ORIGINAL ARTICLE

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

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Revised 15 February 2012
Accepted 28 February 2012
Published Online First 7 July 2012

Linked

▶ http://dx.doi.org/10.1136/gutjnl-2012-303647

ABSTRACT

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

Design In a multicentre cohort study, 372 ETV-treated patients were investigated. Clinical events were defined as development of hepatocellular carcinoma (HCC), hepatic decompensation or death. Virological response (VR) was defined as HBV DNA <80 IU/ml.

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

Conclusion VR to ETV is associated with a lower probability of disease progression in patients with cirrhosis, 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 nucleoside/nucleotide analogue treatment, especially in patients with cirrhosis.

Significance of this study

What is already known about this subject?
- Higher hepatitis B virus (HBV) DNA levels are associated with a higher probability of disease progression.
- Continuous entecavir treatment results in a virological response in the majority of patients with HBV.
- Prolonged entecavir treatment results in regression of fibrosis.

What are the new findings?
- The probability of a virological response during entecavir treatment is not influenced by the severity of liver disease.
- Virological response is independently associated with a lower probability of disease progression in patients with cirrhosis.
- An HBV DNA threshold of 2000 IU/ml is not associated with a lower probability of disease progression.

How might it impact on clinical practice in the foreseeable future?
- Complete HBV DNA suppression by entecavir is associated with a lower probability of disease progression and should thus be aimed for in all patients, especially those with cirrhosis.

INTRODUCTION

An estimated 300–400 million people are chronically infected with hepatitis B virus (HBV) and more than 500 000 patients with chronic hepatitis B (CHB) die annually from complications of (decompensated) cirrhosis and hepatocellular carcinoma (HCC).[1] The ultimate goal of treatment of CHB 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 in HBV DNA to low or undetectable levels has been adopted as an important endpoint for measuring antiviral efficacy in patients with CHB.[3 4]

Entecavir (ETV) showed superior biochemical and virological efficacy compared with lamivudine (LAM) in large phase III trials including patients with CHB with compensated liver disease.[5 6] In addition, Shim *et al* recently showed that 1 year of

ETV monotherapy is similarly effective in patients with decompensated and compensated liver disease.[7] Importantly, after a median of 6 years of continuous ETV treatment, 88% of patients treated with ETV showed improvement in the fibrosis score, including 10 patients with advanced fibrosis or cirrhosis at baseline.[8] In addition, Liaw *et al* showed that LAM treatment 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 the prevention of clinical events such as HCC, decompensation and death.

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

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 adults with CHB (hepatitis B surface antigen (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 centres were included. Patients were excluded if they had viral co-infections (HIV, hepatitis C virus (HCV), hepatitis D virus (HDV)), had an HCC at baseline and if they had undergone liver transplantation before the start of ETV treatment. 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 examinations with biochemical (serum alanine transaminase (ALT), bilirubin, albumin, international normalised ratio (INR), creatinine) and virological (serum HBV DNA level, hepatitis B e antigen (HBeAg), antibody against HBeAg (anti-HBe), HBsAg, antibody against HBsAg (anti-HBs)) assessments were undertaken. Screening for HCC was performed at least yearly by α-fetoprotein and/or ultrasound in cirrhotic and non-cirrhotic patients when other risk factors were present.[10] A 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). A 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 oesophageal 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 either by histocytological examination or was diagnosed if two coincident imaging techniques (ultrasound, CT or MRI) showed a focal lesion >2 cm with arterial hypervascularisation or if one imaging technique showed a focal lesion >2 cm with arterial hypervascularisation in the presence of an α-fetoprotein level >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 normalisation in patients with abnormal ALT at baseline).

