Endpoints of Therapy in Chronic Hepatitis B

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Because clearance of hepatitis B virus (HBV) infection is rarely, if ever, achievable, the goals of therapy necessarily focus on prevention of bad clinical outcomes. Ideally, therapies would be shown to prevent tangible clinical endpoints like development of cirrhosis, end-stage liver disease and hepatocellular carcinoma. However, these endpoints typically take years or decades to occur and are therefore impractical targets for clinical trials which last only 1-2 years. As a result, surrogate biomarkers that are believed to correlate with long-term outcome are used to evaluate therapy. Of the clinical, biochemical, serological, virological, and histological endpoints that have been evaluated, none has been shown to be ideal on its own. Symptoms are uncommon and aminotransferase levels fluctuate spontaneously. Loss of hepatitis B e antigen (HBeAg) has been the traditional therapeutic endpoint; however, the indefinite durability off treatment and the emergence of HBeAg-negative disease have made it inadequate as the sole goal of therapy. Loss of hepatitis B surface antigen is associated with improved clinical outcomes, but it is rarely achieved with current therapies. Suppression of viral replication, as measured by serum HBV DNA levels, has become the major goal of therapy, particularly if maintained off therapy. Although useful, the significance of viral levels depends on the stage of disease, degree of liver damage, and the type of therapy. Finally, liver biopsy, often considered the gold standard, is invasive, prone to sampling error, and may take years to change significantly. At present, there is no ideal biomarker for evaluation of therapies for hepatitis B. Future research should be directed at development and validation of surrogate markers that accurately predict or reflect clinically relevant outcomes of chronic hepatitis B. (Hepatology 2009;49:S96-S102.)

Introduction

The ultimate goal of treatment of any chronic infection is the eradication of the infectious agent, ideally before significant damage has been done to the host. In chronic hepatitis B, complete eradication of hepatitis B virus (HBV) infection is rarely achieved with currently available therapies. Even with prolonged suppression of viral replication for years, most patients remain positive for hepatitis B surface antigen (HBsAg) without antibody to HBsAg (anti-HBs), indicating the persistence of infection. Furthermore, even in those who clear HBsAg, HBV remains in infected hepatocytes in the form of covalently closed circular DNA (cccDNA) likely for the lifetime of the cell if not the individual, thus making it possible for reactivation to occur with the right stimulus. Consequently, true “cure” of hepatitis B with complete viral clearance is an unrealistic therapeutic goal.

Short of viral eradication, the goal of therapy is to prevent the complications of the disease. Unfortunately, in chronic hepatitis B, this goal is also not straightforward. It is estimated that 25%-35% of persons who acquire chronic HBV infection in early childhood will die of liver-related complications. Conversely, at least 65% will not. Thus, progression to cirrhosis and end-stage liver disease is common but far from invariable, and the progression is
slow, generally requiring years or decades to become evident. For these reasons, clinically relevant endpoints in therapy of chronic hepatitis B, such as prevention of cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC), are impractical targets to assess therapy. Most clinical trials last for only 1-2 years during which few if any untreated subjects would be expected to reach one of these clinically relevant endpoints. Furthermore, these are negative endpoints (prevention of cirrhosis or its complications), rather than positive endpoints (loss of HBsAg or reversal of cirrhosis). Consequently, reliable surrogate biomarkers, with clear relevance to long-term outcomes, are needed. Although a number of such markers exist, the question of which markers provide the greatest utility in terms of long-term clinical significance is still unresolved.

Potential therapeutic targets include improvement in clinical, biochemical, virological, serological, or histological surrogate endpoints. Clinical endpoints might include symptoms; yet symptoms in chronic hepatitis B are uncommon and typically mild and nonspecific (fatigue, right upper quadrant discomfort) and are thus difficult to measure and unreliable in assessing benefits of therapy. Biochemical endpoints include serum aminotransferase levels, which are usually elevated in patients with chronic hepatitis B and are reasonable measures of disease activity. However, aminotransferase levels fluctuate considerably over time, and individual values are not useful for assessing long-term effects of therapy. The level of viral replication as reflected by serum levels of HBV DNA has become the predominant metric for assessing new oral treatments. Unfortunately, viral levels also fluctuate and the significance of a given viral level depends greatly on the stage of disease, degree of liver damage, and the type of therapy being used (i.e., oral nucleoside analogs versus peginterferon). The main serological markers used in assessing therapy include hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) and, to a lesser extent, HBsAg and antibody (anti-HBs). Clearance of HBeAg and/or development of anti-HBe are associated with improved outcomes, provided that HBeAg loss is sustained after treatment is discontinued and the patient does not subsequently develop HBeAg-negative chronic hepatitis B with recurrent disease despite loss of HBeAg. Loss of HBsAg is the most convincing beneficial outcome of treatment of hepatitis B, because it is the most robust marker for lasting resolution of disease and is clearly associated with improved outcomes. The only drawback of relying on HBsAg loss is that it occurs very infrequently and therefore is unhelpful for the majority of treated patients. A final possible endpoint for therapy is liver histology. Liver biopsy is considered the gold standard for assessing liver injury and fibrosis; however, it too is not an ideal surrogate endpoint. Like serum aminotransferase levels, histological activity fluctuates over time and can improve or worsen rapidly. Hepatic fibrosis is perhaps a more stable marker for disease progression, but the assessment of fibrosis is problematic, because it is subject to sampling error, slow to change, and requires liver biopsy for accurate assessment. Liver biopsy is costly and invasive and cannot be repeated frequently to assess disease progression.

