A long-term study of liver-related events in Caucasian hepatitis B patients with normal ALT values and high viremia

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Abstract

Background and study aims: **There is ongoing debate whether antiviral therapy should be initiated in hepatitis B e antigen (HBeAg)-negative patients with normal alanine aminotransferase (ALT) levels but high HBV DNA levels >2,000 IU/mL. Since the need for antiviral therapy might be different between Asian and Caucasian patients, we studied the long-term disease outcome in Caucasian patients living in Western Europe.**

Patients and methods: **One hundred sixteen patients with high HBV DNA levels (>2,000 IU/mL) at diagnosis were included in the high viremia group, while those with HBV DNA ≤2,000 IU/mL were used as controls (n = 327). All patients were Caucasian, HBeAg negative, had normal ALT levels and had no significant liver disease at diagnosis.**

Results: **Median follow-up was 7 ± 9.8 years in the high viremia group and this was 10 ± 12.5 years in controls. The cumulative probability of a liver-related event over 10 years was 4.8% vs 0.0% in the control group (p=.008). In multivariable analysis, high viremia group was associated with the occurrence of a liver-related event (hazards ratio (HR) 95% confidence interval (CI): 1.20-11.98, p=.023). In this subgroup, older age at diagnosis (HR 95% CI: 1.01-1.16, p=.023) predicted a higher risk of liver-related event. In the high viremia group, liver-related mortality was 0.9% and none of the patients developed hepatocellular carcinoma.**

Conclusions: **HBV DNA >2,000 IU/mL influences the long-term disease outcome in Caucasian HBeAg-negative patients living in Western Europe. Nevertheless, the risk of liver-related events is low.** (Acta gastroenterol. belg., **2022,** 85, **56-61**).

Keywords: hepatitis B, Caucasian, inactive carrier, ALT, HBV DNA.

Introduction

Chronic hepatitis B virus (HBV) infection represents a serious health problem with approximately 257 million people chronically infected worldwide (1). The natural history of chronic HBV infection can be divided in four phases with a different disease outcome: HBeAg-positive chronic HBV infection (previously termed "immune tolerant" phase), HBeAg-positive chronic hepatitis B ("immune reactive HBeAg positive"), HBeAg-negative chronic infection ("inactive carrier") and HBeAg-negative chronic hepatitis (2-4). Patients with chronic hepatitis suffer from moderate or severe liver necroinflammation with progression of fibrosis and consequently the risk of cirrhosis and hepatocellular carcinoma (HCC) development (2-4). Antiviral therapy in these patients prevents disease progression and improves survival (2-4).

Besides liver necroinflammation, several studies indicate that HBV DNA level is also a predictor for the development of cirrhosis and/or HCC (5,6). HBeAg-negative patients with persistently normal alanine aminotransferase (ALT) levels but with high HBV DNA levels > 2,000 IU/mL do not fit neatly into the criteria for HBeAg-negative chronic infection or HBeAg-negative chronic hepatitis. It is unclear whether patients in this subgroup are at risk for advanced liver disease and/or HCC and would benefit from antiviral therapy (7,8). In fact, the predictive value of high HBV DNA levels (> 2,000 IU/mL) on disease outcome in the setting of persistently normal ALT levels is limited to cohorts of Asian patients (5,6,9-22).

We conducted this long-term cohort study to elucidate the disease outcome in HBeAg-negative Caucasian patients living in Western Europe with normal ALT levels and HBV DNA > 2,000 IU/mL at diagnosis.

Methods

Study design and patients

We carried out a retrospective cohort study of patients with high HBV DNA levels (> 2,000 IU /mL) and persistently normal ALT levels at diagnosis who were under follow-up at one of the participating centres between 1 January 1987 and 31 July 2018. Persistently normal ALT level was defined as ≤ 40 IU/L on three occasions at least two months apart over a period of one year (2-4,8). All patients were of Caucasian descent, negative for HBeAg and positive for HBeAg antibodies (anti-HBe). Supplementary inclusion criteria were HBsAg positivity for at least 6 months and a minimum observation period of one year at the participating centre. Patients were excluded if they were co-infected with hepatitis C, hepatitis D or HIV, had a history of significant alcohol use (more than 14 units and more than

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7 units per week for men and women, respectively), those with signs of significant fibrosis at diagnosis (platelets < upper limit of normal, signs of portal hypertension on ultrasonography, transient elastography values > 9.0 kPa or on liver biopsy), those with coexisting chronic liver disease (e.g. NASH, autoimmune hepatitis), previous antiviral therapy or patients taking antiviral therapy (23,24).

