Chronic Hepatitis B, C, and D

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ABSTRACT Chronic hepatitis B, C, and D virus infections contribute significantly to the morbidity and mortality of immunocompromised individuals. To contextualize discussion of these infections in immunocompromised patients, this paper provides an overview of aspects of infection in normal hosts. It then describes differences in disease, diagnostic testing, and therapeutic management observed in immunocompromised patients.

INTRODUCTION

Viral hepatitis contributes significantly to the morbidity and mortality of at-risk individuals, particularly in chronic, untreated infections. Clinical symptoms vary from none (asymptomatic carriers) to fulminant liver failure. Hepatitis viruses A, B, C, D, and E are responsible for the majority of liver disease, with hepatitis A and E viruses being the least common of the culprits.

This review will provide a general overview of different disease states of chronic hepatitis B, C, and D infections and the management of these infections through diagnostic testing and treatment. It will then focus on aspects of these topics relevant to individuals with quantitative or functional immune deficits due to pharmacologic immunosuppression and human immunodeficiency virus (HIV) coinfection. General characteristics of these viruses that are relevant to resulting liver disease and the management of infection are summarized in Table 1.

HEPATITIS B

Hepatitis B virus (HBV) is highly infectious. Over two billion people have been infected worldwide and most are unaware. Spontaneous resolution occurs in approximately 80% of infected individuals. Chronic hepatitis B (CHB) occurs in the remainder and has reached pandemic proportions of approximately 350 to 400 million cases worldwide.

CHB causes cirrhosis, liver failure, and hepatocellular carcinoma, resulting in approximately 1 million deaths per year (1–3). CHB is widespread in Asia, particularly China. It is also highly prevalent in the Middle East, Africa, and parts of the Americas (1, 4, 6). The prevalence of CHB is lower in the U.S. (~0.5% of the general population); however, prevalence can be as high as 10% in some immigrant populations. Given the global disease burden of this chronic infection, international guidelines recommend screening and management to identify and consider treating CHB to reduce its morbidity and mortality (7, 8).

HBV infection is extraordinarily complex; an understanding of it in normal individuals is therefore useful to better comprehend infection in the immunocompromised patient. Aspects of infection in the normal host that will be reviewed are virion components of diagnostic importance and topics of relevance to CHB, including phases of CHB, immune control, diagnostic testing, treatment, and prevention. Among the different categories of immunocompromised individuals, HBV is most problematic for patients whose antiviral lymphocyte responses are pharmacologically suppressed or impaired by HIV coinfection. In these patients, at-risk
populations and topics relevant to disease (mechanisms, sequelae, treatment, and prevention) will be reviewed.

**Chronic Hepatitis B in the Normal Host**

**Diagnostically important HBV markers, phases of CHB Infection, and testing for the diagnosis of CHB**

HBV markers that are important for understanding CHB include hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), a peripherally secreted protein (HBeAg), and HBV genomic DNA (Fig. 1). These proteins, and/or antibodies against them, along with HBV DNA, are used in the diagnosis of hepatitis B infection (Tables 2–4, also reviewed in reference 9).

HBsAg is a glycoprotein embedded in the host cell-derived lipid membrane of intact virions and in nucleocapsid-free particles that circulate in abundance in individuals with active CHB. HBsAg binds the cellular receptor to initiate infection and is detectable in blood by 4 to 10 weeks following an acute infection; antibody to HBsAg (HBsAb) confers protective immunity to HBV infection (“neutralizing antibody”) and is an indicator of resolved infection. HBsAb is detectable approximately 5 to 6 months following exposure in individuals who spontaneously clear acute infection.

Antibody to the core antigen (anti-HBc) is a host marker of HBV infection. Anti-HBc IgM appears 6 to 8 weeks after infection and remains detectable in the window period between HBsAg clearance (~24 weeks after infection) and development of anti-HBs antibody (~32 weeks after infection). It therefore indicates acute infection in symptomatic individuals in whom HBsAg is no longer detectable. Total anti-HBc IgM+IgG becomes detectable coincident with anti-core IgM (due to anti-core IgM detection) and can persist indefinitely due to detection of anti-core IgG, which can outlast the production of detectable anti-HBs antibody, and is therefore a marker of prior HBV infection. Unlike HBsAb, anti-HBc IgG is not protective. Both anti-HBs and total anti-HBc (IgM and IgG) are positive when infection is resolved by natural immunity. Anti-HBs is the only antibody detectable in vaccinated individuals.

