Hepatol Int (2008) 2:S3–S11
DOI 10.1007/s12072-008-9051-8

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New developments in HBV molecular diagnostics and quantitative serology

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Received: 12 December 2007 / Accepted: 20 January 2008 / Published online: 13 February 2008

Abstract New standardized assays for the quantification of hepatitis B virus (HBV) DNA have yielded insights into the association of HBV DNA levels with the relative risk of developing liver disease. Quantification of HBV DNA has also played a role in the management of chronic hepatitis B by allowing criteria to be established for determining patient eligibility for antiviral therapy, monitoring response, and identifying the development of resistance. In addition to serum HBV DNA levels, the HBV genotype may influence disease progression and response to therapy. However, many of the studies that have included genotype assessment do not compare across the range of genotypes, and current management guidelines do not incorporate genotype determination. More recently, quantitative assays for intrahepatic HBV replicative intermediates, as well as hepatitis B e antigen and hepatitis B surface antigen, indicate that these factors may have promise in identifying patients likely to respond to treatment. Additional work is needed to standardize and validate these assays before they can be considered to be of true diagnostic value. A wide variety of research techniques are being used to investigate chronic hepatitis B. Further evaluation is needed to decide which will have the greatest clinical applicability.

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Keywords Hepatitis B virus · Assay · Genotype · Intrahepatic HBV · HBeAg · HBsAg

Introduction

Assessment of specific serologic and virologic factors plays a pivotal role in the diagnosis and effective management of individuals infected with the hepatitis B virus (HBV). Diagnosis of HBV infection depends in large part upon the accurate interpretation of specific serologic assays. During the course of infection, the viral surface antigens—hepatitis B surface antigen (HBsAg), core protein antigen (HBcAg), and nonstructural secreted "e" antigen (HBeAg)—stimulate the immune system, and the corresponding antibodies (anti-HBs, anti-HBc, and anti-HBe) are produced. The assays for serum HBsAg and its resultant antibody are particularly important because the persistence of HBsAg for longer than 6 months defines chronic infection, and the presence of anti-HBs indicates immunity from vaccination or disease resolution.

Recent advances in molecular technology have enabled the sensitive detection of viral nucleic acid and facilitated investigations into the relationship between serum HBV DNA level and disease progression [1]. Accordingly, for the management of chronic hepatitis B, the quantification of HBV DNA has become a primary tool. Viral load measurements via serial samples taken in studies of infection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), as well as chronic HBV infection, show that there is a dynamic equilibrium between virus production and virus clearance [2, 3]. However, this relationship is more complex for HBV infection than for HIV and HCV infections because of the more variable course of chronic HBV disease. Flares occurring during

seroconversion in the immune clearance phase of chronic HBV infection, for example, can raise serum HBV DNA levels. Similarly, viral loads tend to be higher in HBeAg-positive persons with chronic hepatitis B (10^7 to 10^{10} copies/ml) than in persons with HBeAg-negative disease (viral loads generally $<10^6$ copies/ml) [4].

HBV DNA quantification plays a crucial role in the management of chronic hepatitis B and clinical development of new antiviral therapies. The three major liver societies—the American Association for the Study of Liver Diseases [5], the European Association for the Study of the Liver [6], and the Asia Pacific Association for the Study of the Liver [7]—have all issued guidelines for the management of chronic hepatitis B that specify certain HBV DNA thresholds to define which patients become candidates for antiviral treatment. Viral load measurement is also used in monitoring patients for the effectiveness of therapy, although the respective guidelines differ in the recommended intervals for such testing. During antiviral therapy, an increase in HBV DNA level may signal the development of resistance. In a compliant patient, an increase in HBV DNA level of greater than 1 $\log_{10}$ from nadir is generally considered indicative of virologic breakthrough [8].

Advances in understanding the life cycle of the virus and the natural history of HBV infection have provided evidence that various factors in addition to serum HBV DNA level may warrant investigation for their relationship to disease progression and patients' response to antiviral therapy. These factors include HBV genotype, levels of intrahepatic HBV replicative intermediates, and levels of circulating HBeAg and HBsAg.

