Chronic Hepatitis B Infection

New insights in therapy and predictors of response

Pauline Arends

Kunst

Wat we willen
Momenten Van helderheid

Of beter nog: van grote
Klaarheid

Schaars zijn die momenten
En ook nog goed verborgen

Zoeken heeft dus
Nauwelijks zin, maar
Vinden wel

De kunst is zo te leven
Dat het je overkomt
Die klaarheid, af en toe

Martin Bril, 'Verzameld werk' 2002

CHRONIC HEPATITIS B INFECTION: NEW INSIGHTS IN THERAPY AND PREDICTORS OF RESPONSE

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Chronic Hepatitis B Infection: New insights in therapy and predictors of response

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nieuwe inzichten in behandeling en voorspellers van respons

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3). Arends P, Janssen HL. Behandelmogelijkheden van Chronische Hepatitis B. Nederlands Tijdschrift voor Medische Microbiologie. 2011; 19: nr1: 12-16.

The hepatitis B virus (HBV) was discovered by dr. Baruch Samuel Blumberg when he identified the 'Australia antigen' among an aboriginal in the 1960s. The 'Australia antigen' is nowadays known as the hepatitis B surface antigen (HBsAg). For his work dr. Blumberg was awarded the 1976 Nobel Prize in Medicine. Despite the introduction of safe and effective vaccines in the eighties, HBV infection still constitutes a major burden of disease. Currently, about one third of the world's population has evidence of past or current HBV infection and over 350 million people still being chronically infected.[1] Approximately 45% of the infected population lives in high endemic areas (HBV prevalence over 8%) including Asia and sub-Saharan Africa. Chronic HBV infection remains one of the most serious infectious diseases worldwide with 0.5-1.2 million deaths every year due to long-term sequelae of hepatitis B related chronic liver disease, such as liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC).[2]

THE HEPATITIS B VIRUS

The HBV is one of the smallest enveloped animal double-stranded DNA viruses (virion diameter 40-42 nm) belonging to the family of the *Hepadnaviridae*, which have a strong preference for infecting hepatocytes.[3,4]

The virus particle (virion) consists of an outer lipoprotein envelope containing HBsAg. The hepatitis B core (HBcAg) is located within this outer layer and contains the viral genome and HBV DNA polymerase, which is necessary for viral synthesis. Analysis of the nucleotide sequence of the virus revealed four overlapping open reading frames (ORFs), regions of the genome which may code for viral antigens.[4] The surface (S) ORF encodes the envelope protein, which consists of three separate surface proteins: large (L), middle (M), and small (S) proteins. The polymerase (P) ORF encodes a multifunctional protein that is involved in encapsidation, initiation of minus strand DNA synthesis, reverse transcription, and degradation of pre-genomic RNA. The core (C) ORF encodes both HBcAg, which is a structural nucleocapsid core protein, and hepatitis B e antigen (HBeAg), which is a soluble nucleocapsid protein. The X ORF encodes hepatitis B x protein (HBx), which plays roles in signal transduction, transcriptional activation, DNA repair, and inhibition of protein degradation.[5]

After entry in the hepatocyte, the HBV is converted to covalently closed circular DNA (cccDNA) in the hepatocyte nucleus. The cccDNA forms the key template for transcription of both messenger RNA (for translation of viral proteins), as well as pre-genomic RNA (for reverse transcriptase into genomic DNA). The formed particle can either be excreted via the Golgi apparatus or recycled into the nucleus to form cccDNA.[3] Because the cccDNA is highly resistant to antiviral therapy and the host's immunological

response, complete eradication of HBV from the liver is not yet feasible.[6] The HBV is divided into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and this has been associated with anthropological history, with genotypes A and D as African and/or Caucasian and B and C as South-East Asian genotypes.[7]

PHASES OF INFECTION

Infection with HBV can lead to acute hepatitis which can either resolve spontaneously, become chronic or result into a fulminant hepatitis with decompensated liver cirrhosis. The risk of developing chronic hepatitis B (CHB) depends on the age of the subject at time of infection. Perinatal infection from the infected mother to the child in high endemic countries results in CHB in an estimated 90%, whereas infection with HBV in adults with a mature immune system - mainly through sexual contacts or by (sharing) needles - results into chronic disease in only 5%.[7,8] CHB is characterized by HBsAg positivity for at least six months. Patients with CHB may present in any of four, not necessarily sequential, phases of infection, depending on the presence of HBeAg with/without its antibody anti-HBe and serum levels of HBV DNA and alanine aminotransferase (ALT).[7,9]

Table 1. Phases of chronic hepatitis B infection

Phase	HBeAg	HBV DNA (IU/mL)	ALT
Immune tolerance	Positive	>200.000	Normal
Immune clearance	Positive	>20.000	Elevated
Inactive carrier	Negative	< 2000	Normal
HBeAg negative CHB	Negative	Fluctuating	Fluctuating

During the immunotolerant phase (1), which is common among perinatally infected patients, the virus is not recognized by the host-immune system, resulting in a high serum HBV DNA and HBsAg with a detectable HBeAg, while the serum ALT concentration is within normal range and liver histology shows minimal inflammation. In the immuno-active phase (2), the host immune response results in a decline in HBV DNA and HBsAg level. During this phase HBeAg loss and seroconversion to antibodies to HBeAg (anti-HBe) may occur. However, prolonged hepatic inflammation (as measured by high ALT levels) in this phase is an indication for starting antiviral therapy to reduce the risk of disease progression.[9,10] HBeAg seroconversion is often followed by the inactive carrier state (3), further characterized by a low serum HBV DNA level (<2000 IU/mL) and normalization of ALT. HBsAg also declines further in this state. However, in a significant proportion of HBeAg negative patients viral replication recurs or persists at higher lev-

els, resulting in HBeAg negative active CHB (4). This phase of infection develops through presence of viral strains harbouring mutations in the precore or basal core promotor region that reduce or abolish the expression of HBeAg. Yet, since both HBV DNA and ALT can fluctuate in this phase, distinction from 'inactive carriers' can be difficult, indicating the importance of serial measurements of HBV DNA and ALT, and perhaps also HBsAg, to identify those patients who require treatment.

TREATMENT GOALS

The ultimate goal of anti-HBV treatment in order to prevent the development of long-term sequelae of chronic liver disease is to completely eradicate the HBV from host hepatocytes.[11] However, with the currently available agents this cannot be achieved due to persistence of HBV cccDNA in the liver.[12] Therefore, surrogate outcomes are used as measurements of therapy efficacy and success. Several independent studies have shown that lower levels of HBV DNA and HBsAg, as well as clearance of HBeAg are associated with a lower risk of HBV related chronic liver disease.[13-15] Major endpoints of treatment are therefore, 1) reduction of HBV DNA to undetectable levels (virologic response), 2) loss of HBeAg with or without anti-HBe (serologic response), 3) normalization of ALT levels (biochemical response) and 4) a reduction in necroinflammation with or without improvement of liver histology (histologic response).[10] Although rarely achieved, loss of HBsAg from serum, accompanied by appearance of anti-HBs, is currently considered closest endpoint to clinical cure of disease.[16]

TREATMENT OPTIONS

Currently approved agents include the immune modulating (peg)interferon ((PEG)-IFN) and five viral polymerase inhibiting nucleos(t)ide analogues (NA). Treatment efficacy is evaluated differently as they have different modes of action.

Interferon therapy

Interferon (IFN) alfa has been a continued therapeutic option since it was licensed in the early 1990s for the treatment of CHB. IFN largely acts through enhancement of the immunological response of the host against the virus, although there is also a limited direct antiviral effect on HBV replication.[17] IFN based therapy improved significantly by pegylation of interferon (PEG-IFN) which allowed a more convenient once-weekly dosing interval, with treatment efficacy equal or superior to conventional IFN. Finite treatment (one year) with PEG-IFN injections aims to achieve sustained off-treatment remission of HBV. In HBeAg positive patients the use of a combined serologic and virologic

endpoint (HBeAg loss or seroconversion with concomitant HBV DNA < 2,000 IU/mL) is preferred, since this endpoint is associated with a low probability of relapse[18], a reduction in risk of HCC development[14,19] and a higher probability of subsequent HBsAg loss and seroconversion.[20] In HBeAg negative patients, prolonged suppression of HBV DNA to levels below 2,000 IU/mL combined with ALT normalization is currently considered the optimal definition of response to PEG-IFN, although late relapse beyond six months post-treatment has been described.[9]

Furthermore, it has recently been shown that a decline in serum HBsAg after PEG-IFN treatment reflects the induction of an effective anti-HBV immune response, possibly as an indirect marker of intrahepatic cccDNA decline.[21-24] Moreover, it has been shown that HBsAg expression in the liver evolves significantly after PEG-IFN therapy.[25] However, the effect of PEG-IFN on both intrahepatic HBcAg and HBsAg and its relation with serum HBsAg has not yet been studied.

Nucleos(t)ide analogue therapy

With the introduction of NA since the late nineties considerable progress has been made in the treatment of CHB. NA target the reverse transcriptase of the HBV and are potent inhibitors of viral replication. In contrast to IFN therapy, the most important goal of NA-therapy is on-treatment maintained undetectable HBV DNA level, because persistently detectable HBV DNA during therapy is a major risk factor for the development of viral breakthrough and progression of liver disease.[26,27] While HBeAg seroconversion may herald immune control in untreated patients (spontaneous HBeAg seroconversion) or after treatment with (PEG-)IFN, discontinuation after NA induced HBeAg seroconversion is associated with a higher probability of post-treatment relapse.[28-31] Also in HBeAg negative patients discontinuation of NA will result in relapse in the vast majority of patients.[32] Therefore, continuation of NA therapy until HBsAg loss or seroconversion seems to be the safest. Thus, long-term side effects and costs of NA should also be taken into account when initiating such therapy.

OPTIMAL FIRST-LINE THERAPY FOR CHRONIC HBV-INFECTION

Both PEG-IFN and NA have proven to be effective. However, both treatment options also have substantial disadvantages and important limitations. As a result, current guidelines[9,10] are still lacking clear recommendations as to which treatment strategy should be used as first-line therapy.

PEG-IFN may be a valuable option for those patients with a high likelihood of response. However, clinical use of PEG-IFN is compromised by the suboptimal tolerability, with

a wide spectrum of adverse events, such as flu-like symptoms, emotional lability and bone marrow suppression. Furthermore, only a limited number of patients achieve a response.[33-35] Selection of those patients with the highest probability of response is therefore essential for effective use of PEG-IFN in clinical practice. Recently, several studies have shown that response to PEG-IFN depends upon the infecting HBV genotype, baseline levels of HBV DNA and ALT, presence of precore and core promotor mutants and *IL-28B* genotype and previous failure to response to IFN therapy.[36-40] Despite these important and guiding findings, a reliable prediction of response probabilities for individual patients remains challenging.

The most potent NA (entecavir (ETV) and tenofovir (TDF)) are generally recognized as first line treatment options, and may induce and maintain undetectable levels of HBV DNA in nearly all patients with limited safety concerns and low rates of antiviral resistance through up to five years of treatment.[41-44] ETV and TDF may also improve histological response and result in a better overall clinical outcome in successfully treated patients.[45-47] Moreover, it has been shown in Asian patients that successful treatment with ETV decreases the chance of HCC development.[48]
However, the residual risk of HCC necessitates intensive on-going follow-up of patients with successfully suppressed viral replication.[9] Therapy for decades or possibly even lifelong will be necessary in most patients as high relapse rates have been observed after discontinuation of therapy. Moreover, long-term outcomes regarding viral resistance, also in relation to compliance to therapy, are unknown.[28-31]
Development of antiviral resistance of the first NA has been shown to be a major limitation, leading to reversion of virologic and histological improvement and enhancement of the rate of disease progression.[49] One of the first manifestations of antiviral resistance is a virologic breakthrough which is defined as a > 1 log10 increase in serum HBV DNA from nadir during treatment in a patient who had an initial virologic response. It is usually followed by a biochemical breakthrough with elevated levels of serum ALT.

This thesis contains studies describing how to optimize treatment of CHB. The aims of this thesis were therefore to:

1. Determine the effect of PEG-IFN therapy on intrahepatic HBcAg and HBsAg expression in correlation to on-treatment serum HBsAg decline.
2. Identify whether IFN-related factors can help to predict response to PEG-IFN therapy.
3. Explore the long-term efficacy and safety of NA therapy in a large European real-life cohort of CHB patients. We studied in particular the occurrence of HCC and ALT flares among long-term NA therapy.
4. To evaluate the effect of NA treatment (non-)compliance on treatment outcome.

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CHAPTER 1

Loss of intrahepatic HBsAg expression predicts sustained response to peginterferon and is reflected by pronounced serum HBsAg decline

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ABSTRACT

Background

There is a lack of knowledge regarding the effect of peginterferon (PEG-IFN) on the expression of intrahepatic hepatitis B core and surface antigen (HBcAg and HBsAg) in chronic hepatitis B (CHB) and its relation with response to therapy.

Methods

Fifty-two HBeAg positive and 67 HBeAg negative CHB patients with paired liver biopsies taken at baseline and after 1 year of PEG-IFN therapy were studied.

Results

After PEG-IFN therapy, HBeAg negative patients showed a significant reduction in both intrahepatic HBcAg ($p=0.04$) and HBsAg expression ($p<0.001$). In contrast, a reduction in intrahepatic HBcAg expression was not observed in HBeAg positive patients, while a trend in reduction of intrahepatic HBsAg staining was found ($p=0.09$). Post treatment, 7 (13%) HBeAg positive and 9 (14%) HBeAg negative patients had no expression of intrahepatic HBsAg. Patients without any intrahepatic HBsAg expression post treatment were more likely to achieve a combined response (HBeAg loss with HBV DNA < 2,000IU/mL for HBeAg positive and HBV DNA <2,000IU/mL and normal ALT for HBeAg negative CHB): 71% vs. 5% for HBeAg positive ($p<0.001$) and 60% vs. 16% for HBeAg negative patients ($p=0.004$), respectively. Moreover, a more profound decline of serum HBsAg was observed in patients with absence of intrahepatic HBsAg staining (3.1 vs. 0.4 log IU/mL, $p<0.001$ and 1.7 vs. 0.4 logIU/mL, $p=0.005$ for HBeAg positive and negative CHB, respectively).

Conclusion

PEG-IFN reduces expression of intrahepatic HBsAg. Loss of HBsAg as assessed by immunohistochemistry from the liver predicts a sustained response, and is reflected in a pronounced serum HBsAg decline.

INTRODUCTION

Chronic hepatitis B (CHB) remains a major health problem affecting 350-400 million people worldwide and causing one million deaths every year.[1] To prevent progression of liver disease to cirrhosis, liver failure and hepatocellular carcinoma antiviral therapy reducing viral replication is indicated in a large proportion of patients with both hepatitis B e antigen (HBeAg) positive and negative CHB.[2] Both nucleos(t)ide analogues (NA) and peginterferon (PEG-IFN) have been shown to reduce viral replication. However, unlike NA, PEG-IFN does not only have a direct antiviral effect, but also stimulates the induction of a host immune response against the hepatitis B virus (HBV). An effective immune response may consequently result in a decrease in intrahepatic covalently closed circular DNA (cccDNA), which plays a major role in viral persistence.[3-5] With the introduction of quantitative assays for serum hepatitis B surface antigen (HBsAg) it has been shown that serum HBsAg levels may reflect intrahepatic cccDNA.[6] Recent studies have shown that serum HBsAg levels are higher in HBeAg positive compared to HBeAg negative patients.[7,8] Furthermore, it has been shown that a decline in serum HBsAg after PEG-IFN treatment reflects the induction of an effective anti-HBV immune response, possibly through an association with intrahepatic cccDNA decline.[9-13] However, the exact mode of action remains unclear.

Immunodetection of HBsAg and hepatitis B core antigen (HBcAg) in hepatocytes provides helpful information concerning the replicative status of the hepatitis B virus and is usually performed as part of a histopathological assessment of CHB patients.[14] Intrahepatic HBsAg can be detected by immunohistochemistry in liver tissue of both asymptomatic HBV carriers and in patients with chronic active hepatitis B. Expression of HBcAg in hepatocyte nuclei, detected by immunohistochemical techniques, correlates with the level of viral replication and is generally found in HBeAg positive CHB patients.[15] It has been indicated that there is an association between intrahepatic HBsAg expression and serum HBsAg in Asian CHB patients.[16,17] Furthermore, baseline intrahepatic HBsAg expression appeared to be a predictor for response (i.e. HBeAg loss) to IFN therapy in a recent study.[16] However, intrahepatic HBsAg and HBcAg expression were not linked to other response markers, such as serum HBV DNA, HBsAg and HBeAg decline.

Previous studies have shown that (PEG-)IFN improves liver inflammation and slows progression of fibrosis.[18-22] Moreover, it has recently been shown that HBsAg expression in the liver evolves significantly after (PEG-)IFN therapy.[16,23] However, the effect of PEG-IFN on both intrahepatic HBcAg and HBsAg in a large heterogeneous population has not yet been studied. The aims of this study were therefore to 1) investigate the association between pretreatment intrahepatic expression of HBcAg and HBsAg and response to PEG-IFN therapy, 2) to determine the effect of PEG-IFN therapy on intrahepatic HBcAg and HBsAg expression and 3) to correlate on-treatment serum HBsAg decline with changes in intrahepatic HBsAg expression.

METHODS

Study population

Patients were enrolled from two randomized controlled trials investigating the efficacy of one year of PEG-IFN therapy in HBeAg positive and negative CHB.[20,22] Post treatment follow-up lasted 6 months. HBeAg positive patients were treated with PEG-IFN with or without lamivudine. For the current analysis, only patients treated with PEG-IFN monotherapy were included given the difference of on-treatment HBV DNA suppression in patients treated with or without lamivudine. HBeAg negative patients were treated with PEG-IFN with or without ribavirin. Since ribavirin had no effect on HBV DNA or HBsAg decline at and of FU, nor on end of treatment or off-treatment responses, both treatment groups were eligible for this study.[9] Both studies were conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written informed consent according to standards of the local ethics committees.

Liver biopsies

Patients were eligible for the current study if a liver biopsy was available before and after PEG-IFN therapy. Baseline biopsies were taken at baseline, or if a recent biopsy (taken less than one year before the start of therapy) was available, no new biopsy was required. In HBeAg positive patients a second liver biopsy was taken directly post PEG-IFN therapy (1 year). In HBeAg negative patients a second liver biopsy was taken at the end of follow-up (6 months post treatment). All paired biopsies were blinded and scored by one experienced liver pathologist (PEZ). The adequacy of biopsy samples was judged using its length and the number of portal tracts. Expression of HBcAg and HBsAg in hepatocytes was studied by the avidin-biotin immunoperoxidase method.
Immunohistochemical detection of HBsAg and HBcAg was performed according the standard procedures using a monoclonal anti-HBs antibody (Neomarkers, Fremont, CA, USA) and a polyclonal anti-HBc antibody (Dako, Glostrup, Denmark). The degree of intrahepatic HBsAg and HBcAg expression was scored in a systematic way assessing the proportion of the immunolabeled cells and ranked on a scale of 0 to 5 (0%, 1-10%, 11-25%, 26-50%, 51-75% and >75%, respectively). [15] Furthermore, the degree of necroinflammation and fibrosis was scored according to the Ishak system which includes a necroinflammatory score (0–18) and a fibrosis score (0–6). [24]

Laboratory testing

Alanine aminotransferase (ALT) was assessed locally in accordance with standardized procedures and therefore expressed as times the upper limit of normal (ULN). Serum HBV DNA levels were measured using an in-house developed Taqman polymerase chain

reaction (PCR) assay (lower limit of detection 373 copies/ml) based on the Eurohep standard or the Cobas TaqMan PCR assays (Roche; lower limit of detection 6 IU/mL). Serum HBsAg was quantified using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL).

Statistical analysis

Serum HBsAg and HBV DNA levels were logarithmically transformed for analysis. Comparisons between groups were made using the Chi-2-test and the Mann–Whitney test. Differences in degrees of expression of HBcAg and HBsAg in hepatocytes between HBeAg negative and positive CHB and before and after therapy were analyzed by the Wilcoxon rank sum test. Correlation of levels of HBV DNA, ALT and HBsAg in sera with the degrees of expression of HBsAg in hepatocytes was assessed by Pearson correlation. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

One hundred and nineteen CHB patients with paired liver biopsies were included in this study (Figure 1). Baseline characteristics for both HBeAg positive and negative CHB patients are shown in table 1. Seven HBeAg positive patients (14%) achieved a combined response (HBeAg loss with HBV DNA <2,000 IU/mL) at 6 month post treatment. Of HBeAg negative patients, 15 (22%) developed combined response (HBV DNA <2,000 IU/mL and normal ALT) 6 months post treatment.

Patient characteristics and intrahepatic HBcAg and HBsAg expression

The degree of intrahepatic HBcAg staining was significantly higher in HBeAg positive compared to HBeAg negative CHB ($p<0.001$) (Figure 2A). Only three patients did not have visible intrahepatic HBsAg expression at baseline, two HBeAg positive and one HBeAg negative patient ($p=0.58$). The degree of intrahepatic HBsAg expression did not differ between HBeAg positive and negative patients. (Figure 2B). Interestingly, the degree of pre-treatment intrahepatic HBcAg and HBsAg staining did not correlate with serum HBV DNA, HBeAg or HBsAg in either HBeAg positive or negative CHB (all $p> 0.11$). Among the HBeAg-positive patients, an intrahepatic HBsAg degree >10% was mostly seen in genotype D patients (85%), followed by genotypes A (44%), B (40%) and C (30%) ($p=0.02$); a comparable distribution was observed with serum HBsAg levels at baseline (HBV genotypes A/B/C/D=4.6 ± 0.4, 4.4 ± 0.3, 3.5 ± 1.1 and 4.7 ± 0.5 log IU/mL respectively; $p<0.001$). HBeAg negative patients had predominantly genotype D (81%) and baseline intrahepatic HBsAg staining did not differ between HBV genotypes ($p=0.21$).

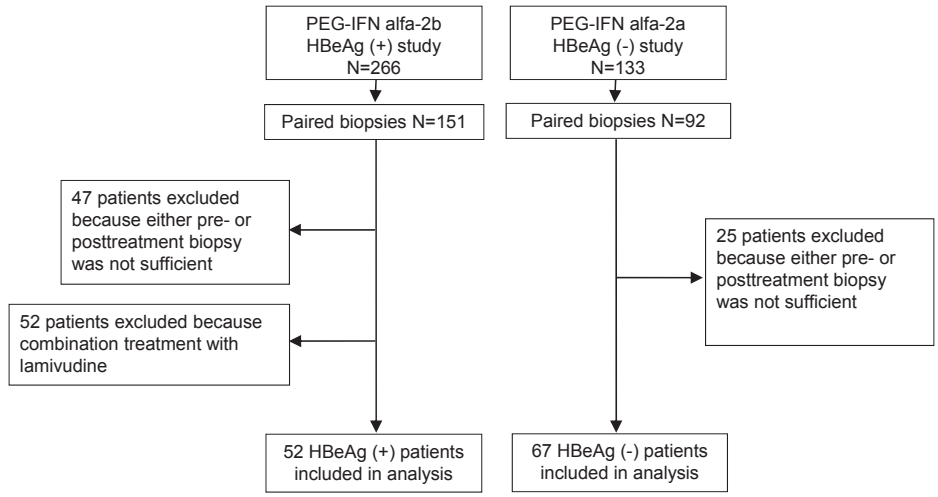

Figure 1. Study population.