**FIGURE 1** Key HBV virion components.

Outer Envelope
Surface antigen HBsAg

HBV DNA
Core Protein HBcAg
Peripherally secreted Protein blood: HBeAg
DNA Polymerase

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**TABLE 1** Characteristics of hepatitis B (HBV), C (HCV), and D (HDV) viruses

<table>
<thead>
<tr>
<th></th>
<th>HBV (221, 222)</th>
<th>HCV (223)</th>
<th>HDV (224)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome</td>
<td>3.2 kb partially double-stranded DNA</td>
<td>9.6 kb positive-strand RNA Replication from negative-strand antigenomic RNA within HCV-induced cytoplasmic double membrane vesicles</td>
<td>1.7 kb negative-sense defective RNA Replication in nucleus via subverted host DNA-dependent RNA polymerase and “double rolling circle” mechanism. Single-stranded RNAs formed by HDV ribozyme-mediated self-cleavage</td>
</tr>
<tr>
<td>Genome replication</td>
<td>Pregenomic RNA transcribed from covalently closed circular (ccc) DNA in nucleus, translocated to cytoplasm where it is reverse transcribed into partially dsDNA. Small amount of cccDNA also made and shuttled back into nucleus</td>
<td>Replication from negative-strand antigenomic RNA within HCV-induced cytoplasmic double membrane vesicles</td>
<td></td>
</tr>
<tr>
<td>Mutation rate Infecivity</td>
<td>Lower mutation rate (10^{-5}) High</td>
<td>Higher mutation rate (10^{-3}) Moderately high</td>
<td>Very high High but requires a hepatitis B infection</td>
</tr>
<tr>
<td>Ability to clear infection with antivirals</td>
<td>Depends on antiviral efficacy against cccDNA. Interferon α: cure rate, 30% HBeAg+, 40% HBeAg- Viral replication is suppressed but not eradicated by nucleos/tide and nonnucleos/tide reverse transcriptase inhibitors because cccDNA is maintained in nucleus</td>
<td>Yes; multidrug direct-acting antiviral regimens produce sustained virologic response</td>
<td>Yes; pegylated interferon-alfa (response rates ~20%) or liver transplant</td>
</tr>
</tbody>
</table>

Cobb and Valsamakis
HBV e antigen (HBeAg) is encoded by the HBV precore/core gene. It is secreted from hepatocytes in which wild-type HBV is actively replicating and therefore serves as a serological marker of viral replication. HBeAg expression can be abrogated by point mutations in the basal core promoter or in the precore coding region. These mutant viruses can still replicate, although at lower levels compared to wild-type virus, and can cause disease progression (HBeAg-negative CHB, see below).

The HBV genome exists in two different forms, partially double-stranded, relaxed circular DNA (rcDNA) found in virions, and supercoiled covalently closed circular DNA (cccDNA) found in the nucleus of infected cells. In newly infected cells, rcDNA from incoming virions is translocated into the nucleus and converted to cccDNA through DNA repair and strand ligation. In the nucleus, cccDNA exists as an episome and serves as the template for synthesis of viral mRNAs and full-length pregenomic RNAs (pgRNAs) that are exported to the cytoplasm where new rcDNAs are synthesized by viral reverse transcriptase using pgRNA as a template. Newly synthesized rcDNAs are then either packaged into virions or transported into the nucleus to form additional cccDNAs.

HBV DNA is released from hepatocytes as a component of intact released virions and as a result of the host necroinflammatory response. Detection of HBV DNA in plasma or serum is therefore an indicator of active infection and underlying liver disease; all guidelines therefore recommend HBV DNA assessment in addition to other markers when establishing the diagnosis of CHB.

