Diagnostic Approach to Hepatitis B Virus (HBV) Infection

Helen S. Te, MD

Abstract

Hepatitis B infection is a global health problem, leading to cirrhosis and hepatocellular carcinoma in some patients and accounting for 6,000 deaths annually. The diagnosis of HBV infection is based largely on the interpretation of serologic markers and hepatitis B DNA levels, which allows establishment of the phase of infection and provides the groundwork for management strategies. More recently, genotyping and detection of genetic mutations that confer drug resistance provide additional data that assist in the therapeutic decisions. Histologic staging also presents important information that allows for individualized management of the disease. This paper reviews the various tests utilized in the diagnosis of HBV infection and their roles in the identification of the different phases of infection and in the determination of the need for further management.
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Key Words: *HBV serology, antigen, antibody, immunologic markers, liver biopsy*

The global burden of HBV is high, with about two billion people exposed worldwide and about 350 million individuals with chronic infection and at risk for complications.[1] Hepatitis B is estimated to be the cause of 30% of cirrhosis and 53% of hepatocellular carcinoma (HCC) worldwide.[2] Acute or chronic HBV infection is estimated to cause 600,000 deaths each year, and about 25% of chronically infected adults later die from cirrhosis or HCC.

In the United States, the overall prevalence of hepatitis B surface antigen (HBsAg) carriers in blood donor studies is 0.2%.[3] However, the Centers for Disease Control and Prevention (CDC) estimated 4.3 - 5.6% of the United States population to have been exposed to HBV, with 800,000 to 1.4 million Americans being chronic carriers of HBV. Annually, HBV is blamed for 3,000 deaths related to chronic liver disease.[4] Despite this high mortality rate, prevention and control of HBV in the United States is inadequate based on the Institute of Medicine Report, due partially to lack of knowledge and awareness about the disease among health care providers, social service providers, and the lay public.[5]

The diagnosis of HBV infection is based largely on serologic markers. In the recent years, molecular tests such as genotyping and detection of genetic mutations that confer drug resistance have provided additional data that assists decision-making in the therapy of HBV. Lastly, histologic staging also provides important information that allows for personalized management of the disease. In this paper, we will review the spectrum of tools utilized in the diagnosis of HBV infection to allow proper identification of the different phases of infection and determine the need for further management.

HBV Serologic Markers

The HBV belongs to the family Hepadnaviridae, genus *Orthoheanavirus*. It has a partially double stranded DNA genome that encodes at least four overlapping open reading frames, including the surface (preS/S), precore/core (preC/C), polymerase (P) and X genes.[6] The major viral proteins include the 1. envelope proteins (small HBsAg, medium and large) located on virion surface that bind cellular receptor to initiate virion entry into host cell, 2. core protein [hepatitis B core antigen (HBcAg)] which encapsidates pregenomic RNA and partially double-stranded DNA genome in cytoplasm, 3. e antigen [hepatitis B e antigen (HBeAg)] which is secreted in the peripheral blood and is involved in immunomodulation and inhibition of HBV replication, 4. DNA polymerase which is a reverse transcriptase enzyme that degrades pregenomic RNA template during reverse transcription, and 5. X protein which is a transcriptional transactivator and cofactor for hepatocellular carcinoma.[7]

There are six HBV serologic and virologic markers in clinical use, namely 1. HBsAg, 2. anti-HBs antibodies (HBsAb), 3. HBeAg, 4. anti-HBe antibodies (HBeAb), 5. anti-HBV core antibodies (HBcAb, including total HBcAb and HBcAb IgM), and 6. HBV DNA.

Hepatitis B surface antigen (HBsAg) and anti-hepatitis B surface antibody (HBsAb). First identified in leukemic patients as the Australia antigen more than four decades ago,[8] HBsAg as detected by radioimmunoassay (RIA) or by enzyme immunoassay (EIA) has become the diagnostic marker of HBV infection. It is the small envelope protein that can be detected within the first 1-10 weeks of exposure and prior to the onset of clinical symptoms or elevations in serum alanine aminotransferase (ALT) (**Figure 1**). The specificity of current HBsAg detection assays is >99.5%, with false

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Helen S. Te, MD
Center for Liver Diseases, Section of Gastroenterology
University of Chicago Medical Center
5841 S. Maryland Ave., MC 7120
Chicago, IL 60637
Email: hte@medicine.bsd.uchicago.edu

positive results rarely observed in pregnant women, autoimmune diseases, chronic liver diseases of other causes, and heparinized, hemolysed or icteric blood specimens.[9-11] In patients who clear the acute infection, HBsAg is expected to disappear after six months. Its persistence for more than six months signifies the evolution into chronic infection, which carries a risk for disease progression and complications. Spontaneous HBsAg clearance in chronic HBV infection occurs at a rate of 0.01-1.0% per year.[12-15]