Table 1. Patient characteristics

	HBeAg positive ***N=52***	**HBeAg negative** ***N=67***	***p-value***
Age (years)	32 ± 10	41 ± 10	*< 0.001*
Male (%)	40 (77%)	45 (67%)	*0.31*
Previous IFN therapy (%)	10 (19%)	8 (12%)	*0.31*
Genotype (%)			*< 0.001*
A	16 (31%)	7 (10%)	
B	5 (10%)	1 (2%)	
C	10 (19%)	2 (3%)	
D	20 (39%)	54 (81%)	
Other	1 (2%)	3 (5%)	
ALT (x ULN)	3.7 (2.3-5.3)	2.0 (1.5-4.0)	*< 0.001*
Log HBV DNA (cop/mL)	9.2 ± 0.8	6.8 ± 1.2	*< 0.001*
Log qHBsAg (IU/mL)	4.4 ± 0.7	3.9 ± 0.5	*< 0.001*
Liver histology			
Necroinflammatory score	5.5 (3.3-7.0)	5.0 (4.0-7.0)	*0.77*
Fibrosis score	2.0 (1.0-3.0)	2.0 (1.0-3.0)	*0.38*
Cirrhosis (%)*	3 (6%)	1 (2%)	*0.31*
No HBsAg expression (%)	2 (4%)	1 (1%)	*0.58*
No HBcAg expression (%)	28 (54%)	56 (84%)	*< 0.001*
Combined response**	7 (14%)	15 (22%)	

Data are represented as mean (± standard deviation) or median (range). * Ishak fibrosis score 5-6. ** Combined response at 6 month posttreatment was defined as HBeAg loss with HBV DNA < 2,000 IU/mL for HBeAg positive and HBV DNA <2,000 IU/mL and normal ALT for HBeAg negative CHB.

Effect of PEG-IFN on intrahepatic HBcAg and HBsAg expression

After PEG-IFN therapy intrahepatic HBcAg was not expressed in 65% of HBeAg positive and 94% of HBeAg negative patients. A significant reduction in intrahepatic HBcAg expression was only observed in HBeAg negative patients (p=0.04) (Figure 2A).

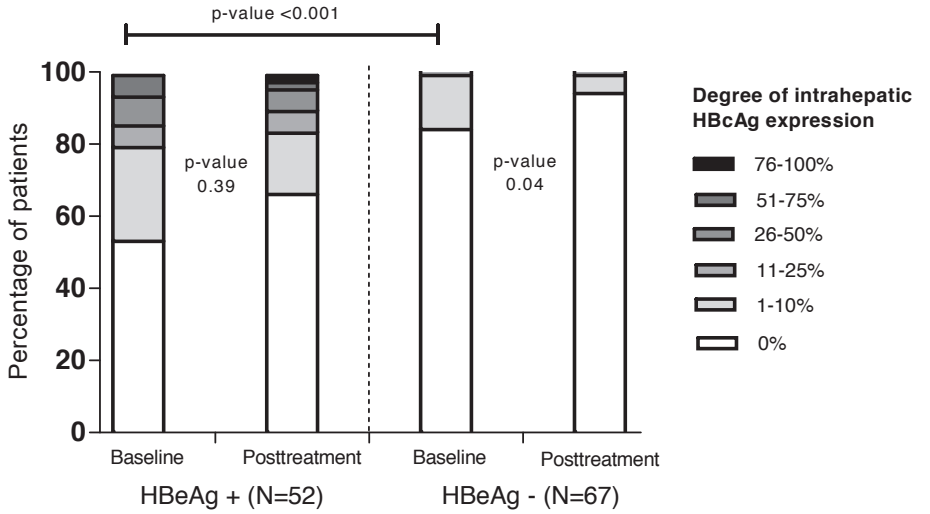

Figure 2A. Changes in degree of intrahepatic HBcAg expression in HBeAg positive and negative CHB before and after peginterferon therapy

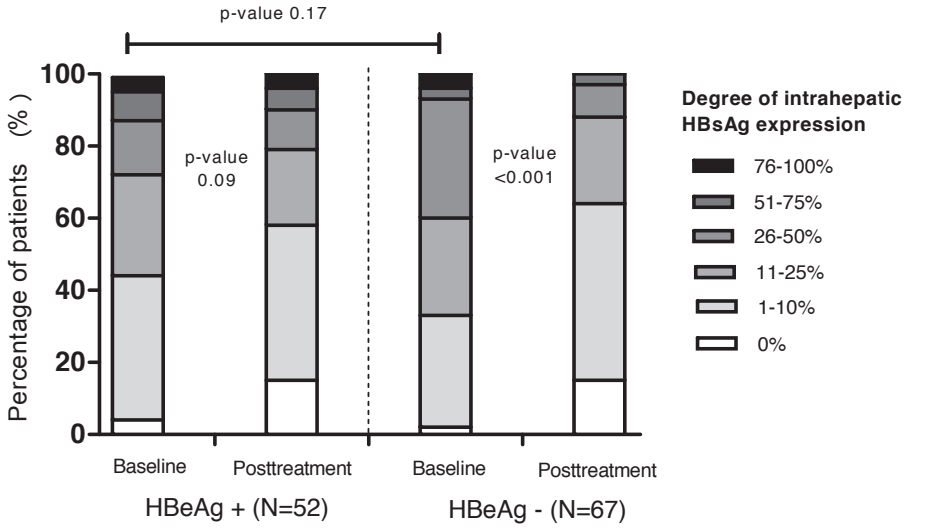

Figure 2B. Changes in degree of intrahepatic HBsAg expression in HBeAg positive and negative CHB before and after peginterferon therapy

Intrahepatic HBsAg expression was absent in 14% of HBeAg positive and 15% of HBeAg negative CHB patients after PEG-IFN treatment. Intrahepatic HBsAg expression significantly reduced both in HBeAg positive patients (p=0.09) as well as in HBeAg negative patients (p<0.001) (Figure 2B).

Relationship between intrahepatic HBcAg and response to PEG-IFN

Baseline degree of intrahepatic HBcAg was not associated with response to PEG-IFN in either HBeAg positive CHB, HBeAg negative CHB or the overall population (p>0.5). In HBeAg positive patients HBeAg loss was observed in 14 out of 34 patients (41%) who cleared intrahepatic HBcAg, and in 4 out of 18 patients (22%) without clearance of HBcAg from the liver (p=0.23). Mean serum HBeAg decline was more profound in patients who showed a decline in the degree of intrahepatic HBcAg compared to patients without a decline in intrahepatic HBcAg (2.0 log IU/mL vs 0.7 log IU/mL, p= 0.03). Patterns of intrahepatic HBcAg staining were comparable in patients with a decline in intrahepatic HBcAg (p=0.21) as well as in patients who showed a more profound decline in serum HBeAg (p=0.07)

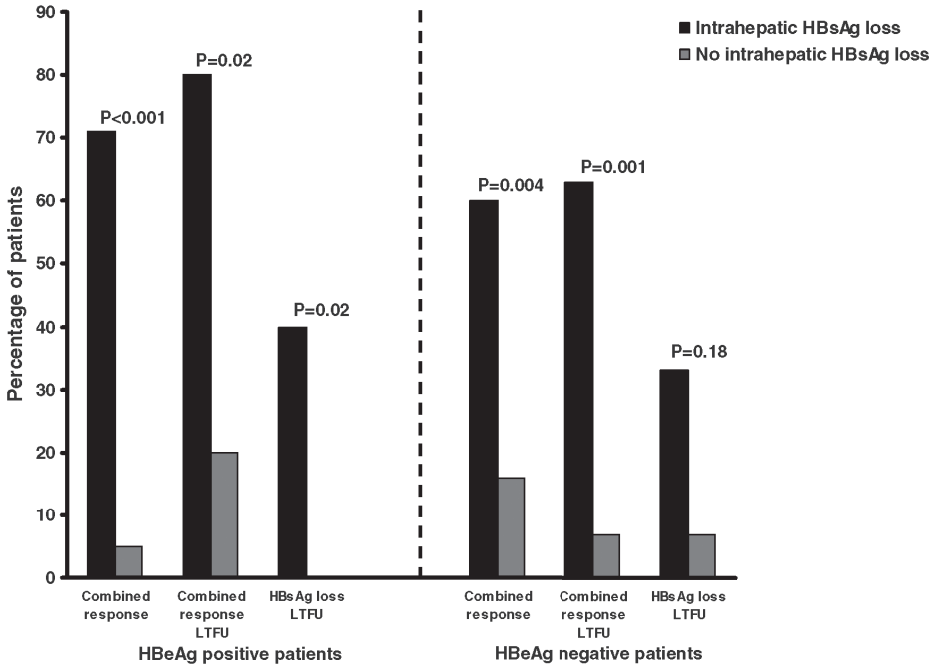

Figure 3. Response rates for patients with and without intrahepatic HBsAg loss after peginterferon therapy.

Relationship between intrahepatic HBsAg and response to PEG-IFN

Baseline degree of intrahepatic HBsAg was not associated with response to PEG-IFN in either HBeAg positive CHB, HBeAg negative CHB or the overall population (p>0.2). Next, we studied the association between clearance of HBsAg from the liver and response. At 6 months post treatment, five out of seven (71%) HBeAg positive patients who cleared intrahepatic HBsAg from the liver achieved a combined response versus 2 out of 43 patients (5%) without clearance of HBsAg from the liver (p<0.001) (Figure 3). These patients

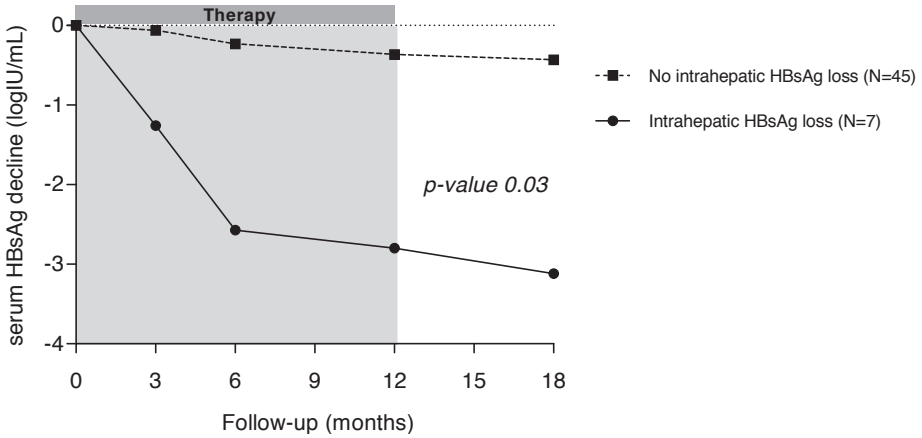

Figure 4A. Serum HBsAg decline in HBeAg positive patients according to the presence of intrahepatic HBsAg loss after peginterferon therapy

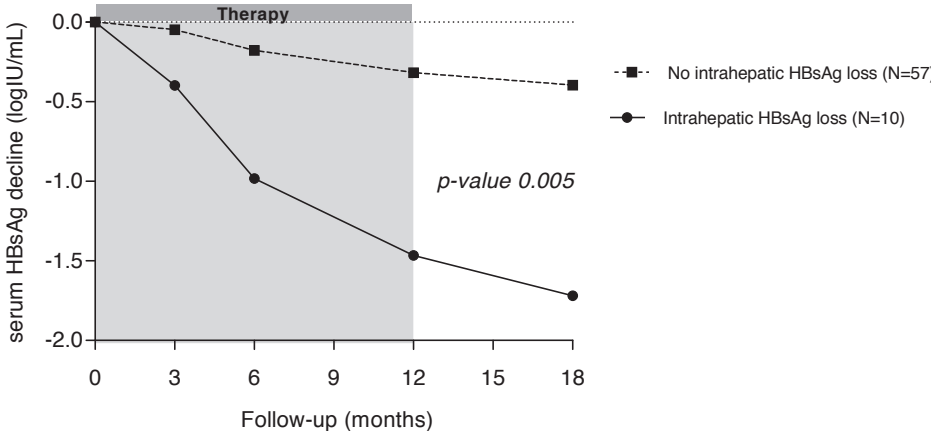

Figure 4B. Serum HBsAg decline in HBeAg negative patients according to the presence of intrahepatic HBsAg loss after peginterferon therapy

also had a more profound serum HBsAg decline (3.1 vs. 0.4 log IU/mL, p=0.03) (Figure 4A).
At 6 months after treatment, HBsAg expression was absent in 10 (15%) HBeAg negative patients, of whom 6 (60%) had a combined response versus 9 (16%) of those who still expressed HBsAg intrahepatically (p=0.004) (Figure 3). In line with our findings in HBeAg positive patients, those without any intrahepatic HBsAg expression post treatment also showed a more profound decline of serum HBsAg (1.7 vs. 0.4 log IU/mL, p=0.005) (Figure 4B).

Response at long-term follow-up and intrahepatic HBsAg expression

Long-term follow-up (LTFU) data (mean 2.8 ± 0.6 years) were available for 35 HBeAg positive patients. Although the number of patients was limited, a remarkable finding was that of 5 patients who cleared intrahepatic HBsAg 4 patients (80%) achieved a combined response at LTFU, compared to 6 (20%) of patients without clearance of HBsAg from the liver (p=0.02). At LTFU, 2 out 5 (40%) HBeAg positive patients without HBsAg staining post treatment achieved HBsAg loss from serum, while HBsAg loss was not observed in those without intrahepatic HBsAg clearance (Figure 3).
In the HBeAg negative population LTFU data (mean 2.9 ± 0.3 years) were available for 50 patients. Of 8 patients without intrahepatic HBsAg expression at week 72, 5 (63%) had a combined response at LTFU versus 3 (7%) of those with intrahepatic HBsAg expression (p=0.001). Two out of six (33%) patients with intrahepatic HBsAg loss also cleared HBsAg from serum compared with one out of 15 (7%) without intrahepatic HBsAg loss (Figure 3).

DISCUSSION

This is the first detailed report on HBV immunohistochemistry in a large population of both HBeAg positive and negative CHB patients treated with PEG-IFN. Important findings are that a one-year course of PEG-IFN therapy significantly reduced the degree of intrahepatic HBsAg expression in the liver. In addition, loss of HBsAg expression after PEG-IFN therapy is associated with high rates of durable off-treatment response to PEG-IFN, and is reflected by a pronounced decline of HBsAg levels in serum. However, intrahepatic expression of HBcAg or HBsAg at baseline is not predictive for response to PEG-IFN.
Recently, on-treatment serum HBsAg has been shown to predict response to PEG-IFN therapy both in HBeAg positive as well as in HBeAg negative HBV patients.[9,10] However, the exact course of action remains unclear. In our study we showed that PEG-IFN therapy reduces the expression of intrahepatic HBsAg in HBeAg negative CHB and appears to do

so in HBeAg positive patients. The importance of a reduction of HBsAg expression in the liver after PEG-IFN therapy was emphasized by our finding that the absence of HBsAg expression in hepatocytes after PEG-IFN treatment was associated with high rates of sustained response. Furthermore, patients without intrahepatic HBsAg expression experienced a strong serum HBsAg decline and were more likely to clear HBsAg from serum during LTFU. These results clarify the association between serum HBsAg after PEG-IFN and sustained response by its relation with intrahepatic HBsAg staining.

Our results confirm the findings of Takkenberg et al. who showed that in a group of 24 HBeAg negative patients the mean proportion of hepatocytes that were positive for HBsAg reduced after PEG-IFN, but also did not find a significant reduction in 16 HBeAg positive patients.[23] Intrahepatic HBsAg expression also reduced in 45 interferon treated HBV patients.[16] However, in contrast to this study from Taiwan and a recent study from Thompson et al. we could not find a correlation between serum HBsAg or HBV DNA and intrahepatic HBsAg expression in either HBeAg positive or negative CHB at baseline.[16,17] These contradictory results may be explained by the fact that their population mainly consisted of Asian patients, infected with genotypes B and C, whereas our cohorts mainly consisted of Caucasians infected with genotypes A and D. It has previously been shown that the influence of HBV genotype on HBsAg levels both in serum as well as intrahepatically may be of significant importance; the highest HBsAg levels have been observed for genotypes A and D, compared to lower HBsAg levels in genotypes B and C.[7,25] We found a comparable distribution of serum and intrahepatic HBsAg expression within the different HBV genotypes.
Finally, we showed in accordance with a previous study[15] that expression of intrahepatic HBcAg differs between HBeAg positive and negative CHB. In the immune control phase, i.e after HBeAg seroconversion, intrahepatic HBcAg is usually undetectable.[26] Loss of detectable HBcAg may also be associated with the emergence of precore stop-codon mutations during periods of cytotoxic T-cell clearance of virus-infected hepatocytes. The occurrence of these core gene mutations is associated with HBeAg negative chronic hepatitis B and active liver disease.[27]

A possible limitation of the current study is related to the fact that the follow-up biopsies were performed at different time points; in HBeAg positive CHB patients a second liver biopsy was performed directly post treatment (1 year), whereas in HBeAg negative CHB patients a second liver biopsy was performed at the end of 6 months of treatment free follow-up). Furthermore, sampling errors may have occurred during scoring of paired liver biopsies.

In summary, we showed that PEG-IFN therapy reduces intrahepatic HBsAg expression. Loss of HBsAg from the liver predicts a sustained response, and is reflected in a pronounced HBsAg decline in serum. Our results provide an important rationale for the use of HBsAg quantification as an easily obtainable estimate for prediction of response to PEG-IFN in both HBeAg positive and negative HBV patients.

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CHAPTER 2

Presence of anti-interferon antibodies is not associated with non-response to peginterferon treatment in chronic hepatitis B

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ABSTRACT

Introduction

Several factors have been related to response to PEG-IFN in chronic hepatitis B (CHB). The occurrence of anti-IFN antibodies are associated with non-response to PEG-IFN in chronic hepatitis C. This study investigated the association between anti-IFN antibodies and response to PEG-IFN in CHB.

Methods

Presence of anti-IFN antibodies was assessed at baseline and at 3 and 6 months post-treatment in 323 CHB patients treated with PEG-IFN for one year.

Results

At baseline anti-IFN antibodies were detected in 112 patients (35%). Prevalence was higher in HBeAg negative compared to HBeAg positive CHB (43% vs. 31%, respectively, p=0.03). Detection of anti-IFN antibodies was not associated with age, sex or HBV genotype. Presence of anti-IFN antibodies at baseline was associated with previous IFN therapy failure (p=0.04), which remained after adjustment for HBeAg status (OR 2.0, 95%CI 1.1-3.7, p=0.03). Presence of anti-IFN antibodies at baseline was not associated with response, nor with HBV DNA or HBsAg decline (all p-values>0.3). Fifty-six of 211 (27%) patients without anti-IFN at baseline developed anti-IFN antibodies after PEG-IFN treatment. Response rates did not differ between patients who developed anti-IFN antibodies and patients who did not develop anti-IFN antibodies during treatment (p=0.1).

Conclusion

Anti-IFN antibodies may frequently be detected in CHB patients, and presence is associated with previous IFN therapy. However, presence or development of anti-IFN antibodies after PEG-IFN therapy is not associated with non-response to PEG-IFN treatment in CHB. There appears to be no future role for anti-IFN antibodies in predicting response to PEG-IFN in CHB.

INTRODUCTION

Chronic hepatitis B (CHB) remains a major health problem, affecting over 350 million people worldwide. Many patients require treatment to prevent progression to cirrhosis, liver failure and hepatocellular carcinoma. Both nucleos(t)ide analogues and peginterferon (PEG-IFN) are registered for the treatment of CHB.[1] Unlike nucleos(t)ide analogues, PEG-IFN does not only have antiviral, but also immunomodulating properties, which may play an important role in the elimination of the virus. Nevertheless, a finite treatment course with PEG-IFN results in a sustained response in only about 25% of patients.[2,3]

Recently, efforts have been made in determining the underlying pathophysiological mechanisms regarding response to PEG-IFN in CHB. Subsequently, several factors that are associated with a high probability of response have been identified which optimized the use of PEG-IFN in CHB. Currently known factors that may be used as baseline predictors are HBV genotype, patient age, HBV DNA and ALT serum levels[4], host *IL28*-B genotype[5] and precore or basal core promoter mutants[6] and serum IP-10 levels.[7] However, the mechanism of (non)-response to PEG-IFN therapy is not well understood. The occurrence of anti-interferon (anti-IFN) antibodies has been reported after PEG-IFN therapy in chronic hepatitis C, and these antibodies may be associated with non-response to PEG-IFN based therapy.[8,9] Anti-IFN antibodies may bind to interferon and interfere with its biological activity by blocking its interaction with its receptor.[10] Previous Chinese studies showed that neutralizing anti-IFN antibodies may influence the effect of interferon in CHB.[11] However, the relation between the presence or development of anti-IFN antibodies and response to PEG-IFN is unknown. Therefore, the aim of this study was to investigate whether presence or development of anti-IFN antibodies is associated with non-response to PEG-IFN in HBeAg positive and negative CHB.

METHODS

Patients

Presence of anti-IFN antibodies was assessed in CHB patients treated with PEG-IFN for one year. The first cohort consisted of HBeAg positive patients treated with PEG-IFN alfa-2b 100µg weekly in combination with placebo or lamivudine (LAM) 100 mg daily for 52 weeks. In- and exclusion criteria for this study have previously been described.[2] The second cohort comprised HBeAg negative patients who were treated with PEG-IFN alfa-2a 180µg weekly, either alone or in combination with ribavirin 1000mg (<75kg) or 1200 mg (≥75kg) daily for 48 weeks. In- and exclusion criteria for this study have previously been described.[3]

Inclusion criteria for the present analysis were completion of the 6 month follow-up phase of the main studies and available serum for detection of anti-IFN antibodies. Of the 404 patients in the initial studies, 323 fulfilled these criteria. Both studies were conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written informed consent according to standards of the local ethics committees.

Laboratory measurements

IFN binding antibodies (including neutralizing) were measured at baseline and at 3 and 6 months post-treatment using a commercially available ELISA assay (Enzyme-linked immunosorbent assay for the quantitative detection of human anti-IFN-alpha, BMS217) in samples that were stored at -20 or -80° Celcius since the original studies. Serum HBV DNA and HBsAg were quantified in samples taken at baseline, during the treatment period and 6 months post-treatment. HBV DNA was measured using an in-house developed TaqMan polymerase chain reaction (PCR) assay (lower limit of quantification 400 copies/mL). HBsAg was measured using the Abbott ARCHITECT HBsAg assay (Abbott laboratories; range 0.05 - 250 IU/mL).

Statistical analysis

Since combination treatment with LAM[2] or ribavirin[3] did not influence response rates, data from the monotherapy and combination arms were pooled for the current analysis. Response was assessed at 6 months post treatment (week 78 in HBeAg positive patients, week 72 in HBeAg negative patients) and was defined as either HBeAg loss with HBV DNA <2000 IU/mL for HBeAg positive CHB or HBV DNA <2000 IU/mL with normal ALT for HBeAg negative patients. Associations between variables were tested using Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents when appropriate. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

Role of the funding source

Financial support was provided by the Foundation for Liver and Gastrointestinal Research (SLO) in Rotterdam, the Netherlands. 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

Baseline characteristics

The characteristics of the enrolled patients are shown in Table 1 by HBeAg status at baseline. Patients were predominantly male (76%), of Caucasian origin (80%) and harboured HBV genotype A in 28%, B in 6%, C in 11%, D in 52% and other genotypes in 3%. At baseline, anti-IFN antibodies were detected (>1 ng/mL) in 112 patients (35%). Prevalence of anti-IFN antibodies was higher in HBeAg negative compared to HBeAg positive CHB (43% vs. 31%, respectively, p=0.03).