We used patients with HBeAg-negative chronic infection seen during this period in the collaborating centres as controls, i.e. patients with persistently normal ALT levels and HBV DNA levels ≤ 2,000 IU/mL at diagnosis (25).

Outcomes and follow-up evaluation

The primary endpoint was the development of a liver-related event. This included compensated advanced stage of liver disease, decompensated cirrhosis, HCC or liver-related mortality. In the absence of clinical signs of decompensation, compensated advanced liver disease was considered on one or more of the following criteria according to the Baveno VI Consensus Workshop: transient elastography value ≥ 10.0 kPa, liver biopsy showing severe fibrosis (F3) or cirrhosis (F4), upper gastrointestinal endoscopy showing gastroesophageal varices or a hepatic venous pressure gradient measurement value of >5 mmHg (26). Decompensated cirrhosis was diagnosed in the presence of complications of portal hypertension (upper gastrointestinal bleeding, ascites, hepatic encephalopathy) (26). HCC diagnosis was based on non-invasive criteria (typical hallmarks of HCC on multiphasic contrast-enhanced computed tomography (CT), multiphasic contrast-enhanced magnetic resonance imaging (MRI), gadoxetic-enhanced MRI or contrast-enhanced ultrasound) or pathology (27).

The secondary endpoint was the progression to chronic active hepatitis (CAH). In line with international practice guidelines and in the absence of other reasons for ALT elevation, CAH was diagnosed when ALT levels increased to more than twice the upper limit of normal on two occasions at least two weeks apart, accompanied by HBV DNA levels > 2,000 IU/mL (2-4).

Body mass index (BMI) was calculated as kg/m^2 and obesity was specified as BMI ≥ 30 kg/m^2. In addition to detailed medical history and physical examination at baseline, all patients underwent blood tests, including ALT levels, HBeAg, anti-HBe and HBV DNA levels and abdominal ultrasound and/or transient elastography and/or liver biopsy to assess liver disease severity. From 2006 onwards, transient elastography was available for the determination of liver stiffness. According to international clinical practice guidelines, patients had regular monitoring assessments, including ALT, HBsAg, HBeAg, HBV DNA and fibrosis assessment (4,28-34). Liver biopsy was performed during follow-up in cases where biochemical results, HBV markers, abdominal ultrasound and/or transient elastography revealed inconclusive results (4,24,35). Moreover, during study period liver biopsy was mandatory for the reimbursement of antiviral therapy. Results from transient elastography and liver biopsies were evaluated according to the METAVIR classification (36).

Serum assays

Serological markers, including HBsAg, HBeAg, anti-HBe, hepatitis C antibodies, hepatitis D antibodies and HIV antibodies were determined using conventional serological assays. Serum samples at baseline were stored at -20°C and up to 2002 quantitative HBV DNA was run at branched DNA signal amplification method developed by Chiron Diagnostics (Emeryville, CA). Later, HBV DNA was measured using a polymerase chain reaction (PCR); ABI Prism 7900HT (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) from 2003-2014 and Abbot RealTime HBV assay (Abbott Molecular Inc, Des Plaines, IL) from 2015 on.

Statistical analyses

Categorical data were expressed as frequencies (%) and continuous variables as median ± interquartile range (IQR). For categorical endpoints, group differences were evaluated using a two-sided Chi-square test or Fisher's exact test. For continuous endpoints, group differences were evaluated using Man-Whitney U test or Kruskal-Wallis H test. Cumulative incidences of liver-related events or CAH were analysed with the use of the Kaplan-Meier method. To avoid statistical repetition in the event that a patient experienced different types of liver-related events at different times, only the initial events were considered for statistical analysis.

Hazards ratio (HR) for the incidence of liver-related event or CAH was determined with Cox proportional hazards regression model. We employed backward multivariable regression model with the following variables: high viremia group (vs controls), age at diagnosis as a continuous variable, male gender (vs female), obesity (yes vs no) and ALT levels as a continuous variable.