Clearly, none of the currently available biomarkers is an ideal measure of treatment efficacy on its own. Perhaps for this reason, approval of new therapies for hepatitis B by licensing authorities has usually depended on demonstration of significant improvements in two or more surrogate markers of disease progression with treatment. Typically, the surrogates are (1) biochemical (aminotransferase levels), (2) virological (HBV DNA levels, HBeAg, HBsAg), and (3) histological (based on histological scoring systems). Furthermore, the timing of assessment of these surrogate markers is defined as either “on-treatment” (maintained) or “off-treatment” (sustained or durable). In the approval of the initial agents for hepatitis B, comparisons were made to placebo-treated controls; more recently, newer agents are compared to standard, licensed therapies.

Evaluation of Biomarkers of Disease Status

Biochemical Endpoint: Serum Aminotransferase Levels. Standard indirect markers of the “activity” of the underlying liver disease are levels of serum alanine and aspartate aminotransferases (ALT and AST). In an analysis of 142,055 Koreans who were 35 to 39 years of age and undergoing routine laboratory testing for insurance purposes, elevations in serum AST and/or ALT of greater than 30 U/L in men was associated with an increased relative risk of death from liver disease during the subsequent 8 years of follow-up.1 The mortality rate in women was too low for a comparable analysis. Those with a family history of liver disease (most likely chronic hepatitis B) had a higher relative risk of liver-related mortality. An observational cohort study of HBV-infected individuals from Hong Kong also observed that subjects with ALT levels below 0.5 × upper limit of normal were at lower risk for developing liver-related complications than those with even minor ALT elevations, in the range of 1× to 2× upper limit of normal.2 These findings suggest that normalization of ALT values has some utility as a surrogate endpoint, but because of the lack of specificity for HBV and the fluctuant nature over time, serum amino-
transferrase levels cannot be used as the only endpoint of therapy.

In addition to the lack of specificity for hepatitis B, ALT levels may also be insensitive. In individuals with advanced liver disease and cirrhosis, with or without signs of liver decompensation, ongoing viral replication may be present, yet not be associated with elevation in ALT. Thus, the context of the measurement of ALT needs to be taken into account, including other laboratory tests, HBV DNA levels, and background liver disease severity.

**HBeAg Status.** Transmission of HBV at birth or in early childhood typically results in chronic infection in the infant, characterized by a pattern of immune tolerance with extremely high levels of HBV DNA in serum, circulating HBeAg, normal ALT levels, and minimal liver injury. Most, but not all such patients, move from the immune-tolerant phase to the immune-active HBeAg-positive phase between the second and fourth decade of life, at which time disease activity and liver injury become evident.

Because of the dynamic nature of the disease, an individual’s HBeAg status at a single point in time cannot, on its own, be considered a marker of disease severity. The REVEAL-HBV (Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HBV) cohort of Taiwanese men aged 30-65 reported a higher relative risk of cancer in those who were HBeAg-positive than in those who were HBeAg-negative at baseline, with a relative risk of HCC of 60.2 for those who were HBeAg-positive compared to 9.6 for HBeAg-negative persons, using HBSAg-negative individuals as the reference. To interpret these data correctly, the age and sex of the patient must be considered. Whether persistent HBeAg-positivity is as clearly related to the absolute risk of cancer in young individuals or women is not known. Indeed, many persons become HBeAg-negative in adolescence or young adulthood (< age 30) and would therefore fall into the “lower risk” HBeAg-negative category at age 30.