Table 1. Baseline characteristics by HBeAg status at baseline

	Overall	**HBeAg positive**	**HBeAg negative**	
	N=323	***N=221***	***N=102***	***p-value***
Age (years)	36 ± 12	34 ± 12	41 ± 10	*< 0.001*
Male (%)	245 (76%)	173 (78%)	72 (71%)	*0.133*
Pretreatment with IFN (%)	53 (16%)	40 (18%)	13 (13%)	*0.31*
Genotype (%)				*< 0.001*
A	89 (28%)	74 (33%)	15 (15%)	
B	20 (6%)	20 (9%)	0 (0%)	
C	34 (11%)	32 (15%)	2 (2%)	
D	169 (52%)	87 (39%)	82 (80%)	
Other	11 (3%)	8 (4%)	3 (3%)	
ALT x ULN (U/l)	3.9 ± 2.9	4.3 ± 3.0	3.3 ± 2.7	0,004
Log HBV DNA (cop/mL)	8.4 ± 1.5	9.1 ± 0.9	6.8 ± 1.2	<0,001
Log qHBsAg	4.2 ± 0.7	4.4 ± 0.6	3.9 ± 0.5	<0,001
Presence of anti-IFN AB	112 (35%)	68 (31%)	44 (43%)	0,03

Detection of anti-IFN antibodies at baseline was not associated with age, sex or HBV genotype. However, anti-IFN antibodies were more often detected in patients treated with IFN in the past; 25 of 53 patients (47%) who were previously treated with IFN versus 87 of 270 IFN-naïve patients (32%) had anti–IFN antibodies at baseline (p=0.04; Figure 1A). After adjustment for HBeAg status, prior treatment with IFN remained associated with the presence of anti-IFN antibodies at baseline (OR 2.0, 95%CI 1.1-3.7, p=0.03).

Association with response to treatment

Sustained response was achieved in 43 (20%) of the HBeAg positive patients, and in 21 (21%) of the HBeAg negative patients. There were no significant differences in response rates between patients with or without anti-IFN antibodies at baseline in either HBeAg positive, HBeAg negative or the overall population (all p-values >0.6; Figure 1B for the

overall population). Also when only IFN-naïve patients (N=270) were studied, presence of anti-IFN antibodies at baseline was not associated with response (23% vs. 21% in patients with or without anti-IFN antibodies at baseline respectively, p=0.8; Figure 1C). Presence of anti-IFN antibodies at baseline was not associated with HBV DNA decline (2.1 vs. 2.0, p=0.7) or HBsAg decline (0.8 vs. 0.8, p=0.8) during follow-up in the overall population. When HBeAg positive, HBeAg negative, IFN-naïve patients or patients within different treatment groups were studied separately no significant differences between the groups were found (all p-values >0.3).

Development of anti-interferon antibodies after PEG-IFN treatment

Of the 211 CHB patients without anti-IFN antibodies at baseline, 56 patients (27%) developed anti-IFN antibodies after PEG-IFN treatment. Although patients who did not develop anti-IFN antibodies showed higher response rates (22%) compared with patients who developed anti-IFN antibodies (11%), this was not significantly different (p=0.1; Figure 1D). Also when HBeAg positive and negative patients were studied separately, no significant differences were found. Furthermore, HBV DNA (2.2 vs. 1.8, p=0.2) and HBsAg decline (0.9 vs. 0.5, p=0.1) during follow up did not significantly differ between both groups, both in the overall population, as well as in HBeAg positive and negative patients separately.

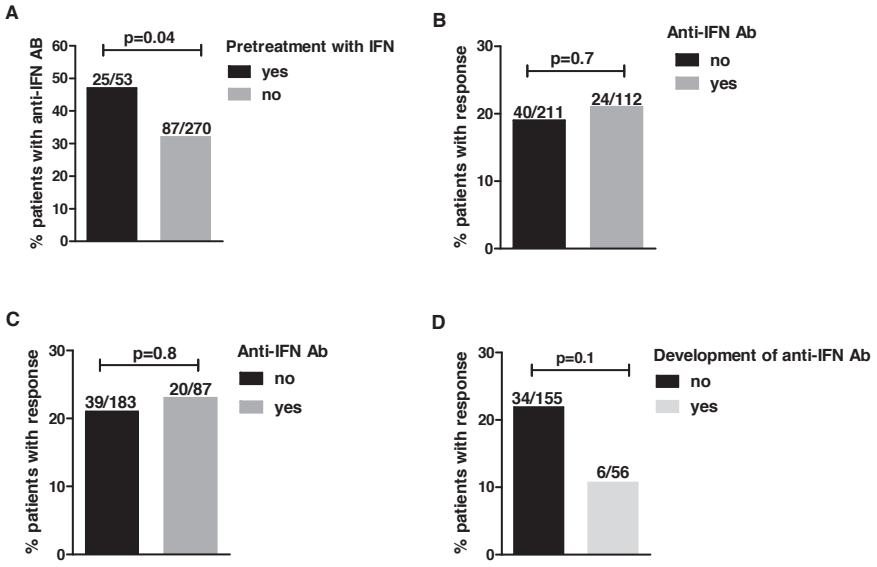

Figure 1. A) Presence of anti-IFN antibodies at baseline by previous interferon treatment
B) Response rates in the entire group by presence of anti-IFN antibodies at baseline.
C) Response rates in IFN-naïve patients by presence of anti-IFN antibodies at baseline.
D) Response rates in patients without anti-IFN antibodies at baseline.

DISCUSSION

This is the first study to describe the association of anti-IFN antibodies and response to PEG-IFN therapy in a large cohort of CHB patients. In our study, we found that a relatively large group of CHB patients had anti-IFN antibodies at baseline which was associated with previous IFN treatment. This is in accordance with previous studies in hepatitis C patients which showed the occurrence of anti-IFN antibodies after (PEG-)IFN therapy.[9,12,13] Interestingly, in contradiction to a previous study in CHB patients treated with interferon, neutralizing anti-IFN antibodies were also found in treatment naïve patients.[11] Natural antibodies to IFN-alfa have previously been reported in patients with cancer and various autoimmune disorders and there are data suggesting that elevated levels of antibodies to IFN are associated with stages of diseases related to disbalances of the immune system.[14] Additionally, Ikeda et al found naturally occurring IgG anti-IFN-alpha 2a in 50% and IgM anti-IFN-alpha 2a in 30% of patients with acute hepatitis B.[15] These antibodies were detectable at the highest frequency three weeks after acute onset and subsequently became negative. However, the appearance of anti-IFN-alpha 2a was not correlated with disease severity and there was no evidence to suggest that anti-IFN-alpha 2a impaired the elimination of the hepatitis virus.

Nevertheless, it is generally recognized that the development of antibodies against any auto-antigen or drug is unwanted. These antibodies may inhibit the pharmacological effects of drugs including exogenously administered interferons. However, in contrast to previous hepatitis C studies [8], we did not find an association between presence of anti-IFN antibodies at baseline and response to PEG-IFN in CHB. Furthermore, unlike a previous study in CHB patients treated with interferon[11], development of anti-IFN antibodies was not associated with non-response to PEG-IFN therapy. However, we should be cautious as the number of patients who developed anti-IFN antibodies in our study was relatively small and a larger group might have shown statistical significance.
Limitations of our study included the fact that we had a rather heterogeneous population of HBeAg positive and negative patients who were treated with different regimens of PEG-IFN. We therefore performed all our analyses in different sub-groups.

Concluding, this study showed that anti-IFN antibodies may frequently be detected in CHB patients, and presence is associated with previous IFN therapy. However, presence or development of anti-IFN antibodies was not associated with non-response to PEG-IFN treatment in CHB and there appears to be no future role for anti-IFN antibodies in predicting response to PEG-IFN in CHB.

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CHAPTER 3

Serum levels of interferon gamma-inducible protein-10 and response to peginterferon therapy in HBeAg-positive chronic hepatitis B

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ABSTRACT

Background & Aims

Serum levels of interferon-gamma inducible protein 10 (IP-10) are a marker for immune activity, and may predict response to peginterferon (PEG-IFN) therapy in chronic hepatitis B.

Methods

IP-10 was measured at baseline and on-treatment week 12 in 210 HBeAg positive patients treated with PEG-IFN for 52 weeks. Response to treatment was assessed at 6 months post-treatment and defined as HBeAg loss, combined response (HBeAg loss with HBV DNA<10,000c/mL) or HBsAg loss.

Results

Median baseline IP-10 levels were 158.2 pg/mL. Higher baseline IP-10 was associated with more HBV DNA, HBeAg and HBsAg decline from week 4 onwards, and IP-10 was higher in patients who achieved HBeAg loss (p=0.001) and combined response (p=0.052). A combination of high IP-10 (>150 pg/mL) with absence of precore (PC) and core promoter (BCP) mutants strongly predicted combined response and HBsAg loss: 48% of patients with high IP-10 and no detectable mutants achieved a combined response (p<0.001). IP-10 decline from baseline to week 12 was very limited, but more pronounced in patients who achieved HBeAg loss (0.05 log pg/mL, versus an increase of 0.05 in patients without HBeAg loss, p=0.04).

Conclusions

Higher pre-treatment IP-10 levels are associated with an increased probability of HBeAg loss after PEG-IFN therapy. A combination of high baseline IP-10 and absence of PC and BCP mutants identified patients with the highest probability of combined response and HBsAg loss. There appears little use for on-treatment quantification of IP-10 for prediction of response to PEG-IFN.

INTRODUCTION

Peginterferon (PEG-IFN) is a first-line treatment option for chronic hepatitis B (CHB), because a finite treatment course may result in a sustained response in about 25% of patients.[1-3] In HBeAg positive patients, HBV genotype, patient age, low baseline HBV DNA and high baseline ALT are independent predictors of response to PEG-IFN therapy.[4] Another recent study suggests that host *IL28B* genotype may also influence the probability of serological response to PEG-IFN,[5] while absence of precore (PC) and basal core promoter (BCP) mutants may predict virologic response after HBeAg clearance.[6] The association of both high ALT and *IL28B* genotype with response to PEG-IFN suggests that successful induction of an immune response with PEG-IFN depends upon a susceptible host in combination with an active immune response, and biomarkers of immune activity may therefore predict response to treatment.

The interferon-gamma inducible protein 10 (IP-10), also known as chemokine C-X-C motif ligand (CXCL-)10, targets the CXC3 receptor, attracts T-lymphocytes and influences T-cell as well as natural killer cell adhesion.[7-9] Therefore, serum levels of IP-10 may be a marker for immune activity.[10] Pre-treatment IP-10 levels appear to predict response to PEG-IFN therapy in chronic hepatitis C patients,[10-13] independent of other known predictors, such as viral load, HCV genotype and stage of liver disease.[10,12,13] Moreover, recent studies have shown that quantification of IP-10 may add substantially to *IL28B* genotyping when aiming to predict a sustained response in hepatitis C patients, possibly through an association with interferon stimulated gene expression.[14-16]

Although the precise role of IP-10 in CHB remains unclear, one previous study showed that IP-10 kinetics are associated with the occurrence of flares in CHB patients. These findings suggest that serum levels of IP-10 could reflect the immune activity of patients, and consequently predict response to PEG-IFN therapy.[17]

The aim of the current study was therefore to investigate the relationship between serum levels of IP-10 and response to PEG-IFN in HBeAg positive CHB patients.

PATIENTS AND METHODS

Patients

In this study, serum levels of IP-10 were measured before treatment initiation and at week 12 of treatment in 210 HBeAg positive CHB patients treated with PEG-IFN alfa-2b within an investigator-initiated multicenter randomized trial.[1,18,19] The inclusion and exclusion criteria for this study have previously been described elsewhere.[1] In summary, patients were eligible if they had been HBsAg positive for at least 6 months before randomization, were HBeAg positive, had elevated serum alanine aminotransferase

(ALT) levels of >2, but <10 times the upper limit of normal (ULN), and had a serum HBV DNA concentration of more than 100,000 copies/mL. Patients were treated with PEG-IFN alfa-2b 100 µg weekly (PegIntron, Schering-Plough, Kenilworth, NJ, USA) in combination with placebo or lamivudine (LAM) 100 mg (Zeffix, GlaxoSmithKline, Greenford, UK) daily for 52 weeks. Inclusion criteria for the present analysis were completion of the 26-week post-treatment follow-up phase of the main study, data on PC / BCP mutants at baseline, and available serum for IP-10 assessment at baseline. Of the 266 patients in the initial study, 210 fulfilled these criteria.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written informed consent according to standards of the local ethics committees.

Laboratory measurements

Serum IP-10 was assessed at baseline and at week 12 of treatment using a commercially available ELISA kit (Alta Analytical Laboratory, San Diego, USA) in samples that were stored at -80° Celcius since the original studies . The presence of PC and BCP mutants was assessed using the INNO-LiPA HBV PreCore assay (Innogenetics, Ghent, Belgium). This very sensitive line probe assay allows for easy detection of PC (at nucleotide position G1896) and BCP (at nucleotide positions A1762 and G1764) mutants.[20] Patients were classified as wildtype (*WT*, only WT virus detectable), or as *non-WT* (when either PC, BCP or both mutants were detected). Serum HBV DNA, HBeAg and HBsAg were quantified in samples taken at baseline, during the treatment period and at 6 months post-treatment. HBV DNA was measured using an in-house developed TaqMan polymerase chain reaction (PCR) assay (lower limit of quantification 400 copies/mL).[21] HBsAg was measured using the Abbott ARCHITECT HBsAg assay (Abbott laboratories; range 0.05 - 250 IU/mL) and HBeAg with the Roche ELECSYS HBeAg assay using a quantitative protocol (Roche Diagnostics, range 0.2 – 100 IU/ml).

Statistical analysis

Response was assessed at 6 months post-treatment (week 78) and was defined as either HBeAg loss, HBeAg loss with HBV DNA <10,000 copies/mL (combined response) or HBsAg loss. Associations between variables were tested using Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents when appropriate. 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.

Role of the funding source

Financial support was provided by the Foundation for Liver and Gastrointestinal Research (SLO) in Rotterdam, the Netherlands. 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 characteristics of the enrolled patients are shown in table 1. Overall, 75 (36%) cleared HBeAg, 39 (19%) achieved a combined response and 17 (8%) cleared HBsAg. Since combination treatment with lamivudine did not influence response rates[1], data from the monotherapy and combination arms were pooled for the current analysis. Treatment allocation was controlled for in multivariate analyses whenever applicable. Median baseline level of IP-10 was 158.2 pg/mL (range: 6.6 – 1500 pg/mL). IP-10 levels were logarithmically transformed for further analysis, and also divided into quartiles: quartile 1 (<2.02 log pg/mL), quartile 2 (2.02 – 2.20 log pg/mL), quartile 3 (2.20 – 2.42 log pg/mL) and quartile 4 (>2.42 log pg/mL).

Relationship between IP-10 levels and baseline characteristics

Baseline IP-10 levels did not significantly differ across the HBV genotypes A through D, across patients with different ethnicities, nor among patients with only WT virus versus those with detectable PC and/or BCP mutants. Baseline IP-10 level did not correlate with baseline HBV DNA, HBeAg, or HBsAg levels, but was significantly associated with patient age ($r=0.23$, $p=0.001$) and correlated strongly with baseline ALT ($r=0.45$, $p<0.001$).

Association of baseline IP-10 with on-treatment decline of HBV DNA, HBeAg and HBsAg

Higher baseline IP-10 level was associated with more HBV DNA ($p=0.001$), HBeAg ($p<0.001$) and HBsAg decline ($p=0.028$) at 6 months after PEG-IFN discontinuation (week 78). Figure 1A-C shows the on-treatment declines in HBV DNA, HBeAg and HBsAg stratified by a baseline IP-10 level of 150 pg/mL (~median). Importantly, a baseline IP-10 level >150 pg/mL independently predicted HBsAg decline at week 78 when adjusted for HBV genotype, presence of only WT virus and baseline HBsAg. Adjusted HBsAg decline for patients with an IP-10 >150 pg/mL was 1.38 log IU/mL, compared to 0.89 for those with an IP-10 <150 pg/mL ($p=0.034$). In similar models, baseline IP-10 level > 150 pg/mL was associated with more HBeAg decline (1.35 vs. 0.83 log IU/mL, $p=0.002$) and HBV DNA decline (3.00 vs. 1.96 log c/mL, $p=0.002$). Combination therapy did not predict HBsAg,

Table 1. Characteristics of the study cohort

Characteristics	
Demography	
Mean (SD) age, years	33.7 (12)
Male	164 (78%)
Previous IFN therapy	38 (18%)
PEG-IFN Monotherapy	102 (49%)
Race	
Caucasian	153 (73%)
Asian	40 (19%)
Other	17 (8%)
Laboratory results	
Mean (SD) ALT*	4.3 (3.0)
Mean (SD) HBV DNA, log c/mL	9.1 (0.89)
Mean (SD) HBsAg, log IU/mL	4.4 (0.60)
Mean (SD) HBeAg, log IU/mL	2.5 (0.70)
Median (range) IP-10, pg/mL	158.2 (6.6-1500)
HBV Genotype	
A	73 (35%)
B	19 (9%)
C	29 (14%)
D	82 (39%)
Other/mixed	7 (3%)
INNO-LiPA result	
Wildtype	76 (36%)
Precore	54 (26%)
Basal core promoter	45 (21%)
Precore and basal core	35 (17%)

*Multiples of upper limit of the normal range

HBeAg or HBV DNA decline at week 78 in these models ($p \geq 0.370$). Importantly, the association between high IP-10 level at baseline (>150 pg/mL) and more pronounced on-treatment decline was apparent as soon as week 4 of treatment for HBsAg ($p<0.001$), HBeAg ($p=0.002$) and HBV DNA ($p<0.001$), when adjusting for HBV genotype, presence of only WT virus, baseline level and combination therapy.

Baseline IP-10 levels and response at 6 months post-treatment

Baseline IP-10 level was higher in patients who cleared HBeAg by week 78 when compared to those who did not (2.34 vs. 2.17 pg/mL, $p=0.001$), as was the case for patients who achieved a combined response versus those who did not (2.32 vs. 2.21 log pg/mL, $p=0.052$). The association between baseline IP-10 level (in quartiles) and probability

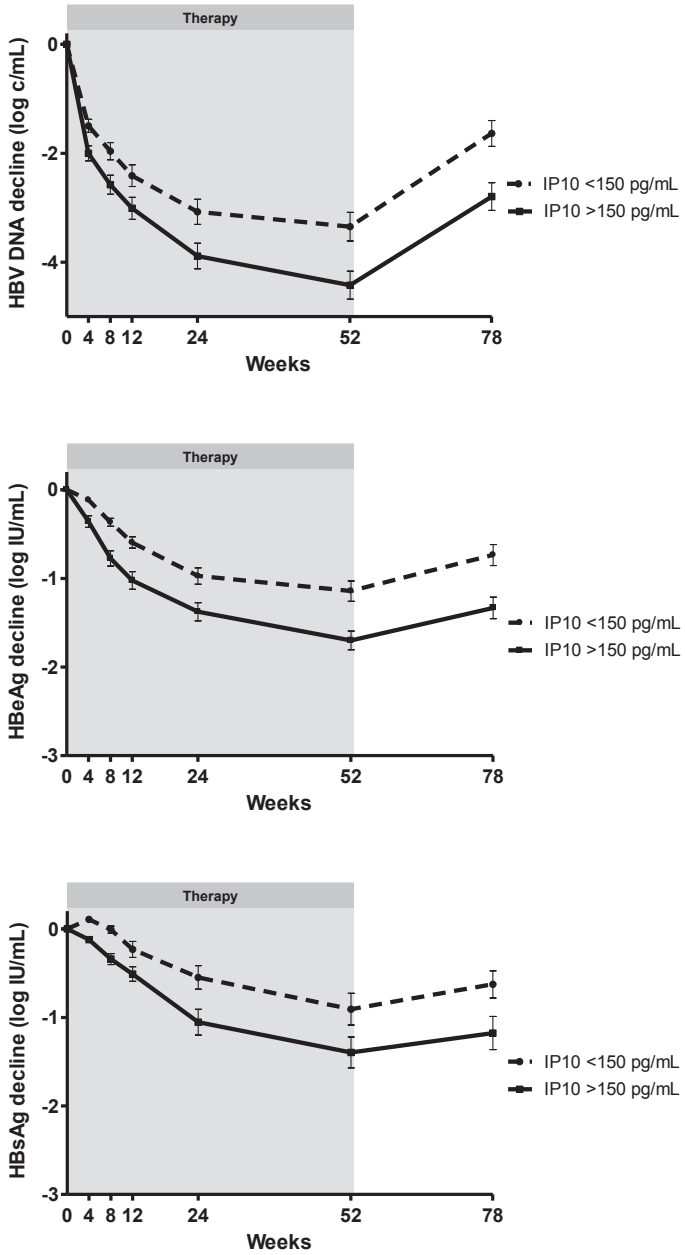

Figure 1. Relationship between baseline IP-10 level and on-treatment viral decline. Decline of serum HBV DNA (A), HBeAg (B) and HBsAg (C) during treatment by baseline IP-10 level (n=210).

of response at 6 months post-treatment is shown in figure 2. In multivariate analysis, IP-10 levels at baseline were significantly associated with the occurrence of HBeAg clearance (adjusted OR: 3.60, 95% CI: 1.15 – 11.22, p=0.024, table 2) when adjusting for HBV genotype, presence of only WT virus, baseline age, HBV DNA and ALT and previous IFN treatment failure. Of note, presence of PC and/or BCP mutants was not an independent predictor of HBeAg loss after PEG-IFN therapy, nor was combination therapy. Interestingly, serum IP-10 at baseline was not significantly associated with the occurrence of a combined response (adjusted OR: 2.48, 95% CI: 0.59 – 10.48, p=0.21, table 2).

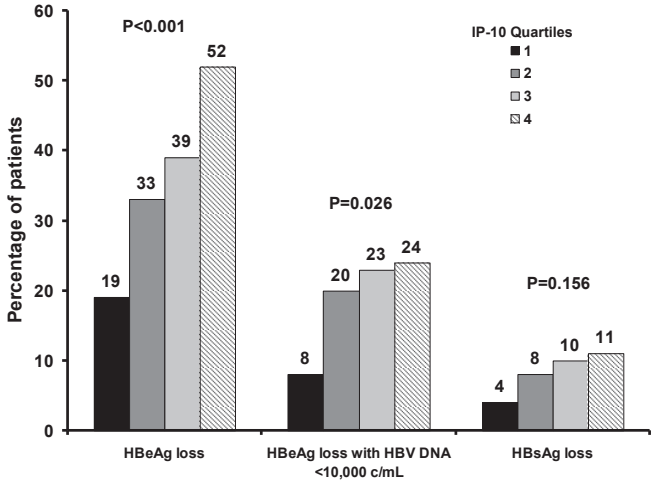

Figure 2. Baseline IP-10 and response at 6 months post-treatment.
Relationship between baseline IP-10 and response to treatment in the HBeAg-positive population in the overall cohort

A combination of IP-10 and presence of only WT virus identifies patients with a high likelihood of response

Since baseline levels of IP-10 was associated with HBeAg loss, but not combined response, whereas presence of only WT virus was previously shown to be associated with achievement of low HBV DNA levels after HBeAg clearance[6], we explored the interplay between these two variables. In a model for combined response, an interaction term between WT virus and baseline IP-10 level was highly significant (p=0.002), indicating that the association of IP-10 levels with response is not the same for patients with WT virus compared to those with detectable mutants at baseline. A similar interaction was also found for WT virus and baseline ALT (p=0.030). Such an interaction was not found when HBeAg loss was considered (p=0.15 for interaction of IP-10 and WT, p=1.0 for ALT

Table 2. Logistic regression model of probability of response to peginterferon in HBeAg positive patients

HBeAg loss week 78			Combined Response week 78		
Variable	**OR (95% CI)**	**p**	**Variable**	**OR (95% CI)**	**p**
IP-10*	3.60 (1.15 – 11.2)	0.024	IP-10*	2.48 (0.59 – 10.5)	0.209
Wildtype	1.11 (0.50 – 2.46)	0.799	Wildtype	3.45 (1.30 – 9.18)	0.011
HBV Genotype		0.001	HBV Genotype		0.048
A	Reference		A	Reference	
B	0.62 (0.18 – 2.13)		B	0.52 (0.12 – 2.19)	
C	0.10 (0.03 – 0.37)		C	0.11 (0.02 – 0.61)	
D	0.45 (0.18 – 1.14)		D	0.33 (0.10 – 1.12)	
ALT#	1.11 (0.99 – 1.25)	0.081	ALT#	1.06 (0.90 – 1.25)	0.528
Age	1.02 (0.99 – 1.05)	0.280	Age	1.04 (1.00 – 1.07)	0.036
HBV DNA**	0.58 (0.38 – 0.88)	0.010	HBV DNA**	0.55 (0.32 – 0.94)	0.031
No previous IFN	3.74 (1.50 – 9.32)	0.003	No previous IFN	5.07 (1.49 – 17.3)	0.004

Combined response was defined as HBeAg loss with HBV DNA <10,000 c/mL at 6 months post-treatment.
*IP-10 in log pg/mL, **HBV DNA in log copies/mL, #ALT in x ULN.

