Clinical Virology of Hepatitis B

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NATURAL HISTORY OF HEPATITIS B VIRUS INFECTION [1, 2, 3]

The natural history of hepatitis B infection varies according to the age that the infection is contracted. In adults with normal immunological status less than five percent of individuals with acute hepatitis B infection develop chronic infection, characterized by persistent circulating viral antigens and viral DNA and most acute infections resolve spontaneously because the immune system eliminates infected cells while developing antibodies to viral surface antigens. However, acute infection is associated with clinical manifestations in approximately one third of patients. On the other hand, the rate of progression to chronic hepatitis is much higher when the infection is acquired in childhood, even though the initial infection is usually clinically asymptomatic. Moreover, progression to chronic hepatitis is more frequent in subjects with immunodepression.

The clinical manifestations of the chronic phase of the disease depend on the immune attack on infected cells, resulting in elevated serum transaminase levels and in some cases clinical symptoms. The natural course of infection is characterized by distinctive phases that differ in the replicative activity of the virus and the intensity of the immune response (Table 1). Generally, in the early stages of infection, the infected cells do not stimulate an immune response and continue to shed viral particles. This immunotolerant phase is characterized by

high serum levels of viral antigens and DNA and normal transaminase levels. Then the immune system mounts an attack on infected hepatocytes. During this immunoactive stage, serum levels of viral DNA fall, transaminase levels rise, and clinical symptoms may appear. If this stage persists because the immune system fails to control viral infection, liver damage will lead to chronic hepatitis. However chronic infection may remain clinically silent for significant periods. In many patients, hepatitis Be antigen (HBeAg) seroconversion occurs and HBe antigens disappear while anti-HBe antibodies appear, although low levels of DNA may persist. Biological markers of hepatic function normalize. This is predictive of a good clinical outcome, and indicates good immunological control of liver infection (Figure 1). In this phase, patients are inactive carriers of hepatitis B virus. As viral supercoiled DNA persists in the liver, episodes of viral reactivation may occur either spontaneously or because of immune suppression. It should be noted that certain individuals carry hepatitis B strains that bear mutations and prevent HBe antigen expression. These individuals may thus present with active chronic hepatitis without serum HBeAg. The prevalence of chronic HBeAg-negative hepatitis varies from region to region, and is more prevalent in the Mediterranean basin, for example, than elsewhere in Europe. There is also some evidence that this form of hepatitis is associated with more severe liver disease than HBeAg-positive hepatitis. In patients with chronic infection, the incidence of cirrhosis and of hepatocellular carcinoma is less than ten cases per hundred patient years. The five-year mortality rate for uncomplicated chronic hepatitis is 0-2%, for chronic hepatitis with compensated cirrhosis is 14-20% and for chronic hepatitis with decompensation is 70-86%.

Infection phase	Serum markers	Clinical risk
Immunotolerant phase	HBV DNA high HBsAg high HBeAg high Transaminases normal	High risk of transmission to the household
Immunoactive phase Chronic hepatitis	HBV DNA decrease HBsAg high HBeAg decrease Transaminases high	Development of cirrhosis and HCC
Low-replicative phase Inactive carrier state	HBV DNA very low HBsAg decrease HBeAg absent, anti-Hbe-positive Transaminases normal	Development of HCC Viral reactivation: wild type or pre-core mutant
HBsAg clearance phase	HBsAg-negative Anti-HBc-positive Anti-HBs-positive or -negative Normal ALT HBV DNA may be positive by PCR	Occult HBV infection Viral reactivation (immune suppression) Transmission of occult HBV (nosocomial infection)

Table 1: Evolution of serum markers in the different phases of chronic hepatitis B virus infection.

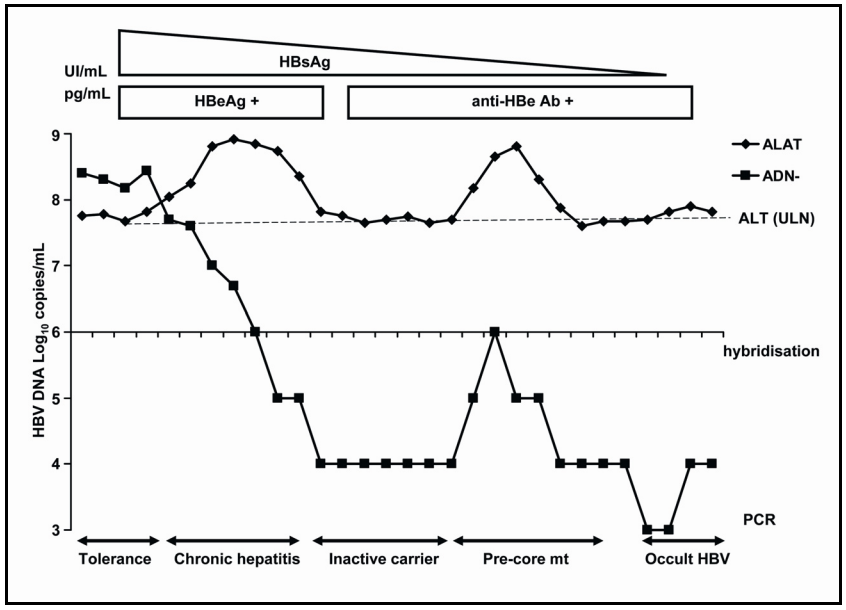

Figure 1: Evolution of virological markers during the natural history of chronic hepatitis B.