To reduce the effect of potential confounders between high viremia group and controls, we supplementary conducted a propensity score-matching analysis including all baseline characteristics (except HBV DNA levels). IBM SPSS Statistics for Windows, version 25 (IBM Corp, Amonk, NY) was used for all analyses. *P* values of < .05 were considered to indicate statistical significance.

Results

Characteristics of patients at baseline

Between 1 January 1987 and 31 July 2018, a total of 443 patients were included in the study: 116 patients with baseline HBV DNA level > 2,000 IU/mL (study group or high viremia group) and 327 patients with HBV DNA

Table 1. — **Baseline characteristics in the study group and controls (n = 443)**

Characteristic	High viremia group HBV DNA > 2,000 IU/mL (n = 116)	Controls HBV DNA < 2,000 IU/mL (n = 327)	*p* Value
Age, years, median + IQR	31 ± 18.0	32 ± 18.0	.883
Male sex, n (%)	63 (54.3)	160 (48.9)	.319
Obesity†, n (%)	14 (12.7)	26 (8.0)	.133
ALT (IU/L), median + IQR	26 ± 11.0	23 ± 12.0	.072
Log10 HBV DNA level (IU/mL), median + IQR	3.9 ± .98	1.7 ± 2.71	<.001

Abbreviations: IQR: interquartile range; ALT: alanine aminotransferase; HBV: hepatitis B virus; HBeAg: hepatitis B e antigen.
† Obesity was specified as body mass index ≥ 30 kg/m^2.

Table 2. — **Disease outcome of the high viremia group and controls at the end of follow-up (n = 443)**

Characteristic	High viremia group HBV DNA > 2,000 IU/mL (n = 116)	Controls HBV DNA < 2,000 IU/mL (n = 327)	p Value
Follow-up, years, median + IQR	7 ± 9.8	10 ± 12.5	.008
Liver-related events, n (%)	6 (5.2)	7 (2.1)	.112
CAH, n (%)	12 (10.3)	7 (2.1)	.001
Compensated advanced liver disease, n (%)	5 (4.3)	7 (2.1)	.314
Decompensated cirrhosis, n (%)	1 (0.9)	0 (0.0)	.262
HCC, n (%)	0 (0.0)	0 (0.0)	...
Liver-related death, n (%)	1 (0.9)	0 (0.0)	.262

Abbreviations: HBV: hepatitis B virus; IQR: interquartile range; CAH: chronic active hepatitis; HCC: hepatocellular carcinoma.

level ≤ 2,000 IU/mL (controls). Comparison of both groups is illustrated in Table 1. Age at diagnosis (p = .883), male (p = .319) and obesity (p = .133) distribution was similar in both patient populations. Moreover, the high viremia group was comparable regarding serum ALT level (p = .072). Median follow-up was 7 ± 9.8 years in the high viremia study group and this was 10 ± 12.5 years in controls.

In the high viremia group, 39/116 (33.6%) had HBV DNA levels > 20,000 IU/mL and 77/116 (66.7%) had HBV DNA levels between 2,000 and 20,000 IU/mL.

Liver-related events

A liver-related event occurred in 6 (5.2%) vs 7 (2.1%) in controls, p = .112 (Table 2). None of the patients developed HCC. The annual incidence rate of a liver-related event was significantly higher in the study group compared with the control group (0.6% vs 0.2%, p = .032). The estimated cumulative probability of liver-related event in the high viremia vs control group was 0.0% vs 0.0% at 5 years and 4.8% vs 0.0% at 10 years (p = .008; Figure 1).

In the high viremia study group, there was no difference in disease outcome between patients with HBV DNA levels > 20,000 IU/mL and those with HBV DNA levels 2,000-20,000 IU/mL (Supplementary file S1). The cumulative incidence of liver-related event at 5 years (0.0% vs 0.0%) and 10 years (0.0% vs 6.5%) was comparable in patients with HBV DNA levels > 20,000 IU/mL and patients with HBV DNA levels between 2,000 and 20,000 IU/mL, p = .850 (Supplementary file S2).