This article provides review of the molecular and serologic tools currently used, or being investigated, in the diagnosis and management of hepatitis B. It describes the different types of assays employed for the quantification of serum HBV DNA, and it discusses the significance of HBV genotypes and HBV covalently closed circular (ccc) DNA in disease pathogenesis and treatment response. The emerging role of quantitation of HBeAg and HBsAg levels in predicting response to antiviral therapy are also discussed.

HBV viral load assays

In HBV DNA testing, one of the deficiencies has been a lack of standardization between assays, along with limitations in the quantification range. Commercial HBV DNA quantification assays available today, using real-time polymerase chain reaction (PCR) technology or improved signal amplification, have overcome some of these problems (Table 1). An international HBV reference standard has been established, allowing assay results to be correlated using International Units (IU) [9].

Signal amplification assays

Assays based on signal amplification rely on enhancing a signal rather than amplifying the target nucleic acid, as in PCR assays. Generally, signal amplification assays are less sensitive than target amplification assays, although they do offer a number of advantages over their PCR-based counterparts (Table 2). One of the first HBV DNA assays widely available was the Digene Hybrid Capture® assay (Digene Diagnostics; Corporation, Gaithersburg, MD) It relied on the hybridization of denatured HBV DNA to an HBV RNA transcript, which, in turn, was captured by an immobilized anti-RNA:DNA antibody. Further amplification led to the generation of a chemiluminescent signal, proportional to the input HBV DNA. In the second-generation Digene Hybrid Capture® 2 assay, the quantification range was 0.5 to 6,000 pg/ml (equivalent to approximately 1.4×10^5 to 1.7×10^9 copies/ml). An ultrasensitive Digene protocol requires a larger sample volume and a centrifugation step (lower limit of quantification, 8×10^3 copies/ml). Although the Digene signal amplification assay was developed prior to the availability of any international

Table 1 Quantification range of HBV DNA load assays

Assay	LLQ[a]	ULQ[b]	Conversion factor (IU > copies)
COBAS HBV MONITOR (Roche Diagnostics)	2×10^2 copies/ml	2×10^5 copies/ml	Not applicable
COBAS TaqMan HBV (Roche Diagnostics)	3×10^1 IU/ml	1.1×10^8 IU/ml	5.82
RealArt HBV PCR (QIAGEN)	2×10^1 IU/ml	10^8 IU/ml	6
VERSANT HBV DNA 3.0 (Siemens Medical Solutions)	3.5×10^2 IU/ml	1.8×10^7 IU/ml	5.7
Real-Time HBV PCR (Abbott Molecular)	1×10^1 IU/ml	1×10^9 IU/ml	3.41

Note that some assays can detect lower amounts of HBV DNA but this is outside the linear quantification range

[a] LLQ, lower limit of quantification

[b] ULQ, upper limit of quantification

Table 2 Advantages and disadvantages of target and signal amplification assays

	Advantages	Disadvantages
Target amplification	Broad dynamic range	Large sample volume
	Rapid turnaround	Need to purify HBV DNA
	Value-add with sequence analysis	Requirement for contamination control
Signal amplification	No sample extraction	Limited dynamic range
	Minimal requirement for contamination control	Relies heavily on controls and standards working
	Small sample volume	

standard, comparative data indicate a good correlation with the upper dynamic range of the newer assays.

The more recently developed and more sensitive VERSANT® HBV DNA 3.0 Assay (bDNA) (Siemens Medical Solutions Diagnostics; Tarrytown, NY), also a signal amplification test, is based on a series of sequential DNA hybridizations. The signal generated is proportional to the HBV load, for which a value is calculated from a standard curve derived from the kit DNA standards. The dynamic range of the assay is 2×10^3 to 1×10^8 copies/ml (3.5×10^2 to 1.8×10^7 IU/ml), and the assay has been standardized by reference to the World Health Organization international standard.