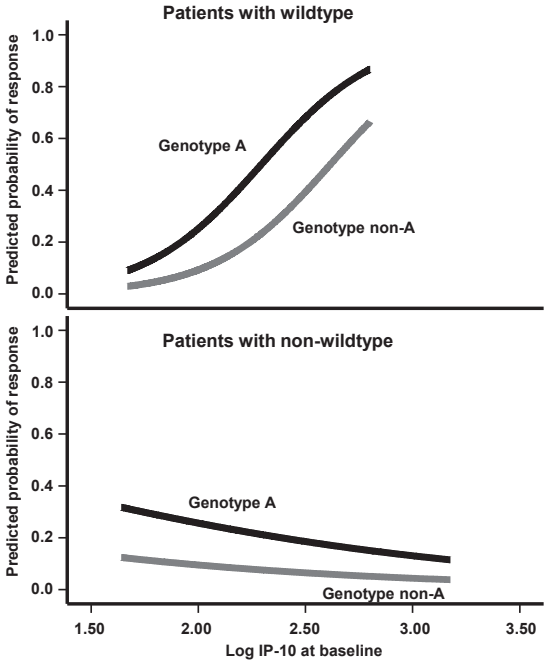

Figure 3. Predicted probability for response as a function of baseline IP-10.
Estimated probability of combined response (HBeAg loss and HBV DNA <10,000 c/mL) stratified by presence of precore and/or core promoter mutants

and WT). Figure 3 shows the estimated probability of combined response as predicted by the prediction model shown in table 2, with addition of an interaction term of WT with IP-10. The probability of response for patients with only WT virus strongly improved with increasing IP-10 level, and similar findings were obtained with ALT. In contrast, patients with detectable PC and/or BCP mutants did not benefit from higher IP-10 or ALT levels. A combination of baseline IP-10 >150 pg/mL and absence of PC and BCP mutants could identify patients with a very high likelihood of response (figure 4). Furthermore, it can also be inferred from figure 4 that high IP-10 level at baseline predisposes to HBeAg clearance after PEG-IFN therapy, but that this did not translate to increased combined response rates or HBsAg loss if PC and/or BCP mutants were present.

On-treatment IP-10 and response to treatment

IP-10 levels remained stable from baseline to week 12; a minimal non-significant decline was observed of 0.015 log pg/mL (p=0.52 compared to baseline). IP-10 decline was more pronounced in patients who achieved HBeAg loss (0.05 log pg/mL decline, versus an increase of 0.05 in patients without HBeAg loss, p=0.04), and the proportion of patients who achieved a decline of IP-10 from baseline was higher among patients with HBeAg loss (59 versus 42%, p=0.024) and combined responders (60 versus 45%, p=0.11). Pa-

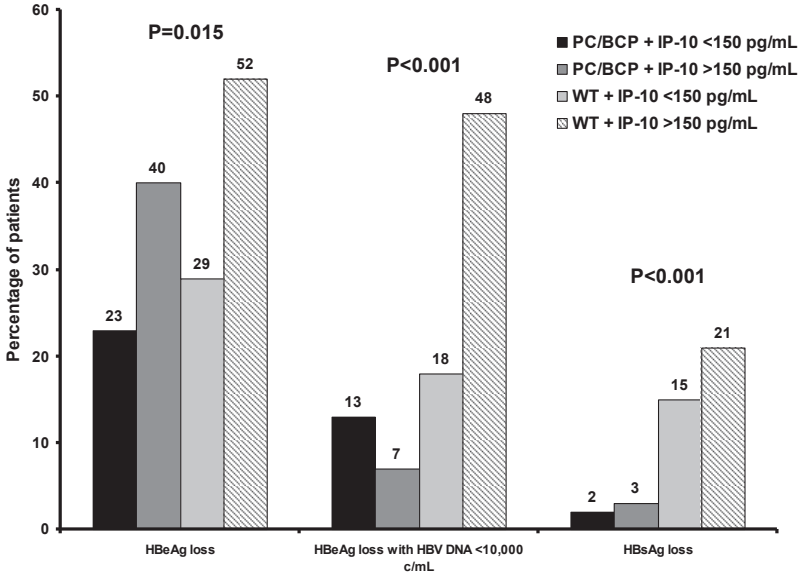

Figure 4. Observed response rates by baseline IP-10 and presence of PC and BCP mutants.
The probability of combined response according to a baseline IP-10 in combination with presence or absence of precore and/or core promoter mutants.

tients with an IP-10 decrease at week 12 achieved more HBV DNA decline (2.55 versus 1.85 log c/mL, p=0.06), HBeAg decline (1.24 versus 0.84 log IU/mL, p=0.029) but not HBsAg decline (1.04 versus 0.75, p=0.26).

DISCUSSION

This is the first study to describe the association of IP-10 level and response to PEG-IFN therapy in CHB. In our study, higher baseline level of IP-10 strongly predicted HBeAg loss after PEG-IFN therapy, and a combination of high IP-10 and presence of only WT virus identified patients with a high likelihood of combined serological and virologic response.

PEG-IFN is a valuable treatment option for CHB, for it is the only agent that can be expected to induce a sustained off-treatment response after a finite treatment course.[22] However, the limited response rates observed in the general CHB patient population necessitate careful selection of patients.[23] Our group recently published a baseline prediction model that can help clinicians identify HBeAg positive patients with a high likelihood of response[4], and extensions with host and viral factors have been proposed.[5,6,24] The current study shows that response to PEG-IFN in HBeAg positive patients also depends upon pre-treatment serum level of IP-10. Importantly, the association of IP-10 with response to treatment is already apparent from week 4 of therapy, as shown by the more pronounced HBV DNA, HBeAg and HBsAg decline observed in patients with higher levels of IP-10 at baseline. Given the strong association of IP-10 with ALT, high levels of IP-10 may be a proxy for an active host immune response, resulting in more active liver inflammation.[25] Importantly, blocking the effects of IP-10 may reduce liver damage in mice,[26] further supporting the association of IP-10 with immune activity. These findings are corroborated by recent data from Cornberg et al, showing more pronounced HBsAg decline in nucleo(s)tide analogue treated patients with high IP-10 levels.[27] The association with immune activity is further strengthened by the observation that IP-10 levels decline during PEG-IFN therapy in patients who achieve a response, mimicking reductions in intrahepatic inflammation previously observed in responders to PEG-IFN.[1] Previous studies have shown that a pre-existing immune response may be a pre-requisite for response to PEG-IFN therapy,[4,28] and the current study shows that serum levels of IP-10 may help identify patients with such favourable characteristics. Nevertheless, the observed IP-10 decline in responders is very limited, restraining the use of IP-10 as an on-treatment predictor of response to PEG-IFN therapy in HBeAg positive CHB. Furthermore, recent studies in HCV infected patients treated with PEG-IFN have shown that a slight increase in IP-10 levels may be observed after PEG-IFN dosing, which may

also reduce the reliability of IP-10 quantification during treatment. The current study therefore does not support the use of IP-10 for on-treatment decision-making in HBeAg positive CHB patients treated with PEG-IFN.[12]

It should be appreciated that baseline and on-treatment IP-10 levels appear to be mainly associated with the probability of HBeAg clearance after PEG-IFN therapy, and less so with a combined serological and virologic response. Persistence of viral replication after HBeAg loss may be accounted for by the presence PC and BCP mutants, which can be detected in a considerable proportion of HBeAg positive patients.[6] Combining levels of IP-10 and presence of PC and BCP mutants showed that both contribute to the achievement of a combined serological and virologic response; patients with both a high baseline level of IP-10 and absent mutants achieved high rates of combined response, whereas patients with a high IP-10 level with detectable mutants progressed to active HBeAg negative CHB. A similar association was found if a combination of baseline ALT and presence of mutants was explored, further illustrating the importance of active inflammation. Based on these findings, we propose that combined serological and virologic response to PEG-IFN in HBeAg positive CHB requires both a susceptible host (high IP-10, high ALT), as well as a susceptible virus (absence of PC and BCP mutants).
Concluding, high levels of IP-10 predict HBeAg loss, and a combination of high IP-10 and absent PC and BCP mutants predicts combined serological and virologic response to PEG-IFN in HBeAg positive CHB. There appears little use for on-treatment quantification of IP-10 for prediction of response to PEG-IFN in CHB.

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CHAPTER 4

Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians

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ABSTRACT

Background

Hepatocellular carcinoma (HCC) risk-scores may predict HCC in Asian entecavir (ETV) treated patients. We aimed to study risk factors and performance of risk scores during ETV treatment in an ethnically diverse Western population.

Methods

We studied all HBV mono-infected patients treated with ETV from 11 European referral centers within the VIRGIL Network.

Results

A total of 744 patients were included; 42% Caucasian, 29% Asian, 19% other, 10% unknown. At baseline, 164 patients (22%) had cirrhosis. During a median follow-up of 167 (IQR 82-212) weeks, 14 patients developed HCC of whom nine (64%) had cirrhosis at baseline. The 5-year cumulative incidence rate of HCC was 2.1% for non-cirrhotic and 10.9% for cirrhotic patients ($p<0.001$). HCC incidence was higher in older patients ($p<0.001$) and patients with lower baseline platelet counts ($p=0.02$). Twelve patients who developed HCC achieved virologic response (HBV DNA<80IU/mL) before HCC. At baseline, higher CU-HCC and GAG-HCC, but not REACH-B scores were associated with development of HCC. Discriminatory performance of HCC risk scores was low with sensitivity ranging from 18-73% and c-statistics from 0.71 to 0.85. Performance was further reduced in Caucasians with c-statistics from 0.54 to 0.74. Predicted risk of HCC based on risk-scores declined during ETV therapy (all $p<0.001$), but predictive performances after one year were comparable to those at baseline.

Conclusion

Cumulative incidence of HCC is low in patients treated with ETV, but ETV does not eliminate the risk of HCC. Discriminatory performance of HCC risk-scores was limited, particularly in Caucasians, both at baseline and during therapy.

INTRODUCTION

The goal of treatment of chronic hepatitis B (CHB) infection is to prevent disease progression to (decompensated) cirrhosis, hepatocellular carcinoma (HCC) and death.[1] Current treatment guidelines consider nucleos(t)ide analogues (NA) or peginterferon (PEG-IFN) as first line treatment for CHB in patients with serum HBV DNA level >2.000 IU/mL in combination with elevated ALT levels (>1-2x ULN) or with moderate to severe liver inflammation and/or fibrosis.[2,3] These guidelines are based on the accepted association between HBV DNA levels and progression to cirrhosis, HCC and liver-related mortality in untreated patients.[4] Entecavir (ETV) inhibits HBV replication in the vast majority of patients and is also able to improve fibrosis scores after continuous therapy in 88% of CHB patients.[5] Furthermore, ETV therapy may reduce the risk of HCC and liver related events, particularly in patients with cirrhosis.[6-8] Nevertheless, the residual risk of HCC necessitates intensive on-going follow-up of patients with successfully suppressed viral replication.[2] Recently, risk scores based on demographic (age and sex), clinical (cirrhosis, ALT, albumin and bilirubin) and virologic (HBeAg status, HBV DNA) characteristics have been developed in order to predict the risk of HCC in treatment-naïve patients. These HCC risk scores were shown to predict HCC in Asian CHB patients treated with ETV as well.[9] However, the performance of these risk-scores in non-Asian patients remains unclear. The aims of the current study were therefore to investigate in this large ethnically diverse European HBV infected population treated with ETV 1) the incidence of, and risk factors for, development of liver related events including HCC and 2) the role of risk-scores for prediction of HCC.

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 CHB patients (HBsAg positive for at least 6 months) treated with ETV monotherapy for at least 3 months between 2005 and May 2013 in 11 large European referral centers were included. Patients were excluded if they were co-infected with HIV, HCV or HDV or if they had an HCC at baseline. Patients' ethnicity was classified as Caucasian, Asian (including only East-Asians from e.g. China, Hong Kong and Thailand) or other (including sub-Saharan Africans). A total of 744 patients were eligible for the current analysis. 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 to six months at the discretion of the local treating physician. At every visit routine examination with biochemical (serum ALT, bilirubin, albumin, INR and creatinine) and virologic (HBsAg, HBeAg, anti-HBe, HBV DNA level) assessments took place. The diagnosis of cirrhosis at baseline was based on histology or ultrasound examinations with signs of cirrhosis (spleen size >12 cm, portal vein>16 mm, or nodules within the hepatic parenchyma).[6] In cirrhotic patients screening for HCC was performed at least yearly by ultrasound and/or alpha-fetoprotein measurement. In non-cirrhotic patients HCC surveillance varied from centre to centre according to local protocols and was only performed when other risk factors were present.[3]

Endpoints

HCC was defined by either i) histological confirmation, or ii) two parallel imaging techniques (ultrasound, computerised tomography, or magnetic resonance imaging) showing a focal lesion larger than 2 cm with arterial hypervascularization, or iii) one imaging technique showing a focal lesion larger than 2 cm with arterial hypervascularization in the presence of an alpha fetoprotein level greater than 400 ng/mL.
Clinical events were defined as a composite endpoint of development of HCC, liver decompensation, or death during the study period. Diagnosis of decompensated cirrhosis was based on the presence of ascites confirmed by ultrasound, jaundice with a serum bilirubin level >2.0 mg/dL, bleeding esophageal varices, or hepatic encephalopathy in cirrhotic patients. Other reported endpoints were virologic response (VR, HBV DNA level < 80 IU/mL), HBeAg loss (in HBeAg-positive patients) and HBsAg loss all during the on-treatment follow-up period.

Laboratory tests

Serum alanine aminotransferase (ALT), bilirubin, albumin levels and international ratio of prothrombin time (INR) 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 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 ten of eleven 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

Data acquisition directly from the patients' charts was performed on site by a single experienced investigator (PA). Data were systematically collected through a standardized clinical record form. 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 (IQR) where appropriate. Follow-up times were calculated from the date of ETV treatment initiation to the date of event or end of follow-up. Components of the HCC risk scores included age, cirrhosis, albumin, bilirubin and HBV DNA level for the CU-HCC risk score[10]; age, cirrhosis, sex and HBV DNA for the GAG-HCC risk score[11]; and age, sex, ALT, HBeAg status and HBV DNA for the REACH-B risk score[12]. The cumulative probability of achieving primary or secondary endpoints was estimated by Kaplan-Meier analysis. Cox's regression analysis was used to study which baseline factors were associated with primary or secondary endpoints. The influence of VR was analyzed as a time-dependent covariate allowing patients to be at risk in either the VR or non-VR group according to HBV DNA level during follow-up. Therefore VR was entered in the model as a time-dependent covariate: all patients started (and thus at risk) within the group without VR and were switched to the group with VR after achieving this endpoint. Sensitivity, negative predictive values (NPVs), and c-statistics of the risk scores to predict HCC were estimated and reported, both within the entire population as well as in a subgroup of Caucasian patients.
All statistical tests were two-sided, and a P value < 0.05 was considered to be statistically significant. SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used for all statistical analyses.

RESULTS

Baseline characteristics

In total, 891 chronic HBV patients treated with ETV were identified. One hundred and forty-seven patients did not fulfill the entry criteria and were excluded; 70 patients were treated for less than 3 months, 19 patients were co-infected with HCV or HDV, 22 patients had an HCC at baseline, two patients had undergone liver transplantation, two patients were HBsAg negative at baseline, 30 patients received concomitant antiviral therapy and two were non-compliant. A total of 744 CHB patients treated with ETV monotherapy were thus eligible and included. Baseline characteristics of the study population are shown in table 1 according to the presence of cirrhosis. Forty-two percent of patients were of Caucasian origin, 29% Asian, 19% other and in 10% ethnicity was unknown. At baseline 164 patients (22%) had cirrhosis (by ultrasound or histology), 239 patients (32%) were HBeAg positive, median ALT was 1.4 x ULN (IQR 0.8 – 2.7) and mean HBV

Table 1. Baseline characteristics

	All (n=744)	cirrhosis (n=164)	no cirrhosis (n=580)	p
Male (%)	569 (77%)	138 (84%)	431 (74%)	0.009
Mean age	44 ± 14	51 ± 13	42 ± 13	<0.001
ETV dosage 0.5mg (%)	640 (86%)	123 (75%)	517 (89%)	<0.001
Race				0.37
Caucasian	316 (42%)	74 (45%)	242 (42%)	
Asian	214 (29%)	41 (25%)	173 (30%)	
Other	139 (19%)	26 (16%)	113 (19%)	
Unknown	75 (10%)	23 (14%)	52 (9%)	
Genotype				0.64
A	100 (13%)	23 (14%)	77 (13%)	
B	48 (7%)	7 (4%)	41 (8%)	
C	78 (11%)	17 (10%)	61 (11%)	
D	186 (25%)	40 (24%)	146 (25%)	
E	52 (7%)	8 (5%)	44 (8%)	
Other	5 (1%)	1 (1%)	4 (1%)	
Unknown	275 (37%)	68 (41%)	207 (36%)	
HBeAg positive	239 (32%)	54 (33%)	185 (32%)	0.92
Mean HBV DNA (log IU/mL)	5.3 ± 2.2	5.4 ± 2.2	5.2 ± 2.2	0.29
Median ALT (xULN)	1.4 (0.8-2.7)	1,5 (1-3,2)	1,4 (0,8-2,5)	0.57
Platelet count (x10E9/L)*	192 ± 72	138 ± 63	210 ± 66	<0.001
Median bilirubin (umol/L)	11 (8-15)	14 (10-20)	10 (7-14)	0.001
Albumin (g/dL)**	4.3 ± 0.5	4.1 ± 0.6	4.4 ± 0.4	<0.001
PT INR^{+}	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	<0.001
Mean CU HCC risk score^{++}	8 ± 9	23 ± 9	4 ± 4	<0.001
Mean GAG HCC risk score	62 ± 18	82 ± 14	56 ± 14	<0.001
Mean REACH-B score	9 ± 3	11 ± 3	9 ± 3	<0.001
NA-naive	569 (77%)	108 (66%)	461 (80%)	<0.001
LAM-naive	617 (83%)	122 (74%)	495 (85%)	0.001
IFN-naive	610 (82%)	138 (84%)	472 (81%)	0.49

Data are represented as mean (± standard deviation) or median (IQR).
Data available for *73%, **75%, $^{+}$63% and $^{++}$69% of patients, respectively

DNA 5.3 log IU/mL (6.6 log IU/mL for HBeAg positive and 4.5 log IU/mL for HBeAg negative patients). Overall median follow-up was 167 weeks (IQR 82-212) and did not differ between cirrhotic and non-cirrhotic patients (p=0.22). Total number of visits was 7160 with a median number of visits per patient of 8 (IQR 5-11), with a median interval of 14 (IQR 12-25)

Virologic response during treatment

HBV DNA < 80 IU/mL (virologic response, VR) was achieved in 655 patients. The cumulative probability of VR was 53%, 76%, 90%, 94%, 97% and 99% at six months and years 1, 2, 3, 4 and 5, respectively. VR was not influenced by the presence of cirrhosis (p>0.2). HBeAg loss was achieved in 85 (36%) of 239 HBeAg positive patients. The cumulative probability of HBeAg loss was 11%, 25%, 36%, 45% and 58% at years 1, 2, 3, 4 and 5 and was higher in patients with cirrhosis (p=0.03). Sixteen patients (2.2%) achieved HBsAg loss. The cumulative probability of HBsAg loss was 0.1%, 1.2%, 1.6%, 2.3% and 4.1% at years 1, 2, 3, 4 and 5.

Development of HCC

Fourteen patients developed HCC (7 Caucasians), after a median duration of 125 weeks (IQR 59-188). The cumulative probability of developing HCC was 2.1% for non-cirrhotic versus 10.9% for cirrhotic patients at year five of follow-up (p<0.001) (Figure 1). Risk of HCC was higher in patients with cirrhosis (p=0.002), older patients (>50 years) (p<0.001), in patients with lower platelet counts (p=0.02), and in patients who were previously treated with lamivudine (p=0.03). When Caucasian patients were studied separately, only age was associated with the occurrence of HCC (HR 1.06, 95% CI 1-1.13, p=0.05). (Table 2)

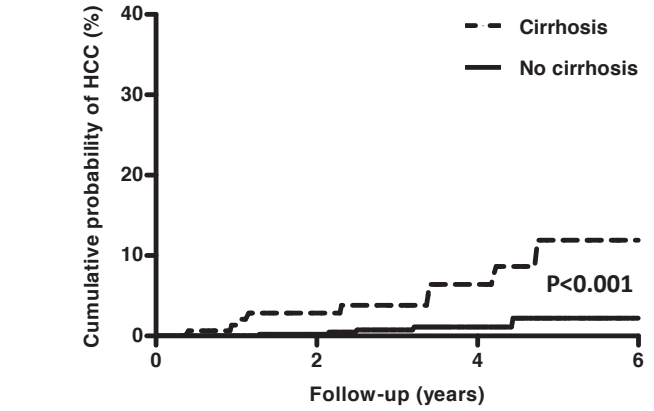

Figure 1. Kaplan-Meier curve for the cumulative probability of developing HCC according to presence of cirrhosis

Table 2. Univariate Cox regression analysis of potential risk factors for developing HCC and clinical events

Risk factor	Overall population (n=744)						Caucasians (n=316)					
	HCC (n=14)			Overall events (n=34)			HCC (n=7)			Overall events (n=20)		
	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p
Age (per year)	1.08	1.04-1.13	<0.001	1.06	1.04-1.09	<0.001	1.06	1-1.13	0.05	1.05	1.02-1.09	0.004
Female	0.22	0.03-1.68	0.15	0.29	0.09-0.96	0.04	0.44	0.05-3.67	0.45	0.50	0.15-1.71	0.27
Caucasian	1.09	0.55-2.14	0.80	1.02	0.80-1.30	0.87	NA	NA	NA	NA	NA	NA
Genotype B	0.94	0.54-1.61	0.81	1.08	0.78-1.49	0.64	0.99	0.47-2.06	0.97	1.18	0.72-1.91	0.52
HBeAg neg	0.81	0.25-2.57	0.72	1.11	0.55-2.34	0.78	0.33	0.04-2.71	0.30	0.48	0.16-1.43	0.19
HBVDNA(log IU/ml)	0.82	0.64-1.05	0.12	1.09	0.94-1.27	0.26	0.78	0.55-1.10	0.15	1.14	0.95-1.38	0.17
ALT (xULN)	0.70	0.41-1.18	0.18	1.02	0.98-1.06	0.40	0.70	0.35-1.41	0.32	1.02	0.98-1.06	0.34
Bilirubin (log umol/L)	2.15	0.36-12.9	0.43	5.18	2.14-12.6	<0.001	0.24	0.01-5.96	0.37	2.44	0.59-10.0	0.25
Albumin (g/dL)	0.93	0.83-1.04	0.18	0.85	0.81-0.89	<0.001	1.01	0.86-1.19	0.87	0.83	0.76-0.90	<0.001
PT INR (per 0.1)	1.00	0.70-1.42	0.99	1.23	1.11-1.36	<0.001	0.70	0.29-1.68	0.42	1.23	1.05-1.45	0.01
Platelet count (per 10x10E9/L)	0.90	0.83-0.98	0.02	0.90	0.85-0.95	<0.001	0.96	0.86-1.08	0.48	0.95	0.89-1.01	0.13
MELD score	1.04	0.87-1.24	0.70	1.13	1.06-1.21	<0.001	0.89	0.53-1.49	0.65	1.15	1.05-1.26	0.003
Cirrhosis	5.82	1.94-17.41	0.002	7.25	3.53-14.89	<0.001	3.70	0.82-16.6	0.09	4.70	1.92-11.52	0.001
Previous NA	0.45	0.16-1.31	0.14	0.52	0.26-1.05	0.07	0.59	0.13-2.63	0.49	1.22	0.44-3.36	0.70
Previous LAM	3.21	1.11-9.26	0.03	2.45	1.21-4.95	0.01	2.60	0.58-11.7	0.21	0.93	0.31-2.79	0.90
Previous IFN	0.63	0.14-2.82	0.55	0.38	0.12-1.23	0.11	0.93	0.18-4.82	0.93	0.43	0.12-1.45	0.17

Abbreviations: HCC, hepatocellular carcinoma; HBV DNA, hepatitis B virus DNA; ALT, alanine aminotransferase; ULN, upper limit of normal; MELD, model for end-stage liver disease; NA, nucleos(t)ide analogue; LAM, lamivudine; IFN, interferon.