MEASURING VIRAL LOAD

Since hepatitis B virus infection can remain clinically silent, it is important to have accurate methods to determine the presence of viral replication to monitor treatment outcomes and to identify changes in viral activity before they provoke clinical symptoms. The most rigorous way of determining viral replication is to measure circulating viral DNA. The threshold for risk of liver damage is around 10^4 virions/mL.

A surrogate measure of viral load is the presence of circulating hepatitis B virus e antigen; this method has been extensively used because it is inexpensive and relative simple compared to DNA measurement. Loss of this antigen and the appearance of anti-HBe

antibody (HBeAg seroconversion) is associated with a decrease in viral DNA titers to below 10^4 copies/mL and clinical remission. However, certain variants of the hepatitis B virus carry mutations in the pre-core region of the HBV genome encoding the HBeAg that prevent determination of serological status.

MONITORING TREATMENT EFFICACY AND RESISTANCE

Patients with hepatitis B treated with antiviral drugs must be closely monitored to assess virological efficacy and to detect any treatment resistance as early as possible. This requires the measurement of viral load, genotyping of viral DNA and assessment of hepatic function (Figure 2).

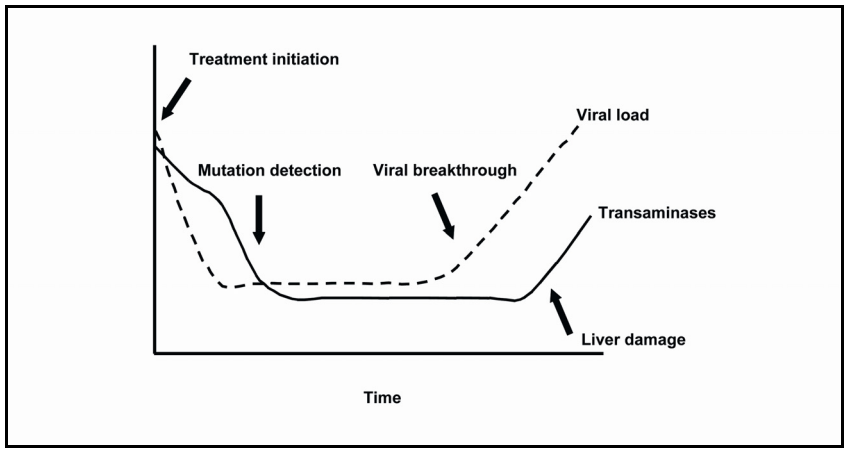

Figure 2: Time-course of emergence of antiviral resistance as measured by evolution of viral load (- -) and serum transaminases (—).

SEROLOGICAL ASSAYS

Regular monitoring of viral markers is necessary to evaluate the virological response to therapy and treatment end-points. In wild type

virus infected patients, HBeAg seroconversion is one of the major aims of antiviral therapy. Usually, it follows a rapid decline in serum viral load that may lead to a restored hepatitis B virus (HBV) specific T cell immune response [4, 5]. In patients infected with a pre-core mutant, hepatitis B surface antigen (HBsAg) clearance is a major endpoint.

VIRAL LOAD

Measuring viral load in serum is important to characterize the early virological response and to detect viral breakthrough. Viral breakthrough is generally defined as an increase in viral load of one log unit compared to the nadir value (lowest viral load obtained during therapy). Breakthrough indicates that the resistant strain has become dominant and that the proportion of hepatocytes infected with this strain and actively shedding the virus has become significant. This is a sign of treatment failure and indicates that the patient is again at risk of developing clinically symptomatic hepatitis (Figure 2).

The most recent assays to quantify intrahepatic viral covalently closed circle DNA (cccDNA) show that the kinetics of clearance during adefovir dipivoxil therapy are much slower than that of serum viral load and total intrahepatic viral DNA. These studies suggest that viral cccDNA decline is mainly a result of the inhibition of intracellular recycling and not of a non-cytolytic process. Mathematical modeling suggests that 14 years of therapy would be required to clear viral cccDNA from the liver [6].

GENOTYPING

Genotyping is the only way to confirm that clinical resistance is due to the emergence of a drug-resistant variant strain of hepatitis B. Genotyping has several functions. First, the use of very sensitive assays is the best way to detect resistant strains before viral breakthrough has taken place. This is because resistant strains mainly infect uninfected cells to become the dominant strain while hepatocytes containing the previously dominant drug-sensitive strain are eliminated from the liver. Thus there is a lag between the appearance of a drug-resistant variant that actively sheds virions, the colonization of the liver by this variant and viral breakthrough. The length of time of this lag period depends on the rate that the immune

system removes the wild type virus infected hepatocytes so that they are replaced with healthy cells. Thus, resistant strains may be detected in plasma before any noticeable change occurs in viral load. The interval between detection of drug-resistant strains in patients treated with lamivudine has been shown to precede viral breakthrough by 4 to 6 months [7]. This is important because suitable treatment may be started to control replication of the resistant strain and to prevent worsening of liver disease.