Laboratory tests

Serum ALT, bilirubin, albumin levels and INR of prothrombin time were measured locally using standardised automated techniques. HBsAg, anti-HBs, HBeAg and anti-HBe were determined using commercially available enzyme immunoassays. Serum HBV DNA levels were measured using a quantitative real-time PCR assay, the COBAS AmpliPrep-COBAS TaqMan HBV test (CAP-CTM; Roche Molecular Systems, Branchburg, New Jersey, USA), with a lower limit of detection of 12 IU/ml, in nine of 10 centres. In one centre serum HBV DNA was measured using Roche Amplicor (linear dynamic range 400–200 000 copies/ml; Roche Diagnostic Systems, Branchburg, New Jersey, 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 were 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 were systematically collected using a standardised clinical research form and entered for analysis in 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 (×ULN). Continuous variables are expressed as means±SD or median (IQR) where appropriate. Follow-up time was calculated as the time interval between start of ETV treatment and diagnosis of a clinical event or the end of follow-up. Patients were censored when the antiviral treatment regimen was adapted. The cumulative probability of achieving primary or secondary endpoints was estimated by Kaplan–Meier analysis. The influence of VR was analysed by a time-dependent analysis. VR was therefore entered in 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 in the univariate analysis were entered in a multivariate model. All statistical tests were two-sided and a p value <0.05 was considered to be statistically significant. SPSS V.15.0 was used for all statistical analysis (SPSS Inc).

RESULTS

Baseline characteristics

A total of 437 patients with chronic HBV treated with ETV were identified. Sixty-five patients did not fulfill the entry criteria and were excluded. Forty patients were treated for <3 months, one patient was <6 months HBsAg positive, nine patients were co-infected with HCV or HDV, five patients had HCC at baseline, two patients had undergone liver transplantation and 18 patients received concomitant antiviral treatment. A total of 372 patients were thus eligible for the analysis. The study population consisted of 274 patients with CHB without cirrhosis, 89

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

	No cirrhosis (N=274)	Cirrhosis (N=89)	Decompensated cirrhosis (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 (×ULN)	1.6 (1.0–3.0)	1.4 (1.0–3.1)	2.8 (1.9–18.2)	0.008
HBV DNA (log_{10} 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 ($10^3/\mu l$)	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

ADV, adefovir; ALT, alanine transaminase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; INR, international normalised ratio; LAM, lamivudine; PEG-IFN, peginterferon; ULN, upper limit of normal.

patients with compensated cirrhosis and nine patients with decompensated cirrhosis. The baseline characteristics of the study population are shown in table 1 according to their initial baseline liver disease severity. Overall median follow-up of the study population was 20 months (IQR 11–32) and did not differ between the three 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 treatment. 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 exposure to LAM and adefovir (ADV)), the probability of achieving a VR was not different between the three 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 was achieved in three HBeAg-positive patients and three HBeAg-negative patients and did not differ between the three groups (p=0.81). Genotypic resistance to ETV was detected in five of 111 (5%) patients previously treated with other nucleoside/nucleotide analogue (NA) regimens. Biochemical response (normalisation 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 three groups (p=0.35). The MELD score did not change significantly in the two groups with cirrhosis at baseline during the study period (compensated cirrhosis: +0.0±2.9, p=0.95; decompensated cirrhosis: −1.4±6.9, p=0.67).

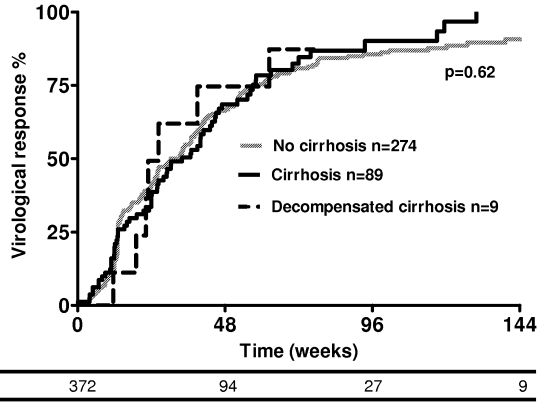
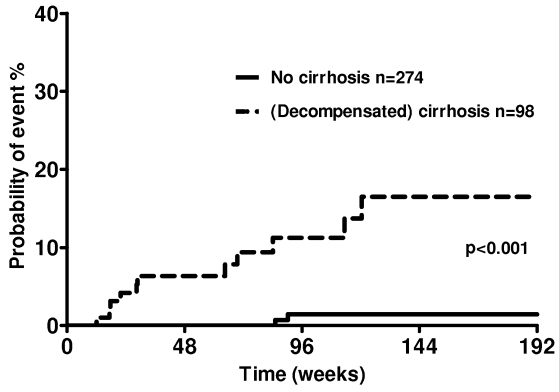


