Changing scene in hepatitis B serology interpretation

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Serological tests have been used in the diagnosis of hepatitis B virus (HBV) infection since the virus was first discovered. Advances in molecular biology and improvements in the understanding of HBV virology have changed the scene of hepatitis B serology interpretation.

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Hepatitis B virus (HBV) infection was first diagnosed by Blumberg et al in 1965 after the discovery of the Australia antigen, now called hepatitis B surface antigen (HBsAg). Over the next 10 years, serological assays for HBsAg and hepatitis B surface antibody (anti-HBs) were developed, and additional HBV antigens and antibodies were subsequently identified. The diagnosis and definition of the natural history of HBV infection has been based on these serological tests (*Table 1*). Over the past decade, the development of the polymerase chain reaction (PCR), permitting the detection of as few as 10 molecules of HBV DNA per millilitre of serum, and sequencing of the entire genome of the HBV virion have provided a new insight into the virology and natural history of HBV infection. The introduction of HBV vaccination and new therapeutics have also changed the way in which the results of HBV serology tests are interpreted.

TABLE 1.
Interpretation of hepatitis B virus (HBV) serology markers

HBV marker	Viral antigens	Clinical interpretations	
HBsAg	Viral envelope and sub-viral particles	HBV infection: acute or chronic	
Anti-HBs	–	Immunity of HBV infection	Recovered HBV infection
			Vaccinated
HBeAg	Secretary viral antigen	Replicative phase: high level of replication and infectivity	
Anti-HBe	–	Low replicative phase: low level of replication and infectivity	
HBcAg	Viral core protein	Not detectable in serum	
Anti-HBc immunoglobulin M	–	Recent HBV infection	
		Exacerbation of chronic HBV infection	
Anti-HBc immunoglobulin G	–	Recovered HBV infection	
		Chronic HBV infection	

Anti-HBc = hepatitis B core antibody; Anti-HBe = hepatitis Be antibody; Anti-HBs = hepatitis B surface antibody; HBcAg = hepatitis B core antigen; HBeAg = hepatitis Be antigen; HBsAg = hepatitis B surface antigen

HEPATITIS B SURFACE ANTIGEN AND ANTIBODY

Natural history

HBsAg is the serological hallmark of HBV infection. It appears in the serum 1–10 weeks after acute exposure to HBV. In patients who subsequently recover from HBV infection, HBsAg usually becomes undetectable after 4–6 months. This is followed by the appearance of anti-HBs. Although anti-HBs is produced early in the course of an acute infection in individuals who subsequently recover, it may not be detectable for several weeks or even months, so that there is a period when neither HBsAg nor anti-HBs can be detected. In most patients, anti-HBs persists for life, conferring long-term immunity. Persistence of HBsAg for more than 6 months implies chronic infection.

Co-existence of HBsAg and anti-HBs

HBV can be classified into genotypes A–G (Norder et al, 1994; Stuyver et al, 2000) and four major subtypes based on the nucleotide and amino acid configuration of the HBV surface region. All HBV subtypes share one common antigenic determinant ('a'), which is a conformational epitope located in the HBsAg. There are two additional pairs of mutually exclusive subtypic determinants ('d' or 'y' and 'w' or 'r') constituting the four major subtypes (adr, ayr, adw and ayw). Antibodies to the 'a' determinant confer protection to all HBV subtypes. In most circumstances, anti-HBs that develop after recovery from acute hepatitis B or immunization with hepatitis B vaccines which consist of recombinant HBsAg are directed against the 'a' determinant, thus providing cross-protection against other subtypes of HBV.

Coexistence of HBsAg and anti-HBs has been reported in about 24% of HBsAg positive individuals (Tsang et al, 1986). In most instances, the antibodies in these individuals are directed against one of the subtypic determinants, and not the common 'a' determinant, and are unable to neutralize the circulating virions. Therefore, the immunity to HBV in these individuals is incomplete and they should be regarded as HBV carriers.

Vaccine escape mutants

After three intramuscular doses of recombinant HBV vaccine, 95–99% of healthy infants, children and young adults develop protective serum titres of anti-HB antibodies (>10 IU/litre) (Lemon and Thomas, 1997). Although the anti-HB titre may fall below 10 IU/litre years after an adequate response to the vaccination, the immunological memory can still launch an anamnestic antibody response to antigen challenge that protects against breakthrough infection (European Consensus Group on Hepatitis B Immunity, 2000). Reappearance of HBsAg after a successful antibody response to vaccination indicates the development of a vaccine escape mutant. The commonest reported vaccine escape mutation involves a single amino acid substitution of glycine to arginine at amino acid 145 of the 'a' determinant of the surface antigen (G145R) (Carman et al, 1990). These mutants are also found among liver transplantation recipients who receive high-dose hepatitis B immunoglobulin as a prophylaxis against HBV recurrence.

