no evidence for the superior efficacy of TDF+FTC in both NA-naïve and NA-experienced CHB patients. Therefore, in **chapter 2** the clinical efficacy and safety of the fixed combination of TDF+FTC was investigated within a European multicenter study of NA-experienced patients. There was a high rate of undetectable HBV DNA and no significant decrease in serum creatinine level up to 2 years of continuous therapy. Although a control arm was lacking, TDF+FTC showed to be highly effective and safe in mostly difficult to treat patients with previous treatment failure and for patients in whom simplification of treatment is desirable and could thus be a useful option for both indications.

CLINICAL OUTCOME DURING NA THERAPY

Phase III studies of patients treated with ETV or TDF indicate that HBV DNA undetectability is achieved in virtually all (naïve) patients during long-term therapy.[15-17] However, there is limited data how this influences the long-term prognosis of these patients. Currently, there is only one randomized trial clearly indicating the clinical advantage of treating patients with NA. Within this study, LAM treatment was associated with a lower rate of liver disease progression and development of HCC then patients treated with placebo.[18] In **chapter 3** the effect of virological response on clinical disease progression (hepatic decompensation, development of HCC or death) was investigated in patients treated with ETV within a large European multicenter study. Virological response rates did not differ between patients without cirrhosis, with compensated cirrhosis or with decompensated cirrhosis at start of ETV therapy. As expected, clinical disease progression was more frequent in patients with (decompensated) cirrhosis. Importantly, virological response in patients with cirrhosis was associated with a significantly lower probability of disease progression compared to those without a virological response, even after correction for important baseline variables. In contrast, a higher threshold of an HBV DNA of 2000 IU/mL (which is generally associated with inactive chronic hepatitis B) was not associated with a lower probability of disease progression. These findings indicate that a lower level of viral replication is indeed associated with a better clinical outcome, but that viral suppression should preferably be below the detection limit of currently available sensitive HBV DNA assays as stated in current guidelines.[1-2] In addition, the protective effect is most pronounced in cirrhotic patients who have the highest probability of clinical disease progression.

SEROLOGICAL OUTCOME DURING NA THERAPY

HBeAg seroconversion occurs in around 20% of patients after 1 year of NA therapy and is still considered a therapeutic endpoint for HBeAg-positive patients treated with both PEG-IFN and NA, as it results in a proportion of patients in remission of liver disease and is associated with a better clinical outcome.[19-20] Whereas a finite course of PEG-IFN aims at achieving HBeAg seroconversion followed by a sustained off treatment response (HBV DNA <2000 IU/mL) and/or HBsAg seroclearance, NA therapy aims on maintained HBV DNA suppression with continuous therapy in most patients. However, prolonged NA therapy may also lead to increasing serological response rates.[16] In **chapter 4** we aimed to compare continuous ETV therapy with a one year course of peginterferon for serological response rates. After correction for possible baseline differences, PEG-IFN resulted both in higher HBeAg seroconversion and HBsAg seroclearance rates compared with ETV up to 3 years of therapy. As to be expected, ETV-treated patients achieved higher rates of undetectable HBV DNA. This indicates that PEG-IFN remains an important first-line therapeutic option when aiming at finite duration of therapy, but careful patient selection seems of vital importance because of the relatively low overall probability of achieving disease remission.

However, stopping therapy after achieving HBeAg seroconversion frequently results in relapse of HBV and therapy should thus probably be continued.[21] Mutations within the precore (PC) and basal core promoter (BCP) region are frequently encountered naturally occurring HBV mutations, which prevent HBeAg production, were thought to be associated with 'active' HBeAg-negative CHB and were associated with HCC development.[22-24] In **chapter 5** the influence of PC and BCP mutations at start of NA therapy on achieving HBeAg seroconversion and disease reactivation after achieving this endpoint was investigated. The detection of PC and/or BCP mutations at baseline by a commercially available line probe assay was associated with a higher probability of HBeAg-seroconversion during NA therapy. In contrast, probability of achieving undetectable HBV DNA after HBeAg seroconversion tended to be lower in patients with a PC mutation, whereas the presence of a BCP mutation was associated with a higher rate of HBeAg relapse. After stopping NA therapy, virological relapse frequently occurred, but did not differ between patients with or without mutants. These findings indicate that probability of HBeAg seroconversion is higher in patients with PC/BCP mutant virus, but this does not result in sustained inactive disease in most patients and therapy should thus probably be continued in the majority of patients until HBsAg seroconversion.