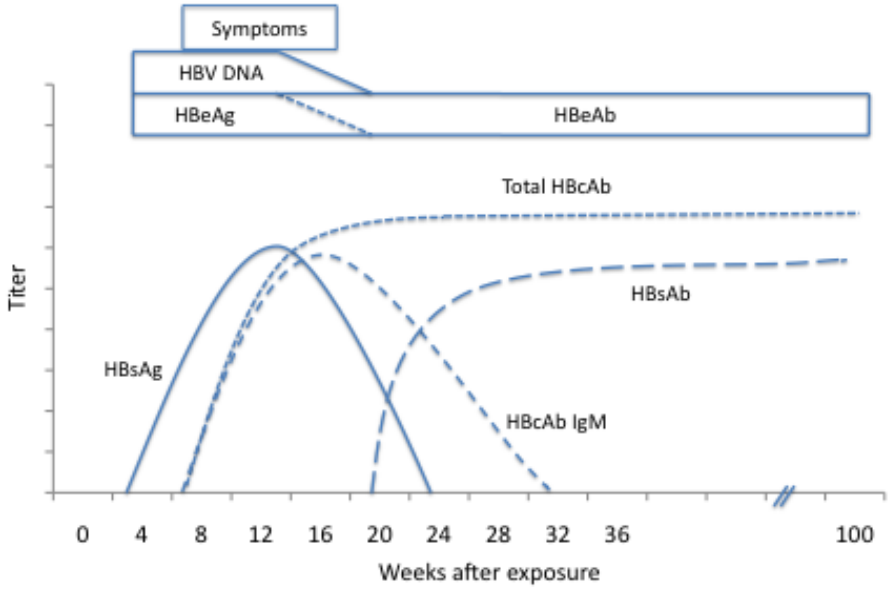

Figure 1. Serological profile in acute hepatitis B with recovery.

While acute infection in infancy and childhood tend to result in chronic HBV infection in up to 90% of cases, only 5% of acute infection in adults goes on to chronic infection. Chronic infection is characterized by the persistence of HBsAg in the serum, which represents ongoing viral presence. Occasionally, HBsAg may not be detectable during chronic HBV infection under the following circumstances: 1. HBsAg titers may be too low to be detected by commercial assays, 2. HBV strains have mutations in the S gene leading to the synthesis of an HBsAg that is not recognized by commercial assays, 3. within the period of HBsAg seroconversion, when HBsAg loss has occurred either spontaneously or in response to treatment, or 4. in co-infected individuals with hepatitis delta virus (HDV), where HDV inhibits HBV replication and HBsAg expression.[16]

Hepatitis B surface antigen quantification can be performed using a chemiluminescent microparticle assay.[17] Its quantity may be a surrogate marker for HBV covalently closed circular (ccc) DNA, which is the persistent intrahepatic form of HBV DNA. Low pretreatment HBsAg levels have been reported to be a better predictor of response to treatment with pegylated interferon plus lamivudine than serum HBV DNA levels.[17-19] HBsAg levels decline in response to therapy and may become undetectable; this may then be followed by the detection of HBsAb, a phenomenon called HBsAg to HBsAb seroconversion.

The HBsAb is a neutralizing antibody that confers long-term immunity against HBV infection. It is detected in individuals who have recovered from HBV infection (when present with HBcAb) or in those who have successfully responded to immunization against HBV (when present by itself with no other markers). Titers must be present at >10 IU/ml to represent adequate immunologic protection following immunization. HBsAb titers may wane several years after recovery from acute HBV or after immunization, but a challenge with one dose of HBV vaccine should elicit detectable titers if an anamnestic response still persists. HBsAg and HBsAb may be observed to coexist in as many as 24% of HBsAg positive individuals.[20] Such coexistence may represent inability of the HBsAb to neutralize the HBV virions, rendering these individuals chronic carriers of HBV.[21]

Hepatiis B core antigen (HBcAg) and anti-hepatitis B core antibody (HBcAb). Hepatitis B core antigen is an intracellular antigen that is expressed in infected hepatocytes but is not detectable in the serum. Its antibody, HBcAb, can be detected in the serum and is indicative of prior or current exposure to HBV, regardless of the HBsAg status. HBcAb appears as IgM within the first month after HBsAg is detected during acute HBV infection (**Figure 1**). Later, the HBcAb-IgG replaces the IgM class to account for all of the detectable total HBcAb (**Figure 2**). HBcAb-IgM is a marker

of acute infection and may be the only detectable HBV serologic marker during the window period between the disappearance of HBsAg and the appearance of HBsAb in the recovery period. It typically disappears within 6 months, but may remain detectable up to two years after the acute infection. In addition, 10-20% of individuals with chronic HBV infection who experience acute exacerbation or hepatitis flares may also have detectable HBcAb-IgM titers, leading to a false diagnosis of acute HBV infection.[22] Such HBcAb-IgM titers seen in acute flares, however, tend to be lower than those seen in acute HBV infection.[23]