**HBeAg Seroconversion to Anti-HBe.** For poorly understood reasons, immune-tolerant HBeAg-positive individuals enter the immune-active phase of disease, during which they eventually lose HBeAg and with time seroconvert to anti-HBe. When this occurs spontaneously, loss of HBeAg is associated with a fall in HBV DNA to low levels (generally <1000 IU/mL), normalization of serum ALT levels, and resolution of active necroinflammatory injury on liver biopsy. When sustained, these changes are associated with excellent long-term prognosis. For these reasons, HBeAg loss has been used as a surrogate marker for treatment efficacy and is considered a potential sign that therapy might be stopped in patients who were initially HBeAg-positive. HBeAg seroconversion (loss of HBeAg and development of antibody to HBeAg [anti-HBe]) has been considered a more reliable marker for a durable disease remission than simple loss of HBeAg; however, this is based on minimal evidence and as a result, most therapeutic trials have used loss of HBeAg as a therapeutic endpoint. Unfortunately, loss of HBeAg with or without HBeAg seroconversion is not always associated with resolution of disease. In some patients, HBeAg reappears in the serum once treatment is withdrawn and the disease returns to its initial level of activity. In other patients, virological, biochemical, and clinical relapse of disease occurs without reappearance of HBeAg (so-called HBeAg-negative chronic hepatitis B), which may be as severe as HBeAg-positive disease. Because HBeAg loss and associated disease quiescence are not always durable, the potential for sustained clinical benefit following this occurrence cannot be relied on, making it an imperfect endpoint in assessing therapy. Relapse in disease either due to return of HBeAg or evolution into HBeAg-negative hepatitis appears to be more common in responders to oral nucleoside analog therapy than in those responding to interferon or peginterferon. Recent data suggest that a fall in HBeAg concentration during peginterferon therapy predicts subsequent HBeAg loss, but HBeAg titers are not widely available. There are also genotypic differences, because patients with genotype C and D HBV infection are more likely to progress to HBeAg-negative hepatitis after HBeAg loss/seroconversion whereas patients with genotype A are more likely to achieve a durable disease remission. Unfortunately HBeAg loss, even with seroconversion to anti-HBe, may only mark the transition from immune-active HBeAg-positive to immune-active HBeAg-negative hepatitis. Therefore, although the presence or absence of HBeAg is still a useful marker in both the natural history and treatment of chronic HBV infection, it is not adequate as an endpoint in and of itself.

**Serum HBV DNA Concentration.** Serum HBV DNA levels reflect the level of hepatic HBV replication but do not necessarily indicate the presence of ongoing HBV-related liver injury. Individuals in the immune-tolerant phase of infection usually have little or no liver injury despite very high levels of viral replication (usually >10^7 IU/mL), while those with HBeAg-negative disease may have progressive liver disease with much lower viral levels (<10^5 IU/mL). Therefore, it is critical to interpret HBV DNA levels in the context of the natural history of infection and other measures of disease activity.

The observation that patients in the REVEAL-HBV study with HBV DNA concentrations >10^4 copies/mL (~20,000 IU/mL) were at increased risk of HCC (odds ratio = 8.9) has led some to suggest that viral suppression
should be the main goal of therapy for all patients, irrespective of other factors. It is critical to recognize that the majority of the REVEAL-HBV cohort were men, all were over the age of 30, probably infected since childhood, and 85% were HBeAg-negative at study entry. The absolute risk of HCC in younger individuals with very high HBV DNA levels is known to be low. Furthermore, none of the REVEAL-HBV observational cohort was treated, and it is important to stress that this study did not show a reduction in HCC with HBV-suppressive therapy. Clearly, HBV DNA level is an important consideration, but different levels have different implications at different times in different individuals. Until a therapy with no toxicity and no risk of resistance is developed, targeting HBV DNA as the only therapeutic endpoint in all infected individuals is not a realistic or appropriate strategy.

Even less clear than the significance of high levels of HBV DNA is the potential importance of lower levels of viremia. As the sensitivity of assays improves, lower and lower levels of serum HBV DNA can be detected. However, whether very low but detectable viral replication leads to progressive liver disease, HCC, or even antiviral resistance in those on oral antiviral therapy is unclear. The outcome of individuals who spontaneously seroconvert to anti-HBe and remain with low level HBV DNA (<10^3 IU/mL) without reactivation is significantly better than that of patients who remain HBeAg-positive. Whether outcomes such as HBsAg loss would improve further with complete viral suppression is not known.

