The role of Hepatitis B Core Antibody: Significance and Clinical practice

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Abstract

Hepatitis B infection remains a global health problem, with progression to acute-chronic hepatitis, severe liver failure, and death making hepatitis B one of the most serious infections worldwide. The disease is most widely transmitted from an infected mother to her baby, after exposure to infected blood or body fluids or unsafe sexual contact. Pregnant women, adolescents, and all adults at high risk for chronic infection are recommended to be screened for hepatitis B. Serological tests allow the distinction between acute and chronic hepatitis. Meanwhile, the molecular tests performed provide detection and quantification of viral DNA, genotyping, drug resistance, and pre-core/core mutation analysis to confirm infection and follow monitoring disease progression in patients with chronic hepatitis B. Because, the current treatment is only based on nucleotide analogs and pegylated interferons that save lives by decreasing liver cancer death, liver transplant, slowing or reversing the progression of liver disease as well as the virus infectivity. In this review, we clearly light the role of Hepatitis B Core Antibody, therefore clinicians understand the need to screen for hepatitis B core antigen (Anti-HBc), proper interpretation of HBV biomarkers, and that "anti-HBc only" indicates HBV exposure, lifelong persistence of cccDNA with incomplete infection control, and potential risk for reactivation.

Keywords*: Hepatitis B virus (HBV), Hepatitis B Core Antibody (Anti-HBc/HbcAb).*

1. INTRODUCTION

Despite the availability of vaccines and robust treatment strategies, infection with the Hepatitis B virus remains a severe worldwide illness because Hepatitis B virus (HBV) infection leads to acute-chronic hepatitis, severe liver failure, and liver cancer with high morbidity and mortality. An estimated 2 billion people worldwide have been infected with the hepatitis B virus in the presence of the hepatitis B core antigen (anti-HBc). In approximately 95% of adults, exposure to HBV leads to an acute infection that rapidly resolves without long-term consequences, while the remaining 5% do not control viral infection, leading to chronic [1, 2]. Over 292 million people are living with chronic hepatitis B (CHB) worldwide Global HBV with surface antigen (HBsAg) positivity was estimated at 3.9% in 2016 [3]. Annually, 887,000 deaths yearly occur from HBV and related diseases, mainly cirrhosis, and advanced cirrhosis. The risk and progression of chronic infection are age-dependent and occur mostly in immunocompromised individuals. It is shown that acute HBV infection is usually cleared in immunocompetent individuals, but chronic HBV infection develops in about 90% of infants, 30 - 50% of children aged five, and 5 - 10% of adults [4].

Occult Hepatitis B infection (OBI) was defined in the group with undetectable HBsAg, defined as the presence of HBV DNA in the liver of HBsAg-negative individuals. OBI has been shown to occur both in the absence and presence of anti-HBc and/or anti-HBs. OBI rates have been reported to be more common in patients at high risk for gastrointestinal infections such as hepatitis C virus (HCV) and HIV infection [5]. OBI is associated with severe liver injury and hepatocellular carcinoma (HCC), and poses a risk to individuals, particularly in transfusion infections, HBV reactivation, chronic liver disease, and HCC [6]. Isolated anti-HBc (IAHBc) is a particular serotype seen in immunocompromised patients. Isolated anti-HBc is determined by negative anti-hepatitis B antigen and positive anti-hepatitis B antibody (whether in the form of IgM or IgG). It is especially important to screen immunocompromised patients for IAHB because HBV replication can be reactivated with the potential for morbidity and mortality [7].

This review describes virological tests, including serological and molecular techniques for the diagnosis of HBV infection, and specifically updates the role of Anti-HBc in clinical practice.

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2. PATHOPHYSIOLOGY OF HBV INFECTION

The Hepatitis B virus belongs to the family Hepadnaviridae. Virions also known as Dane granules, are made by a capsid with an envelope around it, which is entirely infectious. HBV contains viral DNA and two enzymes DNA-polymerase and protein kinase. The HBV genome is an open circular DNA molecule with four open reading frames called P, S, C, and X encode for viral proteins.