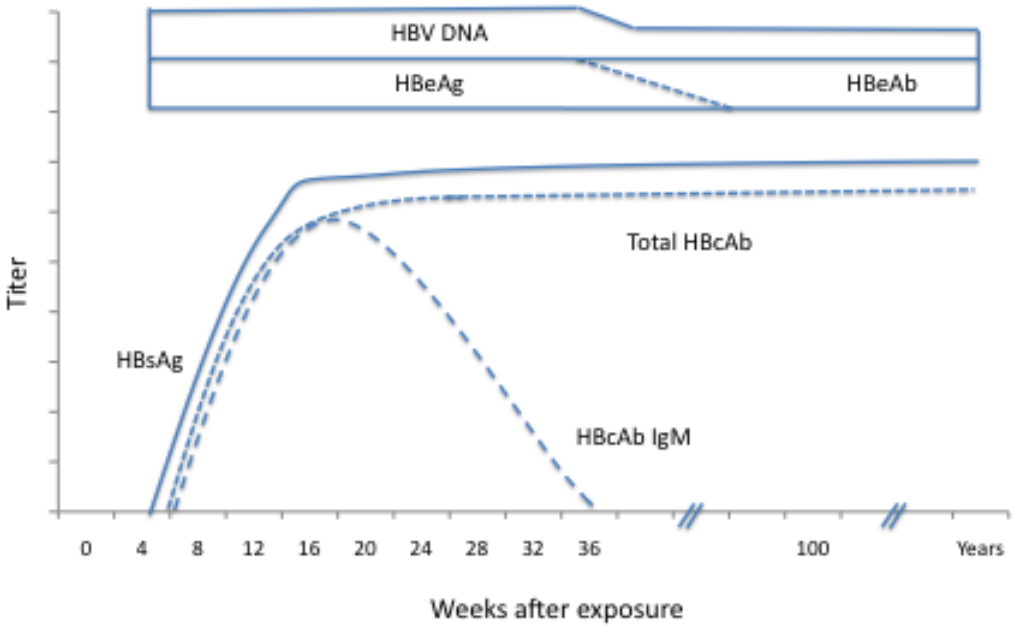

Figure 2. Serological profile in acute hepatitis B that evolves to chronic hepatitis B.

Hepatitis B core antibodies are not neutralizing antibodies and remains detectable lifelong in individuals who have recovered from acute HBV infection and in those with chronic HBV infection. The HBcAb-IgG titer is reported as a component of total HBcAb detection rather than as an individual titer; thus a positive total HBcAb in the absence of a detectable HBcAb-IgM can be attributed to HBcAb-IgG. An isolated HBcAb detected in the absence of HBsAg and HBsAb has been reported in 0.4-1.7% of blood donors in low prevalence areas[24-26] and in 10-20% of the population in endemic areas.[27,28] This isolated HBcAb positivity may represent one of four possible circumstances: 1. during window period of acute HBV infection when only the HBcAb-IgM marker is detectable, 2. in an individual who has recovered from acute HBV infection in whom the HBsAb has dropped to undetectable levels after many years; 3. in an individual with chronic HBV infection for many years in whom the HBsAg titer has dropped below detectable levels by commercial assays, and 4. A false positive result in individuals who have not been exposed to HBV at all. An individual who has recovered from acute HBV but has low and undetectable HBsAb titers is expected to have an anamnestic response to the virus; therefore, a single dose of HBV vaccine should elicit a rise in HBsAb titers to detectable levels in this population.[29,30] Hepatitis B e antibody (HBeAb) may also be detected in this population. Interestingly, HBV DNA has been detected in the serum of 0-20%[31-33] and in the liver of > 50% of individuals with isolated HBcAb who have had previous exposure to HBV.[34,35] Transmission of HBV via blood and organ donors who have an isolated HBcAb have been reported to occur variably at 0.4-78%.[31,36,37] On the other hand, false positive HBcAb results have been observed as frequently as in 50-80% of individuals with isolated HBcAb, based on the absence of a primary HBsAb response to a HBV vaccination in this population.[28,33,38] The false positive result tends to occur more frequently with EIA assays as compared to RIA assays.[38]

Hepatitis B e antigen (HBeAg) and hepatitis B e antibody (HBeAb). Hepatitis B e Ag is a part of the HBe protein, a nonstructural protein encoded by the preC/C gene. Although the HBe protein is not essential for HBV replication, its presence is associated with immune tolerance, high-level viral replication, and high potential for transmission. During acute infection, HBeAg can be detected 6-12 weeks after exposure (**Figure 1**). In patients in whom the acute infection resolves, the HBeAg also clears as the viremia decreases, to be replaced by the HBeAb. In individuals who evolve to chronic infection, HBeAg may persist for several years to cause HBeAg positive infection (wherein the virus' preC/C gene has a wild-type sequence) before an individual may

undergo HBeAg to HBeAb seroconversion (conversion from a high replication state to a low replication state with the appearance of HBeAb, generally associated with a hepatitis flare). While most patients become inactive HBsAg carriers with low HBV DNA <2000 IU/ml, a few undergo selection of variant viruses during the seroconversion process and evolve into the chronic HBeAg negative infection. In HBeAg negative infection, the variant virus has nucleotide substitutions in the precore region and/or in the basal core promoter region of the preC/C gene. The most frequent mutations are G189A in the precore region and A1762T and G1764A in the core region, and these prevent or down regulate HBeAg production.[39] Hence, viral replication may remain very active leading to clinically significant HBV DNA levels, but HBeAg is negative and HBeAb is positive. Similar to quantitative HBsAg, quantitative HBeAg has been reported to be more useful than HBV DNA level for predicting HBeAg seroconversion in patients treated with pegylated interferon therapy.[40]

Table 1. Clinical Interpretation of Hepatitis B Serological Markers.

