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Journal of Hepatology 44 (2006) S71–S76

Journal of Hepatology

www.elsevier.com/locate/jhep

HBV virological assessment

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Management of hepatitis B virus (HBV) infected patients involves serological diagnosis, quantitation of HBV–DNA and measurement of HBV drug resistance. Different serological markers such as HBsAg, anti-HBs, anti-HBc (total and IgM), HBeAg and anti-HBe are assessed by immunoassays in order to define the infection status. The emergence of surface mutants however is a continuous challenge to design more effective immunoassays. Commercially available quantitative HBV–DNA assays with increased sensitivity and wider linear range give a more accurate estimate of viral replication and contribute decisively in the initiation and the monitoring of the response to HBV therapy. Genotypic drug resistance assays are important diagnostic tools, since the administration of nucleoside/nucleotide analogues to HBV infected patients leads to the development of drug resistance patterns very much dependent on the treatment regimen. Special issues have to be taken into consideration regarding HBV/HIV-1 co-infected patients, since concominant HIV and HBV replication results in higher rates of HBV replication. Current efforts are focused on the standardization of HBV–DNA assays (qualitative and quantitative), of HBV drug resistance assays as well as in the development of new assays and markers that will help in the prognosis and management of HBV infection (quantitative detection of pre-core mutants and HBV ccc-DNA assays).

Keywords: **Hepatitis B; HIV; Immunoassays; DNA assays**

1. Introduction

HBV belongs to the hepadnaviridae family of enveloped viruses with double-stranded DNA genome of 3200 bp length. The HBV genome consists of four major overlapping ORFs namely pre-surface/ surface (pre-S/S), core (C), polymerase (P), and X ORF (X) (Fig. 1). HBV that infects humans is classified into eight genotypes (A-H) (Fig. 2) which have a distinct geographical distribution and according to various studies seem to have different biological properties affecting, thus, the clinical outcome of HBV disease.

2. Serological tests for HBV infection

HBsAg (hepatitis B surface antigen) is the primary marker for identification of acute HBV infection (Fig. 3a,b). Typically, HBsAg becomes detectable 6–10 weeks after exposure to the virus. In the past, less sensitive immunoassays failed to identify acute HBV infection resulting in a prolonged window period. Modern automated EIAs are more sensitive and more specific in detecting HBsAg than previous generation tests [1–7]. The currently approved HBsAg tests have shortened the window period by up to 9 days compared to previous tests [8]. Newly designed immunoassays are also effective in detecting surface mutants as well, albeit not with equal effectiveness [9–12]. During resolution of HBV infection after 4–6 months, anti-HBs levels increase whereas HBsAg falls. Anti-HBs guarantees life-long immunity in most people. Anti-HBs also appear in the serum of individuals vaccinated for HBV. The positive predictive value for predicting anti-HBs($\geq$10 mIU/ml which is associated with immunity is 97.6% [13].

Another immunological marker of acute HBV infection which was used during the window-period in the past is IgM anti-HBc (hepatitis B core) which is also detected during flares of chronic hepatitis. IgM anti-HBc appears shortly after HBsAg and persists for 6–24 months. It

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0168-8278/$30.00
doi:10.1016/j.jhep.2005.11.017

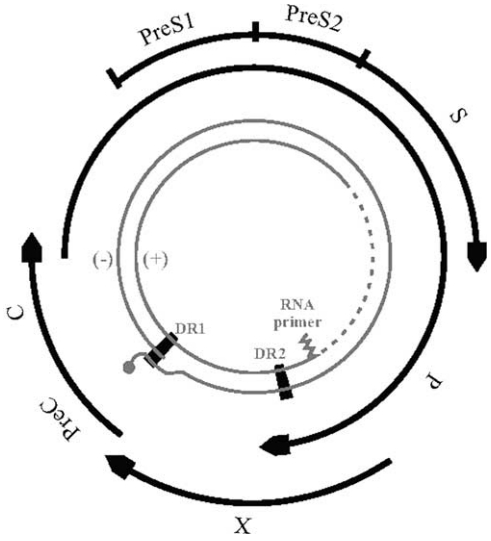

Fig. 1. The organisation of the HBV genome. The pre-S/S gene (1169 bp) codes for the putative hepatocyte-receptor binding site and the HBV surface antigen (HBsAg), pre-core/core gene (C) (638 bp) codes for HBV core antigen and HBV early antigen (HBeAg) while the polymerase gene (P) (2498 bp) codes for the HBV DNA polymerase which is responsible for the reverse transcription step during HBV life cycle. The X gene (464 bp) codes for a protein which plays an essential role in regulation of HBV transcription and is involved in HBV-related hepatocellular carcinoma (HCC). DR1 and DR2 (direct repeat 1 and 2) are sequence modules that mediate HBV reverse transcription (length of genes in brackets correspond to HBV genotype D strain with accession number X02496).