The HBV replication cycle initiates the interaction between HBsAg and hepatocyte surface proteins that help HBV attach to and enter hepatocytes. This is followed by relaxed cyclic DNA (rcDNA) which is changed to Covalently closed circular DNA (cccDNA) and is required for the transcription and production of new RNA and DNA, which is then matured by the viral polymerase (Figure 1). HBV DNA can exist in hepatocytes as cccDNA, unlike rcDNA found in viruses. CccDNA also contributes to core antigen (HBcAg) synthesis in hepatocytes and the production of anti-HBc antibodies, explaining their presence in acute, chronic and resolved HBV infections Normally, there are Four types of responses that have been described in HBV infection. Firstly, there can be a strong immune response leading to the complete and fast elimination of HBV and infected cells, leading to acute hepatitis and/or hepatocellular necrosis. Secondly, the immune response can be useful but not strong, often presenting an asymptomatic infection to resolve progressively. Thirdly, the immune response is inadequate, with a state of tolerance resulting a part in considerable prolonged HBV replication. Lastly, the immune the response may be unstable, which is usually related to the asymptomatic carrier of HBV [8, 9].

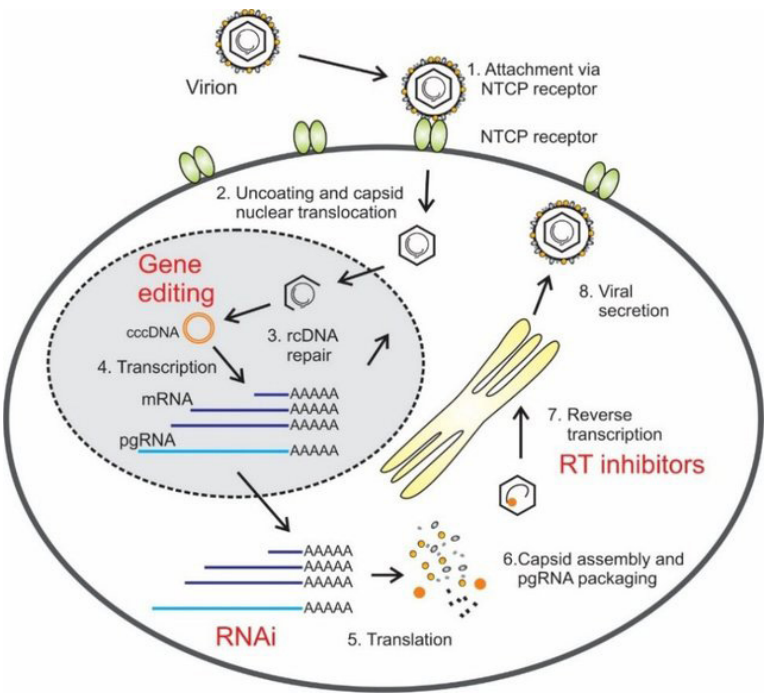

Figure 1. Hepatitis B virus life in hepatocytes [10].

3. LABORATORY DIAGNOSIS OF HEPATITIS B VIRUS

In practice, the assessment of HBV infection begins with the patient history, physical examination, and assessment of liver function, through analysis of various hepatitis serologic markers and/or their combinations such as HBsAg, HBeAg, anti-HBs, anti-HBc (IgM, IgG), anti-HBe, and biochemical parameters, molecular assay [11]. The Hepatitis B Foundation recommends screening all adults for HBV with a triad serological marker that includes HBsAg, anti-HBs, and total anti-HBc. To stage infection in HBV-infected patients, the following should be performed: 1) testing for HBsAg, HBeAg/anti-HBe, HBV DNA; 2) liver blood tests including aspartate aminotransferase (AST), alanine transaminase (ALT), and 3) transient elastography (Fibroscan) as a noninvasive test or needle biopsy of the liver as an

invasive method for the presence of cirrhosis [12].

3.1. HBV serological markers

To assess the patient's HBV status, HBsAg is always tested along with anti-HBc and anti-HBs (Figure 2). A positive HBsAg antigen indicates ongoing infection and infection. Initially, the majority of infections are positive for hepatitis B e antigen (HBeAg). However, with chronic infection, HBeAg is often lost. Loss of HBeAg, whether spontaneous or treatment-induced and with or without anti-HBe seroconversion, represents partial immune control of chronic HBV infection, often resulting in a low replication phase [11]. In later stages of the disease, loss of HBeAg is sometimes caused by viral variants or mutations. It is interesting to note that total anti-HBc is usually lifelong.

