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The predictive effect of serum HBV-RNA and HBcrAg on the disease changes in patients with chronic hepatitis B

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Abstract: Chronic hepatitis B has not been cured until now because covalently closed circular DNA (cccDNA) in the liver cannot be completely eradicated. In the era of continuous medical innovation, nucleoside (acid) analogues (NAs) can make serum HBV deoxyribonucleic acid (HBV-DNA) reach the detection limit, but only represents the interruption of virus replication. Liver biopsy is the gold standard for the expression of cccDNA transcriptional activity. However, it is an invasive detection method, and its clinical popularity is far from enough. Therefore, an alternative and accurate serum marker is needed. Hbv-rna (hepatitis B virus ribonucleic acid), in the form of virions containing pregenomic RNA (pgRNA), can be used to predict early liver fibrosis, viral rebound after NA withdrawal, and to evaluate the efficacy of new antiviral drugs. HBcrAg (Hepatitis B core-associated antigen) is a serum complex viral protein that helps predict HBV reactivation, the risk of hepatocellular carcinoma, the timing of safe drug withdrawal after NA treatment, and HBeAg conversion. Both are new surveillance tools. This article reviews the predictive significance of HBcrAg and HBV-RNA in the development of disease.

1. Current status of chronic hepatitis B

Chronic viral hepatitis B (CHB) is a group of systemic infectious diseases caused by the hepatitis B virus, with liver damage as the main cause, and is one of the major health hazards. According to the World Health Organization (WHO), it affects approximately 240 million people worldwide each year, resulting in 650,000 deaths due to liver failure, cirrhosis and hepatocellular carcinoma caused by chronic hepatitis B. This shows that chronic hepatitis B is a serious threat to human life and health, and poses a great burden of disease to the population. The main drugs currently approved for use in the treatment of chronic hepatitis B are nucleoside (acid) analogues (NAs) and peg-interferon (PegIFN-α). [1-2] NAs therapy inhibits viral replication and reduces the risk of death from liver disease, but drug resistance is the most pronounced, leading to chemical breakthroughs, virological rebound and hepatitis flares, and in a minority of patients, liver failure, acute liver failure and even death; and only a very small proportion of patients can achieve serum HBsAg conversion. [3-5] Therefore, during long-term antiviral therapy, it is important to accurately and timely determine and grasp the viral replication situation to prevent disease progression.

Persistent hepatitis B virus (HBV) infection is mainly due to the inability of covalent closed-loop

DNA (cccDNA) to be completely cleared. Although antiviral drugs can inhibit HBV reverse transcriptase and bring HBV-DNA down to the lower limit of detectable levels, they are only one of many steps in the inhibition of the viral life cycle and do not fully reflect the inhibition of HBV replication. NAs also have little effect on intrahepatic HBVcccDNA, which is why HBV can be controlled but not eradicated. HBVcccDNA serves as a template for viral transcription and can reflect the amount and activity of viral replication, but quantitative detection of intrahepatic cccDNA requires liver tissue puncture biopsy, an invasive test that is not yet widely used as a routine clinical diagnostic indicator, and there is currently. There is no standardised method for the direct detection of cccDNA. There is therefore a need for more sensitive biomarkers to monitor the transcriptional activity of cccDNA in the residual liver where HBV-DNA cannot be expressed [6]. Serum HBV-RNA and HBcrAg are new markers that may have a role in predicting specific clinical outcomes, such as predicting virological relapse and disease progression after cessation of antiviral therapy [7].

