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CLINICAL STUDY

Importance of CHB‘s grey zone: analysis of patients with HBeAg negative chronic hepatitis B virus infection

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ABSTRACT

INTRODUCTION: HBeAg-negative chronic HBV infection is defined by viremia < 2,000 IU/ml (or < 20,000 IU/ml), normal ALT activity and minimal liver fibrosis. Some patients do not meet all the criteria and belong to the so-called grey zone. The aim of the work was to analyse a group of patients with asymptomatic chronic HBV infection, divide them according to the levels of HBV DNA during follow-up and to compare the clinical and laboratory parameters of the patients within the groups.

METHODS: We retrospectively analysed patients with HBeAg-negative chronic HBV infection examined in the Centre for Viral Hepatitis of the Department of Infectology in Košice, Slovakia, from September 2018 to December 2021. Patients were divided into three groups based on HBV DNA levels during long-term follow-up (< 2,000 IU/ml, fluctuating, > 2,000 IU/ ml). We evaluated selected demographic, anamnestic and laboratory data (HBV DNA, ALT, fibrosis stage).

RESULTS: Of the 280 enrolled patients, 160 were men (57.1 %), the average age was 48.0 years, and the mean length of follow-up was 4.7 years. HBV DNA levels were consistently < 2,000 IU/ml in 149 patients, fluctuating levels in 69, and levels consistently > 2,000 IU/ml in 62 patients. 165 patients had normal ALT activity, 74 had fluctuating ALT activity, and permanently increased ALT in 41 patients. 139 patients underwent transient elastography examination, 16 of them had stage F2 fibrosis, two stage F3 and 1 had cirrhosis. When comparing the three groups divided according to HBV DNA, patients with fluctuating HBV DNA had the longest follow-up, but patients with HBV DNA permanently over 2,000 IU/ml were the youngest and the highest proportion of them had elevated ALT activity. 165 patients (58.9%) met the extended criteria of asymptomatic carriers, 115 were in the grey zone.

CONCLUSION: Patients with HBeAg-negative chronic HBV infection often have fluctuating HBV DNA and ALT values during follow-ups. Statistically significantly higher proportion of abnormal ALT activity in patients with HBV DNA > 2,000 IU/ml may suggest higher risk of adverse outcomes. Initiation of treatment in such patients is not always necessary unless they also meet the other indication criteria for treatment. The exact definition of the grey zone is currently absent *(Tab. 2, Fig. 2, Ref. 16)*. Text in PDF *www.elis.sk*

KEY WORDS: HBeAg negative chronic HBV infection, HBV DNA, ALT, liver fibrosis.

Introduction

Approximately 296 million people worldwide are chronically infected with hepatitis B virus (HBV), who are also at increased risk of progressive liver disease that can lead to cirrhosis or hepatocellular carcinoma (HCC) (1). Monitoring the course of chronic hepatitis B (CHB) is essential for individual management in clinical practice. The natural course of the disease in patients with chronic HBV infection is complex and dynamic, which is determined by the interaction of the virus, the host, and the environment (2).

After overcoming the acute form of hepatitis B, 95 % of patients are cured without transitioning to the chronic stage. The high rate of progression of HBV infection to chronicity in neonates and toddlers is largely attributed to the immaturity of the host immune system in these very young age groups (3).

According to the activity of ALT, the level of HBV DNA and the degree of fibrosis, we distinguish 4 clinically important phases of the chronic stage of HBV (Tab. 1) and an additional phase 5, which includes HBsAg-negative and anti-HBc-positive patients with minimal to no viremia (4).

In patients with chronic HBV infection, currently used antiviral drugs are not curative and the main reason is the persistence of covalently closed circular DNA (cccDNA) in infected hepatocytes (5). Therefore, current guidelines recommend starting antiviral therapy mainly in patients with chronic HBV infection and

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Tab. 1. Phases of chronic hepatitis B according to EASL (4).

	HBeAg positive		HBeAg negative	
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
HBsAg	High	High/intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA	> 10^7 IU/ml	10^4–10^7 IU/ml	< 2,000 IU/ml	> 2,000 IU/ml
ALT	Normal	Elevated	Normal	Elevated
Liver disease	None/minimal	Moderate/severe	None	Moderate/severe
Old terminology	Immune tolerant	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative chronic hepatitis

symptoms of necrotic inflammation in the liver. Although current antiviral therapy has good results, few patients achieve the ultimate goal-loss of hepatitis B surface antigen (HBsAg), i.e. functional cure. Since HBeAg-negative chronic HBV infection is the last stage before the loss of HBsAg, the management of patients in this stage together with HBsAg quantification has attracted increasing clinical and research interest (6).