Target amplification assays

The first commercial HBV DNA PCR assay developed was the AMPLICOR® HBV MONITOR® test (Roche Diagnostics; Basel, Switzerland), followed later by the semi-automated COBAS AMPLICOR HBV MONITOR® test (Roche Diagnostics; Basel, Switzerland). Although the latter of these tests had an excellent lower limit of detection of 2×10^2 copies/ml, the upper limit of detection was only 2×10^5 copies/ml, requiring dilution of high-titer samples to obtain an accurate load value. This quantitative end-point PCR assay relied on the co-amplification of a standard of known copy number for estimation of the amount of HBV DNA. The limited upper quantification range is attributable to reagent exhaustion and saturation effects brought about when larger amounts of DNA are amplified.

More recently, so-called real-time PCR assays have been developed for quantifying DNA. These assays are designed to measure the DNA product in the early exponential phase of amplification, when the reaction is at its most efficient, rather than at the end. The ability to monitor accumulating PCR product (amplicons) in real time has been made possible by the use of fluorescent chemistry. This allows a signal to be generated and measured after interaction between the amplicon and a fluorescent label following each thermal cycle. The signal is proportional to the amount of PCR product present. This technology greatly increases the dynamic range of the assay. Furthermore, with real-time PCR, there is no post-PCR processing, thus reducing the chances of contamination by amplicon carry-over.

Several real-time HBV PCR assays are now commercially available. The COBAS® TaqMan® HBV test (Roche Molecular Diagnostics; Pleasonton, CA) uses a dual fluorophore-labeled probe, which is designed to anneal to one strand of the HBV sequence generated from the PCR primers. The reporter fluorophore's emissions are extinguished by a nearby downstream quencher, which prevents a signal from being emitted. However, as the Taq DNA polymerase generates the complementary strand, the nuclease activity of the enzyme cleaves the reporter fluorophore from the probe, and its emissions are no longer quenched; this produces a detectable signal. The signal is proportional to the amount of target originally present. The quantification range of the assay is approximately 30 IU/ml at the lower limit, to an upper limit of greater than 1.1×10^8 IU/ml, depending on the method of DNA purification used (1.7×10^2 to 8.5×10^8 copies/ml). Similarly, the Abbott Real-Time HBV assay (Abbott Laboratories; Abbott Park, IL) also relies on fluorescence detection of a TaqMan probe. The claimed quantification range is 10 to 10^9 IU/ml (34–3.4×10^9 copies/ml).

Another real-time assay, the artus RealART™ HBV LC PCR kit (QIAGEN formally artus GmbH; Hamburg, Germany) contains ready-for-use reagents specifically designed for different real-time platforms. Reagents are provided for use on the ABI Prism instruments 7000/7700/7900 (Applied Biosystems; Foster City, CA) employing the TaqMan technology. For the LightCycler® Instrument (Roche Diagnostics; Basel, Switzerland), the assay uses the fluorescence resonance energy transfer (FRET) technology to monitor the fluorescence intensity. FRET requires two adjoining probes to be used, in which the 3′ end of one probe has a donor fluorophore and the 5′ end of the second probe has an acceptor fluorophore. The probes are designed to hybridize to adjacent sites on a strand of HBV DNA. The RealArt kit for the Rotor-Gene™ instrument (Corbett Research; Sydney, Australia) relies on molecular beacons for detection. Molecular beacons have a donor fluorophore

at one end of the oligonucleotide, whereas the opposite end of the same oligonucleotide contains a quencher molecule. The loop of the hairpin structure is designed to bind to the complementary portion of DNA in the amplified product, while the ends of the oligonucleotide are designed to be complementary to each other and form a stem structure. Quantification is achieved by reference to a standard curve generated from supplied controls. In general, the quantification range of the assay for all of the instruments is approximately 2×10^1 to 10^8 IU/ml (10^2 to 6×10^8 copies/ml).