2. Mechanisms of replication against hepatitis B virus

HBV viral particles infect hepatocytes mainly via the receptor sodium taurocholate cotransport polypeptide (NTCP). Upon entry of HBV into hepatocytes, part of its double-stranded DNA genome is exposed in the cytoplasm, also known as relaxed circular DNA (rcDNA). The rcDNA is then transported to the nucleus of the infected hepatocyte, where it undergoes repair and conversion to cccDNA. Viral RNA is transcribed from HBV cccDNA as the genomic template and contains 3.5kb of prenuclear genome (pcRNA) and pregenome (pgRNA),2.4/2.1kb of viral protein messenger RNAs (mRNAs) and From the nucleus they are transported to the cytoplasm where they are transcribed into HBeAg, HBsAg, HBx protein, HBcAg and viral protein polymerase, or the pregenomic RNA is packaged into capsids to produce rc-DNA by reverse transcription. mature capsids containing rc-DNA can be recycled intracellularly into the nucleus to add to the cccDNA library, or they can be used by The mature capsid containing rc-DNA can be recycled intracellularly into the nucleus to replenish the cccDNA pool, or can be wrapped by viral surface proteins in the endoplasmic reticulum and secreted extracellularly as viral particles. Double-stranded linear DNA (dslDNA) is also produced by HBV reverse transcription and can be converted into viral particle DNA and re-entered into the nucleus to form cccDNA[8-9].

3. Correlation of serum HBV-RNA, HBcrAg and intrahepatic cccDNA

3.1 The relationship between HBV-RNA and intrahepatic cccDNA

HBVpgRNA is directly transcribed from cccDNA in liver tissue, and HBVpgRNA generates HBVRNA, so in theory, HBV-RNA can map cccDNA transcriptional activity. nAs can reduce HBV-DNA to undetectable levels, but have no effect on cccDNA generation of HBsAg, HBcrAg and serum HBV-RNA Wang et al. found that in a group of 47 patients treated with entecavir for between 1 and 10 years, viral replication was effectively controlled and only 12 of these patients had negative serum HBV-RNA levels, but in all patients liver biopsies for intrahepatic cccDNA had positive HBV-RNA levels. Levels were all positive. The association between serum HBV-RNA levels and intrahepatic HBV transcriptional patterns was also analysed using serum-liver paired samples, and serum HBV-RNA levels were found to correlate with the cccDNA ratio, which implies viral transcriptional activity. From these data, it was concluded that serum HBV-RNA levels reflect the level of cccDNA directed viral transcriptional activity [10].

3.2 Relationship between HBcrAg and intrahepatic cccDNA

cccDNA is the viral reservoir in chronic hepatitis B. In recent years, there have been numerous reports that HBcrAg can replace liver tissue biopsy in serving as a valid serum marker of cccDNA transcriptional activity. 89 patients with chronic hepatitis B and positive HBV-DNA were enrolled in a trial by Robin Erken et al. and treated with interferon in combination with adefovir for 48 consecutive The patients were followed up for 144 weeks after 48 weeks of treatment. The trend of HBsAg, HBV-DNA and HBV-pgRNA was also recorded during the treatment period. The results concluded that HBcrAg reflects cccDNA transcriptional activity more strongly than HBsAg, especially when cccDNA transcription was targeted or when the treatment regimen included nucleoside analogues[11].

4. The role of HBV-RNA

4.1 HBV-RNA can predict the risk of viral relapse after discontinuation of NAs treatment

There are now relevant studies showing that [12] when HBV-DNA and HBeAg turn negative and ALT and AST reach baseline levels after NAs treatment, antiviral therapy can be stopped after 1 year of consolidation therapy. Kaewdech et al. included 92 active chronic hepatitis B patients over 18 years of age who were on long-term NA therapy and were tested for HBV-DNA, HBV-RNA and HBcrAg at the end of treatment. Virological relapse was defined as serum HBV-DNA > 2000 IU/mL and clinical relapse as hepatitis B viral DNA > 2000 IU/mL with transaminases elevated to twice the normal level, and biochemical tests and physical examination were performed at 3, 6, 9 and 12 months after discontinuation of treatment. Their results showed a significantly lower rebound rate at the end of treatment in patients who tested negative for serum HBV-RNA than in those who tested positive for HBV-RNA. [13]

