

Part 2

Hepatitis B and D

Chapter 8: Diagnostic tests in acute and chronic hepatitis B

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Introduction

Over the past three decades, laboratory diagnostics of viral infections have become more and more influenced by molecular biology, the field of technology that has grown fastest in this period of time. Classical serologic and virologic tests have advanced and sometimes been replaced by novel detection methods that rely on genome amplification procedures like PCR and NASBA.

Especially for the human hepatitis B virus this technological development has been extremely important. As mentioned in an earlier chapter, in contrast to other viruses, HBV is extremely hard to cultivate as it does not replicate in any cell line used regularly in diagnostic laboratories. Furthermore, earlier techniques were not sensitive enough to detect even small amounts of virus in blood and blood products and consequently failed to avoid unintentional transmission of virus from donors to blood product recipients.

Aims of diagnostics tests in the management of HBV-infected patients

The first diagnosis of an HBV infection has to figure out whether the infection is acute or chronic. Therefore, as standard procedure, the patient with HBV infection diagnosed by clinical symptoms or elevated alanine aminotransferase (ALT) levels needs to test positive for anti-hepatitis B core antigen (HBcAg) antibodies. HBcAg is massively expressed in both acute and chronic infections and is a clear sign of HBV infection. After a positive result for anti-HBcAg antibodies, antibodies reactant to the surface antigen (HBsAg) are looked for. If found, this indicates that the patient is cured from the infection or has been successfully vaccinated.

Based on these initial serologic diagnostics, further efforts to define the status of the infection are made. An anti-HBcAg positive but anti-HBsAg negative patient may be dealing with a chronic infection. In these cases a number of parameters should be investigated, namely early antigen (HBeAg), anti-HBeAg, HBsAg, HBcAg, and finally, the viral load measured as genome equivalents per ml in serum. HBeAg is normally expressed only in case of an acute and/or ongoing infection with active replication. Unfortunately, so-called precore mutants exist that display active replication without expressing HBeAg, still bearing a high risk for progression to hepatocellular carcinoma (HCC). It is worth noting that HBeAg seroconversion occurs in up to 98% of people and that this is not a marker for a cure of the infection, although it does act as a marker for healing.

To sum up, classical serological screenings, using the definitions for chronicity (see Chapter 2), have to be initially performed to analyze the serological status of the HBV infection before more expensive molecular methods are performed. These methods are generally used for monitoring treatment efficacy and treatment compliance, to identify resistant strains, and to identify precore mutant strains of HBV.

Molecular assays in the diagnosis and management of HBV

Utility of quantitative HBV DNA assays

Many scientific societies have published consensus papers and/or guidelines for the management of chronically-infected HBV patients (EASL 2002; de Franchis 2003; Keeffe 2006; Liaw 2005; Lok 2001; Lok 2004a; Lok 2004b). All of them recommend an initial quantification of viral load and continuous measurements during follow-up monitoring. Follow-up is considered important for deciding on initiation of treatment or changes to the patient's drug regimen. Furthermore, sensitive methods for quantification are needed for detection of even low viremia in patients infected with strains bearing a high risk for development of hepatocellular carcinoma such as HBeAg negative strains.

One agreed-upon criterion for chronic HBV infection is a detectable viral load – measured as viral DNA in serum or plasma – for a minimum of 6 months (de Franchis 2003; Keeffe 2006; Liaw 2005; Lok 2001; Lok 2004a; Lok 2004b). In this case, replication is considered to be active if $>20,000$ IU/ml or $>100,000$ copies/ml can be detected. Also, in HBeAg-negative chronic hepatitis B virus infections, HBV DNA is the only marker for viral replication that consequently needs to be monitored. A cutoff limit of 2000 IU/ml differentiates active from inactive replication (Manesis 2003; Zacharakis 2005).

Furthermore, qualitative and quantitative measurement of viral DNA is important to monitor another condition, occult hepatitis. This, by definition, is characterized as HBV infection with measurable DNA levels in the absence of detectable HBsAg. Testing for occult hepatitis B virus infection is recommended if (a) cryptogenic liver disease is observed, (b) prior to immunosuppression, and (c) in solid organ transplant donors with positive HBV serology (HBcAg antibodies) (Conjeevaram 2001; Torbenson 2004; Torbenson 2002). It is recommended that viral load should be measured every 3-6 months (EASL 2002; de Franchis 2003; Liaw 2005), although the therapeutic regimen may influence the decision on the interval lengths.