Occult HBV infection

HBV DNA has been demonstrated in serum and liver tissue of HBsAg-negative patients suffering from chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Brechot et al, 1985; Paterlini et al, 1990). Residual HBV infection has also been shown in patients who had remission of hepatitis and clearance of HBsAg, either spontaneously or as a result of antiviral therapy. Some of these patients may have been carriers with subdetectable levels of HBsAg, some may have integrated HBV DNA into their liver tissues without secreting HBsAg and others may be infected with HBV mutants with alterations in the 'a' determinant that result in the down-regulation of HBsAg production or the production of aberrant HBsAg that cannot be detected by conventional serological assays. In these cases, determination of HBV DNA by sensitive assays would be mandatory for the diagnosis of occult HBV infection, particularly among patients suffering from liver disease with undetermined aetiology in areas where HBV infection is prevalent. This is equally important in patients with fulminant hepatitis B, who may have cleared HBsAg by the time they present.

HEPATITIS B CORE ANTIGEN

Immunoglobulin M antibody

Hepatitis B core antigen (HBcAg) is an intracellular antigen expressed in infected hepatocytes. It is not detectable in serum. In contrast, anti-hepatitis B core (anti-HBc) antibodies can be detected throughout the course of HBV infection. The immunoglobulin (Ig) M anti-HBc antibody usually appears within 1 month of the appearance of HBsAg during acute HBV infection, and its titre declines during convalescence. It is the marker of HBV infection during the serological window, i.e. the time gap between the disappearance of HBsAg and the appearance of anti-HBs. Therefore, the detection of IgM anti-HBc is usually taken as an indication of acute HBV infection.

Low titre IgM anti-HBc persists in most patients with chronic HBV infection. Even in assays with high cut-off values, IgM anti-HBc can be detected in patients with chronic HBV infection during exacerbations of the disease (Maruyama et al, 1994). This may lead to a misdiagnosis of acute hepatitis B in patients who have not previously been known to have chronic HBV infection and an overestimation of the rate of progression to chronicity.

Immunoglobulin G antibody

IgG anti-HBc appears during acute HBV infection. It persists along with anti-HBs in patients who recover from acute hepatitis B and in association with HBsAg in patients who progress to chronic HBV infection. Therefore, the presence of IgG anti-HBc is usually taken as evidence of previous exposure to HBV, and it usually exists in the presence of anti-HBs antibodies.

Isolated anti-HBc

The isolated presence of anti-HBc in the absence of HBsAg and anti-HBs has been reported in 0.4–1.7% of blood donors in low prevalence areas and in 10–20% of the population in endemic countries (Hadler et al, 1984; Chung et al, 1993). Isolated detection of anti-HBc may occur many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels, or after many years of chronic HBV infection when the HBsAg titre has decreased below the cut-off level for detection. The clinical significance of isolated anti-HBc is

unclear. Several studies found that 50–70% of asymptomatic individuals with isolated anti-HBc have false positive test results (Lok et al, 1988; McMahon et al, 1992). HBV DNA has been detected in the serum of individuals with isolated anti-HBc, when tested by PCR assays, at frequencies varying from 0–20%. Transmission of HBV infection has been reported from blood and organ (non-liver) donors with isolated anti-HBc, but the incidence was very low (Japanese Red Cross Non A, Non B Hepatitis Research Group, 1991; Chung et al, 1993). In contrast, the rate of HBV transmission through liver transplantation from donors with isolated anti-HBc ranged from 33–78% (Dickson et al, 1997; Douglas et al, 1997). This suggests that the virus may persist in the liver despite serological resolution of the infection.

To evaluate an individual with isolated anti-HBc detected for the first time, repeat tests for anti-HBc should be carried out, preferably by radioimmunoassays, which show fewer false positive results than enzyme immunoassays. To rule out the window phase of acute HBV infection, the presence of IgM anti-HBc should be tested for, and late immunity should be determined by testing for anti-HBs 1–3 months later. Individuals with evidence of chronic liver disease should be tested for HBV DNA by PCR to exclude low-level chronic HBV infection.

HEPATITIS BE ANTIGEN AND ANTIBODY

Hepatitis Be antigen

Hepatitis Be antigen (HBeAg) is a secretory protein that is processed from the precore protein. It is generally considered to be a marker of HBV replication and infectivity. Epidemiological studies report significantly higher rates of transmission of HBV infection from HBeAg-positive carrier mothers to their babies and from HBeAg-positive patients to health-care workers who sustain needle-stick injuries (Alter et al, 1976; Beasley et al, 1977).