4.2 HBV-RNA can assess the efficacy of new antiviral drugs

Lung-Yi Mak et al. studied 73 patients with HBeAg-positive chronic hepatitis B who were first treated with NVR3-778 after 28 days of treatment. (a CpAM drug) or interferon monotherapy resulted in a greater decrease in serum HBV-RNA levels compared to NVR3-778 combined with interferon treatment.[14] Another study recruited 20 patients with chronic hepatitis B to receive different doses of SB9200 (enalapreviracet) for 12 weeks followed by 12 weeks of continued treatment with tenofovir and observed a dose-dependent decrease in serum HBV-RNA in 11 HBeAg-negative patients and no change in 9 HBeAg-positive patients, whose 11 HBeAg-negative after 24 weeks of treatment patients had undetectable HBV-RNA and two of the nine HBeAg-positive patients had undetectable HBV-RNA.[15] From the above drug trials we can conclude that serum HBV-RNA is a relatively reliable marker for efficacy assessment of emerging antiviral drugs.

4.3 HBV-RNA predicts the risk of developing liver fibrosis in HBeAg-negative hepatitis B patients

Patients with chronic hepatitis B receiving antiviral therapy with NAs can effectively inhibit viral replication but are prone to drug resistance and greatly reduced therapeutic efficacy, resulting in liver damage from inflammation followed by repair and healing to form liver fibrosis. It is therefore particularly important to accurately predict changes in the disease in order to stop its progression in time. An article published by Prof. Wenhong Zhang's team[16] showed that when

treated with NAs, even though HBV-DNA had reached the lower limit of detection, which only meant that the reverse transcription process of the virus was inhibited, these patients still showed signs of inflammation and fibrosis on liver histology; after analysis, serum HBV-RNA levels were found to be significantly correlated with liver inflammation degree score and fibrosis degree score. In a recent study, 145 patients with hepatitis B liver fibrosis, 71 and 74 HBeAg-negative and positive patients respectively, were enrolled and tested for HBVRNA and DNA, liver puncture biopsy, liver function, hepatitis B five and evaluation of liver reserve function. The results suggested that HBV-RNA was significantly lower in HBeAg-positive patients than in HBeAg-negative patients, and the HBV-RNA-positive group had significantly lower liver fibrosis grading, inflammation grading and Child score were significantly higher than those of the HBVRNA-negative group, and the ROC curve showed that HBV-RNA was correlated with hepatitis B liver fibrosis and could be used as a predictor of progression to liver fibrosis in HBeAg-negative patients. [17]

5. Role of HBcrAg

5.1 HBcrAg can predict the risk of progression to hepatocellular carcinoma (HCC) in patients with slow hepatitis B

There are many reasons for HBV progression to HCC, including HBV gene integration, genomic instability due to mutations, and activation of pro-oncogenic signalling pathways, all of which can lead to a transformation from inflammation to tumour [18]. Some studies have reported that the incidence of HCC remains high in hypovirulinaemic patients after NAs treatment. A recent study found a strong association between serum HBcrAg levels and the development of HCC[19]. Of 1031 untreated patients, 78 developed HCC at a mean time of 10.7 years, and the risk of HCC was five times higher in patients with serum HBcrAg >2.9 logU/ml[20]. The author showed that HBcrAg was strongly associated with factors in the development of hepatocellular carcinoma and concluded that higher HBcrAg levels were associated with the development of HCC independent of NA treatment[21].

5.2 HBcrAg predicts the risk of HBV reactivation

Many patients on NA therapy choose to stop treatment when HBV-DNA is undetectable and ALT and AST levels are normal, and more recently, based on serum HBsAg levels. It has been reported that HBcrAg can be reduced under NA therapy and this can be used as a predictor of the risk of HBV reactivation [22]. There are no uniform criteria for HBV reactivation and it is generally judged on the basis of clinical experience. Usually HBV-DNA rises to 10-fold basal normal levels and transaminases to 3-fold when hepatitis B virus-infected cancer patients are on allopathic treatment. Hepatitis B virus activation rarely occurs in chronic hepatitis B virus infection and in inactive HBV carriers, and HBV in a quiescent or low-replicating state is easily activated when chemotherapy or immunosuppressive therapy is administered that blocks the protective effect of lymphocytes and the production of viral suppressor cytokines [23]. Occult hepatitis B (OBI) is HBsAg negative with HBV genome still present in the liver and low viral levels in liver, serum and peripheral blood mononuclear cells (PBMC), but still infectious. Seto et al [24] reported that in a study of serum HBV-DNA below the lower limit of detection, HBsAg negative, anti-hbc positive combined patients with targeted therapy or In 124 patients with bone marrow transplantation, the hepatitis B virus revival rate was 40.4% after 2 years of deposition. Forty-three of these patients were serum HBcrAg-positive and had three times the risk of HBV reactivation than patients with undetectable serum HBcrAg. When there is a risk of HBV reactivation, early antiviral drug treatment is shown in