In contrast to HBeAg seroconversion, HBsAg seroconversion is relatively infrequently achieved under NA therapy. Clearance of HBsAg is the preferred endpoint of therapy as it confers with serological clearance of HBV, is associated with an excellent long-term prognosis and relapse is rarely seen.[25] However, even after achieving this endpoint, the virus persists within the intrahepatic reservoir of covalently closed circular DNA (cccDNA) and could be reactivated when the immune system is suppressed. Quantification of serum HBsAg has been correlated with cccDNA; as were the changes in both parameters during PEG-IFN therapy.[26-27] In addition, different phases of CHB infection have been linked to different levels of serum HBsAg,[28-29] and changes in HBsAg levels during PEG-IFN were shown to be predictive of having a sustained response after stopping of therapy.[27-28] Data on the efficacy of potent NA therapy on HBsAg levels is currently limited, but HBsAg kinetics could be a potential tool in predicting HBsAg seroconversion and thus finite duration of NA therapy. Therefore we investigated in **chapter 6** the effect of prolonged ETV or TDF therapy on HBsAg serum levels. HBsAg steadily declined in HBeAg-positive patients during continuous therapy, and was most pronounced in those with high baseline ALT, older age or achieving HBeAg loss. In contrast, HBsAg decline in HBeAg-negative patients was only very limited. Estimated median time to achieve HBsAg seroclearance was calculated using these declines and was very long for the majority of patients, but shortest for the HBeAg-positive population with a high baseline ALT. HBsAg decline was thus strongest in those having an activated immune response (elevated ALT) at start of NA therapy, which is also supported by data indicating a possible relation with elevated IP-10 (marker for active liver disease) and decline- and clearance of HBsAg.[30] Second, clearance of HBeAg, which could result in immune control of the virus, was also associated with a decrease in HBsAg.[28-29] Unfortunately, prolonged potent NA therapy thus seems necessary for the vast majority of patients.

In **chapter 7** HBsAg kinetics were investigated within a Dutch multicenter cohort of HIV/HBV co-infected patients treated with TDF as part of highly active anti-retroviral therapy (HAART). Clearance of HBsAg could also be of vital importance for these patients as co-infection with HBV is associated with a higher morbidity and mortality.[31-32] As in mono infected patients, HBsAg decline was most pronounced in the HBeAg-positive population, and 6 years of continuous therapy resulted only in a non-significant decline of HBsAg within the HBeAg-negative patients. The HAART induced increase in CD4 cell count within the first year of therapy was shown to be correlated with HBsAg levels within the HBeAg-positive population. In addition, most patients with HBsAg seroclearance achieved this endpoint within the first year of therapy. Consequently, early HBsAg kinetics were shown to be highly predictive for HBsAg clearance. This study thus confirms the importance of immune modulation

for clearing HBV through the correlation between CD4 cell count and HBsAg decline, and the high rate of HBsAg seroclearance within the first year of HAART for HIV/HBV co-infected patients.

CONCLUSIONS

Adaptation of ETV therapy does not seem necessary in the majority of patients with a partial virological response at week 48, as prolonged therapy after week 48 leads to undetectable HBV DNA in the majority of patients. The combination of TDF+FTC could be a useful therapeutic option in patients with previous NA failure. Achieving VR during ETV therapy is associated with a lower probability of clinical disease progression (development of HCC, hepatic decompensation and death) in patients with cirrhosis, underlining the importance of achieving this endpoint especially in those with more advanced liver disease. A one year course of PEG-IFN results in higher HBeAg seroconversion and HBsAg seroclearance rates compared to prolonged ETV therapy. Despite a higher probability of HBeAg seroconversion during NA therapy in patients with PC/BCP mutants, but relapse is frequently seen. Unfortunately, HBsAg decline is limited in the majority of HBV patients. A more pronounced decline of HBsAg is seen in those with a more active immune response at baseline, in patients with CD4 cell count restoration and in those achieving HBeAg seroconversion. However, estimated time to achieve HBsAg seroclearance remains very long and thus indefinite NA therapy seems necessary for the vast majority of patients.