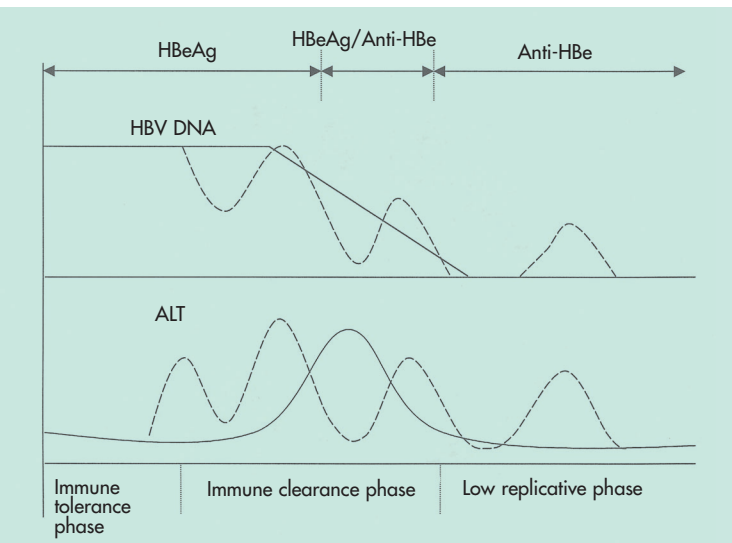

Figure 1. Natural history of perinatally acquired chronic hepatitis B virus (HBV) infection. ALT = alanine aminotransferase; HBeAG = hepatitis Be antigen.

Acute HBV infection

During acute HBV infection, HBeAg appears shortly after the appearance of HBsAg. In patients who recover, HBeAg to anti-HBe seroconversion precedes that of HBsAg to anti-HBs seroconversion. Anti-HBe may persist for many years after resolution of acute HBV infection. The presence of anti-HBe antibodies does not indicate protective immunity.

Chronic HBV infection

In patients with chronic HBV infection, HBeAg may persist for years or even for decades. In adult-acquired HBV infection, positive HBeAg is usually associated with elevated HBV DNA and active liver disease. In patients with perinatally acquired HBV infection, there may be an immune tolerance phase with positive HBeAg, high HBV DNA, normal alanine aminotransferase (ALT) levels and minimal inflammation in the liver (Lok and Lai, 1988) (*Figure 1*). Experiments in mice suggest that transplacental transfer of maternal HBeAg may induce a specific unresponsiveness of T-helper cells to HBeAg in neonates born to HBeAg-positive carrier mothers (Milich et al, 1990). Because HBeAg and HBcAg are highly cross-reactive at the T cell level, deletion of the T-helper cell response to HBeAg results in an ineffective cytotoxic T cell response to HBcAg.

HBeAg seroconversion is frequently accompanied by biochemical exacerbations (abrupt increases in ALT levels) because of a sudden increase in immune-mediated lysis of infected hepatocytes. Seroconversion from HBeAg to anti-HBe is usually associated with the disappearance of HBV DNA in serum and remission of liver disease (*Figure 1*). Not all patients can undergo successful HBeAg seroconversion, and prolonged abortive HBeAg seroconversion is associated with continuous liver damage and increases the risk of liver cirrhosis and possibly hepatocellular carcinoma.

After HBeAg seroconversion, approximately 20–33% of anti-HBe positive patients continue to have active liver disease and detectable HBV DNA in their serum (ter Borg et al, 1998; Chan et al, 2000a). Some patients may have reversion of HBeAg from anti-HBe accompanied by biochemical exacerbations. Precore stop codon

mutant and core promoter mutants have been seen to abolish and downregulate the production of HBeAg respectively, and both these mutants can replicate better than the wild-type HBV in vitro (Scaglioni et al, 1997). However, no clear association can be demonstrated between these HBeAg-negative mutants and disease activity in vivo (Chan et al, 2000b).

Antiviral therapy

HBeAg seroconversion is commonly used as a marker of response to interferon and antiviral agents. However, up to 50% of patients have a relapse after lamivudine treatment is stopped (Song et al, 2000). A sustained virological response is usually defined as HBeAg seroconversion plus the disappearance of HBV DNA, preferably determined by PCR-based assays, lasting for at least 6 months after the cessation of treatment.

CONCLUSIONS

No single serological test can unequivocally diagnose HBV infection. Clinicians should be aware of the various limitations of different serological tests. The interpretation of HBV serology results should be carefully combined with HBV DNA and biochemical results in the context of the clinical situation. **HM**

Conflict of interest: none.

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KEY POINTS

- Positive hepatitis B surface antigen (HBsAg) is the hallmark of hepatitis B virus (HBV) infection.
- Negative HBsAg in patients with chronic liver disease or hepatocellular carcinoma cannot exclude HBV infection.
- The appearance of HBsAg in HBV vaccine responders indicates the development of vaccine escape mutants.
- Immunoglobulin M anti-HBc can be detected in both acute HBV infection and exacerbation of chronic HBV infection.
- The significance of isolated anti-HBc is not certain, but the risk of HBV transmission from donors with isolated anti-HBc in liver transplantation is significant.
- Positive HBeAg reflects high infectivity.
- HBeAg seroconversion to anti-HBe reflects immune clearance of the virus.
- Some patients with negative HBeAg and positive anti-HBe have active liver disease and may revert back to positive HBeAg.