HBsAg	HBcIgM Ab	Total HBc Ab	HBeAg	HBeAb	HBsAb	HBV DNA	Interpretation
+	+	+	-/+	-	-	+/++	Acute HBV Infection
+	-	+	+	-	-	+++	Chronic HBeAg positive infection
+	-	+	-	+	-	++/+++	Chronic HBeAg negative infection
+	-	+	-	+	-	-/+	Inactive HBV carrier
+	-	+	-	+	+	-	Resolved infection with HBV immunity
-	-	-	-	-	+	-	Immune to HBV by vaccination
-	-	+	-	-	-	-	Three possible scenarios: 1. Ongoing chronic HBV infection with very low HBsAg titers 2. Recovered from distant HBV infection with very low HBsAb titers 3. False positive HBcAb test result

Hepatitis B Virus DNA (HBV DNA)

HBV DNA in the peripheral blood represents viral replication, and is detected within a few days after infection. It peaks during the time of acute infection, and declines progressively if the infection resolves, or fluctuates if the infection evolves into chronicity. It is a useful tool to assess the need for therapy, to monitor response or noncompliance to therapy or emergence of virologic resistance to the drug, and to predict future risk of cirrhosis and HCC.[41] Its specificity of detection has significantly improved in the past decade, with dynamic ranges of 10^1 - 10^9 IU/ml being detected at the current time. Most HBV DNA assays use real-time PCR techniques, and results are now more standardized in IU/ml rather than copies/ml. 1 IU is approximately equivalent to 5.2 copies per mL.

The serum HBV DNA level has been established to be the strongest predictor of the risk for progression to cirrhosis and HCC regardless of HBeAg status and serum ALT levels.[42-45] In the treatment arena, a cut-off level of 20,000 IU/ml for HBeAg-positive individuals and 2,000 IU/ml for HBeAg-negative individuals have been established as the threshold for initiation of therapy in the setting of an elevated serum ALT.[46,47] Serum HBV DNA levels are typically high in HBeAg-positive infection and tend to be lower in HBeAg-negative infection. An inactive carrier should have HBV DNA < 2,000 IU/ml to allow for distinction from HBeAg-negative chronic HBV infection.

In terms of therapy, lower baseline serum HBV DNA level is associated with better virological response to interferon-based therapy in HBeAg-positive individuals,[48] although this relationship does not seem to hold fast for the nuclos/tide analogues. However, the rate of viral decline after the onset of treatment appear to be predictive of the HBeAg seroconversion rate in patients treated with lamivudine[49] and of the risk for the development of viral resistance.[50,51]

Serologic Profiles of Phases of HBV Infection

Acute hepatitis B is diagnosed with the detection of HBsAg in the serum which may occur as early as 1-10 weeks after exposure (**Figure 1**). The HBV DNA is also present. This is later accompanied by a rise in the HBcAb-IgM (which also coincides with the appearance of clinical symptoms if present) that lasts for about 4-6 months after which it becomes largely replaced by the HBcAb-IgG which will persist for life. Recovery from the acute infection is heralded by the disappearance of HBV DNA, followed by HBeAg to HBeAb seroconversion, and subsequently, HBsAg to HBsAb seroconversion. Rarely, an individual may be diagnosed during the window period when the HBsAg titers are waning but HBsAb titers are still low, and the only detectable marker of acute HBV is the HBcAb-IgM.

Persistence of HBsAg for six months after the diagnosis of acute HBV represents progression to chronic hepatitis B. The

HBV DNA also persists in the serum. The natural history of chronic HBV infection was defined in the 2000[52] and 2006[52,53] research workshops conducted by the National Institutes of Health (NIH) as consisting of four successive phases: 1. immune tolerance, 2. immune active or immune clearance, 3. inactive HBsAg carrier state, and 4. reactivation as HBeAg-negative infection[54] (See **Figure 1** in Chapter 5).

The immune tolerant phase is characterized by HBeAg-positivity, normal serum ALT levels, and HBV DNA > 20,000 IU/ml. This occurs most commonly in those who acquired HBV infection via perinatal transmission, and is associated with no or minimal hepatic inflammation or fibrosis.[54,55]

The immune active or immune clearance phase is characterized by either HBeAg or HBeAb positivity, elevated serum ALT levels, and HBV DNA >2,000 IU/ml. This may start as an HBeAg-positive infection which undergoes seroconversion to HBeAg-negative infection, but remains in the immune active phase. Individuals who acquired HBV in early adulthood may advance to this phase soon after the acute infection, while those who acquired HBV perinatally may transition into this phase after many years of immune tolerance. There is active inflammatory activity in the liver, with or without fibrosis.[54,55]

The inactive HBsAg carrier phase is characterized by the absence of HBeAg, the presence of HBeAb, normal serum ALT levels, and HBV DNA <2,000 IU/ml. Individuals may transition from the immune active phase into this phase and remain in this phase for years, and histologic activity is expected to remain mild or improve over time if significant inflammation or fibrosis was present at baseline.[54,55]

The reactivation phase refers to the stage when patients who are in inactive phase or even at the stage of resolved hepatitis B infection develop active hepatitis B. This is characterized by elevated serum ALT levels and HBV DNA levels > 2,000 IU/ml with serology most often being HBeAg negative, although the HBV DNA levels are usually lower than those with HBeAg-positive infection.[54,55]