Elevated serum ALT activity indicates active inflammation and hepatocyte damage caused by an antiviral immune response. However, the degree of liver inflammation or fibrosis cannot be predicted only from the degree of ALT activity increase, and clinically significant histological damage cannot be excluded even when ALT levels are normal. A growing number of studies have confirmed that ALT activity is not the only key indicator in the decision to start antiviral treatment (7).

Some patients may have persistently normal ALT activity and be HBeAg negative while the virus is insidiously replicating and the disease is progressing. Such patients are clinically defined as being in the grey zone (GZ) (2). The grey zone is the situation involving patients falling between inactive carrier status and HBeAg-negative chronic hepatitis B (8).

International classification of diseases (ICD) 11 recognizes only two diagnoses for chronic HBV infection, namely chronic hepatitis B with or without Delta virus (B18.0; B18.1) and a diagnosis for carriers of HBV surface antigen HBsAg (Z22.5). In practice, therefore, it is sometimes difficult to adequately classify patients according to diagnoses, since there are fewer of them than groups of chronic HBV infection.

The aim of the work was to analyse a group of patients with asymptomatic chronic infection with the hepatitis B virus registered in the hepatology outpatient clinic from September 2018 to December 2021 with a diagnosis of Z22.5 and to evaluate the fulfilment of the criteria characterizing asymptomatic HBeAg negative chronic HBV infection.

Patients and methodology

We retrospectively analysed a group of 288 patients treated in the hepatology outpatient part of the Department of Infectology and Travel Medicine in Košice with a diagnosis of HBeAg negative chronic HBV infection (hepatitis B virus carrier, code Z22.5 according to ICD), who underwent examination between September 2018 and December 2021. All patients were older than 18 years. Patients with a history of alcohol abuse or co-infection with hepatitis C were excluded. We collected and evaluated demographic data (age, sex), history of diabetes mellitus (DM), immunosuppressive treatment (IS) in patients. From the laboratory parameters, we evaluated HBV DNA, HBeAg, anti HBe, ALT activity and the stage of liver fibrosis assessed by transient elastography (TE) and, depending on availability, we also evaluated stage of steatosis with controlled attenuation parameter (CAP) during the entire follow-up period. We considered values up to 0.60 μkat/l to be normal ALT activity. We compared the obtained data with international criteria for individual groups of the chronic stage of HBV infection.

We then divided the patients into three groups based on the detected levels of HBV DNA. The first group consisted of patients with an HBV DNA level permanently below 2,000 IU/ml, the second included patients with at least one HBV DNA level detected above 2,000 IU/ml in the documentation, and the third group was represented by patients with HBV DNA permanently above 2,000 IU/ml. The above monitored parameters were evaluated in each group.

Continuous variables are presented as mean ± standard deviation. Categorical variables are reported as absolute and relative counts. For statistical comparison of categorical variables, we used the chi square test. We compared continuous variables such as the age and follow-up time of the patients using the ANOVA test or Kruskall–Wallis test where appropriate. We chose $p < 0.05$ as the level of significance.

Results

From the total number of 288 patients, we excluded 8 patients with a history of alcohol abuse or positivity against HCV. In the group of 280 patients, 160 (57.1 %) were men and 120 (42.9 %) were women. The average age of the patients was 48.0 years. Diabetes mellitus was documented in 7 patients, 2 patients were taking immunosuppressive treatment. The mean duration of the follow-up of monitored patients at the Department of Infectology and Travel Medicine was 4.7 years. Out of the entire group, 67 people were examined at the outpatient clinic only once, and 56 people had their HBV DNA value examined only once. All patients were positive for HBsAg and negative for HBeAg (Tab. 2).

Out of 280 patients, 149 (53.2 %) had low HBV DNA values (below 2,000 IU/ml) and 131 patients had an increase in HBV DNA values above 2,000 IU/ml during the duration of the follow-up, which represents 46.8% of patients, of which 62 (22.1 %) had persistently higher HBV DNA levels than 2,000 IU/ml. There were 165 (58.9 %) patients with normal ALT activity during the entire duration of the monitoring. Total of 139 patients completed the transient elastography examination (Tab. 2).