Occurrence of clinical events

Overall, 34 patients developed a clinical event (including 14 HCC) after a median duration of 87 weeks (IQR 49-169). Twenty-three (68%) had cirrhosis at baseline. Of the 14 patients who developed HCC, three patients died. Thirteen patients developed an episode of hepatic decompensation of whom five patients died. Overall, 17 patients died during follow-up, eight liver related and nine of other causes. (Table 3)

Table 3. Distribution of clinical events

	Cirrhosis (n=164)			No cirrhosis (n=580)	
	Decompensation (n)	HCC (n)	Death (n)	HCC (n)	Death (n)
Overall	11	9	3	5	6
Caucasian	6	4	2	3	5
Asian	1	2	0	2	0
Other	4	3	1	0	1

Influence of virologic response and development of HCC and clinical events

In patients without a clinical event median time to VR was 23 weeks (IQR 12-47). Of 14 patients who developed HCC, 12 patients already achieved VR before HCC was diagnosed. The other 2 patients achieved response after the occurrence of HCC. Median time to VR was 24 weeks (IQR 13-41) in patients with HCC. Of the 34 patients with a clinical event, 30 patients achieved VR. Median time to VR in patients who developed a clinical events was 27 weeks (IQR 17-56). In a Cox regression analysis with VR as time-dependent factor HBV DNA < 80 IU/mL was neither significantly associated with the development of HCC (HR 0.87, 95%CI 0.17-4.58, p=0.87), nor with the development of a clinical event (HR 0.70, 95%CI 0.28-1.77, p=0.46).

Performance of HCC risk scores at baseline

At baseline, mean risk-score was 8 for CU-HCC, 62 for GAG-HCC and 9 for REACH-B. Higher CU-HCC and GAG-HCC, but not REACH-B scores were associated with HCC in the overall population.

When established cut-off values for these risk scores were used (5 for the CU-HCC score, 101 for the GAG-HCC score and 8 for the REACH-B score), only CU-HCC and GAG-HCC risk scores were predictive for HCC development (table 4). C-statistics for the overall population were 0.78 for CU-HCC, 0.85 for GAG-HCC and 0.71 for REACH-B risk score. In Caucasians the scores were 0.66, 0.74 and 0.54, for CU-HCC, GAG-HCC and REACH-B, respectively. Negative predictive values (NPVs) at 4 years of therapy for all risk scores at baseline were more than 95% (CU-HCC 84/86, GAG-HCC 184/193 and REACH-B 39/41) with a sensitivity ranging from 18% (2/11) for GAG-HCC, 78% (7/9) for CU-HCC and 82%

Table 4. Performance of HCC risk scores at baseline and after 1 year of ETV treatment

Baseline	Overall (N=744)							Caucasian (N=316)						
	HR	95%CI	p	c	95%CI of c	NPV at 4 yr	Sensitivity at 4 yr	HR	95%CI	p	c	95%CI of c	NPV at 4 yr	Sensitivity at 4 yr
CU-HCC	1.07	1.03-1.11	0.001	0.78	0.65-0.91	-	-	1.04	0.98-1.11	0.23	0.66	0.44-0.88	-	-
GAG-HCC	1.08	1.04-1.12	<0.001	0.85	0.78-0.91	-	-	1.06	1.01-1.11	0.03	0.74	0.60-0.89	-	-
REACH-B	1.18	0.99-1.39	0.06	0.71	0.58-0.85	-	-	1.01	0.78-1.31	0.92	0.54	0.32-0.75	-	-
cirrhosis	1.04	0.79-1.36	0.80	0.63	0.44-0.81	-	-	0.90	0.62-1.32	0.60	0.46	0.20-0.72	-	-
no cirrhosis	1.25	0.91-1.72	0.16	0.69	0.45-0.94	-	-	0.99	0.67-1.46	0.96	0.58	0.37-0.78		-
CU-HCC > 5	4.67	1.26-17.30	0.02	0.70	0.58-0.83	98%	78%	2.44	0.45-13.34	0.30	0.63	0.44-0.82	98%	67%
GAG-HCC > 101	15.95	3.4-74.79	<0.001	0.57	0.47-0.68	95%	18%	28.15	2.81-281.8	0.005	0.61	0.42-0.80	97%	25%
REACH-B > 8	1.09	0.30-3.90	0.90	0.55	0.47-0.63	95%	82%	0.68	0.13-3.51	0.65	0.52	0.36-0.68	96%	75%
cirrhosis	0.50	0.10-2.44	0.39	0.50	0.41-0.60	-	-	0.28	0.03-2.77	0.28	0.55	0.38-0.71	-	-
no cirrhosis	1.43	0.16-12.82	0.75	0.55	0.39-0.71	-	-	0.71	0.06-7.81	0.78	0.55	0.27-0.84	-	-
Year 1														
CU-HCC	1.07	0.98-1.17	0.13	0.73	0.60-0.87	-	-	1.06	0.97-1.15	0.18	0.71	0.59-0.84	-	-
GAG-HCC	1.07	1.03-1.12	0.004	0.84	0.76-0.92	-	-	1.06	1.01-1.11	0.02	0.77	0.64-0.90	-	-
REACH-B	1.27	1.07-1.52	0.008	0.79	0.69-0.89	-	-	1.13	0.87-1.46	0.37	0.65	0.52-0.79	-	-
cirrhosis	1.07	0.72-1.60	0.74	0.54	0.40-0.68	-	-	0.93	0.57-1.52	0.78	0.58	0.36-0.81	-	-
no cirrhosis	1.37	1.10-1.72	0.005	0.91	0.83-0.98	-	-	1.21	0.87-1.67	0.26	0.79	0.66-0.93	-	-
CU-HCC > 5	4.20	0.53-33.20	0.17	0.61	0.51-0.70	98%	89%	3.14	0.38-26.17	0.29	0.59	0.45-0.73	97%	75%
GAG-HCC > 101	13.80	1.65-115.7	0.02	0.58	0.43-0.72	95%	11%	24.76	2.48-247.8	0.006	0.61	0.42-0.80	97%	25%
REACH-B > 8	4.34	1.16-16.23	0.03	0.63	0.45-0.82	97%	50%	1.85	0.31-11.12	0.50	0.49	0.30-0.68	97%	0%
cirrhosis	1.30	0.22-7.92	0.77	0.46	0.25-0.67	-	-	0.74	0.07-8.25	0.81	0.60	0.37-0.83	-	-
no cirrhosis	13.4	1.38-129.7	0.025	0.79	0.59-0.99	-	-	4.08	0.25-65.75	0.32	0.56	0.24-0.89	-	-

(9/11) for REACH-B. Comparable NPVs were found in Caucasians, and also at year three and five . Additionally, when cirrhotic and non-cirrhotic patients were studied separately, only GAG-HCC score remained predictive for the occurrence of an HCC.

Influence of ETV treatment on HCC risk scores

Overall, predicted HCC risk based on CU-HCC, GAG-HCC and REACH-B declined after one year of ETV therapy, both in the overall population, as well as in cirrhotic, non-cirrhotic and Caucasian patients (all p-values < 0.001 for the change during follow-up with baseline). The decline in HCC risk scores from baseline to year 1 was comparable in patients who developed HCC versus those who did not.(Figure 2) Furthermore, hazard ratios of the dynamic risks for development of an HCC by an HCC risk score measurement at a random visit were comparable with those at baseline (Table 4 vs. Figures 2A-C) Despite the observation that the mean calculated risk scores were consistently higher in patients who developed HCC, diagnostic performance remained suboptimal during treatment. Negative predictive values in all patients with a minimum of 4 years of follow-up for all risk scores at year 1 of therapy were more than 95% (CU-HCC 51/52, GAG-HCC 157/165 and REACH-B 124/128) with a sensitivity of 11% (1/9) for GAG-HCC, 89% (8/9) for CU-HCC and 50% (4/8) for REACH-B. Comparable values were found in the Caucasian subpopulation (Table 4) and also when using a single HCC risk score measurement at a random visit .

DISCUSSION

In this European multicenter real-life cohort study we showed that CHB patients treated with ETV remain at considerable risk for developing HCC. The risk of HCC cannot be confidently predicted using HCC risk-scores at baseline nor during therapy, particularly not in Caucasians. Careful follow-up therefore remains necessary even if HBV DNA is adequately suppressed.

ETV therapy effectively suppresses viral replication, and in the current study virtually all patients achieved an undetectable HBV DNA during therapy. Recent studies have shown that a reduction of HBV DNA to low or undetectable levels reduces the risk of liver-related events and HCC.[6-8] However, in the current study we were unable to confirm the association between time to and duration of viral suppression and a reduction in the incidence of HCC or clinical events. Our findings are in line with another large European study which also found considerable rates of HCC despite long-term viral suppression.[13] The reason for the differences between the Asian studies and those conducted in Europe are currently unclear, but may be accounted for by differences in HBV genotype distribution, time since infection, and previous treatment exposure in the Western cohorts.

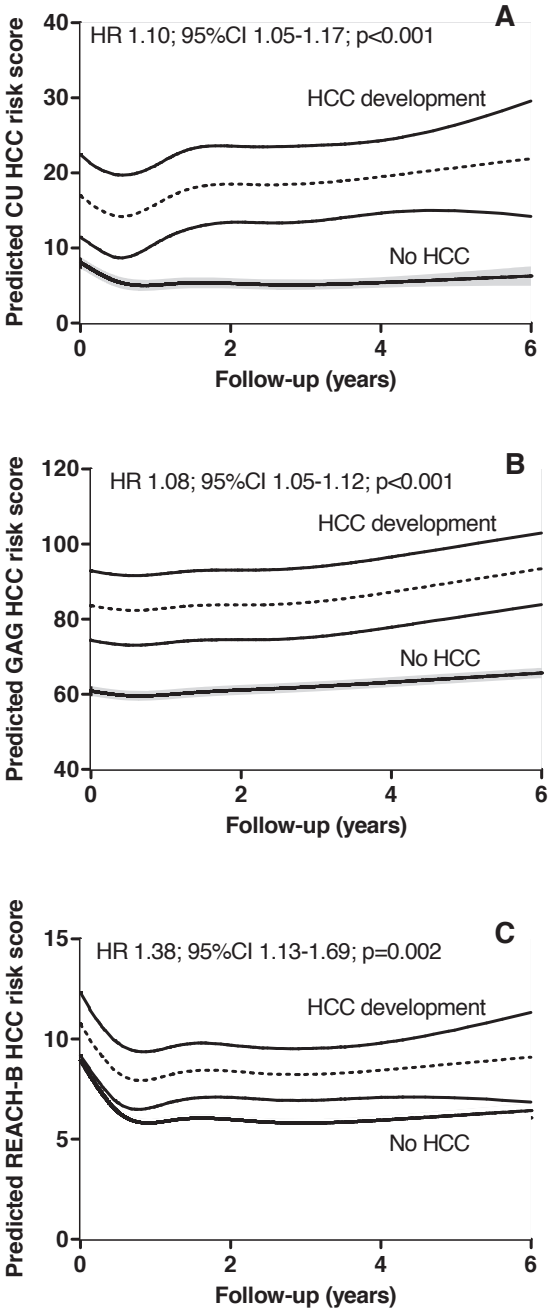

Figure 2. A) CU HCC B) GAG-HCC and C) REACH-B risk scores over time with 95% CI by development of HCC during ETV treatment. HR represents the dynamic risks for development of an HCC by an HCC risk score measurement at a random visit. HR was corrected for duration of therapy and multiple visits per patient.

Considering the residual risk of HCC even in patients with undetectable HBV DNA, careful monitoring remains of vital importance. A recent study from Hong Kong suggests that previously identified risk scores for HCC in untreated patients may also be applied effectively in ETV treated subjects.[9] We were unable to confirm these findings in our ethnically diverse cohort. While baseline GAG-HCC and CU-HCC risk scores were higher in patients that developed HCC, REACH-B scores offered little prognostic help. Furthermore, the discriminatory performance of the risk scores was limited by the low sensitivity observed in the overall population and mainly in the Caucasians. These findings are of major clinical importance because they show that a considerable proportion of patients that will develop HCC is not identified using previously defined risk-score cut-offs. Moreover, this implies that there is little to no additional value of those HCC risk scores to the pre-existing life-time risk of HCC in CHB patients and the clinical relevance for daily practice of these risk scores remains disputable, particularly in Caucasian patients.

Given the fact that ETV effectively suppresses HBV DNA in the majority of patients after a single year of therapy, we considered applying the risk scores at various on-treatment time-points. While we observed a decline in the predicted risk of HCC over time, the patterns were comparable for patients who developed HCC compared to those who did not and predictive performance after one year was therefore comparable to that at baseline. Furthermore, HR did not alter over time when looking at the dynamic risks for development of an HCC by an HCC risk score measurement at a random visit. These findings suggest that there is little reason to continue calculating the risk-scores during therapy. However, studies with longer follow-up may be required to estimate the risk of HCC during therapy beyond 5 years. Despite our large cohort of CHB patients, our study was limited by the fact that we observed a limited number of HCC's. Since the availability of ETV limits our duration of follow-up, future long-term follow-up may help us to understand the long-term effect of ETV on HCC risk. Furthermore, the risk of HCC in non-cirrhotic patients might be underestimated since screening may be less frequent or suboptimal when compared to the cirrhotic population. In conclusion, in this European multicenter real-life cohort study we showed that continuous ETV therapy effectively suppresses HBV DNA in the vast majority of patients. While the risk of HCC in ETV treated patients is low through up to five years of treatment, ETV therapy does not eliminate the risk of HCC. Previously described risk-scores for HCC have limited sensitivity for HCC in Caucasian patients and do not appear to be clinically useful either at baseline nor during therapy. Screening of risk groups therefore remains necessary despite successful ETV therapy, at least during the first years of treatment.

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CHAPTER 5

Early hepatic flares during ETV treatment are rare and do not require treatment adaptation in chronic hepatitis B without cirrhosis

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ABSTRACT

Background

Flares during NA therapy are usually associated with antiviral resistance or cessation of therapy. Since ETV resistance is rare and therapy is rarely stopped, we investigated the frequency and outcome of flares during ETV therapy in CHB.

Methods

All HBV monoinfected patients treated with ETV from 11 large European centers (VIRGIL Study Group) were studied. Flares were defined as an ALT level >3x compared to baseline with an absolute ALT level > 3xULN.

Results

A total of 733 patients were treated for a median of 168 (IQR 84-213) weeks with ETV monotherapy. Nineteen patients (3%) developed a flare after a median of 26 (10-83) weeks. None of the patients developed genotypic resistance and in only one case non-compliance was documented. Flares were relatively mild with a median ALT peak of 7.3xULN (IQR 4.5-10-1). Among patients with flares, one developed HBeAg seroconversion, and one lost HBeAg. Baseline HBeAg status (HR 2.91, 95%CI 1.17-7.23, p=0.02), HBV DNA (HR 1.31, 95%CI 1.06-1.63, p=0.01), platelet count (HR 0.99, 95%CI 0.98-1.00, p=0.04) and albumin (HR 0.91, 95%CI 0.84-0.99, p=0.03) were associated with development of a flare. Nine patients (47%) had a flare which was associated with a decline in HBV DNA, three patients (16%) with a stable HBV DNA and seven (37%) with an increase in HBV DNA. Flares associated with a decline in HBV DNA occurred after a median of 10 weeks (IQR 4-21), which was significantly earlier compared to flares associated with a stable or increase in HBV DNA (76 weeks, IQR 29-149) (p<0.001).

Conclusion

Flares during ETV are rare. Flares in patients without cirrhosis and flares occurring before week 26 of therapy were almost exclusively present during continued decline of HBV DNA. In these patients ETV can be continued under strict monitoring as the majority have a good biochemical- and virologic outcome.

INTRODUCTION

Approximately 400 million people worldwide are chronically infected with the hepatitis B virus (HBV), which can lead to progression of liver diseases with increased risk of cirrhosis, liver failure, and hepatocellular carcinoma.[1]

Currently, peg-interferon (PEG-IFN), tenofovir (TDF) and entecavir (ETV) are first line treatment options for chronic hepatitis B (CHB) infections.[2,3] During IFN therapy, flares of inflammatory activity are often observed. These flares are the result of an increase in particular cytotoxic T lymphocytes, which are important to control HBV but can also induce liver damage, depending on the environment and functional capability, resulting in intrahepatic necroinflammation.[4,5] Flares during IFN treatment can be severe, but have also been associated with virologic response.[6] Flares during nucleos(t)ide analogue therapy are rare and are mostly associated with antiviral resistance, cessation of therapy or non-compliance to therapy.[7,8]

ETV is a cyclopentyl guanosine analogue and showed superior biochemical, virological and histological efficacy and HCC-free survival compared to lamivudine (LAM).[9-13] Moreover, genotypic resistance to ETV is rare in NA-naïve patients through five years of continuous therapy.[14] Current guidelines recommend long-term continuation of ETV treatment, unless there is genotypic resistance or if HBsAg loss occurs. Therefore, therapy is rarely stopped. However, it is unclear whether treatment adaptation is necessary for patients who develop a flare during ETV treatment. The aims of this cohort study were to investigate the frequency and outcome of on-treatment flares during ETV therapy in CHB patients.

METHODS

Study population

Within this investigator-initiated cohort study within the European network of excellence for Vigilance against Viral Resistance (VIRGIL) we studied all consecutive CHB patients (HBsAg positive for at least 6 months) treated with ETV monotherapy for at least 3 months between 2005 and April 2013 from 11 large European referral centers. Patients were excluded if they were co-infected with HIV, HCV or HDV or if they had an HCC at baseline. A total of 733 patients were eligible for analysis. 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 two to four times a year at the discretion of the local treating physician. At every visit routine examination with biochemical (serum alanine aminotransferase (ALT), bilirubin and albumin, international ratio (INR) of prothrombin time) and virologic (Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody against HBeAg (anti-HBe), HBV DNA level) assessments took place. The diagnosis of cirrhosis at baseline was based on histology or ultrasound examinations with signs of cirrhosis (spleen size >12 cm, portal vein>16 mm, or nodules within the hepatic parenchyma).

Endpoints

The primary outcome was the occurrence of a flare. Transaminases were assessed locally and expressed as values representing a ratio to the local upper limit of normal (xULN). In accordance with Flink [6] a flare was defined as a threefold increase in serum ALT compared with baseline levels with an absolute ALT level > 3xULN. Secondary endpoints were virologic response, (HBV DNA levels < 80 IU/mL), HBeAg loss (in HBeAg-positive patients) and HBsAg loss during the on-treatment follow-up period.

Laboratory tests

Serum ALT, bilirubin, albumin levels and INR of prothrombin time were measured locally using standardized automated techniques. HBsAg, HBeAg and 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 ten of eleven 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

Data were systematically collected from the patients' charts through a standardized clinical research form by one investigator (PA). HBV DNA levels were logarithmically transformed. ALT levels are expressed as values representing a ratio to the local ULN. Continuous variables are expressed as means ± SD or median (IQR). Cox's regression analysis was used to study which factors were associated with primary or secondary endpoints. All statistical tests were two-sided, and a P value < 0.05 was considered to be statistically significant. SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used for all statistical analysis.

RESULTS

Baseline characteristics

A total of 733 CHB patients treated with ETV monotherapy were included. Baseline characteristics of the study population according to the occurrence of a flare are shown in Table 1. Overall, median follow-up was 168 (IQR 84-213) weeks (154 weeks (IQR 93-258) for patients who developed a flare and 168 (IQR 83-212) weeks for patients without a flare). At baseline 234 patients (32%) were HBeAg positive, median ALT was 1.4 x ULN (IQR 0.8 – 2.7) and mean HBV DNA 5.3 ± 2.2log IU/ml. Two flare patients had NASH at baseline and none of those had a history of excessive alcohol abuse. Six patients had a BMI > 25, three patients < 25 and BMI was unknown in ten cases.

Virologic and serologic response during treatment

Virologic response (HBV DNA < 80 IU/mL) was achieved in 642 patients (88%). The cumulative probability of virologic response was 52.5%, 75.4%, 89.6%, 94.4% and 98.8% at six months and years 1, 2, 3 and 5, respectively. Virologic response was influenced by baseline HBV DNA (HR 0.77; 95%CI 0.74-0.80; p<0.001) and HBeAg status (HR 0.39; 95% CI 0.33-0.47l p<0.001), but not by baseline ALT (p=0.62). HBeAg loss was achieved in 83 (36%) of 233 HBeAg-positive patients. The cumulative probability of HBeAg loss was 11.2%, 24.8% and 35% at years 1, 2 and 3 and was influenced by ALT at baseline (HR 1.05; 95% CI 1.03-1.08, p<0.001), but was not associated with baseline HBV DNA (p=0.57). Fifteen patients (2%) achieved HBsAg loss.