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Samenvatting en bespreking

BEHANDELING VAN CHRONISCHE HEPATITIS B

De therapeutische opties voor de behandeling van chronische hepatitis B zijn de laatste jaren toegenomen. De huidige therapieën focussen op surrogaat eindpunten als virologische respons (HBV DNA onderdrukking), biochemische respons (ALAT normalisatie), serologische respons (HBeAg en HBsAg verlies of seroconversie) en histologische respons (histologische verbetering), om zo progressie naar (gedecompenseerde) cirrose, hepatocellulair carcinoom (HCC) en overlijden te voorkomen.[1-2] De behandeling van chronische hepatitis B bestaat momenteel uit het immuun modulerende gepegyleerd interferon (PEG-IFN) en uit 5 verschillende nucleos(t)ide-analogen (NA). PEG-IFN is geregistreerd voor de duur van 1 jaar, waarna de medicatie wordt gestopt en de duurzaamheid van de respons wordt geëvalueerd. PEG-IFN wordt eenmaal per week toegediend door middel van een subcutane injectie, heeft een aanzienlijk bijwerkingenprofiel en resulteert bij ongeveer 30% van de patiënten in een duurzame respons.[3] De andere optie voor behandeling is langdurige dagelijkse orale therapie met NA, resulterend in succesvolle HBV DNA onderdrukking bij vrijwel alle patiënten en slechts beperkte bijwerkingen. Momenteel zijn er 5 NA goedgekeurd voor de behandeling van chronische hepatitis B: lamivudine (LAM), entecavir (ETV), telbivudine (LdT), adefovir (ADV) en tenofovir (TDF).[4-6] Alle NA werken door competitieve inhibitie van het hepatitis B virus (HBV) polymerase en onderdrukken dus de virale replicatie.[7]

VIROLOGISCHE EFFECTIVITEIT VAN NA THERAPIE BIJ NA-NAÏEVE PATIËNTEN EN PATIËNTEN VOORBEHANDELD MET NA

Grote cohortstudies met onbehandelde Aziatische patiënten laten zien dat de hoogte van het HBV DNA geassocieerd is met het risico op progressie van de leverziekte en de ontwikkeling van HCC.[8-9] Deze niveaus van virale replicatie werden vervolgens gebruikt in de huidige richtlijnen als een drempelwaarde voor het starten van antivirale therapie. Complete onderdrukking van virale replicatie naar ondetecteerbare HBV DNA waarden wordt momenteel beschouwd als het meest belangrijke surrogaat eindpunt van NA therapie omdat het relatief frequent wordt bereikt. De huidige chronische hepatitis B richtlijnen adviseren om te starten met ETV of TDF monotherapie als eerstelijns NA therapie (wanneer beschikbaar) vanwege hun potentie (snelheid van onderdrukking van de virale replicatie), de hoge genetische barrière tegen resistentie en de beperkte bijwerkingen.[1, 10] Verschillende definities van virologische eindpunten tijdens NA therapie worden ook vermeld binnen deze richtlijnen.[10] Virologische respons is gedefinieerd als een ondetecteerbaar HBV DNA gemeten met een sensitieve

test. Virologische doorbraak is gedefinieerd als een bevestigde stijging in HBV DNA van tenminste 1 log ten opzichte van het laagst bereikte HBV DNA niveau en is meestal geassocieerd met therapie-ontrouw of verminderde gevoeligheid van het virus als gevolg van de ontwikkeling van genotypische resistentie. Gebaseerd op de genetische barrière tegen resistentie werd een detecteerbaar HBV DNA op week 24 (LAM en LdT) of week 48 (ADV, ETV en TDF) gedefinieerd als partiële virologische respons. In het geval van een partiële virologische respons suggereerden de Europese richtlijnen van 2009 om de behandeling aan te passen, ondanks het gebrek aan bewijs voor patiënten behandeld met ETV of TDF.[10-13] Daarnaast komt de meeste data betreffende de langetermijneffectiviteit van ETV en TDF momenteel van registratiestudies waabij patiënten werden geïncludeerd, behandeld en gevolgd volgens strikte criteria, wat het belang van cohort studies uit de klinische praktijk onderstreept.[7-8]

In **hoofdstuk 1** wordt de invloed onderzocht van een partiële virologische respons op de virologische uitkomst bij NA-naïeve patiënten behandeld met ETV binnen een Europees cohort. Partiële virologische respons werd vastgesteld bij 21% van de patiënten na 48 weken ononderbroken ETV therapie. Echter, virologische respons werd bereikt met voortdurende ETV therapie bij en grote meerderheid van deze patiënten zonder aanpassing van de behandeling, vooral bij patiënten met een laag HBV DNA (<1000 IU/mL) gemeten op week 48. Daarnaast resulteerde langdurige ETV monotherapie niet in de ontwikkeling van genotypische resistentie tegen ETV. Andere bevindingen van deze studie zijn dat voorbehandeling met ADV of ADV resistentie niet resulteerde in een significant lagere kans op een virologische respons, maar dat patiënten met LAM resistentie wel een lagere kans hebben op het bereiken van een ondetecteerbaar HBV DNA. Daarnaast ontwikkelden 4 NA voorbehandelde patiënten (1 alleen met ADV) mutaties geassocieerd met genotypische resistentie tegen ETV waarna de behandeling werd aangepast. Persisterende replicatie (detecteerbaar HBV DNA) op week 48 is dus niet geassocieerd met een ongunstige virologische uitkomst bij ETV behandelde NA-naïeve patiënten, wat verschilt van eerdere data over oudere NA als ADV, LAM en LdT. Deze discrepantie wordt waarschijnlijk veroorzaakt door een hoge genetische barrière tegen resistentie (meer mutaties zijn nodig om resistentie te ontwikkelen) in vergelijking met oudere NA. Vanwege de lage kans op resistentie bij behandeling met ETV en TDF is het moeilijk om gevalideerde richtlijnen te ontwikkelen om deze therapieën aan te passen in de klinische praktijk. Bovendien onderstrepen deze bevindingen dat lessen uit studies over NA met een andere potentie en een andere genetische barrière voorzichtig moeten worden toegepast op patiënten behandeld met ETV of TDF.
Volgens kruisresistentie patronen van de momenteel beschikbare NA zou een TDF bevattend regime moeten worden gestart als redding therapie voor patiënten met