Various immunoassays can be used to detect HBV serological markers (reviewed in reference 9). False-negative antibody results can occur depending on when testing is performed during infection and in the setting of immunosuppression. Quantitative HBsAg quantifi-

### TABLE 2 Serologic markers of hepatitis B virus infection

<table>
<thead>
<tr>
<th>Marker</th>
<th>Description</th>
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<tbody>
<tr>
<td>HBeAg</td>
<td>Viral protein of uncertain function produced during replication. Presence indicates high levels of replication and high infectivity. Viral mutants in precore region that fail to express HBeAg evolve during CHB and are still pathogenic (“HBeAg-negative CHB”).</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>Antibody produced against HBeAg. Can indicate resolution of acute and chronic infection but is also observed in HBeAg-negative CHB.</td>
</tr>
<tr>
<td>HbsAg</td>
<td>Viral envelope glycoprotein found in hepatocyte cell membrane, virion envelope, and circulating, highly abundant virions and noninfectious viral particles. Indicates current infection and is detected in acute and chronic HB. Persistence for longer than 6 months typically indicates chronic infection.</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Total antibody (IgM+IgG) produced against HBsAg. Appearance after infection most commonly indicates resolution of disease. Also observed in rare chronic infections with viral mutants that fail to express HBsAg. Anti-HBs is a neutralizing antibody indicating protection from reinfection and successful immunization after vaccination.</td>
</tr>
<tr>
<td>Anti-HBc (IgM only, and total IgM+IgG)</td>
<td>Antibody produced against core antigen. Detectable anti-HBc IgM and total IgM+IgG indicates acute infection. In acute resolving infection, these markers are positive in the window period between HBsAg clearance and anti-HBs detection, 24 to 32 weeks after infection. Anti-HBc IgM+IgG in the absence of anti-HBc IgM indicates remote exposure to HBV and is observed in resolved and chronic infection.</td>
</tr>
</tbody>
</table>

### TABLE 3 Serologic and molecular test results in typical acute, resolved, and chronic hepatitis B

<table>
<thead>
<tr>
<th>Status of Infection</th>
<th>HBsAg</th>
<th>Anti-HBc IgM</th>
<th>Anti-HBc total (IgM+IgG)</th>
<th>Anti-HBs</th>
<th>HBV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not infected</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acute infection</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Chronic infection</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+/−</td>
</tr>
<tr>
<td>Resolved infection</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>−</td>
</tr>
<tr>
<td>Vaccination</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

−, not detected; +, detected; +/−, may be detected. See reference 2 for specific tests, methods, assay performance characteristics, and additional interpretive issues in immunocompetent hosts.

For HBsAg-positive results, confirmation with an approved HBsAg neutralization test is recommended.

HBV DNA may be detected 1 to 2 weeks prior to appearance of HBsAg during acute infection. HBsAg is detectable within 2 to 4 weeks of exposure and persists until approximately 24 weeks after infection. Anti-HBc IgM and total anti-HBc IgM+IgG are detectable 6 to 8 weeks after infection and remain detectable until approximately 32 weeks, persisting as markers of acute infection during the window period between HBsAg disappearance and detection of anti-HBs antibody.

Detectable serologic markers can vary and HBV DNA levels can fluctuate depending on the phase of CHB, as shown in Table 4.

Decades after resolution of acute infection, anti-HBs antibody may become undetectable, leaving total anti-HBc as the only detectable serologic marker of infection. In this phase, hepatocytes still harbor HBV genomes and viral replication can remitiate during periods of immunosuppression.
cation has been studied intensively; however, no tests have received regulatory approval and there are no current recommendations for their use. Highly sensitive quantitative real-time PCR tests are used to detect and measure HBV DNA, and these approved tests have similar analytical performance characteristics (reviewed in reference 9). HBV DNA levels above 2,000 IU/ml or increasing trends indicate evidence of active replication that may be clinically significant. However, the significance of persistent or intermittently low HBV DNA levels (either detected but below the lower limit of quantification, LLOQ, or a low value near the LLOQ) should be interpreted in conjunction with serological and other markers of disease. Importantly, low HBV DNA levels have been transiently detected in individuals who have recovered from acute infection but are at increased risk of hepatocellular carcinoma (HCC), suggesting potential utility of cancer screening in this population (10).

HBV is genetically diverse. Eight distinct genotypes have been characterized (A to H); the genotype distribution throughout the world is shown in Table 5. There is currently no diagnostic utility to HBV genotype determination.