Biochemical response during treatment

At baseline, 451 patients had an ALT level above the upper limit of normal. During follow-up biochemical response (normalization of ALT) was achieved in 378 of those 451 patients (84%) after a median duration of 24 weeks (IQR 13-49). The cumulative probability of biochemical response was 47%, 66%, 80%, 88%, 90% and 96% at six months and years 1, 2, 3, 4 and 5, respectively. Biochemical response rates were influenced by baseline HBeAg status (HR 0.70, 95% CI 0.56-0.86, p=0.001), and baseline ALT (HR 1.03, 95% CI 1.01-1.04, p<0.001), but not by HBV DNA (HR 0.99, 95% CI 0.95-1.05, p=0.92).
Among the total population 659 patients (90%) achieved or maintained an normal ALT level after a median duration of 25 weeks (IQR 0-32). Two hundred and two HBeAg positive patients (87%) achieved biochemical response (median 26 weeks, IQR 4-53) and 457 HBeAg negative patients (91%) achieved biochemical response (median 11 weeks, IQR 0-24). Biochemical response rates in the total population were influenced by baseline HBeAg status (HR 0.67, 95% CI 0.57-0.79, p<0.001), and HBV DNA (HR 0.91, 95% CI 0.88-0.94, p<0.001), but not by baseline ALT (HR 0.99, 95% CI 0.98-1.01, p=0.47)

Table 1. Baseline characteristics

	All (n=733)	Flare (n=19)	No flare (n=714)
Male (%)	562 (77%)	16 (84%)	546 (77%)
Mean age	44 ± 14	41 ± 11	44 ± 14
Race			
Caucasian	313 (43%)	11 (58%)	302 (42%)
Asian	209 (29%)	5 (26%)	204 (29%)
African	137 (19%)	1 (5%)	136 (19%)
Missing	74 (10%)	2 (11%)	72 (10%)
Genotype			
A	99 (14%)	5 (26%)	94 (13%)
B	47 (6%)	1 (5%)	46 (6%)
C	77 (11%)	2 (11%)	75 (11%)
D	185 (25%)	7 (37%)	178 (25%)
E	50 (7%)	0 (0%)	50 (7%)
other	5 (7%)	1 (5%)	3 (1%)
unknown	270 (37%)	3 (16%)	268 (38%)
Cirrhosis	163 (22%)	6 (32%)	157 (22%)
HBeAg positive	233 (32%)	12 (63%)	222 (32%)
Mean HBV DNA (log IU/mL)	5.3 ± 2.2	6.6 ± 2.1	5.2 ± 2.2
Median ALT (ULN)	1.4 (0.8-2.7)	1.3 (1-2.1)	1.4 (0.8-2.8)
Platelet count (x10E9/L)	192 ± 72	153 ± 63	194 ± 72
Median bilirubin (umol/L)	11 (8-15)	9 (8-17)	11 (8-15)
Albumin (g/dL)	43 ± 5	41 ± 6	43 ± 5
PT INR	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
NA-naive	561 (77%)	12 (63%)	549 (77%)
LAM-naive	610 (83%)	15 (79%)	595 (83%)
IFN-naive	600 (82%)	18 (95%)	582 (82%)

Data are represented as mean (± standard deviation) or median (IQR).

ALT flares

Nineteen patients (3%) developed a flare (ALT > 3x ULN compared to baseline ALT, and an absolute ALT level >3xULN) after a median of 26 (10-83) weeks. The flares were relatively mild with a median ALT peak of 7.3 (IQR 4.5-10.1) xULN. Of the 19 flare patients, eight patients had a flare with an ALT level between 5-10x ULN and six patients with an ALT level > 10x ULN. In univariate cox regression analysis, baseline HBeAg status (HR 3.66, 95%CI 1.44-9.29, p=0.01), HBV DNA level (HR 1.31, 95%CI 1.06-1.63, p=0.01), platelet count (HR 1.0, 95%CI 0.98-1.00, p=0.04) and albumin level (HR 0.91, 95%CI 0.84-0.99, p=0.03) were associated with development of a flare. Other baseline characteristics (including ALT level, age and presence of cirrhosis) were not associated with the development of a flare

(Table 2). The cumulative probability of a flare was 3.5%, 4.5% and 8.3% at years 1, 3 and 5 for HBeAg positive versus 0.6%, 1.7% and 1.7% for HBeAg negative patients (p=0.003; Figure 1). Probability of a flare was not influenced by previous therapy. Poor adherence to ETV therapy was only documented in one flare patient.

Table 2. Baseline factors associated with a flare

		Flares (n=19)	
Risk factor	HR	95%CI	p
Age (per year)	0.99	0.95-1.02	0.47
Female	0.58	0.17-2.00	0.39
Caucasian	0.55	0.26-1.15	0.11
Genotype B	0.93	0.66-1.33	0.70
HBeAg pos	3.66	1.44-9.29	0.01
HBVDNA(log IU/ml)	1.31	1.06-1.63	0.01
ALT (xULN)	0.94	0.78-1.12	0.47
Bilirubin (umol/L)	1.00	0.99-1.01	0.38
Albumin (g/dL)	0.91	0.84-0.99	0.03
INR	2.07	0.20-22.04	0.55
Platelet count (x10E9/L)	1.0	0.98-1.00	0.04
Cirrhosis	1.54	0.58-4.06	0.38
Previous NA	0.54	0.21-1.36	0.19
Previous LAM	1.22	0.41-3.69	0.72
Previous IFN	0.23	0.03-1.73	0.15

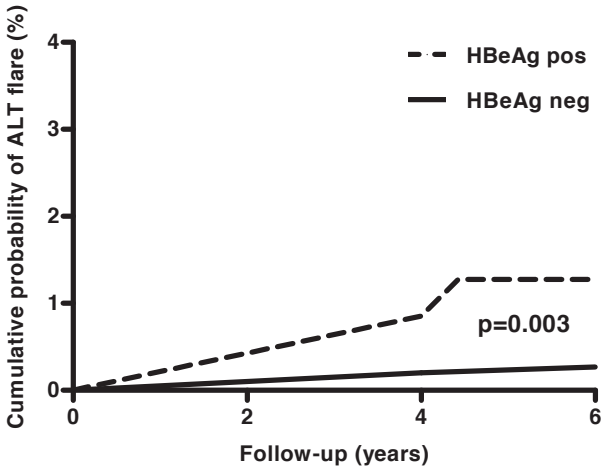

Figure 1. Kaplan-Meier curve for the probability of achieving an ALT flare according to HBeAg status at baseline.

ALT flares and outcome

Figure 2 shows individual HBV DNA and ALT levels during ETV therapy of six patients who developed a flare. Overall, nine patients (47%) had a flare associated with a decline in HBV DNA, three patients (16%) with a stable HBV DNA and seven (37%) with an increase in HBV DNA. One patient with HBV DNA decline also achieved HBeAg seroconversion and one patient with HBV DNA increase achieved HBeAg loss. Of the patients with a stable

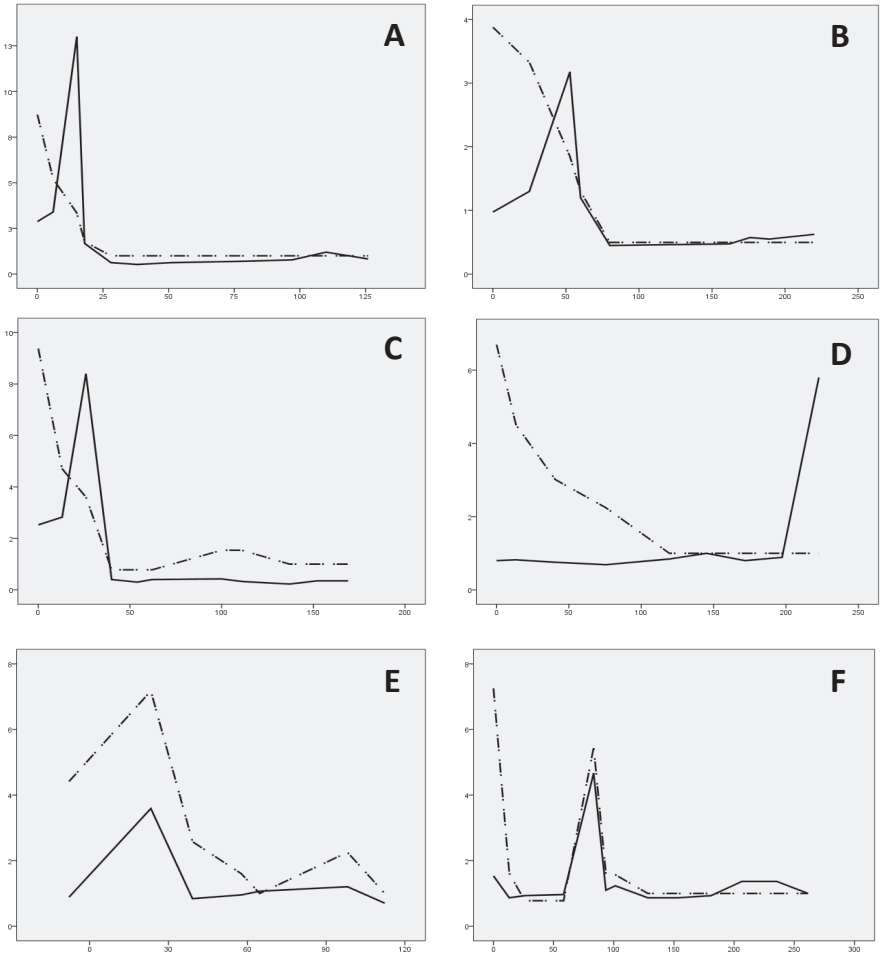

Figure 2 Kinetics of HBV DNA and ALT levels in six patients who developed a flare during ETV treatment.
--- = ALT levels (xULN); - - - = HBV DNA (log IU/mL);
A-C) HBV DNA decline; C) achieved HBeAg seroconversion; D) HBV DNA stable; developed hepatic decompensation and died consequently; E) HBV DNA increase, achieved HBeAg loss; F) HBV DNA increase, poor compliance

HBV DNA, one patient developed an HCC and two patients developed decompensated cirrhosis and died consequently. All three patients had cirrhosis at baseline. (Table 3) Flares associated with a decline in HBV DNA occurred after a median of 10 weeks (IQR 4-21), which was significantly earlier compared to flares associated with a stable or increase in HBV DNA (76 weeks, IQR 29-149) (p<0.001). Moreover, patients with a flare

Table 3. Characteristics of all 19 patients who developed a flare

Flare week	PEAK ALT (xULN)	Cirrhosis	HBeAg	Viral load	ALT normalization	Virologic response	Remarks
4	10.1	No	Pos	HBV DNA decline	Yes	No	
4	7.7	No	Pos	HBV DNA decline	Yes	Yes	
15	14.0	No	Pos	HBV DNA decline	Yes	No	
15	13.0	Yes	Pos	HBV DNA decline	Yes	Yes	
10	4.5	No	Neg	HBV DNA decline	Yes	Yes	
4	8.5	No	Neg	HBV DNA decline	Yes	Yes	
53	3.2	No	Pos	HBV DNA decline	Yes	Yes	
4	8.2	No	Pos	HBV DNA decline	Yes	Yes	
26	8.4	Yes	Pos	HBV DNA decline	Yes	Yes	HBeAg SC week 54
67	6.7	Yes	Pos	HBV DNA stable	No	No	HCC week 21
223	5.8	Yes	Pos	HBV DNA stable	No	No	Decomp + death week 222
124	44.3	Yes	Neg	HBV DNA stable	No	No	Decomp + death week 117
83	4.7	No	Neg	HBV DNA increase	Yes	Yes	Poor compliance
100	3.7	No	Neg	HBV DNA increase	Yes	Yes	
12	3.7	No	Neg	HBV DNA increase	Yes	Yes	
30	16.9	No	Pos	HBV DNA increase	Yes	Yes	
234	5.5	Yes	Pos	HBV DNA increase	Yes	No	
23	3.6	No	Pos	HBV DNA increase	Yes	Yes	HBeAg loss week 39
69	7.3	No	Neg	HBV DNA increase	Yes	Yes	

within 26 weeks of treatment were more likely to have a flare associated with a decline in HBV DNA, compared to patients with a flare after 26 weeks (78% vs 20%, p=0.02).
The cumulative probability of virologic response was comparable between patients with or without a flare (p=0.09). Also in a cox regressions analysis with virologic response as a time-dependent factor HBV DNA decline was not associated with the development of a flare (HR 0.43; 95%CI 0.13-1.43; p=0.17).

Sixteen patients (84%) achieved ALT normalization after the flare. All three patients who were not able to achieve ALT normalization had cirrhosis at baseline, compared to only three patients (19%) who achieved ALT normalization (p= 0.02). After the flare, 13 (68%) patients achieved HBV DNA undetectability without treatment adaptation; one of them achieved HBsAg loss. Three patients died, two because of decompensation and one because of gastric cancer. One patient was switched to TDF therapy with good response. None of the patients developed ETV resistance.

DISCUSSION

In our cohort of 733 CHB patients treated with ETV we showed that the risk of a flare is low, with an overall cumulative probability of 4% at year 5 of therapy. Patients who were HBeAg positive and patients with a higher viral load or lower albumin or platelet level at baseline were more susceptible to developing flares. Interestingly, flares in patients without cirrhosis and flares occurring before week 26 of ETV therapy were almost exclusively present during continued decline of HBV DNA.

Flares are the result of an increase in intrahepatic necroinflammation associated with expanded numbers of intrahepatic lymphocytes.[4,5] They may be observed both spontaneously, as well as during treatment. Previous studies have described flares during LAM therapy. Those flares were mostly caused by viral resistance in which case LAM had to be discontinued and patients had to be switched to other antiviral treatment regimens.[8,15] Nevertheless, flares do not occur more often in NA treated patients than in the natural course of CHB [8] or perhaps even less often.[15] In addition to Manns et al we found that only 3% of all ETV treated patients developed a flare.[16]

Flares during (PEG-)IFN treatment have been associated with virologic and serologic response, with a good clinical outcome.[6] In the study of Manss et al it appeared that most flares during ETV treatment were also associated with a reduction in HBV DNA.[16] Nevertheless, we showed a more distinct pattern of the flares which could be associated with either a decline or increase in HBV DNA levels. Flares occurring before week 26

of ETV therapy were almost exclusively present during continued decline of HBV DNA. However, late flares were more often associated with an increase in HBV DNA and consequent unfavorable treatment outcome, such as decompensation and death. Only two (11%) of the flare patients achieved either HBeAg loss or seroconversion.
Hence, it remains very questionable whether flares during NA therapy can be compared with flares during (PEG-)IFN treatment.

Development of resistance during treatment with NA - resulting in an increase in HBV DNA levels with or without an increase in ALT levels - has been associated with an adverse treatment outcome.[17-19] Therefore, EASL guidelines suggest treatment adaptation in these patients.[2] However, resistance to ETV is rare through five years of continuous therapy.[14] In our study none of the patients who developed a flare did develop genotypic resistance. However, an increase in HBV DNA was observed in some patients, possibly through unknown non-compliance. Nevertheless, in our study poor adherence to ETV therapy was only documented in one patient.

Increase in ALT levels can occur because of several other causes, such as hepatitis A, hepatitis E, autoimmune hepatitis and excessive alcohol consumption and non-alcoholic steatohepatitis (NASH). However, only few patients had a high BMI or NASH and none of the patients had a history of excessive alcohol abuse. Since the flares were rather mild, most patients were not tested for other (acute) liver diseases.

Interestingly, patients who were HBeAg positive and patients with a higher viral load or lower albumin or platelet level at baseline were more susceptible to developing flares. HBeAg positivity has been related to development of flares in the natural course of CHB.[8] However, it is not clear why low albumin and platelet levels are associated with an increased risk of a flare. Possibly, this can be explained by the fact that these patients were also more likely to have (beginning) cirrhosis and thus are more prone to develop decompensated liver disease.

In conclusion, our study showed that ALT flares during ETV are rare. Flares occur more frequently in patients who are HBeAg positive and patients with a higher viral load or lower albumin or platelet level at baseline. Flares in patients without cirrhosis and flares occurring before week 26 of ETV therapy were almost exclusively present during continued decline of HBV DNA. We therefore recommend to continue ETV therapy in these patients as the majority have a good biochemical- and virologic outcome. Furthermore, the importance of treatment compliance should be stressed to all HBV patients on oral anti-HBV agents.

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CHAPTER 6

Real life adherence of chronic hepatitis B patients to entecavir treatment

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Submitted

ABSTRACT

Background and aims

Real-life prospective data on adherence to nucleos(t)ide analogues (NA) in chronic hepatitis B patients (CHB) are scarce. We investigated adherence to entecavir (ETV) therapy in relation to viral response.

Methods

We provided 100 consecutive CHB patients with a medication dispenser that monitors ETV intake real time during 16 weeks therapy. HBV DNA was measured at baseline and at the end of the study. Quality of life (SF-36) and beliefs about medicines (BMQ) were evaluated using questionnaires.

Results

Adherence over 16 weeks averaged 85±17%, with 70% of patients exhibiting ≥80% (i.e. good) adherence. Maximum time between two consecutive doses was 3 days (median, range 1-53 days). Patients with <80% (i.e. poor) adherence were significantly younger (p=0.01). An accepting attitude towards ETV was associated with good adherence while an indifferent attitude was associated with poor adherence (p=0.03). Viral breakthrough did not occur during the study period. Mean adherence in patients with HBV DNA after 16 weeks >20 IU/mL and ≤20 IU/mL (n=18 and n=81 respectively) was 83% and 91% respectively (p=0.19). In multivariate analysis, adherence was not a significant predictor of HBV DNA negativity (adjusted OR 1.02 (95% CI 0.98-1.07), p=0.34), after adjustment for duration of ETV treatment (adjusted OR 18.8, p<0.001) and HBeAg status (adjusted OR 11.9, P=0.001).

Conclusions

Seventy percent of our CHB patients exhibited good adherence to ETV therapy, with younger patients and those with an indifferent attitude being more prone to poor adherence. Poor adherence was not an independent predictor of virologic response.

INTRODUCTION

Chronic hepatitis B (CHB) infection is a worldwide problem with approximately 350 million people being chronically infected.[1,2] Although most CHB patients remain asymptomatic, patients are at risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma.[3] Antiviral treatment with nuleos(t)ide analogues (NA) may enhance survival by preventing progressive disease.[4] NA are administered orally and have minimal side effects. Since they generally do not eradicate but only suppress hepatitis B virus (HBV), the large majority of patients requires long-term and possibly lifelong treatment.

Treatment failure in CHB patients on NA is uncommon. Virologic breakthrough, defined as re-appearance of HBV DNA at levels at least 10-fold higher than the lower limit of detection after achievement of undetectable HBV DNA or HBV DNA increase by >1 log from nadir, is generally the first clinical manifestation of treatment failure. During long-term follow-up of registration studies of currently available highly potent NA such as entecavir (ETV) or tenofovir (TDF), emerging resistance was low or absent.[5-7] It has recently been suggested that in addition to genotypic viral resistance, patient non-adherence is a major cause of treatment failure.[8-10] Poor adherence to long-term treatment for other chronic diseases such as hypertension or hypercholesterolemia is a frequent phenomenon, especially in asymptomatic patients.[11,12] It has also been shown that adherent patients on antihypertensive or anti-HIV medication are less likely to have suboptimal treatment responses.[13-16] Since CHB patients generally are asymptomatic, adherence to long-term antiviral treatment could also be suboptimal in these patients. Nevertheless, only few studies have investigated adherence to NA. Since no reliable assays are currently available to determine ETV plasma levels, one needs to rely here on alternative methods. Previous studies have either used pharmacy refill claims or patients self-report to measure adherence. Nevertheless, pharmacy claims provide only a gross estimation of patient adherence, whereas patient- and especially physician-reported adherence rates are well known to overestimate adherence considerably.[17] In contrast, real time medication intake monitoring is the most reliable new methodology currently available to assess patient adherence.

The aim of the current prospective, open-label study was therefore to evaluate adherence rates in 100 CHB patients on ETV treatment using real time medication monitoring. Furthermore, we aimed to relate adherence to HBV DNA levels and evaluate potential risk factors for suboptimal adherence.

METHODS

Study population

All consecutive adult CHB patients treated with ETV (indications according to EASL guidelines[18]) at two academic hospitals in the Netherlands were asked to participate in the study. Both naive and treatment-experienced patients were allowed to participate, as well as patients with impaired renal function requiring dose reduction of ETV and patients receiving ETV as prophylaxis during chemotherapy or immunosuppressant use. Patients co-infected with HIV, patients without understanding of Dutch or English language and patients unable to provide written informed consent were not eligible for the study. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written informed consent according to standards of the local ethics committees.

Study design

All consecutive CHB patients visiting the outpatient clinic of two academic hospitals in the Netherlands between December 2011 and August 2012 were asked to participate in this prospective, open-label study. After written informed consent, patients received a Sensemedic medication dispenser which monitored medication intake during at least 16 weeks. Patients were asked to fill out a questionnaire at baseline and at one follow-up visit (end of study). At both baseline and end of study visits, routine laboratory tests (including full blood count, ALT, albumin, creatinine) as well as quantitative serum HBV DNA testing (Cobas Taqman, Roche diagnostics, Almere, the Netherlands: real-time PCR, lower limit of detection 20 IU/mL) were performed. Also, charts of the patients were reviewed to extract data on HBV (treatment) history, duration of ETV therapy, (psychiatric) co-morbidities, number of concomitant drugs and (previous) substance abuse.

Sensemedic dispenser

The Sensemedic medication dispenser (Evalan, Amsterdam, the Netherlands, https://real.evalan.com/sensemedic_research), monitors medication intake real-time; when the patient opens the dispenser, data are directly transferred to a central server. Adherence data were available for the investigators through a secured internet account. However, there was no intervention by the study team when the patient did not open the dispenser. Patients used the Sensemedic medication dispenser until the next scheduled visit to the out-patient clinic department, with a minimum period of 16 weeks.

We assumed all patients used the Sensemedic medication dispenser during the total study period of 16 weeks. When no opening of the Sensemedic dispenser was registered on a study day, this was classified as non-adherence. When two openings of the Sensemedic dispenser were registered on the same day, this was classified as one intake

unless no intake was registered on the previous day and consecutive doses on the same day were ≥12 hours apart. We assumed in this situation, that the patient forgot the first ETV dose, but catched up the next day.

Questionnaires

Patients were asked to fill out a questionnaire at baseline and at the follow-up visit. The baseline questionnaire contained questions on demographics, education, side effects of ETV, and quality-of-life. The follow-up questionnaire also contained questions on patients' beliefs about medicine, self-reported adherence and patient experiences regarding the Sensemedic system.

Quality of life was assessed using the validated medical outcomes Study 36-item Short-Form General Health Survey (SF-36).[19] The SF-36 is composed of 36 questions, and contains four domains in the area of physical health (Physical Component Summary) and four domains in the area of mental health (Mental Health Summary). SF-36 scores range from 0 to 100, with higher scores indicating better health.

The Beliefs About Medicines Questionnaire (BMQ) consists of two sections: the BMQ-Specific assesses beliefs about the necessity of a specific medication and concerns about the potential adverse effects whereas the BMQ-General assesses beliefs about the harmfulness and overuse of medicine in general.[20] The BMQ uses a 5-point Likert scale ranging from strongly disagree (=1) to strongly agree (=5). The BMQ-Specific comprises two 5-item scores (Necessity and Concerns), the BMQ-General has two 4-item scores (Harm and Overuse). Mean scores for each subscale were calculated with scores ranging from 1 to 5. According to the balance between scores for Necessity and Concerns, patients can be subdivided into 4 attitudinal groups.[21] Self-reported adherence was measured using the Medication Adherence Report Scale (MARS)[20], which is a brief self-report instrument, assessing five separate non-adherent behaviors using a 5-point Likert scale. Scores of the separate items are summed, scores can range from 5 to 25 with higher scores representing higher levels of self-reported adherence. The BMQ and the MARS questionnaires have been used and validated in patients with various chronic diseases, including asthma, HIV, inflammatory bowel disease and psychiatric illnesses.[21-24]

Definitions and endpoints

The primary endpoint of the study was adherence to ETV during 16 weeks. Adherence was expressed as percentage and calculated using the formula: (no. of treatment days – no. of missed doses) / no. of treatment days. The total study period was divided in 4 periods of 4 weeks to investigate whether adherence changed over time. Patients were subdivided into two groups (good adherence vs. poor adherence) using a cut-off of 80% adherence.[25] Secondary endpoints of the study were the maximum number of days without any dose and the virologic response of the patients (HBV DNA levels).