resistentie tegen LAM, LdT of ETV en een ETV bevattend regime voor patiënten die falen op ADV of TDF.[14] Huidige richtlijnen beschouwen alleen NA monotherapie als een eerstelijnsoptie voor patiënten met chronische hepatitis B, omdat het voordeel van combinatietherapie nog niet overtuigend is aangetoond bij NA-naïeve patiënten.[1, 10] In het geval van genotypische resistentie wordt switchen naar een ander middel zonder kruisresistentie of het toevoegen van een nieuw middel (combinatietherapie) aanbevolen. De vaste combinatie van TDF en emtricitabine (FTC, structureel vergelijkbaar met LAM) wordt momenteel veel gebruikt bij HIV-geïnfecteerde patiënten en zou een simpele en elegante optie kunnen zijn voor HBV patiënten met eerder falen van therapie vanwege de combinatie van 2 middelen in 1 tablet. Er is momenteel echter geen bewijs voor de superioriteit van TDF+FTC in zowel NA-naïeve als voorbehandelde chronische hepatitis B patiënten. In **hoofdstuk 2** wordt daarom de klinische effectiviteit en veiligheid van de vaste combinatie TDF+FTC onderzocht in een Europese studie met NA voorbehandelde patiënten. Er was een hoge kans op virologische respons en geen significante daling in het serum kreatinine tijdens de eerste 2 jaar van therapie. Hoewel er geen controle groep was, liet TDF+FTC zien een zeer effectieve en veilige optie te zijn bij vaak moeilijk te behandelen patiënten met eerder falen van therapie en bij patiënten waar een versimpeling van therapie wenselijk is. TDF+FTC zou dus een nuttig middel kunnen zijn voor beide indicaties.

KLINISCHE UITKOMST TIJDENS THERAPIE MET NA

Registratiestudies van patiënten behandeld met ETV en TDF wezen uit dat ondetecteerbaar HBV DNA wordt bereikt bij vrijwel alle (NA-naïeve) patiënten tijdens langetermijntherapie.[15-17] Echter, er is beperkte data hoe dit de langetermijnprognose van deze patiënten beïnvloedt. Momenteel is er slechts één gerandomiseerde studie die duidelijk het klinische voordeel laat zien bij patiënten die worden behandeld met NA. In deze studie werd behandeling met LAM geassocieerd met een lagere kans op leverziekte progressie en ontwikkeling van HCC dan behandeling met placebo.[18] In **hoofdstuk 3** wordt het effect van virologische respons op klinische ziekteprogressie (decompensatie, ontwikkeling van HCC of overlijden) onderzocht bij patiënten behandeld met ETV binnen een grote Europese cohort studie. De kans op een virologische respons verschilde niet tussen patiënten zonder cirrose, met gecompenseerde cirrose of met gedecompenseerde cirrose bij de start van ETV therapie. Zoals verwacht werd klinische ziekte progressie vaker gezien bij patiënten met (gedecompenseerde) cirrose. Virologische respons bij patiënten met cirrose werd geassocieerd met een significant lagere kans op ziekteprogressie in vergelijking met patiënten zonder virologische respons, zelfs na correctie voor belangrijke andere variabelen. Een hogere

concentratie van 2000 IU/mL HBV DNA (welke is geassocieerd met inactieve chronische hepatitis B) daarentegen was niet geassocieerd met een lagere kans op ziekte progressie. Deze bevindingen laten zien dat een lager niveau van virale replicatie inderdaad geassocieerd is met een betere klinische uitkomst. Bovendien dient het virus te worden onderdrukt tot bij voorkeur beneden de detectielimiet van de momenteel beschikbare gevoelige HBV DNA testen, zoals ook aangegeven in de huidige richtlijnen.[1-2] Daarnaast is het beschermende effect het meest geprononceerd bij patiënten met cirrose die de hoogste kans hebben op het ontwikkelen van klinische ziekteprogressie.