clinical practice to be more effective than treatment when HBV-DNA tests positive, and can prevent disease progression to some extent.

5.3 HBcrAg levels can predict when antiviral therapy is safe to discontinue when partial cure is achieved

Different serum markers such as HBV-RNA and HBcrAg have been reported to predict virological relapse after discontinuation of treatment for NA[25]. A study in 45 hbeAg-negative patients showed that serum HBcrAg >3.7 log IU/mL at the end of treatment was associated with a 3.7-fold risk of virological relapse within one year of NA discontinuation[26]. Partial cure was defined as persistent DNA below the lower limit of detection after discontinuation of consolidation therapy, no relapse, and HBV-RNA to the extent that it was also consistently undetectable. As serum HBcrAg reflects the activity of liver tissue cccDNA, it would be useful if serum HBcrAg levels could be used to predict the risk of relapse prior to drug discontinuation, in order to identify patients who have achieved virological response and can continue to be maintained after drug discontinuation as "partially cured", and thus achieve safe drug discontinuation[25].In a trial of 34 patients with chronic hepatitis B treated with lamivudine, high HBcrAg levels, despite HBV-DNA negativity for 6 months, were shown to predict viral relapse[27].

5.4 HBcrAg predicts HBeAg conversion

HBeAg seroconversion is a marker signal for treatment endpoints in HBeAg-positive patients and is the primary prerequisite for HBsAg conversion or achieving functional cure[28].Among 127 HBeAg-positive patients, 35 were treated with NAs antiviral therapy, 14 with interferon and the rest with a combination of both, and the decrease in HBcrAg levels from baseline to 6 months and from baseline to 12 months was defined as ΔHBcrAg as a predictor of serological conversion of HBeAg when ΔHBcrAg concentrations were greater than 0.75 log10 U/ mL, seroconversion of HBeAg occurred within 6 months, and within 12 months when ΔHBcrAg concentrations were greater than 2.05 log10 U/mL[29].In a retrospective analysis of 118 patients treated with at least one antiviral drug, HBeAg seroconversion occurred in 36.4% of patients, and there was a difference in treatment kinetics between HBcrAg levels before and after HBeAg seroconversion, with a high predictive value of HBcrAg for seroconversion in HBeAg-positive patients at 6 and 12 months [30].

6. Shortcomings and outlook

CHB remains one of the life-threatening and incurable infectious diseases. As several studies continue to be updated, drugs with good antiviral efficacy and few or no side effects have been introduced, leading to an expansion of the treatment goals and their therapeutic endpoints for chronic hepatitis B. Clinicians are not only limiting their treatment to the control of viral replication and normalization of basic indicators such as liver function, but are also more vigilant in the control of disease progression and related complications. The presence of intrahepatic cccDNA makes eradication of hepatitis B an insurmountable challenge, but novel testing tools, serum HBcrAg and HBV-RNA, offer significant advantages in expressing cccDNA transcriptional activity. It has significant advantages in predicting the treatment and progression of slow hepatitis B. Theoretically, multiple relevant variables are superimposed to predict more comprehensively and accurately, therefore, if the two are used together, they can reflect cccDNA more accurately and reliably. but the shortcoming is that there is no standardised assay for HBV-RNA and HBcrAg, so their use has not yet been commonly promoted in the clinic. It is hoped that more in-depth, systematic studies in the future will reveal a test for both indicators that is widely used in clinical practice, which could

then be a major step towards a complete cure.

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