Statistics

Baseline characteristics of adherent vs. non-adherent patients as well as patients with or without adequate virologic response were compared using the Student's t-test or Mann Whitney-U test for continuous variables and the chi-square test for dichotomous variables. Potential risk factors for inadequate virologic response were evaluated by multivariate logistic regression analysis. Factors with a p-value <0.2 in univariate analysis were entered in a subsequent multivariate analysis. IBM SPSS Statistics, version 20.0.0 (IBM, Armonk, New York, United States) was used for statistical analysis. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

One hundred thirty-six consecutive CHB patients on ETV treatment were asked to participate in this study. Of those, 100 patients provided written informed consent. Reasons for not participating in the study were unwillingness to participate in a research project (n=15), unwillingness to use the Sensemedic medication dispenser (n=13) and language discordance (n=8). Baseline and treatment history characteristics of the 100 included patients are given in Table 1, whereas treatment history characteristics are summarized in Table 2. Mean age of the patients was 45 years, with the vast majority of patients being male and often of Asian origin. Twenty-nine percent of patients exhibited cirrhosis, based on liver biopsy, transient elastography (Fibroscan®, Echosens, Paris, France) and/or abdominal ultrasound and 27% of patients were treated with any other NA before start of ETV. At inclusion in the study 64% of patients were HBeAg negative and 67% had been treated with ETV for at least one year.

Adherence

Adherence of the 100 included patients during the total study period of 16 weeks averaged 85 ± 17%, with a median of 91% (range 25-100%). The percentage of patients with ≥70%, ≥80%, ≥90%, ≥95% and ≥99% adherence was 81%, 70%, 52%, 43% and 25% respectively (Figure 1A). The maximum time between two consecutive doses was 3 days (median, range 1-53 days) (Figure 1B), which correlated with overall adherence (Spearman's ρ -0.87, p<0.001). As shown in Figure 2, adherence decreased over time.

Table 1. Characteristics of 100 CHB patients on entecavir treatment.

BASELINE CHARACTERISTICS	Chronic hepatitis B *(n=100)*
Male gender	76%
Mean age, years ±SD (range)	45 ± 14 (18-80)
Body weight, kg ±SD (range)	75 ± 15 (44-130)
Presence of cirrhosis	29%
Result most recent Fibroscan®#	
F0-F1, n	33 (48%)
F2, n	20 (29%)
F3, n	6 (9%)
F4, n	10 (14%)
Years since diagnosis HBV, median [IQR]	8 [3 – 13]
HBe negativity	
Before initiation of treatment	51%
At start of study	64%
Elevated AST and/or ALT	31%
HBV genotype	
A	18%
B	11%
C	14%
D	20%
Other / unknown	37%
Mode of transmission	
Vertical	29%
Sexual	13%
Other / unknown	58%
≥1 co-morbidity	54%
Gastro-intestinal / hepatobiliary disease	19%
Hypertension	15%
Cardiovascular disease	11%
Diabetes mellitus	10%
Hepatocellular carcinoma	6%
Other malignancy	8%
Renal insufficiency	5%
History of depression / psychiatric illness	17%
Number of concomitant oral medications	
≥1	47%
median [IQR]*	2 [1 – 6]
History of i.v. drug use	0%
Current alcohol use >5 units/week	11%

based on the 69 patients with Fibroscan® result

* based on the 47 patients with ≥1 oral medication

Table 2. Treatment characteristics of 100 patients with chronic HBV infection on entecavir therapy.

TREATMENT HISTORY	
Previous HBV treatment	
(Peg-)interferon	29%
Any NUC	27%
Lamivudine	19%
Adefovir	16%
Tenofovir	6%
Duration of entecavir treatment	
Months, median [IQR]	24 [5 – 48]
≥1 year	67%
Indication for entecavir treatment	
Therapeutic	96%
Prophylactic	4%
Entecavir dose	
0.5 mg/day	87%
1.0 mg/day	8%
Other[$]	5%

$ dose adjustment due to renal insufficiency

Questionnaires

Results of the questionnaires are given in Table 3. Patients reported a median adherence score of 24 on the MARS scale (range 15-25), which correlated with adherence as measured by the electronic medication dispenser (Spearman's ρ 0.37, p=0.003). However, self-reported adherence was higher than adherence as measured by the electronic dispenser. Sixty-seven percent of patients said that their adherence to ETV did not change since they started using the Sensemedic dispenser and 52% of patients believed that the Sensemedic medication dispenser could be useful for other patients.

According to the BMQ Specific questionnaire, virtually all patients (94%) had strong beliefs in the necessity of ETV treatment for maintaining their health. However, most patients (59%) had also strong concerns about the potential adverse effects of ETV. When patients were categorized into the 4 attitudinal groups, 59% of patients were "ambivalent" (high necessity, high concerns), 35% were "accepting" (high necessity, low concerns), 6% was "indifferent" (low necessity, low concerns) and none of the patients was "sceptical" (low necessity, high concerns). When asked about medication in general, 55% of patients had strong beliefs about the harmfulness of medicines and 79% of patients had strong beliefs about the overuse of medicines.

Predictors of poor adherence

In univariate analysis (Table 4) a significant association between age and percentage of patients with less than 80% adherence was found with younger age being associated with poor adherence (p=0.01). Furthermore, an "accepting" attitude towards ETV was

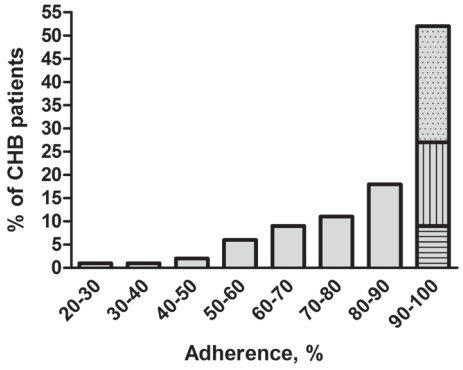

Figure 1A. Adherence in 100 CHB patients during entecavir therapy
The bar indicating 90-100% adherence is divided into a part with horizontal stripes (90-95% adherence (n=9)), a part with vertical stripes (95-99% adherence (n=18)) and a part with dots (99-100% adherence (n=25)).

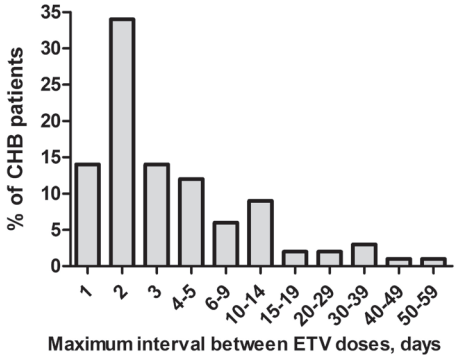

Figure 1B. Maximum interval between two consecutive entecavir doses

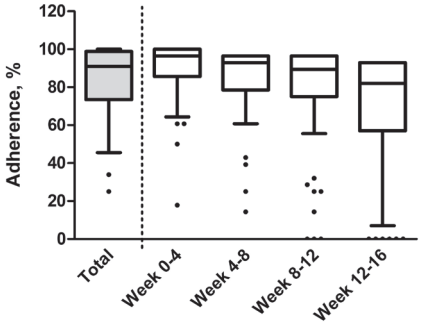

Figure 2. Trends in entecavir adherence during 16 weeks of follow-up
Boxes represent the inter-quartile range (IQR): the boundaries of the box indicate the 25th and the 75th percentile, the horizontal line in the middle of the box represents the median. The vertical lines from the ends of the box indicate the highest respectively lowest value observed within 1.5 IQR, circles represent outliers.

Table 3. Questionnaire results of 100 CHB patients on entecavir treatment

	Chronic hepatitis B *(n=100)*
Quality of life (SF-36)	*Median [IQR]*
Physical functioning	85 [50-95]
Social functioning	75 [50-88]
Role-physical	100 [50-100]
Role-emotional	100 [0-100]
Mental health	68 [52-80]
Vitality	50 [35-65]
Bodily pain	80 [47-100]
General health	50 [40-70]
Physical health summary	74 [52-88]
Mental health summary	69 [45-82]
Country of origin	
Asia	29%
Netherlands	24%
Turkey / Northern Africa	17%
Surinam	12%
Sub-Saharan Africa	6%
Other	12%
Marital status	
Married / in a relationship	54%
Single	29%
Divorced / widowed / unknown	17%
Employment status	
Paid employment	52%
Disabled	15%
Retired	12%
Other	21%

significantly associated with good adherence (≥80%) whereas patients with an "indifferent" attitude were more prone for poor adherence (<80%) (p=0.03). No significant differences in gender, comorbidities, previous HBV treatment history, duration of ETV treatment, quality of life and any other covariates were observed between patients with good and poor adherence. In multivariate analysis, both age (adjusted OR 1.03, 95% CI 0.99 – 1.07, p=0.18) and attitude towards ETV (p=0.12 for total subgroup) were no independent predictors of poor adherence.

Virologic response

One patient died after the follow-up period of 16 weeks but before HBV DNA measurement due to myocardial infarction following elective coronary artery stent placement and could therefore not be included in the analysis regarding virologic response. Of the 99 patients with HBV DNA results after 16 weeks, 66 patients (67%) had undetectable HBV

Table 4. Predictors of ≥80% (good) adherence in 100 CHB patients on entecavir treatment

	Adherence ≥80% (n=70)	**Adherence <80%** (n=30)	**P-value**
Male gender	73%	83%	0.26
Age, years	47±13	40±15	0.01
Marital status: married	56%	52%	0.72
Country of origin:	27%-26%-47%	33%-20%-47%	0.75
Asia vs. NL vs. other			
SF-36: physical health*	69 ± 24	68 ± 27	0.88
SF-36: mental health*	64 ± 23	62 ± 25	0.82
BMQ Specific: attitude			0.03
Accepting	42%	18%	
Ambivalent	56%	64%	
Indifferent	2%	18%	
BMQ General: Harm#	56%	53%	0.81
BMQ General: Overuse#	77%	82%	0.65
BMI, kg/m²	25.4±4.5	25.4±4.0	0.95
Presence of cirrhosis	29%	30%	0.76
HBe negativity	67%	60%	0.52
Psychiatric comorbidity	16%	20%	0.60
Any comorbidity	57%	47%	0.34
≥ 1 other medication	51%	37%	0.18
History of PEG-IFN	29%	30%	0.89
History of other NUC	29%	23%	0.58
Entecavir ≥ 1 year	70%	60%	0.33
HBV DNA ever undetectable	79%	78%	0.97
Alcohol >5units/wk, present	11%	10%	0.83
Alcohol >5units/wk, past	19%	23%	0.59

*also no significant differences in the separate SF-36 scales (data not shown)
percentage of patients with average score >2,5 on the subscale, also no significant differences in mean scores (data not shown)

DNA levels, 15 patients (15%) exhibited positive but unquantifiable HBV DNA (<20 IU/mL) and in 18 patients (18%) HBV DNA levels >20 IU/mL were measured. Of those, 11 patients had an HBV DNA level between 20 and 200 IU/mL, whereas in 7 patients HBV DNA levels above 200 IU/mL were observed. In none of the patients a virologic breakthrough was observed. When comparing the 81 patients with either undetectable or unquantifiable HBV DNA with the 18 patients with HBV loads >20 IU/mL, some differences in patient characteristics and adherence were observed. (Table 5) Patients with high viral loads were significantly younger, were less frequently treated with other NA before start of ETV and duration of ETV treatment was shorter compared to patients with HBV DNA loads <20 IU/

mL. Furthermore, the vast majority of patients with high viral loads was HBeAg positive and exhibited HBV DNA > 20 IU/mL at inclusion in the study. Adherence tended to be lower among patients with high viral loads: 83% vs. 91% (p=0.19). However, after adjustment in multivariate analysis for duration of ETV treatment (adjusted OR 18.8 (4.1-87.0, p<0.001) and HBeAg status (adjusted OR 11.9 (2.6-53.6), P=0.001), adherence was not a significant predictor of HBV treatment response (adjusted OR 1.02 (0.98-1.07), p=0.34).
We also compared adherence rates between patients with HBV loads 20-200 and >200 IU/mL to evaluate if more pronounced differences in adherence could be found between these subgroups. Duration of ETV treatment (3 [2-18] vs. 2 [0-7] months, p=0.10), age (38 vs. 30 years, p=0.13) and also adherence (95% vs. 71%, p=0.10) tended to be less in patients with HBV DNA >200 IU/mL, whereas no differences in HBeAg status and presence of cirrhosis were observed. Given the small number of patients in these subgroups multivariate analysis was not performed.

Table 5. Covariates associated with virological response (univariate analysis) in 100 CHB patients on entecavir treatment

	HBV DNA <20 IU/mL (n=81)	**HBV DNA >20 IU/mL (n=18)**	**OR (95% CI)**	**P-value**
Male gender	62 (77%)	13 (72%)	1.26 (0.40-3.97)	0.70
Age, years	47 [39-56]	32 [27-45]	1.06 (1.01-1.11)	0.001
Marital status: married	46 (58%)	8 (44%)	1.69 (0.60-4.74)	0.32
Presence of cirrhosis	22 (27%)	6 (33%)	0.63 (0.20-1.95)	0.42
HBe negativity	58 (72%)	5 (28%)	6.05 (0.92-19.12)	0.002
Any comorbidity	44 (54%)	9 (50%)	1.19 (0.43-3.31)	0.74
≥ 1 other medication	38 (47%)	8 (44%)	1.11 (0.40-3.09)	0.85
History of PEG-IFN	25 (31%)	4 (22%)	1.56 (0.47-5.22)	0.47
History of other NUC	25 (31%)	2 (11%)	3.57 (0.76-16.72)	0.11
Entecavir Tx, months	29 [13-54]	3 [2-13]	1.08 (1.03-1.14)	0.002
Entecavir ≥ 1 year	61 (75%)	5 (28%)	7.93 (2.52-25.01)	<0.001
Alcohol, past	10 (12%)	4 (22%)	0.86 (0.25-2.97)	0.81
HBV DNA at incl <20 IU	73 (91%)	2 (11%)	83,4 (15.8-439.7)	<0.001
Adherence, %	91 [75-99]	83 [60-97]	1.02 (0.99-1.05)	0.19
Adherence ≥80%	58 (72%)	11 (61%)	1.61 (0.55-4.65)	0.38
Adherence ≥90%	44 (54%)	7 (39%)	1.87 (0.66-5.31)	0.24
Max interval between doses	3 [2-4]	4 [2-11]	0.99 (0.94-1.04)	0.62

DISCUSSION

In this study we assessed adherence to ETV treatment in 100 consecutive CHB patients using real-time medication monitoring. Our main findings are that adherence averaged 85% during 16 weeks and that even in case of poor adherence, virologic response often appeared to be sufficient.

Our results on adherence are comparable with the few previous studies that assessed adherence in CHB patients. In a recent systematic review we concluded that mean adherence to NA therapy in CHB patients ranged from 81% to 99%, with 66% to 92% of patients being 100% adherent.[25] Adherence in those studies was measured by self-report, pharmacy refill data or pill count. Of special interest is the study by Chotiyaputta *et al.*[26], who - based on pharmacy claims of 11.100 patients - reported an adherence rate of 88% for various NA. Of the 2434 patients on ETV, 59% exhibited >90% adherence, compared to 52% in our study. Poor adherence seems to occur less frequently in CHB patients than in patients with other chronic (asymptomatic) diseases. For example, adherence rates after 6 months and 3 year of statins for secondary prevention of cardiovascular disease were 71% and 45%, respectively, while for primary prevention this was 65% and 35%, respectively.[12] Similar results were obtained for antihypertensive medication.[11]

In our study, poor adherence was associated with younger age, which is in line with previous research.[26-28] No other demographic or treatment-related predictors of non-adherence could be identified. However, patients' attitude towards medication appeared to be significantly associated with adherence, with patients with an "indifferent" attitude (low necessity as well as low concerns score) being more prone to poor adherence and patients with an "accepting" attitude (high necessity score, low concerns score) being more prone to good adherence. We are the first to assess CHB patients' beliefs about medicine using the BMQ-questionnaire. In other chronic diseases it has been shown that attitude towards medication is an important predictor of non-adherence.[21-24]

We also assessed potential consequences of poor adherence for virologic response. The frequency of virologic breakthrough during ETV therapy has previously been estimated at 1-3% during 3-5 years of follow-up.[7,8] However, virologic breakthrough did not occur in our study, which is possibly related to the relatively low number of included patients and the relatively short follow-up. Also, we did not measure HBV DNA levels at the end of the interruption periods (up to 53 days interruption). Adherence tended to be lower among the 18% of patients that exhibited HBV DNA >20 IU/mL after 16 weeks of treatment in our study, compared to patients with undetectable HBV DNA. However, after adjustment for duration of ETV treatment and HBeAg status, adherence was not found to be a significant predictor of HBV treatment response. This finding suggests that virologic response can often be maintained even in case of poor adherence. Previous studies have reported a significant association between adherence and virologic

response.[27-30] However, none of these previous studies focused exclusively on ETV which is a potent NA with a high barrier to resistance, at least in treatment-naive patients.

Our study has several strengths and some limitations. We are the first to provide prospectively collected adherence data in CHB patients using the Sensemedic medication dispenser, which is the most reliable method to measure adherence. Furthermore, our patient cohort is representative for the total cohort of CHB patients on NA treatment, since we did not exclude patients based on, for example, previous NA treatment or co-morbidities. A possible limitation of our study could be that adherence rates may have been influenced by participation in a study. In our opinion, it seems unlikely that participation in the study would have affected adherence results during the entire 16 weeks. It would be very interesting to correlate plasma levels with electronic adherence data. Unfortunately, measurement of ETV plasma levels is currently not possible. Last, one could hypothesize that opening of the medication dispenser is no guarantee for medication intake by the patient. Also, absence of a signal indicating opening of the dispenser does not have to indicate non-adherence. However, it has been shown that mismatches between electronic detection of opening of the medication dispenser and actual dosing are rare.[31,32]

In conclusion, 70% of our CHB patients exhibited good adherence to ETV therapy, with younger patients and those with an indifferent attitude being more prone to poor adherence. Adherence and patient attitude were not independent predictors of virologic response.

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SUMMARY

Chronic hepatitis B (CHB) remains one of the most prevalent infectious diseases.[1] Infection with the hepatitis B virus (HBV) can lead to acute hepatitis which can either resolve spontaneously, become chronic or result into a fulminant hepatitis with liver failure.[2,3] Patients with CHB may present in any of four phases of infection, depending on the presence of hepatitis B e antigen (HBeAg) with/without its antibody anti-HBe and serum levels of HBV DNA and alanine aminotransferase (ALT).[3]
To prevent progression to cirrhosis, liver failure, hepatocellular carcinoma and liver-related death many CHB patients require antiviral therapy. The potential of antiviral therapy for CHB increased enormously over the last years as a result of the introduction of better nucleos(t)ide analogues (NA) and of a pegylated form of interferon-α (PEG-IFN).

PREDICTING RESPONSE TO PEGINTERFERON THERAPY

Since an off-treatment sustained response can be achieved in a considerable number of patients after a finite treatment course, PEG-IFN remains an important first-line treatment option for CHB.[4-8] Response to (PEG-)IFN-based therapy is accompanied by increasing rates of hepatitis B surface antigen (HBsAg) seroconversion, a reduced incidence of hepatocellular carcinoma and prolonged survival.[7,9-11]
It has previously been shown that serum HBsAg levels at baseline and during PEG-IFN treatment can predict response.[12-14] However, there is a lack of knowledge regarding the effect of PEG-IFN on the expression of intrahepatic hepatitis B core antigen (HBcAg) and HBsAg in CHB. In **Chapter 1** we showed in a group of 119 CHB patients with paired biopsies taken at baseline and after one year of PEG-IFN therapy that PEG-IFN reduces the expression of intrahepatic HBsAg. More importantly, loss of HBsAg as assessed by immunohistochemistry from the liver predicted sustained response, and was reflected in a pronounced serum HBsAg decline. These results provide an important rationale for the use of HBsAg quantification as an easily obtainable estimate for prediction of response to PEG-IFN in both HBeAg positive and negative CHB.

Yet, since treatment with PEG-IFN is often accompanied by the occurrence of side-effects such as flu-like symptoms, headache, myalgia, fatigue and local reactions at the site of injection, the clinical use of PEG-IFN is compromised.[15] Selection of patients with the highest probability of achieving a response to PEG-IFN is therefore essential to successful application of this agent. Several factors have been related to a favorable response to PEG-IFN therapy in CHB, such as HBV genotype A and B, low baseline HBV DNA, high baseline ALT, older age, female sex, no previous IFN therapy and absence of precore (PC) and basal core promotor (BCP)mutants.[16-18] Nevertheless, discrimination remains

limited and identification of other baseline or on-treatment factors that influence the probability of response is required in order to develop a better prediction model.

In **Chapter 2**, we therefore investigated whether presence of anti-interferon antibodies affects the probability of response to PEG-IFN in CHB patients. The occurrence of anti-IFN antibodies has been associated with non-response to PEG-IFN in chronic hepatitis C.[19,20] We showed in this study of 323 CHB patients treated with PEG-IFN that presence of anti-IFN antibodies was associated with previous IFN therapy failure. However, presence or development of anti-IFN antibodies during or after PEG-IFN therapy was not associated with non-response to PEG-IFN treatment in CHB. Thus, there appears to be no role for measurement of anti-IFN antibodies in predicting response to PEG-IFN in CHB.

It has been shown that both viral as well as host factors influence response to PEG-IFN therapy.[16-18] We therefore hypothesized that the most optimal response to PEG-IFN therapy can be expected in the presence of both a susceptible host and a susceptible virus. In **Chapter 3** we showed that high levels of interferon gamma inducible protein (IP)-10 predict HBeAg loss and that a combination of high serum levels of ALT or IP-10 (both markers of an active immune response), together with absence of PC and BCP mutants identified patients with a very high likelihood of response to PEG-IFN therapy. Combined use of the described predictors of response to PEG-IFN may help select patients with the most favourable characteristics for PEG-IFN therapy. This will possibly result in a more attractive cost-benefit ratio for this treatment option in the future.

NUCLEOS(T)IDE ANALOGUE THERAPY AND CLINICAL OUTCOME

With the introduction of NA in the early nineties, the landscape of treatment for CHB has undergone great changes. Large cohort studies with untreated patients have shown that HBV DNA levels are associated with risk of liver disease progression and HCC development.[21,22] Therefore, antiviral treatment with NA aims at competitively inhibiting viral polymerase activity.[23] As the most recently approved NA Entecavir (ETV) and Tenofovir (TDF) can effectively maintain suppression of HBV DNA levels for prolonged periods of time in the vast majority of patients [24-28], these are recommended as first-line treatment in current guidelines. Recently, it has also been shown that ETV and TDF may improve fibrosis scores after continuous therapy and reduce the risk of HCC and liver related events, particularly in patients with cirrhosis.[27,29-32]

In order to predict the risk of HCC in treatment-naïve patients, HCC risk scores have been developed. Recently, these HCC risk scores were shown to predict HCC in Asian CHB

patients treated with ETV as well.[33] However, the performance of these risk-scores in non-Asian patients is unknown. In **Chapter 4** we studied in a large ethnically diverse European HBV infected population treated with ETV the incidence of, and risk factors for development of liver-related events including HCC, and the role of risk-scores for prediction of HCC. In our cohort of 744 CHB patients treated with ETV we showed that continuous ETV therapy effectively suppressed HBV DNA in the vast majority of patients. However, while the risk of HCC in ETV treated patients was low through up to five years of treatment, ETV therapy did not eliminate the risk of HCC. Furthermore, the previously described risk-scores for HCC appeared not to be clinically useful either at baseline nor during therapy, particularly in Caucasians. Despite successful ETV therapy, we therefore recommend to continue screening of risk groups.