SEROLOGISCHE RESULTATEN TIJDENS NA THERAPIE

HBeAg seroconversie vindt plaats bij ongeveer 20% van de patiënten na 1 jaar NA therapie en wordt nog steeds beschouwd als een therapeutisch eindpunt voor HBeAg-positieve patiënten behandeld met zowel PEG-IFN als NA, omdat het resulteert in remissie van de leverziekte bij een deel van de patiënten en wordt geassocieerd met een betere klinische uitkomst.[19-20] Waar de behandeling met PEG-IFN gedurende 1 jaar streeft naar het bereiken van HBeAg seroconversie gevolgd door een blijvende respons na het staken van behandeling (HBV DNA <2000 IU/mL) en/of HBsAg verlies, streeft NA therapie naar een blijvende HBV DNA suppressie onder voortdurende therapie. Echter, langdurige NA therapie zou ook kunnen leiden tot een toenemende kans op serologische respons.[16] In **hoofdstuk 4** is er getracht de serologische uitkomsten te vergelijken van voortdurende ETV therapie en een kuur PEG-IFN van 1 jaar. Na correctie voor mogelijke verschillen, resulteerde PEG-IFN zowel in een hogere kans op HBeAg seroconversie als HBsAg verlies vergeleken met 3 jaar ETV therapie. Zoals verwacht bereiken ETV behandelde patiënten vaker een ondetecteerbaar HBV DNA. Dit indiceert dat PEG-IFN een belangrijke eerstelijns therapeutische optie blijft wanneer men streeft naar een eindige therapieduur. Echter, zorgvuldige selectie van patiënten lijkt hierbij van vitaal belang vanwege de relatief lage kans op het bereiken van blijvende ziekteremissie.

Het stoppen van NA therapie na het bereiken van HBeAg seroconversie resulteert echter nog vaak in terugval van HBV en therapie zou dus waarschijnlijk moeten worden gecontinueerd.[21] Mutaties binnen de precore (PC) en basal core promoter (BCP) regio zijn vaak geziene natuurlijk voorkomende HBV mutaties die HBeAg productie voorkomen, in verband worden gebracht met ‘actieve’ HBeAg-negatieve chronische hepatitis B en ook zijn geassocieerd met de ontwikkeling van HCC.[22-24] In **hoofdstuk 5** is de invloed onderzocht van PC en BCP mutaties bij het starten van NA therapie

op het bereiken van HBeAg seroconversie en op reactivatie na het bereiken van dit therapeutisch eindpunt. De detectie van PC en/of BCP mutaties bij de start van therapie door een commercieel beschikbare test werd geassocieerd met een hogere kans op HBeAg seroconversie tijdens NA therapie. De kans op het bereiken van een ondetecteerbaar HBV DNA na HBeAg seroconversie was echter lager bij patiënten met een PC mutatie en de aanwezigheid van een BCP mutatie leek geassocieerd met een hogere kans op HBeAg terugval. Na het stoppen van NA therapie, was er frequent sprake van virologische terugval, dit verschilde niet tussen patiënten met of zonder mutanten. Deze bevindingen wijzen er op dat de kans op HBeAg seroconversie hoger is bij patiënten met een PC/BCP mutant, maar dat dit bij de meeste patiënten niet resulteert in inactieve ziekte en NA therapie dus waarschijnlijk zal moeten worden gecontinueerd bij de meerderheid van de patiënten tot HBsAg seroconversie.