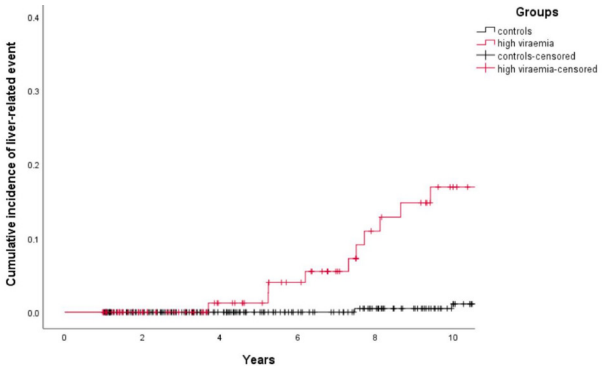

Figure 1. — Cumulative probabilities of liver-related events in high viremia group vs controls (n = 443). Person-years were censored on the date of liver-related event, death or the last outpatient clinic visit, whichever came first.

Chronic active hepatitis

There was a higher number of patients who developed CAH (10.3%) in the study group compared to controls (2.1%), p = .001 (Table 2). All patients with CAH initiated antiviral therapy. Last platelet level was < 150 x 10^9/L in 3/19 (15.8%) patients who had developed CAH. The annual incidence rate of CAH development was 1.2% for the high viremia group and 0.2% for the controls, p < .001. The estimated cumulative incidence

Table 3. — **Factors predictive of liver-related event (n = 443)**

Variables	Univariate analysis		Multivariable analysis	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Liver-related event				
High-viremia group	1.33-11.83	.014	1.20-11.98	.023
Age, years	1.01-1.10	.012	1.00-1.09	.048
Male sex	1.11-22.56	.037	1.03-22.34	.045
Obesity†	1.31-14.07	.016	.95-13.11	.059
ALT level	1.03-1.20	.007	.97-1.15	.208

Abbreviations: HR: hazards ratio; CI:confidence interval; ALT: alanine aminotransferase. † Obesity was specified as body mass index ≥ 30 kg/m^2.

of CAH in the study group vs control group was 1.4% vs 0.0% at 5 years and 18.2% vs 1.2% at 10 years ($p < .001$).

Within the high viremia study group, the occurrence of CAH was 3 (7.7%) in patients with HBV DNA levels > 20,000 IU/mL and 9 (11.7%) in patients with HBV DNA levels 2,000-20,000 IU/mL, $p = .547$. (Supplementary file S1).

Factors predictive of liver-related event and chronic active hepatitis

In the total population, high viremia group (hazards ratio (HR) 95% confidence interval (CI): 1.20-11.98, $p = .023$), age at diagnosis (HR 95% CI: 1.00-1.09, $p = .048$) and male sex (HR 95% CI: 1.03-22.34, $p = .045$) were independently associated with a significantly higher risk of liver-related events (Table 3).

A selected analysis was conducted in the high viremia group (n = 116). In this subgroup 6 (5.2%) patients developed a liver-related event and older age at diagnosis (HR 95% CI: 1.01-1.16, $p = .023$) was the only independent predictor of a higher risk of liver-related event (Supplementary file S3).

In the multivariable analysis, the risk of CAH development was significantly higher in the high viremia group compared with controls (HR 95% CI: 2.67-19.00, $p < .001$).

Propensity score-matched analysis

In the propensity score-matched cohort, patients in the high viremia and control group did not differ significantly in baseline characteristics, except for HBV DNA levels (Supplementary file S4). A significantly higher risk of liver-related events (5.2% vs 2.7%, $p = .033$) and CAH development (10.3% vs 1.8%, $p < .001$) was shown in the high viremia group compared with controls.

Discussion

As illustrated in this study, patients with HBeAg-negative chronic infection and HBV DNA levels ≤ 2,000 IU/mL have an excellent long-term prognosis and accordingly they are no candidates for antiviral treatment (2-4). However, there is still an ongoing debate regarding the management of HBeAg-negative patients with persistently normal ALT levels but high viremia (HBV DNA levels > 2,000 IU/mL) (2-4,8). Much of the data concerning the management of this high viremia group is derived from studies done in Asia (5,6,9-22). We found that in Caucasian patients with high viremia the estimated 10-year risk of a liver-related event was significantly higher (4.8%) than the patients with HBV DNA levels ≤ 2,000 IU/mL (0.0%).