HBsAg: HBsAg is an envelope protein expressed on the surface of infectious virions called Dane particles. The detection of positive HBsAg in the serum indicates the current HBV infection condition [11]. The incubation stage for hepatitis is around 60–150 days after exposure to HBV, and HBsAg appears in the blood between 1–10 weeks after initial exposure to HBV [11]. During the immune window, HBsAg can disappear rapidly without the appearance of Anti-HBs, and IgM Anti-HBc is the only evidence of infection during this period. If HBsAg positivity persists after 6 months, it means the progression of a chronic HBV infection. Quantitative immunofluorescence analysis was performed to assess the HBsAg level of chronic HBV patients and is a useful marker for interferon-alfa-treated chronic HBV patients with HBeAg-negative [12].

Anti-HBs/HBsAb: The presence of anti-HBs in serum indicates convalescence and immunity against HBV infection by an infected virus or by immunized HBV vaccine. People with first-degree relatives such as a parent-child or a partner who are chronic carriers are recommended to be vaccinated if their triple sera screening test is negative [12]. The titer of anti-HBs should be ≥10 mIU/ml for protection [13].

HBeAg and anti-HBe: HBeAg stands for Hepatitis B Envelope Antigen. The presence of HBeAg correlated with active viral replication is indicative of the infectivity of patients with HBV. Meanwhile, the appearance of anti-HBe indicates low viral replication and is strong evidence for infection resolution [14]. These tests are commonly used to determine the stage of chronic HBV infection [12].

Anti-HBc/HBcAb: Anti-HBc also known as HBcAb is an antibody against the core antigen of the hepatitis B virus, this antibody appears very early and persists for life. Detection of anti-HBc antigens confirms HBV exposure and indicates acute, chronic, or resolved infection but not vaccine immunity [15]. The presence of IgM Anti-HBc, along with HBsAg positivity, usually indicates an acute infection, which usually lasts no more than six months [16]. Anti-HBc-positive and Anti-HBs-negative individuals have chronic infections and show a reduced risk of HBV reactivation. However, there is also no clinical benefit of vaccination for individuals who are only Anti-HBc positive due to HBV exposure or who are anti-HBc and anti-HBs positive due to immune control [17].

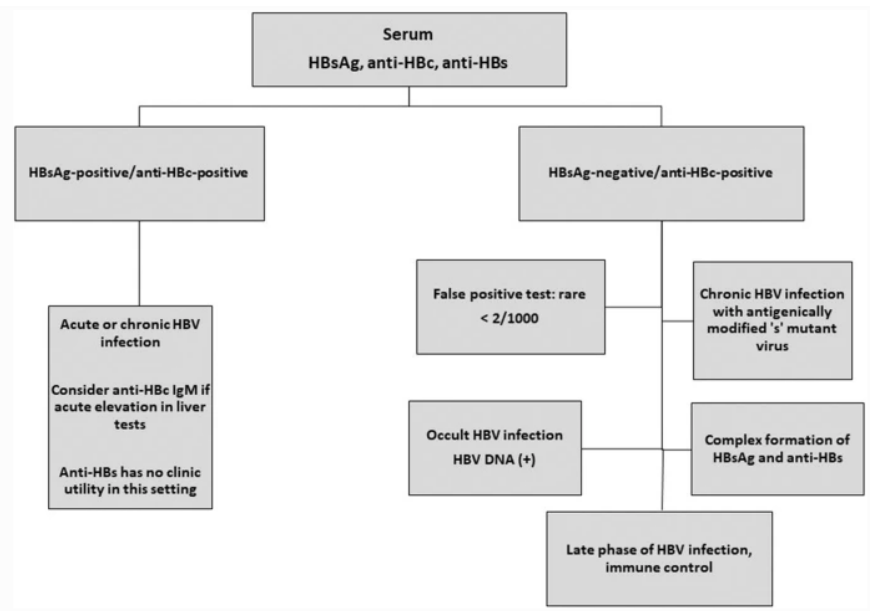

Figure 2. Interpretation of HBsAg, Anti-HBs and anti-HBc test results [12]