Individuals who develop chronic HBV infection initially harbor HBeAg and high HBV DNA levels, which may continue for several years. Eventually, some individuals lose HBeAg at a rate of 8-12% per year,[56-58] although this tends to be lower in the immune tolerant phase.[59,60] An individual who undergoes HBeAg seroconversion may potentially follow one of these courses: 1. remain in immune active phase as HBeAg-negative hepatitis (10-30%),[57,61] 2. revert to HBeAg-positive infection one or more times (20%),[58] 3. transition to inactive HBsAg carrier state (70-80%) where he may remain for life or where he may experience reactivation as HBeAg-negative hepatitis.[57,61] About 0.1-1.0% of chronically infected individuals spontaneously clear HBsAg per year.[12-15]

Hepatitis B Genotypes

There are eight HBV genotypes (A to H) that differ by 8%-15% in their genome sequence. They may further be subdivided into several geno-subtypes (adr, adw, ayr, and ayw) which differ by > 4% from each other.[62,63] Hepatitis B genotypes have a distinctive geographical distribution. Genotype A is more prevalent in northwestern Europe, North America, India and sub-Saharan Africa, and less commonly in some regions of South America. Genotypes B and C are endemic to Asia, while genotype D predominates in the Mediterranean region and Eastern Europe, although it can also be found all over the world.[64] Genotype E is characteristic of Western Africa, genotype F of South America, and genotype H of Central America. Lastly, genotype G has been reported in France, Germany, Central Amercia, Mexico and the United States.[63-67] Individual countries and its local regions, as well individual population groups at risk, may harbor specific genotypes at varying prevalence rates,[64] but the largest report of HBV genotype distribution in the United States identified genotypes A (35%) and C (31%) to be the most common. Genotype A was more common among Caucasians and African Amercians, while genotypes B and C were mostly seen in Asians.[68]

At the present time, HBV genotypes may have clinical significance in terms of treatment outcomes, with patients infected with genotype A and B having better response to interferon than those with genotype C and D.[63] The cumulative rate of spontaneous HBeAg seroconversion occurred more often in patients with genotype B than those with genotype C.[69-74] The association of specific HBV genotype with disease progression and risk for HCC is less consistent, varying by the country where it was studied. Genotype C, for example, portends a more severe disease in Taiwan while genotype B is associated with the development of HCC in young, noncirrhotic patients; however, genotype B has a relatively good prognosis in Japan and China with no strong association to HCC. In India, genotype D is associated with more severe liver disease and HCC in young patients than genotype A.[75] Likewise, the relationship between HBV genotype and chronicity of infection is also not well-established, although studies in Japan suggested that genotype A was more likely to cause chronic infection than genotype C.[76-78] Another study in Switzerland also supported a higher likelihood to chronicity for genotype A as compared to genotype D.[79] Despite the growing knowledge on HBV genotypes, its role in standard clinical practice remains limited at the present time other than as a tool to aid the choice of therapeutic options.

Histology

To date, the liver biopsy remains as the gold standard for evaluating hepatic pathology and can be useful in confirming most disease etiology while excluding others. It is also used to assess disease severity, despite its shortcoming of being such a minute representation of the entire adult liver at 1:50,000 ratio. Since the diagnosis of HBV infection can be based on serology alone, the use of the liver biopsy in this disease is limited mostly to the appraisal of the degree of hepatic injury in acute HBV infection or a flare of chronic

HBV infection, staging of hepatic inflammation and fibrosis in chronic HBV infection, and the exclusion of other concomitant liver diseases such as fatty liver disease or iron overload.

Acute HBV infection is histologically characterized by lobular disarray, ballooning degeneration, apoptotic bodies, Kupffer cell activation, and lymphocytic lobular and portal inflammation. While a liver biopsy is typically not obtained in acute HBV infection, massive hepatocyte necrosis may lead to fulminant hepatic failure, and occasionally a liver biopsy is useful in assessing the degree of necrosis and in predicting the likelihood of hepatic recovery. These histologic features of acute HBV may also be seen on liver biopsies obtained from individuals with chronic HBV who have an acute flare of disease.

Chronic HBV infection is characterized by the presence of predominantly lymphocytic infiltrates in the portal tracts, but interface hepatitis and spotty lobular inflammation may also be seen. This pattern of chronic hepatitis is not specific for chronic HBV infection, being also present in other chronic liver diseases such as chronic hepatitis C infection and autoimmune hepatitis. However, hepatocytes that contain a large amount of HBsAg in the cytoplasm may appear as "ground-glass" hepatocytes, although again, this feature may also be seen in other conditions such as drug-induced endoplasmic reticulum hypertrophy, cyanamide toxitiy and storage diseases.[80] Hepatic inflammation is minimal in the immune tolerant state, but may be significant in the immune clearance or reactivation phase. HBeAg seroconversion is accompanied by significant improvement or resolution of histologic activity, regardless of the severity of the histologic disease at baseline.[81]

Immunostains for HBsAg and HBcAg may be obtained to confirm the presence of HBV in the hepatocytes. HBsAg is usually not expressed in acute HBV infection, but may be expressed as cytoplasmic and/or membranous particles in chronic HBV infection; HBcAg expression may be nuclear and/or cytoplasmic as well.[80] In the immune tolerant phase, large amounts of membranous HBsAg and nuclear HBcAg may be seen with little inflammatory activity.[82,83] On the other hand, the detection of HBsAg but not HBcAg may represent an inactive carrier state.[84]

Therapy is typically recommended for individuals with high HBV DNA and serum ALT levels by all treatment guidelines without the need for a liver biopsy. The use of a liver biopsy in the management of chronic HBV infection is advocated in individuals who are >40 years and have active viral replication but have normal or near normal serum ALT.[46,47,85,86] Age over 40 years has been associated with significant necroinflammatory activity and advanced fibrosis,[87] while10-30% of patients with normal serum ALT have been found to have significant fibrosis and may be at risk for progression of disease.[88-91] The finding of significant histologic disease in this setting is an indication to initiate therapy.[46,47,85,86] A liver biopsy would also be useful in individuals who are suspected to have cirrhosis, as they are at higher risk for HCC and poorer outcomes.[92] Overall, the use of liver biopsy in the management of HBV is highly individualized.