Viral genotypes

Genetic variability in HBV was originally described by means of antigenically defined subtype determinants, subsequently found to be specified by different amino acids in the surface protein, together with the main antigenic determinant *a* [10–12]. Eight HBV genotypes (A–H) have been defined based on nucleotide sequence divergence of >8% over the genome [12–14]. Some of the genotypes have a wide distribution, while others are more geographically restricted (Table 3). Major genotypes A to D are found worldwide; genotypes B and C are the predominant forms in Asia and Oceania. Genotype G is limited to a small number of people in Europe and the United States. Genotype E is largely restricted to West Africa, genotype F to native Americans and Polynesians, and genotype H to Central and South America. As direct sequencing can be time consuming, a number of methods have been developed to facilitate more rapid genotyping. Most involve prior PCR amplification, and they include a line probe assay (LiPA), restriction fragment length polymorphism analysis, and discrimination using genotype-specific primers in multiplex PCR [15, 16].

Important pathogenic and therapeutic differences do exist among HBV genotypes [3, 16, 17]. For example, in Taiwan, genotype C is associated with more severe liver disease than is genotype B, while in India, genotype D is associated with more severe liver disease than is genotype A. Response to antiviral therapy may also be influenced by genotype. Patients infected with genotype A HBV appear to respond better to interferon therapy than do those infected with genotype D, while patients infected with genotype B respond better than those infected with genotype C. Similarly, some preliminary reports suggest there may be genotype-specific differences in patients' response to therapy with nucleoside and nucleotide analogs. Determining the clinical relevance of genotypes and their relationship to response to therapy is further complicated by the existence of multiple HBV co-infections and HBV genotype B recombinants which have acquired some genotype C sequences from the precore/core region [18]. A drawback of many of the genotype studies is the small sample size involved or the selection of patients used. Further evaluation involving large-scale longitudinal studies is required to provide convincing evidence of the effect of genotype on clinical outcome. Current management and treatment guidelines do not recommend a role for genotype determination.

Antiviral resistance

In addition to interferon, several nucleoside and nucleotide analogs have been approved for the treatment of chronic hepatitis B. The primary mode of action of these agents is

Table 3 Overview of the eight genotypes of HBV

Genotype	Genome length (nucleotides)	Frequency of mutation[a]		Global distribution
		PC	BCP	
A	3221	Uncommon	Common	Western Europe, USA, Central Africa, India
B	3215	Common	Common	Japan, Taiwan, Indonesia, China, USA
Bj	3215	Common	Uncommon	Japan
Ba	3215	Common	Uncommon	China, Taiwan, Indonesia, Vietnam
C	3215	Common	Common	East Asia, Taiwan, Korea, China, USA, Japan, Polynesia
D	3182	Common	Common	Mediterranean region, India, USA
E	3212	ND	ND	West Africa
F	3215	Uncommon	ND	Central & South America, Polynesia
G	3248	Very common	ND	USA, Europe
H	3215	ND	ND	Central, South America

ND, not described

[a] PC, Precore mutations such as G1896A; BCP, basic core promoter mutations such as A1762T, G1764A; very common (most isolates); common (up to 50% of isolates); uncommon (less than 10% of isolates)

specific inhibition of the HBV polymerase and reverse transcriptase. Generally, monotherapy results in rapid suppression of viral replication, but this is often not sustained because of the emergence of drug-resistant HBV variants. Antiviral resistance may manifest itself in several different ways. Very recently, the Hepatitis B Virus Drug Resistance Working Group published a report aimed at standardizing nomenclature and defining genotypic, phenotypic, and clinical resistance [19].

For the detection of drug resistance, a large variety of methods have been used [19, 20]. The most common methods in use today are direct sequencing of PCR product after amplification of a portion of the polymerase gene and detection with the commercially available reverse-phase hybridization line probe assay, INNO-LiPA® DR (Innogenetics; Ghent, Belgium) [21]. Direct sequencing lacks sensitivity in detecting minor populations of emerging drug-resistant virus, but it has the advantage of detecting mutations which may not yet be correlated with drug resistance. The INNO-LiPA assay has proven to be more sensitive than direct sequencing for the detection of minor populations of virus with known drug resistance, but it cannot detect new mutations that may be associated with drug resistance [20]. The plethora of different methods for identifying changes associated with antiviral resistance reinforces the need for standardization [19].