Furthermore, the measurement of viral load after starting therapy is a useful standard tool to help identify therapy non-responders (Schildgen 2004; Schildgen 2006; Sirma 2007; Volz 2007). Non-response to therapy can be induced by host factors, viral resistance, or non-compliance (reviewed by Tillman 2007). For quantification of the HBV viral load, several assays are commercially available, each having advantages and disadvantages (reviewed by Valsamakis 2007).

Utility of HBV genotyping

Genotyping of the HBV genome can be useful. First, the viral genotype influences the success of therapy, e.g., patients with an HBV genotype B infection have a better chance for a more favorable outcome than those patients infected with genotype C. Furthermore, genotype-specific response to drugs has also been observed for some antiviral compounds (Chen 2004; Colombo 2003; Enomoto 2006; Erhardt 2005; Flink 2006; Fung 2004; Guettouche 2005; Kao 2002; Kao 2003; Kao 2000; Kobayashi 2002; Liu 2002; Peteres 2004; Sanchez-Tapias 2002; Zhang 1996).

Second, genotyping is the simplest method for identification of resistance mutations known to be associated with viral non-response to nucleoside and nucleotide analogues. For those mutations that develop during treatment, genotyping is the method of choice for orientation and subsequent phenotyping (Volz 2007; Sirma 2007; Schildgen 2004; Schildgen 2006).

Third, genotyping plays an important role in the identification of infection chains in a nosocomial setting or if transmission by blood donations or blood products has occurred. Genotyping can be performed by in-house or commercial sequencing. PCR followed by INNO-LiPA hybridization has recently been developed and covers newly described mutations in the most recent product release (Hussain 2003; Hussain 2006; Osioy 2003; Osioy 2006). INNO-LiPA has the major advantage in being able to detect mixed infections as well.

Utility of antiviral resistance testing

With the introduction of more and more antiviral compounds into clinical practice over the last decade the option for new and combination treatments of HBV infections has increased greatly, and will continue to grow. As a side effect of the number of novel antivirals the development of resistance mutations has also started to increase, and it can only be assumed that the problem of antiviral resistance in HBV will become as complicated as what is happening for HIV treatments. After a genotypic analyses as mentioned above mutations already known can be identified and associated with resistance. Major evidence for resistance is if such mutations, like the mutations in the polymerase YMDD motif, evolve during ongoing therapy. The real question for virologists is if none of the known mutations is observed at failure. In such cases it has to be estimated how far other novel mutations not yet associated with resistance or novel mutations may contribute to the therapy failure.

In that case, *in vitro* phenotyping procedures established in a rather small number of HBV laboratories need to be performed (see Chapter 10). Unfortunately, known mutations can be detected by commercial methods whereas novel mutations remain speculative and thus, undetected or underestimated. Most recently, a project funded by the German Ministry for Education and Research that covers the first systematic geno2pheno approach for HBV has started. The major aim is to analyze clinical histories, corresponding genotypes, and the respective phenotypic profiles of 200-500 people with chronic HBV with respect to their individual drug regimens. By making use of a combined machine learning approach this project will result in a novel diagnostic tool that is combined with a genotypic assay and will rank the likelihood of success of a new drug or drug combination for a given RT sequence.

Host-related treatment failure, *i.e.*, non-response despite proven compliance combined with a lack of resistance-associated mutations is an increasing problem in resistance testing. This panorama may be due to mal-functioning host mechanisms (see chapter 10) or to resistance mutations in the periphery of the postulated active site of the RT polymerase. The latter can be solely investigated by full length sequencing of a large number of clinical samples and subsequent phenotypic testing, until a crystal-structure is available that allows credible molecular modeling.

Utility of core promotor and precore mutation detection assays

Today, the diagnosis of HBeAg-negative chronic hepatitis B virus infection is based on the assessment of a combination of infection markers, namely positive HBsAg, negative HBeAg, and detectable viral DNA, in concert with anti-HBeAg antibodies and the evidence for liver injury measured by elevated liver enzymes or histopathological findings. Assays are commercially available in formats of PCR+hybridization, INNO-LiPA hybridization and sequencing, and the Affigene HBV mutant VL19 test (Olivero 2006; Qutub 2006).

Conclusion and ways forward

The major challenge for HBV diagnostics in the future will be the increasing number of mutations and the immune escape mutants, occult hepatitis and HBeAg-negative chronic hepatitis. Novel tools like those already established for HIV that help in the interpretation of laboratory results may help overcome these problems. However, the main problem will remain, namely the costs that tend to explode with every newly approved drug and the accompanying number of laboratory investigations that are essential to avoid suboptimal treatments while trying to find the optimal drug regimen.

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