These results were consistently observed in our multivariable and propensity-score matched analyses. In the high viremia group, 39/116 (33.6%) patients had HBV DNA levels > 20,000 IU/mL and these patients had a comparable disease outcome to those patients with HBV DNA levels 2,000-20,000 IU/mL.

Natural history studies in mainly Asian patients have found a significant association between HBV DNA levels and a liver-related event irrespective of ALT levels (5,6). This raises the issue whether our patients with high viremia but normal ALT levels would benefit from antiviral therapy. Yet, there were no long-term studies in the Caucasian population living in Western Europe demonstrating a worse prognosis in the high viremia group compared to controls with HBeAg-negative chronic infection according to strict criteria and the use of high sensitive PCR-based assays, which have been introduced only recently in the last decade. Given the lack of evidence on long-term, our data may provide novel information on long-term disease endpoints. From the few short-term studies in Caucasian HBeAg-negative patients with persistently normal ALT levels, only Papatheodoridis and colleagues (37) determined that those patients with HBV DNA level > 2,000 IU/mL had a higher probability of progression to CAH when compared to those with HBV DNA level ≤ 2,000 IU/mL (37-42). Nonetheless, this study was limited by a short follow-up of 3 years and a small sample size of only 20 patients with baseline HBV DNA levels > 2,000 IU/mL.

Although a 10.3% prevalence of CAH in our high viremia group is not negligible, liver-related mortality was 0.9% and none of the patients developed HCC. Therefore, stringent follow-up by specialist physician is recommended but since the long-term liver-related event is very low, antiviral therapy seems not be indicated in these patients. Older age at diagnosis was an independent predictor of a liver-related event in our study. Therefore, further studies are warranted to identify other predictors

of compensated advanced stage of liver disease, decompensated cirrhosis, HCC or liver-related mortality to allow a more targeted recommendation for antiviral treatment.

With regards to the absence of HCC development in our Caucasian study population living in Western Europe, one could question the recommendation of a strict screening protocol for the development of HCC with periodical abdominal ultrasound in these patients (2-4). In this regard, FIB-4 score is a non-invasive scoring system that has been developed to estimate liver fibrosis and has also been associated with HCC development during 5 years of follow-up (43,44). PAGE-B score, which is based on a patient's platelet, age and gender, has been developed to estimate the progression to HCC in treated Caucasian patients (5,45,46). However, more research is needed whether non-invasive scores could also predict the probability of HCC development in Caucasian patients with HBeAg-negative chronic infection and subsequently the need of stringent screening with abdominal ultrasound.

This study had some limitations. Due to the retrospective design of this study, liver biopsy was not conducted on a large scale and the histological changes remain mostly undefined. A transient elastography or liver biopsy to exclude significant fibrosis at diagnosis was available for the majority 324/443 (73%) of the study population. Intrinsic to the long-term evaluation from the year 1987 to 2018, international scientific societies have changed the follow-up programme of chronic HBV patients during the past 40 years. In addition, other risk factors such as quantitative HBsAg levels, HBV genotypes, HBV variants (basal core promoter; precore) were not available to evaluate if they might be associated with long-term outcome in Caucasian HBeAg-negative patients. However, these tests are not routinely available in standard practice as this study aimed to assess the long-term outcome and their predictors among Caucasian hepatitis B patients in real-life daily practice.

In conclusion, the level of HBV DNA affects the long-term disease outcome in Caucasian HBeAg-negative patients living in Western Europe despite the absence of liver necroinflammation and significant liver disease at diagnosis. Nonetheless, the risk of liver-related events is too low to favor antiviral therapy but stringent follow-up for disease progression is recommended.

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Author contributions

ÖK, JV, GK, RB, DB, MG, GR and FN contributed to the conception and design of the study. FN, GK and GR supervised ÖK to collect data. Following statistical analysis of data, ÖK drafted the first version of the paper, the co-authors critically revised the article and approved the final version to be submitted, including the authorship list.

Ethical statement

Following Belgian and Dutch regulation, ethical approval was waived because of the retrospective character of our study.

Conflicts of interest and source of funding:

None declared.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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