Assays for intrahepatic HBV replicative intermediates

Quantification of serum HBV DNA provides clinically relevant information for the management of chronic hepatitis B, including assessment of the efficacy of antiviral therapy. However, the absolute level of HBV DNA found in serum may not always accurately reflect the state of disease in the liver. In recent years, several investigators have attempted to quantify the various intrahepatic HBV replicative forms—in particular, the HBV cccDNA and pregenomic RNA (pgRNA)—to gain further insights into the natural history of HBV infection as well as to potentially gain an additional tool for evaluating the efficacy of specific antiviral therapy.

Formation of cccDNA

The HBV cccDNA is generated after the partially double-stranded open circular genomic DNA of the infecting virion is transported to the hepatocyte nucleus, where host cell enzymes convert it to a relaxed circular fully double-stranded molecule. The HBV cccDNA remains in the cell nucleus and serves as the transcriptional template for HBV RNA production. Viral replication proceeds by the production of multiple copies of a terminally redundant replicative RNA, the pgRNA. Viral mRNAs, including those coding for the multifunctional polymerase protein, core protein, and envelope proteins, are transported to the cytoplasm for translation. The pgRNA becomes encapsidated in precursors of the virion core particle and is reverse transcribed by the viral polymerase to form a minus-sense single-strand DNA. Subsequently, the pregenome is degraded, and the minus-strand DNA acts as a template for synthesis of a plus-strand DNA of variable length. Following assembly, nucleocapsids can follow either of two pathways: they can associate with the envelope proteins to produce virions and be secreted from the cell, or, as part of a regulatory pathway, they can be recycled back to the nucleus to maintain a pool of cccDNA molecules in the form of viral minichromosomes [22].

The HBV cccDNA pool provides a difficult obstacle for antiviral therapy to overcome. HBV replication does not employ a semiconservative mechanism, and therefore any nucleoside or nucleotide analog-based therapy can only indirectly affect the pre-existing cccDNA template. The likely reason for the relapse seen after completion of antiviral therapy for hepatitis B infection is the persistence of the HBV cccDNA, which can re-establish pretreatment replication levels.

Quantification of cccDNA

The development of methods for quantification of HBV cccDNA was initially hindered by technical difficulties, but these were overcome by the introduction of sensitive and reliable real-time PCR assays and, more recently, an invasive signal amplification assay (Invader® HBV DNA; Third Wave Technologies; Madison, WI) [23–26]. Several groups have performed investigations of intrahepatic HBV cccDNA, and a number of common themes have emerged. Patients with HBeAg-positive chronic hepatitis B have higher levels of cccDNA than do their HBeAg-negative counterparts [23, 25–27]. Laras et al. further expanded on this finding by also measuring intrahepatic pgRNA levels and determining replicative activity (pgRNA copies per cccDNA copies) in HBeAg-positive and HBeAg-negative patients [23]. They reported that, for HBeAg-negative patients, not only were the levels of HBV cccDNA lower, but also the levels of pgRNA were substantially lower, indicating a reduced replicative activity.

Similarly, changes in level of intrahepatic HBV cccDNA during antiviral therapy has been investigated [25, 27, 28]. With adefovir, 48 weeks of therapy resulted in a 0.8 $\log_{10}$ decrease in HBV cccDNA levels, whereas a similar duration of lamivudine and entecavir resulted in a 1 $\log_{10}$ in cccDNA levels [25, 28]. A larger decrease in

cccDNA levels was noted before and at 48 weeks of combination therapy using pegylated interferon plus adefovir in HBeAg+ patients who lost HBeAg than those without HBeAg loss [27]. Histologic analysis from the adefovir monotherapy and adefovir-plus-interferon studies revealed some differences in the possible mechanisms of HBV cccDNA reduction. In the monotherapy study, there was no reduction in the number of cells expressing HBsAg and HBcAg after 48 weeks, implying a depletion of the cccDNA pool by suppression of HBV DNA replication, rather than by elimination of HBV cccDNA-containing cells [25]. In contrast, with interferon-plus-adefovir combination therapy, the number of antigen-positive cells was substantially reduced, indicating clearance of infected cells by cytolytic means [27].