3.2. Biochemical parameters

The severity of liver damage as inflammation and fibrosis is assessed using biochemical parameters, including AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) which are enzymes released from the liver in response to damage and liver disease. Besides, There are other biochemical parameters including gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, serum albumin gamma globulin, and prothrombin time (PT) for diagnosing liver disease [12]. But, in case biochemical and HBV markers are not sure, then invasive and noninvasive methods are chosen to determine the stage of liver injury [12]. Because liver biopsy is an invasive, expensive, and hurtful technique compared to the other techniques, many non-invasive techniques are safe to identify the stage of the presence of fibrosis in chronic HBV patients. Therefore, the APRI index is calculated according to the formula = [AST/AST upper limit of normal (ULN) × 100/platelet count] [18] of The WHO recommends estimating the stage of liver fibrosis [11]. It has been recommended that 40 IU/ml as the AST ULN value should be used in the APRI formula. In addition, ALT levels must also be evaluated in chronic HBV patients as it is relative to the severe disease. According to the WHO guidelines, the ULN ALT levels are below 30 U/l for men and 19 U/l for women [11].

3.3. Molecular assays

Molecular diagnostic methods are used for HBV DNA quantification, genotype, determining mutations of drug resistance, and analysis of pre-core/core mutation [19]. Currently, various molecular assays are recommended by FDA (2019) for the diagnosis of HBV infection such as the UltraQual HBV PCR technique, COBAS AmpliScreen HBV technique, Procleix Ultrio test, Procleix Ultrio Plus technique, and COBAS TaqScreen MPX technique, nucleic acids amplification tests (NATs).

HBV DNA quantification: HBV DNA by NATs is recommended to evaluate the infectivity of HBsAg-positive individuals and HBsAg-positive pregnancy to prevent transmission risk and lead to a conclusion of whether to treat HBV disease. And HBV DNA quantification with molecular assays allows early detection of risk people with HBV acute disease before HBsAg emerges and excludes OBI [20]. The methods of HBV DNA are also used to follow the treatment response in chronic HBV infection patients [11]. The HBV DNA concentration reflects the disease evaluation, the long-term following of chronic HBV infection, and the treatment's effectiveness to prevent the evolution of HCC (Hepatocellular carcinoma). The viral concentration of HBV is often evaluated by IU/ml or by copies/ml. The metering of the level of HBV DNA is recommended to be used with a more sensitive method rt-PCR assay with a 10 IU/ml detection limit [12].

Until now, HBV genotyping, drug resistance, and pre-core/core mutations have been confirmed. There are 10 genotypes of HBV, from A to J, and more than 40 sub-genotypes [21], which may be related to the concentration of viral in the host, risk of progressive fibrosis, responses of antiviral treatment, and follow the mutations progress in clinical practice [6]. Although HBV genotyping is not necessary for diagnosis at the first step, also sequencing the determination of HBV genotypes and resistance drug mutations is a useful parameter for patients at risk of developing HCC to monitor an effective therapy [12].

4. THE ROLE OF HEPATITIS B CORE ANTIBODY

The HBV core antibody (anti-HBc) serological markers test is chosen for monitoring a history of the infection. IgM Anti-HBc is the earliest antibody of the body to perform in the immune response to HBV infection. Meanwhile, IgG Anti-HBc typically persists for life after 6 months of the HBV infection. Thus, IgM anti-HBc is a marker of acute infection while IgG anti-HBc is a marker of chronic infection. It is interesting that the total anti-HBc (IgM anti-HBc and IgG anti-HBc) usually lasts a lifetime. Therefore, screening for hepatitis B core antigen (anti-HBc) helps to clarify the role of HBV serological markers including anti-HBc and "anti-HBc only" status in confirming HBV infection, but not due to vaccination and moreover, the indefinite existence of cccDNA in host hepatocytes resulting in infection control not completely, and the potential of risk for HBV reactivation as described analyzed earlier in this review [22].

4.1. Anti-HBc Test: Effective and Meaningful

Historically, although there were many versions of tests anti-HBc markers with various different specificities, most countries, and labs in the world now are using the FDA-cleared method and technique. Currently, these anti-HBc marker tests are really effective with a specificity of more than 99.8% for healthy donors' blood and leading to confirmation of residual disease (cccDNA) in the host and a necessary consideration is whether to HBV vaccinate against HBV or not [23, 24].