In tegenstelling tot HBeAg seroconversie, komt HBsAg seroconversie relatief weinig voor tijdens NA therapie. Klaring van het HBsAg is het gewenste eindpunt van therapie bij chronische hepatitis B omdat het de definitie is van serologische klaring van het virus, een uitstekende langetermijnprognose heeft en terugval zeldzaam is.[25] Echter, ook na het bereiken van dit eindpunt blijft het virus detecteerbaar in het intrahepatische reservoir van covalent gesloten circulair DNA (cccDNA) en zou kunnen worden gereactiveerd wanneer het immuunsysteem wordt onderdrukt. De concentratie van het HBsAg in serum is gecorreleerd met cccDNA en ook de veranderingen in beide parameters tijdens PEG-IFN therapie zijn gecorreleerd.[26-27] Verder zijn de verschillende fases van de chronische hepatitis B infectie gekoppeld aan verschillende niveaus van serum HBsAg[28-29] en zijn veranderingen in HBsAg waarden tijdens PEG-IFN voorspellend voor het hebben van een blijvende respons na het stoppen van therapie.[27-28] Data over de effectiviteit van potente NA therapie op HBsAg waarden is momenteel gelimiteerd, maar HBsAg kwantificatie zou een potentieel hulpmiddel kunnen zijn in het voorspellen van HBsAg seroconversie en dus het stoppen van NA therapie. In **hoofdstuk 6** is daarom het effect van langdurige ETV of TDF therapie op serum HBsAg onderzocht. Het HBsAg daalde gestaag bij HBeAg-positieve patiënten tijdens voortdurende therapie en was meest uitgesproken bij patiënten met een hoog ALAT bij start, bij oudere patiënten en bij patiënten die HBeAg negatief werden. HBsAg daling bij HBeAg-negatieve patiënten was echter beperkt. Geschatte mediane tijd tot HBsAg verlies werd berekend met behulp van deze dalingen en bleek erg lang voor de meeste patiënten, maar het kortst voor de HBeAg-positieve populatie met een hoog ALAT bij start van therapie. HBsAg daling is dus het sterkst bij patiënten met een actieve immuunrespons (verhoogd ALAT) bij het starten van NA therapie, wat ook ondersteund wordt door data wijzend op een mogelijke relatie tussen IP-10 (waarde voor actieve leverziekte) en daling en klaring van het HBsAg.[30] HBeAg verlies, wat zou

kunnen resulteren in immunologische controle over het virus, was ook geassocieerd met een daling in het HBsAg.[28-29] Langdurige potente NA therapie lijkt helaas dus noodzakelijk voor een grote meerderheid van patiënten.

In **hoofdstuk 7** is de HBsAg kinetiek onderzocht binnen een Nederlands cohort van HIV/HBV co-geïnfecteerde patiënten behandeld met TDF als onderdeel van hun antiretrovirale therapie (HAART). Klaring van het HBsAg zou van vitaal belang kunnen zijn voor deze patiënten aangezien co-infectie met HBV geassocieerd wordt met een hogere morbiditeit en mortaliteit.[31-32] Net als bij patiënten geïnfecteerd met alleen HBV, was de HBsAg daling het meest uitgesproken bij de HBeAg-positieve populatie. Zes jaar ononderbroken therapie resulteerde slechts in een niet significante daling van het HBsAg bij de HBeAg-negatieve patiënten. De door HAART geïnduceerde stijging in CD4 cel getal in het eerste jaar van therapie was gecorreleerd met HBsAg waarden binnen de HBeAg-positieve populatie. Daarnaast bereikten de meeste patiënten met HBsAg verlies dit eindpunt binnen het eerste jaar van therapie. Vroege HBsAg kinetiek was derhalve zeer voorspellend voor HBsAg verlies. Deze studie bevestigt dus de importantie van immuunmodulatie om het HBV te kunnen klaren door de correlatie tussen CD4 cel getal en HBsAg en de hoge kans op HBsAg verlies in het eerste jaar van HAART bij HIV/HBV co-geïnfecteerde patiënten.

CONCLUSIES

Aanpassing van ETV therapie lijkt niet noodzakelijk bij de meeste patiënten met een partiële virologische respons op week 48, omdat het continueren van de behandeling na week 48 leidt tot een ondetecteerbaar HBV DNA bij de meerderheid van de patiënten. De combinatie van TDF+FTC zou een nuttige therapeutische optie kunnen zijn bij patiënten met eerder falen van NA therapie. Het bereiken van een virologische respons tijdens ETV therapie is geassocieerd met een lagere kans op klinische ziekte progressie (ontwikkeling van HCC, decompensatie of overlijden) bij patiënten met cirrose. Dit onderstreept het belang van het halen van een virologische respons, vooral bij patiënten met meer gevorderde leverziekte. Een PEG-IFN kuur resulteert in een hogere kans op HBeAg seroconversie en HBsAg verlies in vergelijking met langdurige ETV therapie. Ondanks een hogere kans op HBeAg seroconversie tijdens NA therapie bij patiënten met PC/BCP mutanten, komt terugval frequent voor. HBsAg daling is helaas beperkt bij de meerderheid van HBV patiënten. Een meer uitgesproken daling van het HBsAg wordt gezien bij HBeAg-positieve patiënten met een actieve immuun respons bij starten van therapie, bij HIV/HBV patiënten met een herstel van het CD4 cel getal en bij patiënten met HBeAg verlies. Echter, de geschatte tijd om het HBsAg

te klaren blijft erg lang en dus lijkt langdurige NA therapie noodzakelijk voor de grote meerderheid van patiënten.