ETV is a cyclopentyl guanosine analogue and it has shown superior biochemical, virological and histological efficacy and HCC-free survival compared to first generation NA, such as lamivudine (LAM).[34,35] Moreover, genotypic resistance to ETV is rare through five years of continuous therapy and ETV resistance in LAM-naïve patients has only been described in few reports.[36-38] Avoiding viral resistance is a cornerstone of CHB treatment as resistance is associated with a worsened outcome.[39] After achieving an undetectable HBV DNA the risk of developing resistance to potent ETV and TDF is thought to be minimal.

Current guidelines recommend long-term continuation of NA treatment, unless there is genotypic resistance or HBeAg or preferably HBsAg seroconversion.[40,41] Therefore, therapy is rarely stopped. However, it is unclear whether treatment adaptation is necessary for patients who develop a flare during NA treatment. Flares during IFN treatment can be severe, but have also been associated with virologic response.[42] Flares during NA therapy are rare and are mostly associated with antiviral resistance or cessation of therapy.[43,44] In **Chapter 5**, we showed that only 19 of 733 patients (3%) experienced a flare during ETV therapy. More interestingly, flares occurring before week 26 of therapy and in patients without cirrhosis were almost exclusively present during continued decline of HBV DNA. We therefore recommend to continue ETV therapy in these patients as the majority had a good biochemical and virologic outcome. Interestingly, none of the patients with a flare developed antiviral resistance.

Non-compliance has also been proposed as a possible factor of non-response and development of antiviral resistance or flares during ETV therapy.[45,46] Therefore, in **Chapter 6** we investigated data on adherence to NA in CHB patients using real-life prospective data including real-time medication monitoring (RTMM) during 16 weeks of follow-up. We showed in a group of 100 CHB patients treated with ETV that 70% of CHB patients

exhibited good adherence (>80%) to ETV therapy, with younger patients and those with an indifferent attitude (according to the "beliefs about medicines" questionnaire (BMQ)) being more prone to poor adherence (<80%). Interestingly, adherence and patient attitude were not independent predictors of virologic response. No viral break-through occurred during the study period.

It has previously been shown that stopping NA results in a relapse of HBV infection in the majority of patients.[47] It must therefore be outlined that NA should most likely be given indefinitely to the vast majority of patients. Thus, long-term side effects and costs of NA should also be taken into account when initiating such therapy.

CONCLUSIONS

For CHB patients who require treatment both PEG-IFN and NA are still considered first line treatment. PEG-IFN should only be given in patients with the highest probability of response. For prediction of response to PEG-IFN in CHB patients serum HBsAg levels can be used as an easily obtainable estimate. Furthermore, high ALT and high IP-10 - indicators of a susceptible host - are associated with a higher probability of response to PEG-IFN. However, measurement of anti-IFN antibodies before or during PEG-IFN therapy does not predict response and is therefore not recommended. Treatment with ETV results in undetectable HBV DNA in most patients and is associated with a low risk of development of HCC or a flare. However, known risk-scores for HCC are not clinically useful either at baseline nor during therapy, particularly in Caucasians. Moreover, as patients remain at risk to develop HCC or ALT flares, we recommend to continue intensive follow-up. Finally, it must be anticipated that NA should be given indefinitely in the great majority of patients. Thus, long-term side effects and costs of NA should also be taken into account when initiating such therapy.

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SAMENVATTING

Chronische infectie met het Hepatitis-B-virus (HBV) blijft, ondanks de introductie van veilige en effectieve vaccins ongeveer twee decennia geleden, een veelvoorkomend probleem. De wereldwijde incidentie wordt geschat op zo'n 350 miljoen patiënten en zelfs een derde van de wereldbevolking heeft ooit een infectie met HBV doorgemaakt.[1] Infectie met het HBV kan leiden tot een acute hepatitis. Deze kan vervolgens spontaan genezen, of kan resulteren in een chronische vorm of een ernstige fulminante hepatitis met leverfalen als gevolg.[2,3]

Om progressie naar cirrose, leverfalen, hepatocellulair carcinoom (HCC) en lever-gerelateerde sterfte te voorkómen, heeft een groot deel van de chronische hepatitis B (CHB) patiënten antivirale behandeling nodig. De laatste jaren is er veel bekend geworden over het natuurlijk beloop van de ziekte, alsook de nieuwe therapeutische mogelijkheden.

Behandeling van CHB is geïndiceerd wanneer er sprake is van actieve replicatie van het HBV (serum HBV-DNA>2,0 X 10^E4 IU/ml) met daarbij activiteit van leverziekte, weergegeven door de hoogte van het serum ALAT en/of de ernst van necro-inflammatie in het leverbiopt.[4-6]

Doel van behandeling is de kwaliteit van leven te verbeteren en de kans op overleven van patiënten met CHB te vergroten door het voorkómen van progressie van ziekte. Daarbij is het verlies van hepatitis B surface antigen (HBsAg) en de vorming van de betrokken antistof anti-HBs het ultieme doel. Aangezien dit echter slechts in enkele gevallen wordt bereikt, zijn surrogaat eindpunten gedefinieerd, zoals virologische respons (HBV DNA onderdrukking), biochemische respons (ALAT normalisatie), serologische respons (HBeAg-verlies en –seroconversie) en histologische respons (histologische verbetering).[4]

HUIDIGE THERAPIEËN

Momenteel zijn er zeven middelen geregistreerd voor de behandeling van CHB. Enerzijds zijn er de immuun-modulerende interferonen en anderzijds de direct anti-virale nucleoside analogen (NA's), die weer in drie klasse onderverdeeld kunnen worden, te weten L-nucleosides (Lamivudine, Telbivudine), deoxyguanosine analogen (Entecavir) en acyclische nucleoside fosfonaten (Adefovir en Tenofovir).

Voorspellen van respons op Peginterferon therapie

Het (secundaire) antivirale effect van de immuunmodulerende interferonen werd voor het eerst beschreven in 1957 en sinds de jaren '80 wordt Interferon-alfa toegepast voor de behandeling van CHB. Sinds een aantal jaren is door het binden van een

polyethyleenglycol(PEG)-molecuul aan interferon (PEG-IFN) de effectiviteit van behandeling toegenomen. PEG-IFN wordt wekelijks toegediend door middel van een subcutane injectie, wat resulteert in een redelijk gelijkmatige IFN spiegel gedurende dit interval.
Zowel voor HBeAg-positieve als HBeAg-negatieve patiënten blijft PEG-IFN een eerstelijns behandeloptie aangezien het bij ongeveer 30% van de patiënten resulteert in blijvende respons na één jaar behandeling.[7-11] Daarnaast is respons op PEG-IFN therapie geassocieerd met toenemende kansen op HBsAg seroconversie, een lager risico op HCC en langere overleving.[10,12-14]

Het is recent aangetoond dat de hoeveelheid serum HBsAg voor het starten van en tijdens PEG-IFN behandeling voorspellers zijn van respons.[15-17]

Het effect van PEG-IFN op de expressie van intrahepatisch hepatitis B core antigen (HBcAg) en HBsAg is echter niet bekend. In een groep van 119 CHB patiënten met gepaarde biopten toonden wij in **Hoofdstuk 1** aan dat na een jaar behandeling met PEG-IFN de expressie van intrahepatisch HBsAg verlaagt. Daarnaast bleek het dat verlies van intrahepatisch HBsAg, gemeten met behulp van immunohistochemie, een voorspeller was van blijvende respons, en werd gereflecteerd in een daling van het HBsAg in het serum. Een eenvoudig uit te voeren serum HBsAg bepaling lijkt dan ook correct gebruikt te kunnen worden als voorspeller van respons op PEG-IFN in zowel HBeAg positieve als negatieve CHB patiënten.

Het klinisch gebruik van PEG-IFN wordt echter bemoeilijkt door de vaak optredende bijwerkingen, zoals griepachtige verschijnselen, lokale reacties op de injectieplaats, beenmergsuppressie en psychische problematiek.[18] Selectie van patiënten met de hoogste kans op respons is daarom essentieel voor succesvol gebruik van PEG-IFN.
Verschillende factoren zijn de afgelopen jaren naar voren gekomen als mogelijke voorspellers van respons. Het is gebleken dat een lage virale load, een hoog serum ALAT, infectie met HBV genotype A of B, een hogere leeftijd, vrouwelijk geslacht, niet eerder behandeld zijn met IFN en afwezigheid van *precore* (PC) en *core promotor* (BCP) mutanten de kans op succes van behandeling vergroten.[19,20] Helaas is echter de betrouwbaarheid van voorspelling op individueel niveau beperkt en rest er een grote onzekerheid welke patiënt succesvol met PEG-IFN behandeld kan worden. Identificatie van extra factoren die respons kunnen voorspellen is daarom van essentieel belang.
In **Hoofdstuk 2** onderzochten wij of de aanwezigheid van anti-interferon (anti-IFN) antilichamen invloed had op de responskansen op PEG-IFN in CHB patiënten. De aanwezigheid van anti-IFN antilichamen is in de behandeling van chronische hepatitis C met PEG-IFN geassocieerd met non-response.[21,22] In een groep van 323 CHB patiënten, die behandeld werden met PEG-IFN, hebben we aangetoond dat de aanwezigheid van

anti-IFN antilichamen geassocieerd was met eerder falen van (PEG-)IFN behandeling. De aanwezigheid of ontwikkeling van anti-IFN antilichamen tijdens of na PEG-IFN behandeling was echter niet geassocieerd met non-response op PEG-IFN behandeling in CHB patiënten. Het bepalen van anti-IFN antilichamen om respons kans op PEG-IFN in CHB patiënten te voorspellen lijkt dan ook niet zinvol.

Eerder onderzoek van onze groep toonde aan dat de aanwezigheid van PC en BCP mutanten invloed heeft op een blijvend hoog serum HBV DNA na HBeAg verlies. Het lijkt er dan ook op dat succesvolle behandeling met PEG-IFN afhangt van zowel de gevoeligheid van de drager alsook de gevoeligheid van het virus.[19,20,23] In **Hoofdstuk 3** toonden wij aan dat hoge waarden van *interferon gamma inducible protein-10* (IP-10) HBeAg verlies voorspelden en dat een combinatie van een hoog ALAT of IP-10 (beide markers van een actieve immuun response), samen met de afwezigheid van PC- en BCP-mutanten kan helpen in het identificeren van patiënten met een grote kans op respons op PEG-IFN behandeling. Het combineren van de beschreven voorspellers van respons op PEG-IFN kan derhalve helpen alleen die patiënten te selecteren met de meest gunstige karakteristieken voor PEG-IFN therapie en dus een hoge kans op succes van therapie. Dit zal mogelijk ook resulteren in een betere kosten-baten ratio voor PEG-IFN behandeling in de toekomst.

Nucleoside analogen en lange termijn respons

De laatste decennia is met de introductie van NA's grote vooruitgang geboekt in de behandeling van CHB. Door de directe onderdrukking van de virale replicatie - door remming van het virale polymerase - leidt behandeling met NA's bij vrijwel alle patiënten na één jaar tot een daling van HBV DNA en ALAT en in stijgende mate daarnaast tot verbetering van de leverhistologie. NA's moeten dagelijks oraal worden ingenomen en hebben weinig bijwerkingen.

Grote cohort studies met onbehandelde Aziatische patiënten hebben laten zien dat HBV DNA levels geassocieerd zijn met het risico op progressie van leverziekte en de ontwikkeling van HCC.[24,25] Complete onderdrukking van virale replicatie naar ondetecteerbare HBV DNA waarden wordt momenteel dan ook beschouwd als één van de belangrijkste surrogaat eindpunten van NA therapie.[26] De meest recent goedgekeurde NA's Entecavir (ETV) en Tenofovir (TDF) worden in de huidige richtlijnen geadviseerd als eerstelijns monotherapie vanwege hun potentie (snelheid van onderdrukking van virale replicatie), de hoge genetische barrière tegen resistentie en de beperkte bijwerkingen.[27,28] Zowel ETV als TDF therapie kunnen langdurige onderdrukking van HBV DNA levels bewerkstelligen in vrijwel alle CHB patiënten.[28-33] Het is daarnaast recent aangetoond dat continue ETV en TDF therapie leverfibrose kunnen verminderen alsook het risico op HCC en levergerelateerde sterfte kunnen reduceren, met name in patiënten met cirrose.[31,34-37]

Om het risico op HCC te voorspellen in onbehandelde patiënten zijn er HCC risico scores ontwikkeld, welke recent hebben laten zien ook het risico op HCC in Aziatische CHB patiënten die behandeld worden met ETV te kunnen voorspellen. [38] Het is echter niet bekend of deze HCC risico scores ook geschikt zijn in niet-Aziatische patiënten.

In **Hoofdstuk 4** onderzochten wij de incidentie van, en de risicofactoren voor het ontwikkelen van lever-gerelateerde events inclusief HCC en de rol van HCC risico-scores in het voorspellen van HCC in een groot Europees cohort van CHB patiënten. In ons cohort van 744 CHB patiënten, die behandeld werden met ETV toonden we aan dat continue ETV therapie HBV DNA onderdrukt in vrijwel alle patiënten. Daarnaast bleek dat het risico op HCC in ETV behandelde patiënten laag was (1.9%). Behandeling met ETV kon echter niet het risico op HCC volledig doen verdwijnen. Daarnaast bleek dat de eerder beschreven risico-scores voor HCC niet klinisch bruikbaar waren aan het begin of gedurende de behandeling met ETV, met name niet in de Kaukasische populatie. Het wordt dan ook aangeraden om ook patiënten met succesvolle ETV behandeling frequent te blijven vervolgen en HCC screening te continueren, met name in risicogroepen.

ETV is een cyclopentyl guanosine analoog welke superieure biochemische, virologische en histologische effectiviteit en HCC-vrije overleving heeft laten zien in vergelijking met eerste generatie NA's, zoals lamivudine (LAM).[39,40] Daarnaast is gebleken dat antivirale resistentie gedurende ETV zeer zeldzaam is, tot vijf jaar na het starten van de behandeling.[41-43] Het voorkómen van antivirale resistentie is een belangrijk onderdeel van de behandeling van CHB patiënten, aangezien antivirale resistentie geassocieerd is met een slechtere uitkomst.[44]
Huidige richtlijnen adviseren NA's langdurig en misschien wel levenslang te continueren, en alleen te wijzigen als er sprake is van antivirale resistentie of te staken in geval van HBsAg seroconversie.[4,5] Aangezien dit zeer sporadisch voorkomt, wordt behandeling met NA's vrijwel nooit gestaakt. Het is echter onbekend of verandering van behandeling ook nodig is bij patiënten die een flare (stijging van het serum ALAT tot boven drie keer de normaal waarde) ontwikkelen tijdens behandeling met NA's. Het is bekend dat tijdens behandeling met IFN ernstige flares kunnen ontstaan. Deze kunnen echter ook geassocieerd zijn met virologische en/of serologische respons.[45] Flares tijdens NA behandeling zijn zeldzaam en meestal geassocieerd met antivirale resistentie of staken van therapie.[46,47]

In **Hoofdstuk 5**, lieten we zien dat slechts 19 van de 733 patiënten (3%) een flare ontwikkelden tijdens behandeling met ETV. Daarnaast bleek dat flares die ontstonden in het eerste half jaar van therapie en in patiënten zonder cirrose vrijwel altijd geassocieerd waren met een persisterende daling van het serum HBV DNA. Het valt daarom aan te

bevelen om ETV behandeling te continueren in deze groep patiënten, omdat de meerderheid een goede biochemische en virologische uitkomst had. Daarnaast ontwikkelde geen van deze patiënten antivirale resistentie.

Therapieontrouw wordt als een mogelijke oorzaak van non-response en het ontwikkelen van antivirale resistentie of flares tijdens ETV therapie beschouwd. In **Hoofdstuk 6** beschreven wij het effect van ETV therapietrouw in een groep van 100 CHB patiënten die behandeld worden met ETV. Hiervoor maakten wij gebruik van een speciaal medicijndoosje dat medicijngebruik real-time registreert. Wij toonden daarbij aan dat tijdens de studieperiode van 16 weken 70% van de CHB patiënten therapietrouw was (gedefinieerd als ≥80% therapietrouw). Daarnaast bleek dat CHB patiënten met slechtere therapietrouw (<80%) jongere patiënten waren en vaker een negatieve attitude ten opzichte van ETV hadden (gebaseerd op de BMQ-vragenlijst, een gevalideerde vragenlijst op het gebied van medicatiegebruik). Geen enkele patiënt kreeg een virologische doorbraak tijdens de studieperiode. Therapietrouw en patiëntenattitude waren echter geen onafhankelijke voorspellers voor virologische respons. Het wordt wel aanbevolen om tijdens behandeling met NA's patiënten initieel frequent te vervolgen (eenmaal per drie maanden) om de therapietrouw te monitoren en eventuele antivirale resistentie tijdig te detecteren om zodoende een biochemische doorbraak te voorkómen.

Conclusies

Met de beschikbaarheid van nieuwe antivirale middelen is het momenteel voor vrijwel alle CHB patiënten haalbaar HBV medicamenteus onder controle te houden door het induceren van een blijvende respons met PEG-IFN, of het onderhouden van een respons door middel van langdurige NA therapie.

Behandeling met PEG-IFN moet alleen gegeven worden aan die patiënten met een hoge kans op respons op PEG-IFN. Om respons op PEG-IFN te voorspellen kunnen serum HBsAg levels gebruikt worden. Daarnaast is gebleken dat een hoge IP-10 waarde – als marker voor gevoegheid van de drager - geassocieerd is met een grotere kans op respons.

De aanwezigheid of het ontwikkelen van anti-interferon antilichamen heeft echter geen invloed op respons op PEG-IFN en het wordt dan ook niet aangeraden deze te bepalen.

Ten aanzien van NA behandeling is gebleken dat ETV therapie in vrijwel alle patienten resulteert in een ondetecteerbaar HBV-DNA en daarnaast geassocieerd is met een laag risico op het ontwikkelen van een HCC of een flare. Bekende HCC risicoscores bleken echter klinisch niet bruikbaar voor het voorspellen van HCC voor of tijdens ETV behandeling, met name niet in Kaukasische patiënten. Langdurige follow-up blijft dan ook van belang, ondanks goede respons op ETV.

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2. **Arends P**, Janssen HLA. *Behandelmogelijkheden van Hepatitis B*. Nederlands Tijdschrift voor Medische Microbiologie; 2011 March (1): 16-20.

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4. Sonneveld MJ, **Arends P**, Boonstra PA, Hansen BH, Janssen HLA. *Serum levels of interferon gamma inducible protein-10 and response to peginterferon therapy*. Journal of Hepatology. 2013 May; 58 (5): 898-903.

5. **Arends P,** Sonneveld MJ, Janssen HLA. *HBV treatment: which patient should be treated with interferon?* Clinical Liver Disease. 2013 Jan.

6. **Arends P**, Janssen HLA. *Response guided treatment for peginterferon in chronic hepatitis B*. Current Hepatitis Report. 2013 June.

7. **Arends P**, Sonneveld MJ, van der Eijk A, Hansen BE, Janssen HLA, Haagmans B. *Presence of anti-interferon antibodies is not associated with non-response to peginterferon in chronic hepatitis B*. Antiviral therapy 2013; Dec 3, Epub ahead of print.

8. **Arends P.** Rijckborst V, Zondervan PE, Buster E, Cakaloglu Y, Ferenci P, Tabak F, Akarca US, Simon K, Sonneveld MJ, Hansen BE, Janssen HL. *Loss of intrahepatic HBsAg expression predicts sustained response to peginterferon and is reflected by pronounced serum HBsAg decline*. Journal of Viral Hepatitis. 2014 Jan 20, Epub ahead of print.

9. **Arends P,** Sonneveld MJ, Zoutendijk R, Carey I, Brown A, Fasano M, Mutimer D, Deterding K, Reijnders JGP, Oo Y, Petersen J, van Bömmel F, de Knegt RJ, Santantonio T, Berg T, Wezel TM, Wedemeyer H, Buti M, Pradat P, Zoulim F, Hansen B, Janssen HLA for the VIRGIL Surveillance Study Group. *Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians.* GUT. 2014 July 10, Epub ahead of print.

CURRICULUM VITAE

Pauline Arends werd geboren op 18 december 1984 te Rotterdam. Na het behalen van haar gymnasiumdiploma aan de Christelijke Scholengemeenschap Johannes Calvijn te Rotterdam, startte zij in 2002 met de studie geneeskunde aan de Erasmus Universiteit. In juli 2006 behaalde zij na haar onderzoek op de afdeling Chirurgie het doctoraalexamen. Alvorens zij in april 2007 aan haar co-assistentschappen begon, deed zij een periode vrijwilligerswerk in India en Australië. Tijdens haar co-assistentschappen liep zij onder andere stage in het Tygerberg Ziekenhuis te Kaapstad, Zuid-Afrika.
In juli 2009 behaalde zij haar artsexamen, waarna zij tot en met september 2010 als arts niet in opleiding tot specialist (ANIOS) werkte op de afdeling Interne Geneeskunde van het Reinier de Graaff Gasthuis te Delft (opleider: dr. E. F. M. Posthuma).
In oktober 2010 startte zij met haar 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 en dr. B. E. Hansen.
Sinds oktober 2013 is zij in opleiding tot Maag-, Darm en Leverarts in het VU Medisch Centrum (opleider: dr. R. A. de Vries), thans in vooropleiding in het Sint Lucas Andreas ziekenhuis (opleider: dr. C. E. M. Siegert).

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2013 Diagnosis and treatment of chronic hepatitis B. Third year Erasmus MC medical students participating in a 4-week Gastroenterology and Hepatology training program. Rotterdam, The Netherlands.

ABBREVIATIONS

ALT	Alanine aminotransferase
Anti-HBe	Antibody against HBeAg
Anti-HBs	Antibody against HBsAg
Anti-IFN	Anti-interferon
BCP	Basal core promotor
BMQ	Beliefs about medicines questionnaire
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
CI	Confidence interval
ETV	Entecavir
FU	Follow-up
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus DNA
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D (delta) virus
HIV	Human immunodeficiency virus
HR	Hazard ratio
IFN	Interferon
*IL*28-B	*Interleukin* 28-B
IP-10	interferon-gamma inducible protein 10
LAM	Lamivudine
LTFU	Long-term follow-up
MARS	Medication Adherence Report Scale
MELD	Model for end-stage liver disease
NA	Nucleos(t)ide analogues
OR	Odds ratio
ORF	Open reading frame
PC	Precore
PCR	Polymerase chain reaction
PEG-IFN	Pegylated interferon
PT	Prothrombin time
qHBsAg	Quantitative hepatitis B surface antigen
RTMM	Real-time medication monitoring

SF-36	Study 36-item Short-Form General Health Survey
TDF	Tenofovir
ULN	Upper limit of normal
VR	Virologic response
WT	Wildtype

“Lernen Sie ernstnehmen, was des Ernstnehmens wert ist,
und lachen über das ander“

Hermann Hesse