There are four phases of CHB (Fig. 2, Table 4). The first is the “immune tolerance phase,” which is characterized by high HBV DNA concentrations in blood, relatively normal serum transaminase levels, and detectable HBeAg. This phase is prolonged after perinatal and early childhood infection but is brief or nonexistent after acute infection in adults. The “immune clearance phase” occurs with the onset of a robust anti-HBV immune response. In this phase, HBV DNA levels in blood are initially high and then fluctuate as the immune system gains some control over the virus. Most individuals transition from this phase into the third, “inactive carrier” phase, in which HBV DNA levels decline to low or undetectable levels and HBeAg seroconversion occurs (disappearance of HBeAg/emergence of detectable anti-HBe antibody). Greater rates of disease progression and higher annual incidence of cirrhosis are observed if immune clearance is prolonged and HBeAg persists. In contrast, in inactive carriers, liver inflammation is minimal to mild and rates of disease progression are low. HBV replication can recur during the inactive carrier phase. In these individuals, HBV mutations that abrogate HBeAg expression occur. HBV replication in this fourth “HBeAg-negative” phase of CHB induces a necroinflammatory response leading to detectable HBV DNA in blood, a rise in serum transaminases, and liver damage that can progress to cirrhosis.

Prevention of HBV infection
HBV infection can be prevented by immunization of uninfected individuals with hepatitis B vaccine (11) that consists of purified HBsAg expressed in yeast. HBV vaccination programs have significantly reduced the incidence of perinatal infection, especially in developing countries, but only a small number of adults worldwide are immunized. Among adults, screening programs are critical for identifying uninfected individuals who would benefit from vaccination and for diagnosing chronically infected individuals who may benefit from treatment.
to prevent transmission and progression of liver disease (12). Despite high levels of newborn vaccination in the U.S., HBV screening is still important due to ongoing high transmission rates in selected populations (13).

**Therapeutic management of CHB**

HBV treatment can significantly reduce the risk of HCC and prevent death. Two classes of drugs are available: immunomodulators (interferon alfa and pegylated interferon alfa) and nucleos(t)ide analogs that inhibit HBV reverse transcriptase (Table 6). Interferon alfa administration for a defined interval, typically 24 to 48 weeks, inhibits viral replication and can result in the loss of HBeAg and HBsAg followed by the development of anti-HBe (“HBeAg seroconversion”) and anti-HBs antibodies (“HBsAg seroconversion”). In chronically

**TABLE 6** Current treatments for CHB

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>HBV Antiviral activity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon alpha-2b (Schering-Plough Corp)</td>
<td>Immunomodulator</td>
<td>Weak</td>
<td>None</td>
</tr>
<tr>
<td>Lamivudine (GlaxoSmithKline)</td>
<td>Nucleos(t)ide analog</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>Adefovir dipivoxil (Gilead Sciences, Inc.)</td>
<td>Nucleos(t)ide analog</td>
<td>Weak</td>
<td>Moderate</td>
</tr>
<tr>
<td>Entecavir (Bristol-Myers Squibb)</td>
<td>Nucleos(t)ide analog</td>
<td>Weak</td>
<td>Low</td>
</tr>
<tr>
<td>Peginterferon alfa-2a (Hoffmann-La Roche Inc.)</td>
<td>Immunomodulator</td>
<td>Weak</td>
<td>None</td>
</tr>
<tr>
<td>Telbivudine (Novartis)</td>
<td>Nucleos(t)ide analog</td>
<td>No</td>
<td>High</td>
</tr>
<tr>
<td>Tenofovir (Gilead Sciences, Inc.)</td>
<td>Nucleos(t)ide analog</td>
<td>Yes</td>
<td>Low</td>
</tr>
</tbody>
</table>
infected individuals, clearance of HBsAg and development of anti-HBs positivity can be observed spontaneously in roughly 0.5 to 2% of patients per year (14–16). The nucleos(t)ide analog-induced HBsAg clearance rate is similar, 0.33% per year (17). Despite this relatively low rate, HBsAg clearance is durable and associated with favorable outcomes. Among these seroconverters, disease progression and HCC is limited to those with extensive liver damage prior to viral clearance.

HBsAg seroconversion represents resolution of infection and is thought to occur through the induction of intracellular interferon-response effectors that degrade HBV RNAs and DNAs, including cccDNA, thereby shutting down viral replication and eradicating virus from infected hepatocytes (18).