Figure 1 Kaplan–Meier curve for the cumulative probability of achieving a virological response for entecavir-treated patients stratified according to severity of liver disease at baseline.

Factors associated with clinical events in patients treated with entecavir

Thirteen patients in the study population developed a clinical event: six patients developed an episode of hepatic decompensation (three of whom died), three patients developed HCC and seven patients died. The cumulative probability of an event was 0%, 2%, 2% and 2% at years 1, 2, 3 and 4 for non-cirrhotic patients and 6%, 11%, 17% and 17%, respectively, for patients with cirrhosis (p<0.001, figure 2). The higher cumulative probability of an event in patients with cirrhosis remained when decompensated patients were excluded (p<0.001). Two patients, both without cirrhosis, developed an ALT flare (ALT >10×ULN) on treatment, but neither of these patients subsequently experienced clinical disease progression. Three of nine decompensated patients had an ALT flare at baseline, all of whom achieved ALT normalisation. In univariate analysis, patients with an event tended to be older (p=0.12, table 2) and more



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

Figure 2 Kaplan–Meier curve 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.

often had cirrhosis (p<0.001). Virological breakthrough was observed in 18 patients, two of whom developed a clinical event. In eight (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 one 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 (one non-cirrhotic patient, three patients with compensated cirrhosis and one patient with decompensated cirrhosis) developed a clinical event after achieving VR (figure 3), with a median time to VR of 32 weeks (IQR 15–53) and a median time to event of 65 weeks (IQR 28–88). To investigate the clinical effect of response to ETV, we studied the influence of VR on developing clinical events with VR as a time-dependent covariate within a Cox model (table 2 and figure 4A). Patients with a VR during ETV treatment had a lower probability of developing a clinical event (HR 0.29, 95% CI 0.08 to 1.00, p=0.05). This effect was significant among all patients with cirrhosis (HR 0.22, 95% CI 0.05 to 0.99, p=0.04, figure 4B) and among those with compensated cirrhosis alone (HR 0.15, 95% CI 0.03 to 0.81, p=0.03). For the nine patients with decompensated cirrhosis, VR was not significantly associated with a lower probability of disease progression (95% CI 0.06 to 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 patients without cirrhosis (HR 0.24, 95% CI 0.02 to 3.76, p=0.27).

Importantly, when age or other significant baseline variables were separately included in a multivariate model, VR remained significantly associated with a lower probability of events for the patients with cirrhosis (table 3). Owing to the limited number of events within the study population, we could only include one baseline variable besides VR in the models. The influence of VR in the patients with cirrhosis 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 to 0.77, p=0.02 and HR 0.07, 95% CI 0.01 to 0.48, p=0.006, respectively).

Since an HBV DNA <2000 IU/ml is associated with immune control in natural history and peginterferon studies, we

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

Risk factor	HR (95% CI)	p Value
Age (per year)	1.03 (0.99 to 1.07)	0.12
Female gender	0.52 (0.12 to 2.35)	0.52
Asian ethnicity	0.88 (0.24 to 3.21)	0.85
ALT (×ULN)	1.00 (0.92 to 1.08)	0.90
HBV DNA (log_{10} IU/ml)	1.07 (0.82 to 1.39)	0.62
HBeAg negativity	1.60 (0.54 to 4.75)	0.40
Genotype A	0.99 (0.21 to 4.67)	0.99
Bilirubin (mg/dl)	1.00 (0.99 to 1.01)	0.54
Albumin (g/dl)	0.74 (0.67 to 0.83)	<0.001
INR	38.53 (6.12 to 242.62)	<0.001
Platelet count	0.99 (0.98 to 1.00)	0.02
Cirrhosis	15.41 (3.42 to 69.54)	<0.001
Decompensated cirrhosis	16.76 (4.58 to 61.29)	<0.001
Previous treatment with PEG-IFN	0.23 (0.03 to 1.80)	0.16
Previous treatment with LAM	2.85 (0.96 to 8.49)	0.07
Previous treatment with ADV	2.52 (0.83 to 7.71)	0.11
Virological response*	0.29 (0.08 to 1.00)	0.05