The second reason to use genotyping to identify resistant strains rather than waiting for viral breakthrough is to determine the genetic variant, and to choose the most appropriate alternative treatment in relation to the sensitivity of the variant to antiviral drugs. This will become increasingly relevant as new antiviral drugs with different resistance profiles become available. Furthermore, genotyping confirms the diagnosis of drug resistance by identifiying specific viral mutants thus ruling out any questions about patient compliance.

Different methods are available for genotyping viral strains (Table 2). Direct sequencing identifies all possible mutations including previously unknown mutations. This is particularly pertinent in patients receiving new nucleoside analogs that do not yet have any resistance profiles. However, genotyping can only be used if the proportion of the strain in the total viral population is significant, around 20%. In addition, if multiple mutations are identified direct sequencing cannot determine if they originate from one or multiple strains. This problem can be solved by cloning individual viral strains before sequencing, although this technique is too labor intensive to be used in routine screening. Hybridization with oligonucleotide probes or restriction enzyme polymorphism can be easily automated, but these techniques only identify previously characterized mutations. Hybridization with specific oligonucleotide probes is more sensitive than direct sequencing of Polymerase Chain Reaction (PCR) products, since a minor mutant can be detected even if it is as little as 5% of the total viral population [8]. Although it is only under development for the moment, DNA chip technologies would allow large scale screening of multiple mutations in viral sequences (Table 3).

Direct sequencing using RT-PCR
Sequencing of cloned viral DNA
Hybridization using specific oligonucleotide probes (Lipa)
Size polymorphism of fragments after restriction enzyme digestion
DNA chip technology

Table 2: Available methods for genotyping of hepatitis B viral strains.

	Lamivudine	**Emtricitabine**	**Adefovir dipivoxil**	**Entecavir**	**Telbivudine**
Resistance mutations	M204V M204I	M204V M204I	N236T A181V/T	S202G S202I T184G M250V	M204I
Compensatory mutations	V173L L180M				
Drugs active on resistant mutants	Adefovir Tenofovir Entecavir +/–	Adefovir Tenofovir Entecavir +/–	Lamivudine Emtricitabine Entecavir		Adefovir Tenofovir

Table 3: Main polymerase mutants responsible for drug resistance.

PHENOTYPING ASSAYS

The phenotype of HBV clinical isolates can now be analyzed. Rapid cloning of the entire HBV genome or the polymerase gene of the virus in appropriate vectors allows transfection of single or multiple HBV

clones in hepatoma cell lines to study their replication capacity and sensitivity to drugs [9, 10]. These assays can be used to show that the M204I and L180M+M204V polymerase mutants confer resistance to lamivudine and have a lower replication capacity than the wild type virus and to characterize the newly identified N236T polymerase mutant that causes adefovir resistance [11]. These assays may become critically important as more drugs become available and more resistant mutants are selected by therapy. They also show that combined therapy with compounds that do not have cross-resistance should prevent or significantly delay the selection of drug resistant viruses.

BIOCHEMICAL ASSAYS

Serum transaminases should be monitored as a marker of hepatic function. Elevated transaminases suggest that the immune system is no longer controlling the infection and that extensive lysis of hepatocytes is occurring. This is a signal that a new round of clinical symptoms may occur, since severe acute exacerbation of the disease can be observed in some patients in association with viral resistance. However, alanine aminotransferase (ALT) flares are usually preceded by an increase in viral DNA titers, suggesting that close monitoring of viral markers is required for optimal management of patients receiving antiviral therapy.

MONITORING PROTOCOLS

The choice of a monitoring protocol should take into account the clinical status and history of the patient (for example, HBeAg seroconversion, other risk factors for hepatic disease, immunological status) [12, 13]. The cost of monitoring is significant and will influence the public health policy on the type and frequency of tests when allocating resources. Treatment cost will also be considered in the future, but it should be weighed against the rate of drug resistance for each drug as well as the cost of management of drug resistant patients.

An optimal monitoring regime would be to follow the emergence of mutations using genotyping while measuring viral load, serological status and transaminases. This would provide information about the emergence of potentially resistant strains before intrahepatic spread of the mutant and viral breakthrough, and allow a suitable treatment

strategy to be begun. However, DNA sequencing is resource-intensive and may not be considered cost-effective for routine monitoring, although this may change if DNA chip technology could be used for automated screening. To guarantee consistency, DNA sequencing may also need to be performed by reference centers. The risk of emergence of resistant strains with currently available antiviral drugs may also affect the decision on the need for regular DNA sequencing. Although genomic assays are obviously of interest, there is no consensus on the optimal frequency of these tests.

The frequency of monitoring depends on the severity of hepatitis and the treatment duration. If treatment has been stopped, monitoring of biochemical and virological markers should be continued because of the risk of viral reactivation.

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