Conclusion

The diagnosis of HBV infection remains largely based on interpretation of a composite of HBV serologic markers. While one can generally easily distinguish acute from chronic infection, chronic infection can transition from one phase to the next (immune tolerance, immune clearance, inactive HBsAg carrier state, and reactivation) or vice versa. A horizontal follow-up of serologic markers and serum ALT over several months is essential to allow for proper identification of the phase of chronic HBV infection and of consequent need for therapy. HBV genotypes are now increasingly used to predict the response to interferon in those individuals who are candidates for the treatment, but its ability to predict the course of the infection and future complications still remains unclear. Liver biopsies are typically obtained in patients with high HBV DNA levels but normal serum ALT to assist in the management decisions.

Disclosure

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References

1. World Health Organization. Hepatitis B. World Health Organization Fact Sheet 204 (Revised August 2008). http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed August 1, 2010. 2008.
2. Perz JF, Armstrong GL, Farrington LA, et al. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45(4):529-538.
3. Glynn SA, Kleinman SH, Schreiber GB, et al. Trends in incidence and prevalence of major transfusion-transmissible viral infections in US blood donors, 1991 to 1996. Retrovirus Epidemiology Donor Study (REDS). JAMA. 2000;284(2):229-235.
4. US CDC. Disease burden from hepatitis A, B and C in the United States. http://www.cdc.gov/Hepatitis/Statistics.htm#section1. Accessed August 1, 2010.
5. Institute of Medicine. Hepatitis and Liver Cancer: A National Strategy for Prevention and Control of Hepatitis B and C. http://www.iom.edu/Reports/2010/Hepatitis-and-Liver-Cancer-A-National-Strategy-for-Prevention-and-Control-of-Hepatitis-B-and-C.aspx. Accessed August 1, 2010.
6. Robinson WS, Clayton DA, Greenman RL. DNA of a human hepatitis B virus candidate. J Virol. 1974;14(2):384-391.
7. Valsamakis A. Molecular testing in the diagnosis and management of chronic hepatitis B. Clin Microbiol Rev. 2007;20(3):426-439.
8. Blumberg BS, Alter HJ, Visnich S. A "New" Antigen in Leukemia Sera. JAMA. 1965;191:541-546.
9. Weber B, Bayer A, Kirch P, et al. Improved detection of hepatitis B virus surface antigen by a new rapid automated assay. J Clin Microbiol. 1999;37(8):2639-2647.
10. Weber B, Dengler T, Berger A, et al. Evaluation of two new automated assays for hepatitis B virus surface antigen (HBsAg) detection: IMMULITE HBsAg and IMMULITE 2000 HBsAg. J Clin Microbiol. 2003;41(1):135-143.
11. Weber B, Van der Taelem-Brule N, Berger A, et al. Evaluation of a new automated assay for hepatitis B surface antigen (HBsAg) detection VIDAS HBsAg Ultra. J Virol Methods. 2006;135(1):109-117.
12. Liaw YF, Sheen IS, Chen TJ, et al. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. Hepatology. 1991;13(4):627-631.