*According to a Cox model with this variable as a time-dependent covariate.
ADV, adefovir; ALT, alanine transaminase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; INR, international normalised ratio; LAM, lamivudine; PEG-IFN, peginterferon; ULN, upper limit of normal.

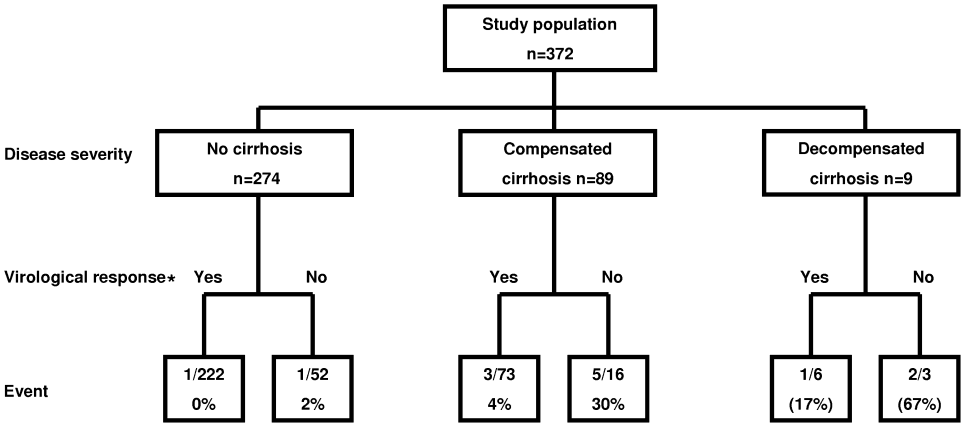


Figure 3 Distribution of clinical events within the study population according to the severity of liver disease at baseline and achievement of a virological response (HBV DNA <80 IU/ml). *Virological response at time of event or censoring.

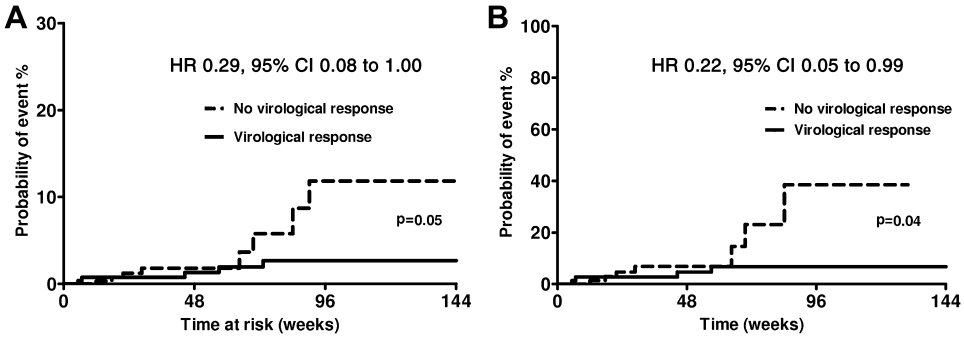


Figure 4 Cumulative probability of developing a clinical event during entecavir treatment in (A) all patients and (B) patients with cirrhosis, according to virological response. For this analysis, all patients started within the group without virological response and were censored and switched to the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

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 in 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 to 1.10, p=0.10). In contrast, for a threshold of 200 IU/ml (HR 0.12, 95% CI 0.03 to 0.58, p=0.007), the association with probability of disease progression remained significant.