Normally, most the normal immune system individuals are protected from the infected HBV,

but these cases can also have reactivation under various conditions [25, 26]. Because a clearance cure for HBV infection may not be completed because the DNA of HBV is in the nucleus as the cccDNA form and is integrated into the host genome for surface antigen (HBsAg) production. Even the people who have recovered after the acute HBV infection stage, cccDNA can still be found in the liver cells, the existence of these cccDNAs explains that when these recovered individuals are profoundly immunosuppressed for any reason, there is a reactivation of HBV replication [27]. This explains why, in patients with HBV infection who have resolved or become chronic, the available prophylactic hepatitis B vaccine has not shown any clinical benefit [28, 29]. People with a history of past HBV infection, whether they are currently infected or not, may be at risk for hepatitis reactivation during and after immunosuppressive therapy. HBV reactivation has the potential to lead to fulminant liver failure and/or death [30]. Therefore, it is important to identify individuals who may be at risk of reactivation prior to immunosuppressive therapy.

4.2. Isolated anti-HBc (IAHBc) and Occult Hepatitis B Infection (OBI)

Isolated anti-HBc (IAHBc) is a specific HBV serological sample, defined as both HBsAg and anti-HBs negative but anti-HBc positive (whether IgM or IgG). The prevalence of this sera profile may be high, particularly in patients infected with hepatitis C virus (HCV), patients with human immunodeficiency virus (HIV), and other immunocompromised patients, such as cancer patients. Although this pattern may be a serological false positive, it can also be found in patients with Occult Hepatitis B Infection (OBI). However, it is particularly important to screen immunocompromised patients for IAHBc to prevent HBV replication that could be reactivated with the potential for severe illness and death [7].

Occult Hepatitis B Infection (OBI) is defined as HBsAg-negative hepatitis B with detectable HBV DNA in the liver or blood [31]. The definition of OBI is not limited to an isolated anti-HBc pattern. OBI can be the association of negative HBsAg with positive HBV DNA in the blood and/or the liver [32], or positive HBV DNA in the liver whatever the level in the blood [33]. However, the criterion in European and American guidelines [12, 15], the positive blood HBV DNA is still kept for confirming OBI. The concentration of anti-HBc may help differentiate OBI from other cases of positive anti-HBc. Indeed, anti-HBc is produced by the immune system against HBcAg, a viral nucleocapsid protein that is the most immunogenic component of HBV in the cell liver. These antibodies are known as the marker of a stage HBV infection or exposition, and they can persist for 10–20 years or more after viral clearance. The level of these antibodies is known to fluctuate depending on the stage of HBV infection [34]. It has been reported that the levels were higher in cases of chronic HBV hepatitis than in OBI, and higher in OBI than in cases of past/cured HBV infection [35]. In patients with an isolated anti-HBc profile, a cut-off of 6.6 IU/mL was associated with a sensitivity of 60.7% and a specificity of 75.3% for discriminating OBI and the past infection. Finally, a quantitative assessment of anti-HBc might be a straightforward means of screening the different stages of HBV infection.

5. CONCLUSION AND FUTURE PERSPECTIVES

In summary, It is necessary for clinicians to understand the importance of screening patients to evaluate the anti-HBc status, proper interpretation of HBV biomarkers, and that “anti-HBc only” indicates exposure to HBV, lifelong persistence of cccDNA with incomplete control of infection, and risk for reactivation under strong immunosuppression. It is also very important to expand understanding among both clinicians and patients that HBV reactivation is a risk in individuals requiring HCV therapy, chemotherapy, or potent immunosuppressive therapy. Despite the substantial body of evidence demonstrating the risk of reactivation, this too often goes unaddressed in clinical settings where patients are not screened for HBV infection prior to initiation of such therapies and thus do not receive appropriate prophylaxis. Careful screening of liver transplant recipients and organ donors for anti-HBc, with appropriate follow up including transplant recipient vaccination where appropriate, is also necessary. As the usefulness of anti-HBc levels is confirmed, the anti-HBc test should also be considered expanded in research settings and clinical practice.

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