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Soli Deo Gloria!

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Curriculum vitae

De auteur van dit proefschrift werd geboren op 16 oktober 1983 te Dirksland. In 2002 behaalde hij zijn V.W.O. diploma aan de Christelijke Scholengemeenschap Prins Maurits te Middelharnis, om vervolgens te starten met de opleiding Geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraal examen werd behaald in 2006 en het artsexamen in 2008. Van januari tot juli 2009 werkte hij als arts niet in opleiding tot specialist (ANIOS) Spoedeisende Hulp Geneeskunde in het Vlietland Ziekenhuis te Schiedam. Hierna begon hij aan zijn promotieonderzoek op de afdeling Maag-, Darm-, en Leverziekten van het Erasmus MC te Rotterdam (afdelingshoofd: prof. dr. E.J. Kuipers) onder supervisie van prof. dr. H.L.A. Janssen. Door de Nederlandse Vereniging voor Hepatologie (NVH) kreeg hij in die periode de 'Young Hepatologist Award' uitgereikt voor beste klinisch hepatologisch wetenschappelijk artikel van 2011. Per juli 2012 is hij gestart met de opleiding tot Maag-Darm-Leverarts (opleider: dr. R.A. de Man). De vooropleiding Interne Geneeskunde volgt hij gedurende twee jaar in het Ikazia Ziekenhuis te Rotterdam (opleider: dr. A.A.M. Zandbergen). Hij is gehuwd met Sanne Zoutendijk-Kraaijeveld en woonachtig in Rotterdam.

PhD Portfolio

Summary of PhD training and teaching
Name PhD student: Roeland Zoutendijk
Erasmus MC Department: Gastroenterology and Hepatology
PhD period: 2009-2012
Promotor: Prof. Dr. H.L.A. Janssen

1. PHD TRAINING

Presentations and workshops	Year	Workload
Entecavir treatment is effective in patients with previous adefovir treatment: results from an international multicenter cohort study. Early morning workshop: Nucleoside analogues – is a finite duration of therapy possible? 46th Annual meeting of the European Association of the Study of the Liver, Berlin, Germany.	2011	32 hours
Documenting the journey – Importance of real-patient data. The patient journey: Documenting optimal long-term treatment of CHB. BMS educational meeting, Barcelona, Spain.	2011	12 hours
Ensuring a clear path: optimising treatment for all patients – An interactive workshop. The patient journey: Documenting optimal long-term treatment of CHB. BMS educational meeting, Barcelona, Spain.	2011	6 hours
Living the patient journey – An interactive workshop. The patient journey: Documenting optimal long-term treatment of CHB. BMS educational meeting, Barcelona, Spain.	2011	6 hours
Virological response to entecavir is associated with a lower probability of disease progression: results from 377 chronic hepatitis B patients. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2011	12 hours
Virological response to entecavir is associated with a lower probability of disease progression: results from 377 chronic hepatitis B patients. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, The United States of America.	2011	36 hours
Hepatitis B infectie. 9e Post-AASLD symposium, Erasmus Liver Day, Rotterdam, the Netherlands.	2011	18 hours

Kwantitatieve HBsAg bepaling in de kliniek, Abbott gebruikersdag, Utrecht, the Netherlands.	2012	4 hours
Treatment of chronic hepatitis B: a real-life European perspective, BMS Satellite symposium, 18th Annual Meeting of the Korean Association for the Study of the Liver, Seoul, Republic of Korea.	2012	12 hours

Awards

Young Investigator Bursary for best abstract (European Association for the Study of the Liver).	2011
Presidential poster of distinction (American Association for the Study of Liver Diseases).	2011
Young Investigator Bursary for best abstract (European Association for the Study of the Liver).	2012
Young Hepatologist Award 2011 (Nederlandse Vereniging voor Hepatologie) voor beste klinisch hepatologisch wetenschappelijk artikel van 2011.	2012

Statistical training

Classical methods of data analysis. Netherlands Institute for Health Sciences, Rotterdam, the Netherlands.	2009	104 hours

Research Integrity

BROK course.	2010	30 hours

Poster presentations

Treatment adaptation is not needed for the majority of partial virological responders to entecavir: results from 326 chronic hepatitis B patients in an international multicenter cohort study. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2010	32 hours