Although HBV DNA alone cannot be used to determine the need for therapy, it may be a useful marker to follow during oral antiviral therapy. With suppression of viral replication, liver biochemistry, and more importantly, histology, both inflammation and fibrosis improve. Furthermore, failure to achieve viral suppression increases the risk of the development of antiviral resistance. However, although viral suppression is a necessary condition, it is not sufficient on its own as a lasting treatment endpoint. For HBeAg-positive patients who do not achieve HBeAg loss/seroconversion, HBV DNA and disease activity will rebound promptly if treatment is discontinued. Unfortunately, the pattern of decline of HBV DNA during antiviral therapy in patients with HBeAg-positive hepatitis does not reliably predict HBeAg loss/seroconversion whether interferon or nucleoside analog-based therapy is administered.9,10 If HBeAg loss occurs with nucleoside analog treatment, guidelines suggest a period of consolidation before stopping therapy. However, even with a further 6 months of treatment, reactivation with or without reappearance of HBeAg may occur in a proportion of patients.11 Reactivation can be severe, and therefore all patients should continue to be followed after stopping treatment, particularly those with advanced fibrosis. In HBeAg-negative disease, reactivation if treatment is stopped is almost universal even after prolonged viral suppression, unless HBsAg loss occurs. In one small study, HBsAg loss was reported in 18 of 33 (55%) patients treated with adefovir for 4-5 years.12 Thus, sustained suppression of HBV DNA is necessary for full clinical benefit, but is not a sufficient measure of treatment efficacy unless it is sustained and durable when therapy is stopped.

**Drug Resistance and HBV DNA.** Maintained suppression of HBV DNA on treatment with oral antiviral agents is required to minimize the risk for selecting drug resistance mutations. Rise of HBV DNA on antiviral therapy (excluding interferon) suggests either drug resistance or noncompliance (which may increase the likelihood of resistance). Genotypic evaluation of HBV DNA is needed to establish the presence of mutations in the polymerase/reverse transcriptase gene. Presence of resistance mutations can limit treatment efficacy and if allowed to persist, can be associated with worsening of disease (occasionally associated with fatality).13,14

**Liver Biopsy.** Liver biopsy has, to date, been a prerequisite for almost all trials designed to evaluate new antiviral agents in chronic hepatitis B prior to licensing. Pretreatment and end-of-treatment liver biopsies have been required to demonstrate that treatment improved histological activity and was not associated with worsening of fibrosis (little attention has been paid to regression of fibrosis). The size of the liver biopsy specimen is important in assessing fibrosis, because biopsies less than 2.5 cm can lead to an underestimation of hepatic fibrosis. In most clinical trials, a minority of biopsies meet this standard. There are a number of scoring systems designed to report the degree of fibrosis and inflammatory activity. Although the scores are numerical, it is incorrect to assume that they are linear; a change in METAVIR activity from 1 to 2 does not necessarily have the same implication as a change from 2 to 3. The scoring system with the greatest range of values, namely the Ishak score (18 points for necroinflammation and 6 for fibrosis), is more discriminating by virtue of the larger range, and thus it is more sensitive for detecting smaller degrees of change. However, the increase in sensitivity may come at the expense of a decrease in reproducibility.

The development of cirrhosis greatly increases the risk of HCC and the other complications of advanced liver disease, and therefore prevention of this outcome is an important and clinically meaningful goal of therapy. By extrapolation, it seems reasonable to assume that improvement in lesser degrees of fibrosis or inflammation might be beneficial; but this has been difficult to demon-
Noninvasive Markers of Hepatic Fibrosis. Several serum markers have been developed and compared to liver biopsy. Many of these tests were developed initially for chronic hepatitis C. These tests may not be as reliable in chronic hepatitis B, where serum aminotransferase levels can fluctuate widely. Noninvasive approaches to assessing fibrosis using conventional blood tests such as the AST-to-platelet ratio index (APRI), Forns, Fibrotest, ELF (European Liver Fibrosis Group), and the platelet count have been evaluated in hepatitis B (Table 1).