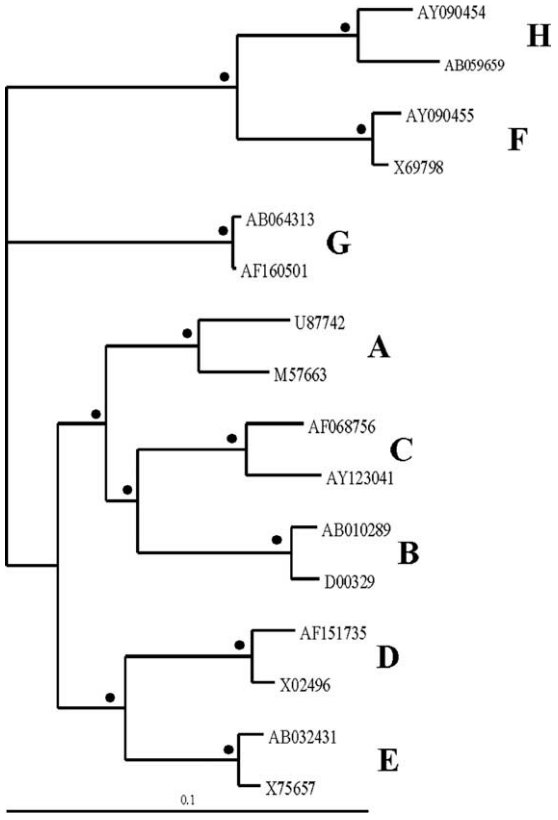

Fig. 2. Phylogenetic tree depicting the eight known HBV genotypes infecting humans. Two strains belong to a different HBV genotype if sequence divergence in the entire genome is greater than 8%. Bullets represent statistically significant clustering.

then gives place to IgG anti-HBc during the resolution of HBV infection. Commercial assays detect total anti-HBc and IgM anti-HBc. IgG anti-HBc presence is therefore inferred when anti-HBc total is detectable and IgM anti-HBc is undetectable.

There are certain issues regarding HBsAg immunoassays: a negative HbsAg test on a few occasions does not exclude an active HBV infection; up to 5% of healthy blood donors have an isolated anti-HBc result; and an isolated anti-HBc result is more common in HBV patients co-infected with HIV-1 (see also special issues regarding HIV-1/HBV co-infected patients) [14,15]. Several studies have shown that 0.5–1% of HBsAg negative/anti-HBc reactive samples contain low HBV DNA levels [16–18]. Another possible explanation for an isolated anti-HBc positive result could be co-infection with HCV, super-infection with or emergence of surface mutants which occur during therapy with nucleoside inhibitors, or false positive results of anti-HBs assays [19,20].

Patients that are negative for HBsAg, but positive for HBV DNA, with or without the presence of HBV antibodies, correspond to 'occult' HBV infection [21]. In occult HBV infection, ultra-sensitive PCR assays with detection limits of less than 10 copies of HBV–DNA per reaction should be employed. [22].

Finally, HBeAg (hepatitis B early antigen) is a serum secreted protein which can be invariably detected by commercial immunoassays during acute HBV infection 6–12 weeks after exposure to HBV and is connected with high transmissibility, infectivity and active viral replication. Clearance of HBeAg is associated with concomitant seroconversion to anti-HBe. Persistance of HBeAg for three to four months signifies advance to chronic HBV infection. When seroconversion to anti-HBe occurs in chronic infection, HBV–DNA may drop.

A precore mutation (G1896→A, codon 28) that results in defective synthesis of HBeAg through the creation of a stop codon can occur [23,24]. Three other mutations (at nt positions 1817, 1874 and 1897) have been reported to cause truncations in the HBeAg. Additional changes that affect the initiation codon have been described at nt 1814, 1815 and 1816. Precore mutants are associated with increased virulence for severe liver disease and fulminant hepatitis B and prevail in the Mediterranean countries where genotype D is predominant [25–29].

The BCP (basal core promoter) mutations such as A1762T plus G1746A may be found isolated or in conjunction with precore mutations. The double A1762T/G1746A mutation leads to a decrease of HBeAg