For each patient in the set, we evaluated the minimum and maximum level of HBV DNA as well as the minimum and maximum value of ALT activity during the duration of their follow-

Tab. 2. Comparison of observed parameters of patients in groups according to HBV DNA levels.

	All patients	Groups according to HBV DNA levels			Statistical significance (p)
		permanently <2,000 IU/ml	at least once >2,000 IU/ml	permanently >2,000 IU/ml	
Number of patients	280	149	69	62	
Men (%)	160 (57.1)	77 (51.7)	41 (59.4)	42 (67.7)	0.09
Average age	48.0±11.3	50.6±12.0	47.3±10.7	42.7±7.6	<0.001
Follow-up (years)	4.7	4.2	7.9	2.6	<0.001
DM	7	6	1	0	0.189
IS	2	2	0	0	NA
ALT (%)					
normal	165 (58.9)	98 (65.8)	38 (55.1)	29 (46.8)	0.002
fluctuating	74 (26.4)	28 (18.8)	27 (39.1)	19 (30.6)	
elevated	41 (14.7)	23 (15.4)	4 (5.8)	14 (22.6)	
TE performed (%)	139 (49.6)	48 (32.2)	45 (65.2)	46 (74.2)	
F0–F1	120 (86.4)	40 (83.2)	41 (91.1)	39 (84.8)	F0–F1 vs F2
F2	16 (11.5)	6 (12.5)	4 (8.9)	6 (13.0)	0.764
F3	2 (1.4)	1 (2.1)	0 (0.0)	1 (2.2)	F0–F2 vs F3–F4
F4	1 (0.7)	1 (2.1)	0 (0.0)	0 (0.0)	0.385
CAP performed (%)	114 (40.7)	38 (25.5)	39 (56.5)	37 (59.7)	
S0	34 (29.8)	10 (26.3)	13 (33.3)	11 (29.7)	0.844
S1	20 (17.5)	5 (13.2)	9 (23.1)	6 (16.2)	
S2	22 (19.3)	8 (21.1)	7 (17.9)	7 (18.9)	
S3	38 (33.4)	15 (39.4)	10 (25.7)	13 (35.2)	

*diabetes mellitus (DM), immunosuppression (IS), transient elastography (TE), controlled attenuation parameter (CAP)

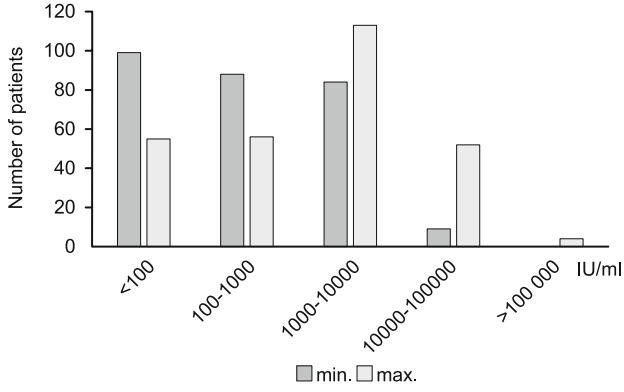

Fig. 1. Minimal and maximal levels of HBV DNA.

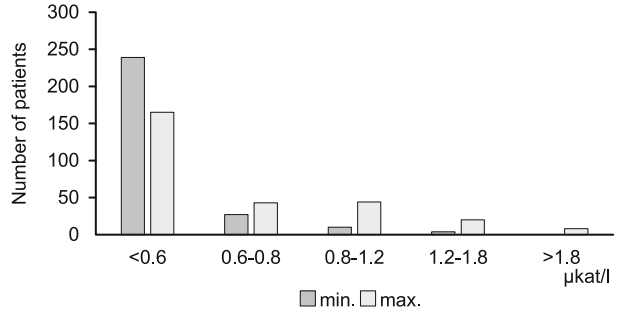

Fig. 2. Minimal and maximal levels of ALT activity.

up. The level of HBV DNA when evaluating the minimum HBV DNA ranged from 0 IU/ml to 69,900 IU/ml (median 366 IU/ml) and between the maximum levels ranging from 0 IU/ml to 363,900 IU/ml (median 1,843 IU /ml) (Fig. 1).