13. Fattovich G, Farci P, Rugge M, et al. A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HBeAg. Hepatology. 1992;15(4):584-589.
14. Nam SW, Jung JJ, Bae SH, et al. Clinical outcomes of delayed clearance of serum HBsAG in patients with chronic HBV infection. Korean J Intern Med. 2007;22(2):73-76.
15. Wu TT, Hsu HC, Chen DS, et al. Clearance of hepatitis B surface antigen (HBsAg) after surgical resection of hepatocellular carcinoma. J Hepatol. 1987;4(1):45-51.
16. Shukla NB, Poles MA. Hepatitis B virus infection: co-infection with hepatitis C virus, hepatitis D virus, and human immunodeficiency virus. Clin Liver Dis. 2004;8(2):445-460, viii.
17. Ozaras R, Tabak F, Tahan V, et al. Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment. Dig Dis Sci. 2008;53(11):2995-2998.
18. Akhan SC, Yulugkural Z, Vahaboglu H. Response to interferon-alpha in chronic hepatitis B patients with and without precore mutant strain and effects on HBsAg titers. Chemotherapy. 2007;53(6):402-406.
19. Chan HL, Wong VW, Tse AM, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol. 2007;5(12):1462-1468.
20. Tsang TK, Blei AT, O'Reilly DJ, et al. Clinical significance of concurrent hepatitis B surface antigen and antibody positivity. Dig Dis Sci. 1986;31(6):620-624.
21. Tabor E, Gerety RJ, Smallwood LA, et al. Coincident hepatitis B surface antigen and antibodies of different subtypes in human serum. J Immunol. 1977;118(1):369-370.
22. Maruyama T, Schodel F, Iino S, et al. Distinguishing between acute and symptomatic chronic hepatitis B virus infection. Gastroenterology. 1994;106(4):1006-1015.
23. Huang YW, Lin CL, Chen PJ, et al. Higher cut-off index value of immunoglobulin M antibody to hepatitis B core antigen in Taiwanese patients with hepatitis B. J Gastroenterol Hepatol. 2006;21(5):859-862.
24. Chevrier MC, St-Louis M, Perreault J, et al. Detection and characterization of hepatitis B virus of anti-hepatitis B core antigen-reactive blood donors in Quebec with an in-house nucleic acid testing assay. Transfusion. 2007;47(10):1794-1802.
25. Hadler SC, Murphy BL, Schable CA, et al. Epidemiological analysis of the significance of low-positive test results for antibody to hepatitis B surface and core antigens. J Clin Microbiol. 1984;19(4):521-525.
26. Joller-Jemelka HI, Wicki AN, Grob PJ. Detection of HBs antigen in "anti-HBc alone" positive sera. J Hepatol. 1994;21(2):269-272.
27. Kao JH, Chen PJ, Lai MY, et al. Occult hepatitis B virus infection and clinical outcomes of patients with chronic hepatitis C. J Clin Microbiol. 2002;40(11):4068-4071.
28. Lok AS, Lai CL, Wu PC. Prevalence of isolated antibody to hepatitis B core antigen in an area endemic for hepatitis B virus infection: implications in hepatitis B vaccination programs. Hepatology. 1988;8(4):766-770.
29. Draelos M, Morgan T, Schifman RB, et al. Significance of isolated antibody to hepatitis B core antigen determined by immune response to hepatitis B vaccination. JAMA. 1987;258(9):1193-1195.
30. McIntyre A, Nimmo GR, Wood GM, et al. Isolated hepatitis B core antibody--can response to hepatitis B vaccine help elucidate the cause? Aust N Z J Med. 1992;22(1):19-22.
31. Chung HT, Lee JS, Lok AS. Prevention of posttransfusion hepatitis B and C by screening for antibody to hepatitis C virus and antibody to HBcAg. Hepatology. 1993;18(5):1045-1049.
32. Douglas DD, Taswell HF, Rakela J, et al. Absence of hepatitis B virus DNA detected by polymerase chain reaction in blood donors who are hepatitis B surface antigen negative and antibody to hepatitis B core antigen positive from a United States population with a low prevalence of hepatitis B serologic markers. Transfusion. 1993;33(3):212-216.
33. Silva AE, McMahon BJ, Parkinson AJ, et al. Hepatitis B virus DNA in persons with isolated antibody to hepatitis B core antigen who subsequently received hepatitis B vaccine. Clin Infect Dis. 1998;26(4):895-897.
34. Cacciola I, Pollicino T, Squadrito G, et al. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N Engl J Med. 1999;341(1):22-26.
35. Koike K, Kobayashi M, Gondo M, et al. Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients. J Med Virol. 1998;54(4):249-255.
36. Dickson RC, Everhart JE, Lake JR, et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. Gastroenterology. 1997;113(5):1668-1674.
37. Hoofnagle JH, Seeff LB, Bales ZB, et al. Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. N Engl J Med. 1978;298(25):1379-1383.
38. McMahon BJ, Parkinson AJ, Helminiak C, et al. Response to hepatitis B vaccine of persons positive for antibody to hepatitis B core antigen. Gastroenterology. 1992;103(2):590-594.
39. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008;48(2):335-352.
40. Fried MW, Piratvisuth T, Lau GK, et al. HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. Hepatology. 2008;47(2):428-434.
41. Chen CJ, Yang HI, Iloeje UH. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. Hepatology. 2009;49(5 Suppl):S72-84.
42. Chen CJ, Iloeje UH, Yang HI. Long-term outcomes in hepatitis B: the REVEAL-HBV study. Clin Liver Dis. 2007;11(4):797-816, viii.
43. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295(1):65-73.
44. Iloeje UH, Yang HI, Jen CL, et al. Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol. 2007;5(8):921-931.
45. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130(3):678-686.
46. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: 2008 update. Clin Gastroenterol Hepatol. 2008;6(12):1315-1341; quiz 1286.
47. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50(3):661-662.
48. Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. N Engl J Med. 1990;323(5):295-301.
49. Yuen MF, Hui CK, Cheng CC, et al. Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: The effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. Hepatology. 2001;34(1):139-145.
50. Yuen MF, Sablon E, Hui CK, et al. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology. 2001;34(4 Pt 1):785-791.
51. Lai CL, Gane E, Liaw YF, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med. 2007;357(25):2576-2588.
52. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. Gastroenterology. 2001;120(7):1828-1853.
53. Hoofnagle JH, Doo E, Liang TJ, et al. Management of hepatitis B: summary of a clinical research workshop. Hepatology. 2007;45(4):1056-1075.
54. Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. Hepatology. 2006;43(2 Suppl 1):S173-181.
55. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49(5 Suppl):S45-55.
56. Hoofnagle JH, Dusheiko GM, Seeff LB, et al. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med. 1981;94(6):744-748.
57. Lok AS, Lai CL, Wu PC, et al. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology. 1987;92(6):1839-1843.
58. McMahon BJ, Holck P, Bulkow L, et al. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med. 2001;135(9):759-768.
59. Chang MH, Hsu HY, Hsu HC, et al. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special

