Molecular Testing in Assessment and Management of Hepatitis B

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Molecular testing of hepatitis B consists of two categories of assays: HBV DNA quantification assays that measure the amount of HBV DNA in peripheral blood, which reflects the level of HBV replication in the liver; and assays that identify sequences or motifs of clinical or pathophysiological importance in the HBV genome.

HBV DNA Quantification

HBV DNA quantification has been based for many years on the use of poorly sensitive assays based on hybridization and/or signal amplification methods. The implementation of polymerase chain reaction (PCR) assays has improved detection sensitivity, but the most frequently used assays provide limited ranges of dynamic quantification.

More recently, the introduction of techniques based on real-time PCR technology has allowed accurate quantification over a broad range of HBV DNA levels, making it possible to accurately quantify high HBV DNA values in the untreated patient, as well as monitor the antiviral efficacy of highly potent antiviral drugs. Real-time PCR technology should become the standard technology for monitoring hepatitis B therapy, both in clinical trials (including registration ones) and in clinical practice. The current real-time PCR assays, however, raise several issues, including technical issues and issues related to their use in the management of chronic hepatitis B.

1. Technical issues:
    a. The analytical sensitivity and specificity of current real-time PCR assays are good and allow for accurate quantification over a range of approximately 7-8 logs. However, this range is not sufficient to quantify the very high HBV DNA levels that can be encountered in certain HBV-infected patients, which necessitates retesting these samples after dilution, a factor of quantification errors.
    b. Equal quantification of all HBV genotypes and robustness of quantification in case of nucleotide polymorphisms has not been validated so far for the current commercial real-time PCR assays. Anecdotal, unpublished data suggest that some genotypes may be underquantified, and this may have important consequences in the management of therapy in these patients. In this respect, hybridization-based assays using several different probes, although less sensitive, provide accurate quantification of all HBV genotypes.
    c. There is currently no uniform tendency to report HBV DNA levels in standardized units. As a result, the levels given in nonstandardized units (such as copies/ml or genome equivalents/ml) cannot be

compared among different assays and, thus, between different studies. This emphasizes the absolute and urgent need for expressing all HBV DNA levels in international units/ml (IU/ml), provided that the calibration of the individual assays in IU/ml has been appropriately done and validated.

d. Not all assays are currently registered for use with both plasma and serum.

2. Issues related to the use of HBV DNA assays in the assessment of HBV disease and monitoring of therapy:
    a. HBV DNA has been shown to be an important predictor of liver disease severity and progression. However, no precise thresholds have been established that could guide medical decisions.
    b. Different therapies are associated with different endpoints. Short-term therapy with interferon alpha or nucleosidic/nucleotidic inhibitors in the HBeAg-positive patient is aimed at achieving an HBe (and eventually an HBs) seroconversion. In this case, HBV DNA monitoring is of little utility, although a faster decrease could be a predictor of sustained seroconversion. In contrast, in patients receiving long-term suppressive therapy with nucleos(t)ide analogs, monitoring of treatment efficacy and failure is primarily based on the use of HBV DNA quantification.
    c. The response to antiviral therapy can be assessed by different means. The antiviral potency of a drug or a drug combination can be measured as a log reduction at various time points. This is the most accurate assessment of drug antiviral potency, provided that HBV DNA remains detectable so it can be measured.
    d. Achieving “undetectable HBV DNA” is an attractive endpoint in theory, but it is difficult to standardize in clinical practice. It indeed depends on the lower limit of detection of the used assay. With highly sensitive assays, HBV DNA can be detected in virtually all HBsAg carriers, including during antiviral therapy; whereas with less sensitive assays, any patient with an efficient antiviral response may fall below the lower limit of detection and be “undetectable.” Therefore, a better endpoint is to achieve “an HBV DNA level of less than” a certain amount, expressed in standardized units, which can be the lower limit of detection of the assay or not. Given the lower limit of quantification of current assays, achieving an HBV DNA level of less than 50-60 IU/ml is an acceptable endpoint for therapy.
    e. It is important to use sensitive enough assays to be able to detect a virologic breakthrough early enough to make the decision to modify therapy, i.e., before the patient experiences an ALT flare.

HBV Sequence Analysis

Various assays can be used to analyze the HBV sequence. They are based on direct sequence analysis of the HBV genome after PCR amplification or on reverse hybridization by means of probes specific for the sought mutations or sequence motifs. Three different types of assays can be used: genotyping assays, assays detecting precore and core promoter mutations, and assays detecting resistance mutations.

1. Genotype determination: different HBV genotypes have been identified in different areas of the world. The genotype could influence the natural outcome of HBV disease and the outcome of antiviral therapy. However, HBV genotype determination has not proven to be useful in clinical practice so far.
2. Assessment of precore and core promoter mutations: precore and core promoter mutations have been shown to be involved in the expression and secretion of the HBe protein and to influence the outcome of liver disease. However, the assessment of the HBeAg status is still based on the use of serological assays.
3. Resistance mutation testing:
    a. Until recently, resistance mutation testing was not necessary because only one drug was approved for HBV treatment, lamivudine, and the resistance profile could be guessed without further testing in the presence of a virological breakthrough in an adherent patient.
    b. The advent of multiple options for the treatment of hepatitis B and the emergence of multi-drug resistant HBV following sequential treatment will probably make it necessary to identify resistance mutation profiles in the patients during therapy. However, identification of resistance mutation profiles can be envisaged in clinical practice only if consensus treatment algorithms are derived early in order to help clinicians to make the appropriate treatment decision for each possible case.

Practical Use of Molecular Markers for Diagnosis, Assessment, and Monitoring During and After Therapy

Although there currently is no consensus on the ideal use of molecular markers for diagnosis, assessment, and treatment monitoring, guidance will be proposed during the conference.

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