ALT activity when assessing its minimum activity ranged from 0.06 ukat/l to 1.67 ukat/l (median 0.36 ukat/l). When evaluating the maximum activity of ALT, the values were from 0.10 ukat/l to 9.50 ukat/l (median 0.56 ukat/l) (Fig. 2).

Patients with HBV DNA permanently below 2,000 IU/ml

Of the 149 patients in this group, 77 (51.7 %) were men and 72 were women (48.3 %). The average age was 50.6 years, the duration of follow-up 4.2 years. A total of 6 patients in the group had diabetes mellitus and 2 patients were immunocompromised. ALT activity was persistently normal in 98 patients, 28 patients had ALT activity increased at least 1x, and 23 patients had permanently increased ALT activity. TE examination was performed in 48 patients, 6 patients had fibrosis stage F2 and advanced fibrosis stage was detected in 2 patients. The patient with the degree of fibrosis F3 had overcome hepatitis. In the past, he had been infected with the hepatitis B virus in early childhood. The patient with fibrosis grade F4 had documented cirrhosis of the liver, he was a morbidly obese patient with concomitant steatohepatitis.

Patients with HBV DNA output above 2,000 IU/ml

In the group of 69 patients, 41 (59.4 %) were men, 28 (40.6 %) were women. The average age was 47.3 years, the duration of follow-up was 7.9 years, one patient had diabetes mellitus and no one had a detected immune disorder. ALT activity was normal in 38 patients, 27 patients had ALT activity increased at least 1 time,

and 4 patients had permanently increased ALT activity. Forty-five patients underwent TE examination, fibrosis stage F2 was detected in 4 patients, no patient had advanced stage of fibrosis F3–F4.

Patients with HBV DNA permanently above 2,000 IU/ml

The group of 62 patients consisted of 42 men (67.7 %) and 20 women (32.3 %). The average age in this group was the lowest, 42.6 years. The length of follow-up was 2.6 years. None of the patients had diabetes mellitus or was immunocompromised. Almost half of the patients (29 out of 62) had a consistently normal ALT activity, 19 patients had elevated ALT activity at least once, and 14 patients had elevated ALT activity at every check-up. Forty-six patients were examined by transient elastography, 6 of them had fibrosis stage F2. Fibrosis stage F3 was found in 1 patient in this group, however, this patient's ALT activity was consistently normal and HBV DNA level was below 20,000 IU/ml in the long term.

When comparing all three groups, we observed statistically significant differences in the following variables: average age of patients, duration of follow-up and ALT activity. The other observed variables do not differ statistically significantly (Tab. 2).

Discussion

The aim of this analysis was to evaluate a group of patients with HBeAg negative chronic HBV infection monitored at the Department of Infectology and Travel Medicine. According to the EASL classification, the HBeAg-negative phase of chronic HBV infection is characterized by HBV DNA values below 2,000 IU/ml, low ALT activity and absent signs of liver fibrosis. In our group, 98 out of 280 patients meet all criteria, which represents 35.0 %. However, the EASL classification also allows HBV DNA values up to 20,000 IU/ml for the chronic HBeAg negative phase of HBV infection. There were 67 (23.9 %) of such patients with values of HBV DNA in the range of 2,000–20,000 IU/ml in our group, therefore more than half of our patients (58.9 %) meet the extended EASL criteria. Despite the fact that this is a high proportion of patients, other authors have also observed a much higher representation, for example in the group in the study of Sanai et al (81.2 %) (15).

The remaining 41.1 % of patients meet these criteria only partially, but also do not meet the criteria of HBeAg negative chronic hepatitis B. These patients are in the grey zone. In a study according to Duan et al up to 59.0 % of patients were in the GZ, in study according to Sanai et al there was a total of 18.8 % of patients (13, 15).

The term 'grey zone' is used when referring to patients who don't completely fulfil given criteria for each phase of chronic hepatitis B, therefore, those patients may be HBeAg positive or negative. Yao et al performed a retrospective study of 4,759 untreated HBV-infected patients, both HBeAg positive and negative, in China. A substantial proportion of patients with chronic B infection who did not fall into any of the defined phases were considered as patients in the GZ. The studies focused on investigating the distribution and characteristics of GZ in a large cohort of CHB patients. CHB stages were defined based on the AASLD 2018 hepatitis B guideline. As many as 1,322 (27.78 %) patients did not fit into any of the defined phases and were defined as GZ. Older age, HBeAg-positive status, and higher ALT levels were independent risk factors for advanced disease in patients with CHB on GZ. Therefore, the results revealed that more than a quarter of patients with CHB were classified as GZ (2).