DISCUSSION

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

Current antiviral treatment focuses on established surrogate endpoints such as HBV DNA suppression as well as HBeAg and HBsAg seroconversion to assess and compare antiviral treatment in the short term as these endpoints are easy to measure and occur relatively frequently.[2] Treatment with the most potent antiviral agents such as ETV and tenofovir (TDF) led to a VR in the vast majority of patients, both in registration trials and in 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, ADV has been 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 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 treatment reduced the risk of disease progression and development of HCC in the absence of genotypic resistance compared with patients treated with placebo.[9] A second observation in this study was that patients developing LAM resistance were at increased risk of developing liver-related complications.[9] This underlines that, especially in patients with advanced liver disease, first-line treatment 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 treatment for patients with cirrhosis 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 in a multivariate model. In contrast, for patients without cirrhosis 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 treatment is not always lifesaving in patients with decompensated cirrhosis. An initial high rate of disease progression was also seen in our nine 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 did not find a significant association between achieving this virological threshold and developing clinical events. This suggests, on the one hand, 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 minimise the risk of developing complications.

In conclusion, our study shows the beneficial effects of a VR to ETV in patients with cirrhosis in preventing liver disease

Table 3 Multivariate Cox models including virological response and different baseline variables

	HR (95% CI) virological response	p Value
Model 1		
Virological response and age	0.22 (0.05 to 0.99)	0.04
Model 2		
Virological response and albumin	0.02 (0.00 to 0.44)	0.01
Model 3		
Virological response and INR	0.15 (0.02 to 1.00)	0.04
Model 4		
Virological response and platelets	0.11 (0.02 to 0.75)	0.01

For 98 patients with (decompensated) cirrhosis at baseline treated with entecavir.
Virological response was included in all models as a time-dependent covariate.
INR, international normalized ratio.

progression for patients with CHB. A VR during potent NA treatment is achieved in the majority of patients and could minimise the risk of developing complications.

Acknowledgements The authors would like to thank Pierre Pradat, Ye Oo, Florian van Bommel and Marc Luetgehetmann for their assistance in data collection.

Contributors RZ: acquisition of data, analysis and interpretation of data, drafting and finalising the article. JR: acquisition of data, drafting and finalising the article. FZ, AB, DJM, KD, JP, WPH, MB, MF, TB, HW: acquisition of data, critical revision of draft of article and approval of final version. MJS: interpretation of data, drafting of the article and approval of final version. BEH: analysis and interpretation of data, critical revision of draft of article and approval of final version. HLAJ: study concept and design, study supervision, analysis and interpretation of data, drafting and finalising the article.

Funding The study was sponsored by the Foundation for Liver and Gastrointestinal Research Rotterdam (SLO) and conducted with support from the European Network of Excellence for Vigilance against Viral Resistance (VIRGIL) and a research grant from Bristol-Myers Squibb (BMS). BMS has not been involved in the design of the study, data collection, writing of the manuscript or decision to publish.

Competing interests RZ has received speaker's honoraria from Roche and Bristol-Myers Squibb (BMS); JR has received speaker's honoraria from Novartis and BMS; FZ is consultant for BMS, Gilead, Roche and Novartis; AB advises MSD, Roche, BMS, Gilead and Novartis; DJM has received honoraria and grants from BMS; JP is consultant for Gilead, Novartis, MSD, BMS, Roche and Janssen-Cilag and received research support from Roche, BMS, Novartis and Glaxo Smith Kline; MB has received speaker's honoraria and advisory board fee from Gilead, MSD, BMS and Novartis; TB is a consultant for and on the speakers' bureau of Gilead, Roche, Novartis and MSD; HW received research grants and speaker's honoraria from BMS, Gilead, Roche and Novartis; HLAJ received grants from and is consultant for BMS, Gilead Sciences, Novartis, Roche and Merck. The remaining authors have nothing to disclose.

Ethics approval The study was approved by the medical ethics committee of Erasmus MC Rotterdam.

Provenance and peer review Not commissioned; externally peer reviewed.

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