emphasis on the clearance of hepatitis B e antigen before 3 years of age. Hepatology. 1995;22(5):1387-1392.
60. Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. Hepatology. 1988;8(5):1130-1133.
61. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. Gastroenterology. 1984;86(2):230-235.
62. Norder H, Courouce AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004;47(6):289-309.
63. Schaefer S. Hepatitis B virus: significance of genotypes. J Viral Hepat. 2005;12(2):111-124.
64. Schaefer S. Hepatitis B virus genotypes in Europe. Hepatol Res. 2007;37(S1):S20-26.
65. Devesa M, Pujol FH. Hepatitis B virus genetic diversity in Latin America. Virus Res. 2007;127(2):177-184.
66. Fung SK, Lok AS. Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology. 2004;40(4):790-792.
67. Lai CL, Ratziu V, Yuen MF, et al. Viral hepatitis B. Lancet. 2003;362(9401):2089-2094.
68. Chu CJ, Keeffe EB, Han SH, et al. Hepatitis B virus genotypes in the United States: results of a nationwide study. Gastroenterology. 2003;125(2):444-451.
69. Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology. 2002;122(7):1756-1762.
70. Kao JH, Chen PJ, Lai MY, et al. Hepatitis B virus genotypes and spontaneous hepatitis B e antigen seroconversion in Taiwanese hepatitis B carriers. J Med Virol. 2004;72(3):363-369.
71. Nakayoshi T, Maeshiro T, Nakasone H, et al. Difference in prognosis between patients infected with hepatitis B virus with genotype B and those with genotype C in the Okinawa Islands: a prospective study. J Med Virol. 2003;70(3):350-354.
72. Ni YH, Chang MH, Wang KJ, et al. Clinical relevance of hepatitis B virus genotype in children with chronic infection and hepatocellular carcinoma. Gastroenterology. 2004;127(6):1733-1738.
73. Sumi H, Yokosuka O, Seki N, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology. 2003;37(1):19-26.
74. Yuen MF, Sablon E, Yuan HJ, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology. 2003;37(3):562-567.
75. Kao JH. Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J Gastroenterol Hepatol. 2002;17(6):643-650.
76. Kobayashi M, Arase Y, Ikeda K, et al. Clinical features of hepatitis B virus genotype A in Japanese patients. J Gastroenterol. 2003;38(7):656-662.
77. Kobayashi M, Arase Y, Ikeda K, et al. Clinical characteristics of patients infected with hepatitis B virus genotypes A, B, and C. J Gastroenterol. 2002;37(1):35-39.
78. Suzuki Y, Kobayashi M, Ikeda K, et al. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. J Med Virol. 2005;76(1):33-39.
79. Mayerat C, Mantegani A, Frei PC. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? J Viral Hepat. 1999;6(4):299-304.
80. Mani H, Kleiner DE. Liver biopsy findings in chronic hepatitis B. Hepatology. 2009;49(5 Suppl):S61-71.
81. Ruiz-Moreno M, Otero M, Millan A, et al. Clinical and histological outcome after hepatitis B e antigen to antibody seroconversion in children with chronic hepatitis B. Hepatology. 1999;29(2):572-575.
82. Naoumov NV, Portmann BC, Tedder RS, et al. Detection of hepatitis B virus antigens in liver tissue. A relation to viral replication and histology in chronic hepatitis B infection. Gastroenterology. 1990;99(4):1248-1253.
83. Walewska-Zielecka B, Madalinski K, Jablonska J, et al. Composition of inflammatory infiltrate and its correlation with HBV/HCV antigen expression. World J Gastroenterol. 2008;14(25):4040-4046.
84. Martinot-Peignoux M, Boyer N, Colombat M, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. J Hepatol. 2002;36(4):543-546.
85. European Association For The Study Of The L. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol. 2009;50(2):227-242.
86. Liaw YF. 2008 APASL guidelines for HBV management (Provisional). http://www.apasl.info/pdf/GuidelinesHBV.pdf. Accessed August 9, 2010. 2008.
87. Cadranel JF, Lahmek P, Causse X, et al. Epidemiology of chronic hepatitis B infection in France: risk factors for significant fibrosis--results of a nationwide survey. Aliment Pharmacol Ther. 2007;26(4):565-576.
88. Dixit VK, Panda K, Babu AV, et al. Asymptomatic chronic hepatitis B virus infection in northern India. Indian J Gastroenterol. 2007;26(4):159-161.
89. Kumar M, Sarin SK, Hissar S, et al. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology. 2008;134(5):1376-1384.
90. Lai M, Hyatt BJ, Nasser I, et al. The clinical significance of persistently normal ALT in chronic hepatitis B infection. J Hepatol. 2007;47(6):760-767.
91. Tsang PS, Trinh H, Garcia RT, et al. Significant prevalence of histologic disease in patients with chronic hepatitis B and mildly elevated serum alanine aminotransferase levels. Clin Gastroenterol Hepatol. 2008;6(5):569-574.
92. Taylor BC, Yuan JM, Shamliyan TA, et al. Clinical outcomes in adults with chronic hepatitis B in association with patient and viral characteristics: A systematic review of evidence. Hepatology. 2009;49(5 Suppl):S85-95.