In HBeAg-negative patients with chronic HBV infection, HBV DNA levels usually do not exceed the level of 2,000 IU/ml, in our group more than half of the patients (149 out of 280) meet this condition, as well as in the study by Sanai et al, where more than half of the patients (58.5 %) had long-term HBV DNA levels below 2,000 IU/ml (15).

From our entire set of patients, only 3.2 % of patients also had minimum HBV DNA values higher than 10,000 IU/ml, and 1.1 % of patients had minimum HBV DNA values higher than 20,000 IU/ml. In the study by Sanai et al, there were no patients with HBV DNA values consistently higher than 20,000 IU/ml, but there were 4.7 % of patients with fluctuating HBV DNA values that exceeded 20,000 IU/ml during monitoring (15).

ALT activity was permanently normal in almost 60 % of patients in the monitored group, and only 1.4 % of patients also had a minimum ALT activity more than 2 times the norm. In study by Zhou et al, ALT activity was consistently normal in 43 % of patients, and 12 % of patients had ALT activity greater than 2 times the normal (9). On the contrary, in the study by Deng et al up to 96 % of patients had long-term normal ALT activity (14). In study by Sanai et al 18.8 % of patients had higher ALT activity (15).

Advanced fibrosis was documented in 2.2 % of patients in our cohort, but these patients had an associated cause of liver damage other than CHB – non-alcoholic steatohepatitis. In some studies, the proportion of patients with advanced liver fibrosis was much higher than in our group, namely 15.0 % in the study by Sanai et al up to 27.1% in study according to Duan et al and 51.6% in study by Wu et al (12, 13, 15).

Other studies report recurrent and occasional small-scale liver tissue damage in HBeAg-negative patients with chronic HBV infection and normal ALT activity, whereby fibrotic changes are more pronounced than inflammation. In a study of 327 such patients, Zhuang et al found that 193 (59.0 %) were in the GZ, 106 (32.4 %) had HBV DNA ≥ 2,000 IU/mL, and 122 (37.3 %) had significant liver inflammation. Significant liver fibrosis occurred in 174 (61 %) patients (7). In a study by Yao et al, 117 HBeAg-negative patients with chronic HBV infection, normal ALT activity, and HBV DNA positivity, liver biopsy results showed that 57.2 % had severe liver disease that met the indications for antiviral therapy (16).

By comparing groups of patients divided on the basis of HBV DNA values, we observed statistically significant differences in the duration of follow-up, average age and ALT activity. We did not observe significant differences in other monitored parameters. During the monitoring of HBeAg-negative patients with chronic HBV infection, there is a frequent fluctuation of HBV DNA values, usually up to 20,000 IU/ml, as well as ALT activity, so immediate treatment is not always necessary, unless these patients also meet other indication criteria for starting of treatment (5,6).

Some studies found that after 4–5 years of follow-up, 37–54.3 % of HBeAg-negative patients with chronic HBV infection were

still in the GZ, of which 43.5–85 % had converted to the stage of HBeAg negative chronic HBV infection and 2.2–15 % of them to the stage of HBeAg-negative chronic hepatitis B. The results showed that HBeAg-negative patients with chronic HBV infection and normal ALT activity were not a homogeneous population. Some of them were inactive HBsAg carriers who may stay untreated, while others may be in the calm or mildly active phase of HBeAg-negative chronic HBV infection, which is a grey zone of the disease progression (9, 10, 11).

Bonacci et al, in their study evaluated the activity of the ALT enzyme of 287 HBeAg-negative patients. It was found that 45 % of patients classified as in GZ progressed to the stage of inactive carriers by the end of a median follow-up period of 8.2 years, and that the results of these patients were very similar to those of inactive HBsAg carriers in terms of the rate of HBsAg loss, although they more often developed HBeAg-negative chronic hepatitis B (8).

The results of our analysis, as well as the results of published studies, demonstrated the occurrence of patients in the GZ. A more precise definition of the grey zone and the possible correct timing of treatment in such patients should also be included in the recommendations for the management of patients with chronic hepatitis B virus infection. It is important to identify, monitor, and reassess initiation of antiviral therapy as needed. However, more studies and research are needed to assess the benefit of antiviral treatment in such patients.

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