At the present time, in patients receiving long-term nucleoside analog therapy for chronic HBV infection, it is not known whether the pretreatment level of intrahepatic HBV cccDNA will be a predictor of response. Equally unknown is whether it will be possible to establish a threshold level of HBV cccDNA to aid in the decision of when to stop therapy. Some evidence suggests that measuring this parameter may be useful. In studies of combination interferon-plus-adefovir therapy, patients showing HBeAg loss or seroconversion had a significantly lower HBV cccDNA level before and after treatment than did patients who showed no HBeAg change [27]. Hui et al. have identified a threshold level of HBV cccDNA for predicting HBV reactivation in patients with lymphoma treated with cytotoxic chemotherapy [29]. In addition, in patients treated with pegylated interferon plus lamivudine or with lamivudine monotherapy, levels of intrahepatic total HBV DNA and cccDNA proved to be superior to serum HBV DNA as predictors of sustained virologic response [30].

Serum versus intrahepatic cccDNA

One of the drawbacks to measuring intrahepatic HBV intermediates is the requirement to use liver tissue. Only limited assessment of the value of intrahepatic HBV cccDNA levels can be made on the basis of pretreatment and end-of-treatment liver biopsies. Of great interest, therefore, was the discovery of HBV cccDNA in the serum compartment [26]. In a study using the Invader assay, approximately 75% of both HBeAg-positive and HBeAg-negative patients were found to have HBV cccDNA in serum, with the loads being higher in the HBeAg-positive patients [26]. Further evaluation of serum HBV cccDNA was carried out in patients treated with lamivudine for 52 weeks [31]. At weeks 24 and 52, the median cccDNA level was reduced by 2 $\log_{10}$, which was significantly greater than that of controls (0.2 log, $P < 0.001$). Lamivudine resistance was associated with a lower median reduction of cccDNA. The levels of HBV cccDNA that have been found in serum are surprising. It would be expected that a small amount of HBV cccDNA might leach into the serum compartment from the lysis of hepatocytes and that there would be some correlation with levels of alanine aminotransferase. Consistent with this supposition, some investigators have found much lower levels in serum using PCR-based assays [23].

The measurement of intrahepatic HBV markers such as HBV cccDNA and pgRNA could offer a new paradigm for predicting treatment response and determining treatment cessation. Eradication of HBV cccDNA may not be necessary to control chronic hepatitis B if its replicative activity, as measured by the pgRNA level, is low or quiescent. The importance of accurately measuring these intrahepatic replicative markers highlights the need for standardization, as contrasting results from different studies may reflect the different methodologies used in quantification.

Quantitative HBeAg and HBsAg assays

A correlation between the levels of circulating HBsAg and the levels of intrahepatic HBV cccDNA has led to renewed interest in the development of sensitive quantitative assays for HBsAg as well as HBeAg [32, 33]. Although population distributions have been defined for patients having chronic hepatitis B [32–36], it is clear that much more relevant data can be obtained than by standard qualitative serology. Thus, the use of quantitative HBeAg and HBsAg assays may allow fine-tuning of treatment protocols.

Quantitative HBeAg assays

The therapeutic endpoint for HBeAg-positive chronic hepatitis B is HBeAg seroconversion. This predicts long-term viral suppression, normalization of serum aminotransferase values, remission of hepatic inflammation, and good prognosis [37–39]. A potential role for quantitative HBeAg titer in guiding management algorithms is emerging. A large study using pegylated interferon alfa-2a for the treatment of HBeAg-positive chronic hepatitis B found that seroconversion was more likely to occur in the setting of a low HBeAg titer pretreatment or if there was a rapid decline of HBeAg titer during therapy [40]. Conversely, the failure of HBeAg to fall during treatment predicted nonresponse. In this study, the predictive power of HBeAg titer was greater than that of viral load, measured using a sensitive PCR assay.