Prediction of HBsAg loss using HBsAg decline after long-term virological response to nucleos(t)ide analogue therapy for chronic hepatitis B. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2010	32 hours
Efficacy and tolerance of tenofovir disoproxil fumarate plus emtricitabine combination in patients with chronic hepatitis B – A European multicenter study.	2010	32 hours
Tenofovir treatment for up to eight years results in pronounced HBsAg decline in HBeAg-positive HIV/HBV coinfected patients. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2011	32 hours
Peginterferon is superior to continuous entecavir for serological response in HBeAg-positive chronic hepatitis B. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2011	32 hours
Relationship between precore/base core promoter mutants and serological or virological response in HBeAg-positive chronic hepatitis B treated with nucleos(t) ide analogues. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2011	32 hours
Intrahepatic NK cell phenotype and function is dependent on clinical phase of chronic hepatitis B and presence of fibrosis.	2011	32 hours
Prospective monitoring of the intrahepatic lymphocyte compartment during antiviral treatment of chronic hepatitis B shows increased activation of liver NK cells.	2011	32 hours
Relationship between precore/ core promoter mutants, HBeAg levels and serological response in HBeAg-positive chronic hepatitis B treated with nucleos(t)ide analogues. 47th Annual Meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain.	2012	32 hours
Finite peginterferon results in higher serological, but not virological, response rates when compared to continuous entecavir in HBeAg-positive chronic hepatitis B. 47th Annual Meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain.	2012	32 hours

A European field study of the efficacy and safety of Tenofovir disoproxil fumarate (TDF) as monotherapy in patients with prior failure to other nucleoside/nucleotide analogues. 47th Annual Meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain.	2012	32 hours
ALT flares during entecavir treatment are associated with a favourable outcome in chronic hepatitis B. 63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2012	14 hours

International conferences

The Liver Meeting 2009, 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2009	28 hours
45th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria.	2010	28 hours
The Liver Meeting 2010, 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2010	28 hours
46th Annual Meeting of the European Association for the Study of the Liver (EASL). Berlin, Germany.	2011	28 hours
The Liver Meeting 2011, 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA, United States of America.	2011	28 hours
47th Annual Meeting of the European Association for the Study of the Liver (EASL). Barcelona, Spain.	2012	28 hours

Attended seminars and workshops

Tweede Lagerhuisdebat Hepatitis B en C. Amsterdam, the Netherlands.	2009	2 hours
7th Post-AASLD symposium. Rotterdam, the Netherlands.	2009	2 hours

Virus – Host interactions and the regulation of viral immunity, Molecular Medicine Postgraduate School, Rotterdam, the Netherlands.	2010	3 hours
8th Post-AASLD symposium. Rotterdam, the Netherlands.	2010	2 hours
De 24-uur van De Vanenburg. Putten, the Netherlands.	2011	6 hours
9th Post-AASLD symposium. Rotterdam, the Netherlands.	2011	2 hours

Referee scientific journal

Hepatology. Official Journal of the American Association for the Study of Liver Diseases.	2012

2. TEACHING

Lecturing

Treatment of hepatitis B. Education for general practitioners. Rotterdam, the Netherlands.	2009	6 hours
Hepatitis B. 3rd year Erasmus MC students participating in a 4-week Gastroenterology and Hepatology training program. Rotterdam, the Netherlands.	2010	8 hours
Treatment of chronic hepatitis B and case discussion. Education for residents in Internal Medicine, Rotterdam, the Netherlands.	2010	8 hours
Hepatitis B. 3rd year Erasmus MC students participating in a 4-week Gastroenterology and Hepatology training program. Rotterdam, the Netherlands.	2011	4 hours
Hepatitis B. Master students 'Infection & Immunity'. Rotterdam, the Netherlands.	2011	4 hours
Treatment of hepatitis B, cases. Minor students from the Erasmus MC. Rotterdam, the Netherlands.	2011	4 hours
'Allemaal beestjes'. Hepatitis B. Education for general practitioners. Rotterdam, the Netherlands.	2011	6 hours
Treatment of hepatitis B, cases. Minor students from the Erasmus MC. Rotterdam, the Netherlands.	2012	4 hours

Abbreviations

ADV	adefovir dipivoxil
ALT	alanine aminotransferase
anti-HBe	antibody against HBeAg
BCP	base core promoter
BMI	body mass index
cccDNA	covalently closed circular DNA
CHB	chronic hepatitis B
CI	confidence interval
ETV	entecavir
FTC	emtricitabine
HAART	highly active anti-retroviral therapy
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis delta virus
HIV	human immunodeficiency virus
HR	hazard ratio
IFN	interferon
LAM	lamivudine
LdT	telbivudine
NA	nucleos(t)ide analogues
PC	precore
PCR	polymerase chain reaction
PEG-IFN	peginterferon
PVR	partial virological response
TDF	tenofovir disoproxil fumarate
ULN	upper limit of normal
VR	virological response
WT	wildtype