More recently, transient elastography has been developed as a means of assessing hepatic fibrosis. This approach depends on a device that measures the speed at which ultrasonic waves of low amplitude and frequency move through the liver, which can be measured with a high degree of reproducibility and which reflect hepatic “elasticity”. Scores obtained with elastography have been compared to results of liver biopsy and serum-based noninvasive scores and have been found to be equally if not more reliable than the serum-based tests. In some individuals (about 10%), reliable elastography measurements cannot be made, particularly in persons who are overweight or obese. Transient elastography scores can be affected by edema and acute hepatic inflammation as occurs in acute hepatitis, disease relapses, and even acute cholangitis. Clearly, measurement of hepatic elasticity shows great promise as a noninvasive means of assessing hepatic fibrosis. To date, most studies have been conducted in patients with chronic hepatitis C, and its utility in evaluating outcomes of therapy and longitudinal progression in hepatitis B await further definition. Transient elastography measurements may be particularly helpful in assessing persons with normal results on standard blood tests. In a study from China, 18% of subjects with normal results on standard blood tests were destined to progress to advanced liver fibrosis if left untreated.

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<th>Table 1. Use of Serum Markers to Assess Hepatic Fibrosis</th>
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<td>APRI (AST-to-Platelet Ratio Index)</td>
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Platelet Count

*Values determined based on cohorts of patients with hepatitis C infection.

AUC, area under receiver operating characteristic curve; GGT, gamma glutamyl transferase; NPV, negative predictive value; PIINP, N-terminal propeptide of Type III collagen; PPV, positive predictive value; TIMP1, tissue inhibitor of matrix metalloproteinase 1.

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<th>Table 2. The Child-Turcotte-Pugh Score (CTP)</th>
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CTP score = sum of all five individual scores, categorized as A = ≤6, B = 7–9, or C = ≥9.

MELD score = 3.8 × ln (bilirubin mg/dL) + 11.2 × ln (INR) + 9.6 × ln (creatinine mg/dL).
values for ALT, AST, and gamma glutamyl transpeptidase had elastography scores in the range associated with cirrhosis. Unfortunately, transient elastography ultrasound systems have not been approved for use and licensed in North America, whereas serum markers are both inexpensive and widely available.

**Indirect Measures of Disease Complications.** In patients with advanced liver disease, measures of portal hypertension and hepatic synthetic dysfunction can be assessed as markers of progression of disease. These range from simple evaluations such as the decline in platelet count over time as a measure of progressive portal hypertension, to calculation of either the Child-Turcotte-Pugh (CTP) or Model for End-Stage Liver Disease (MELD) scores. The CTP and MELD scores have been repeatedly validated as predictors of hepatic decompensation and survival. The CTP score incorporates clinical (ascites and encephalopathy) and laboratory (albumin, international normalized ratio, bilirubin) parameters that are objective markers of hepatic synthetic dysfunction, portal hypertension, and liver failure (Table 2). As a result, the use of such measures as endpoints in clinical trials has the advantage of demonstration of clear clinical utility; however, they are only useful in populations of patients with advanced disease at baseline.

**HBsAg Status.** Natural history studies of chronic hepatitis B stretching over many decades have shown that loss of HBsAg is associated with improved survival and reduced risk of HCC (particularly if the loss occurs before the age of 50). Recent data indicate that measurement of HBsAg concentration may be a reliable predictor of HBsAg loss while an individual is on antiviral therapy. Furthermore, long-term treatment and follow-up of patients treated with interferon or nucleoside analogs indicate that rates of HBsAg loss can rise to 10%-20% over time. It is unclear whether development of anti-HBs is an essential element in documenting effective disease resolution. Reactivation of chronic hepatitis B is possible even after HBsAg loss but only with profound and sustained immune suppression. Furthermore, in rare occasions, clinical outcomes including HCC can arise in patients with chronic hepatitis B after they have cleared HBsAg.

Although loss of HBsAg is clearly a reliable marker of resolution of clinically significant HBV-related disease and is therefore a useful surrogate endpoint, its relatively rare occurrence limits its utility for evaluation of new therapies. To date, it has been difficult to determine the treatment-related predictors of HBsAg loss because it occurs too infrequently in even relatively large cohorts. Because HBsAg loss should be the goal of therapy, a better
understanding of the factors predicting this occurrence should be a focus of future research.

Conclusions

The avoidance of progressive liver disease and the development of HCC are the true goals of anti-HBV therapy; however, the time necessary to observe an improvement in these outcomes, in the absence of advanced liver disease, makes them unrealistic endpoints for studies or clinical practice. The alternative to using these hard clinical endpoints are various surrogates ranging from serologic markers and viral levels to measures of hepatic fibrosis. These surrogates, while imperfect, are useful as indicators of the need for and efficacy of antiviral therapy. Unfortunately none, with the exception of HBsAg loss, can be considered an accurate and reliable clinical outcome on its own (Table 3). Until therapy improves rates of HBsAg loss or better surrogates are developed, a combination of existing markers of disease activity will have to suffice.

References