Beyond interferon-based therapy, it is likely that measurement of HBeAg titers will also be applied to therapy with nucleoside or nucleotide analogs, both for predicting seroconversion early as well as for monitoring patient response. Nucleoside and nucleotide analogs rapidly suppress HBV viral load to undetectable levels, decreasing the utility of HBV DNA level as a monitoring tool for HBeAg seroconversion. Thus far, the HBeAg titer has been shown to predict both seroconversion and nonresponse during lamivudine therapy [35, 36, 41–45]. In one study, the HBeAg titer also predicted lamivudine resistance prior to virologic breakthrough (using the Digene signal amplification assay for HBV DNA; lower limit of detection, 0.5 pg/ml) [42]. The clinical utility of quantitative HBeAg measurements may be greatest when these data are used in conjunction with virologic sequencing of the HBV precore or basal core promoter region. Both basal core promoter and precore variants, which occur as quasispecies in HBeAg-positive chronic hepatitis B, are associated with lower HBeAg titer. Stratification according to the dominant virus may be useful. Further studies with larger numbers of patients are required to validate HBeAg titer as a valuable tool for the clinic. Unfortunately, there is no commercial assay currently available for the quantitative measurement of HBeAg. It therefore remains a research tool, modeled after the method of Perrillo et al., with the level expressed by establishing a standard curve using the Paul-Ehrlich HBeAg reference standard [43].

Quantitative HBsAg assays

HBsAg seroconversion is believed to reflect clearance of HBV from the liver and to represent cure. Although uncommon, it remains the ultimate goal in the management of chronic hepatitis B. HBsAg titers have been shown to correlate with intrahepatic levels of cccDNA and serum levels of HBV DNA in the different phases of disease [32, 33]. Furthermore, HBsAg titer appears to decline along with decline of cccDNA during treatment [25, 27]. Quantitative HBsAg titer may therefore be a useful surrogate marker for cccDNA and have clinical utility in predicting treatment response both in HBeAg-positive and HBeAg-negative patients. Mathematical modeling of HBsAg decline during treatment has recently been used to predict the duration of therapy required for HBsAg seroconversion, for both interferon-based and nucleoside or nucleotide analog-based therapies [46]. The first assay for quantitative determination of HBsAg titer was described by Deguchi et al. [32], but a number of such assays are now commercially available [25, 27, 46]. Although larger, prospective studies will be required to define the exact role for measurement of HBsAg titer in actual clinical practice, it shows promise as a tool for monitoring antiviral therapy, as it is a less invasive alternative to measurement of intrahepatic HBV DNA replicative forms.

Conclusion

The past decade has seen major improvements and new developments in the tools used for the clinical assessment of patients with chronic hepatitis B. Sensitive and standardized quantification assays for HBV DNA have allowed the evaluation of the association between viral load and the risk of disease progression. The recent introduction of several new antiviral agents into the marketplace has made it all the more important to have accurate ways for determining patients' eligibility for treatment and for monitoring treatment efficacy and drug resistance. Current treatment algorithms, which rely on HBV DNA monitoring, will undoubtedly be fine tuned as additional data accumulate on the predictive value of other markers. Closer examination of the HBV life cycle utilizing measurement of intrahepatic replicative intermediates may provide further insights into the natural history of chronic hepatitis B and identify additional predictors of antiviral treatment response. The lack of standardization and need for liver tissue in the case of intrahepatic cccDNA are barriers for adoption of these techniques in the clinical setting. As yet, the clinical utility of the quantitative HBsAg and HBeAg assays that have been developed is not known. Continuing evaluation of the expanded range of research tools will identify those that have the most clinical applicability.

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