Nucleos(t)ide analogs inhibit reverse transcriptase and suppress HBV replication but have no effect on clearance of cccDNA or eradication of infection. Discontinuation of nucleos(t)ide analog therapy leads to a rebound in viral replication that can result in hepatic flare (17, 19–21); therefore, these drugs must be administered indefinitely to sustain suppression of viral replication and maintain therapeutic efficacy. Drugs with high barriers to resistance are now available. They are easily dosed and have a favorable safety profile compared to interferon alfa, which must be administered subcutaneously and has significant side effects such as psychiatric side effects (depression, psychosis) and flu-like symptoms. Therefore, although indefinite therapy may be required, nucleos(t)ide analogs are used preferentially over interferon alfa.

The decision to initiate treatment has been based historically on elevated alanine aminotransferase (ALT) and high HBV DNA levels (>20,000 IU/ml) (22) because these abnormalities correlate with increased disease progression risks and rates (23–25). However, significant fibrosis can also occur despite normal ALTs and low levels of HBV DNA (<2000 IU/ml) in HBeAg-positive and HBeAg-negative CHB. Therefore, recent recommendations outline a more nuanced approach consisting of treatment for individuals with abnormal ALTs and HBV DNA levels >2000 IU/ml and fibrosis assessment in those with normal ALT regardless of HBV DNA level (26). Treatment should be undertaken if liver assessment through transient elastography or biopsy demonstrates underlying disease; otherwise ALT and HBV DNA can be monitored every 6 to 12 months.

The primary endpoint of therapy is prevention of disease progression (cirrhosis, HCC) through suppression of HBV replication indicated by undetectable HBV DNA in peripheral blood as determined with a sensitive nucleic acid amplification test (NAAT) using plasma or serum. Other endpoints such as HBeAg seroconversion and HBsAg seroconversion are desirable but more difficult to achieve. Stopping rules for nucleos(t)ide analog therapy, while of great interest recently, have yet to be established (27, 28).

HBV DNA viral load measurement plays an important role in the management of CHB because it is used in the decision to initiate therapy, to assess response during treatment, and, in the case of interferon alfa, to determine durability of response after treatment (22, 29, 30). HBV DNA levels are determined at baseline and then at regular intervals (typically every 3 months) throughout the duration of nucleos(t)ide analog treatment.

Pharmacologic immunosuppression and reactivation of hepatitis B

The major risk of hepatitis B disease in pharmacologically immunosuppressed patients is due to reactivation of prior HBV infection. Treatments that are of particular concern are drugs used as preparative agents for hematologic stem cell transplant (HSCT) for leukemias and lymphomas, for the treatment of solid tumors and autoimmune diseases, and to prevent allograft rejection after solid organ transplant (31–33). In contrast, acute HBV infection in this setting occurs rarely due to the use of screened blood products during treatment and vaccination of HBV-seronegative patients prior to or after immunosuppression, as directed by current recommendations (34, 35).

The degree of reactivation risk is associated with the state of HBV infection at the time of immune suppression and with immunosuppressive drug regimens (Table 4, Table 7, Fig. 3). Individuals with detectable HBsAg at the time of immune suppression can have acute exacerbations of hepatitis. The etiology of the exacerbation is readily determined by documentation of high or increasing HBV DNA levels (Table 7). In individuals with serologic evidence of recovery from hepatitis B (no detectable HBsAg, detectable anticoagulant antibody, with or without surface antibody), immunosuppression can cause reactivation of HBV replication that is diagnosed by the findings of reappearance of HBsAg, termed “reverse seroconversion,” and rising HBV DNA levels (Table 7). These patients have a lower, but definite, risk of immune suppression-associated reactivation compared to individuals with detectable HBsAg (Fig. 3).

HBV replication is curbed normally by multiple immune mechanisms including key modulators of innate immunity such as interferon gamma and TNF-alpha, and components of adaptive immunity such as CD8+ T cells.
that clear HBV from infected hepatocytes by cytolytic and noncytolytic mechanisms (36). The disruption of this immune control leads to a resurgence of HBV replication, which by itself is noncytolytic. Hepatitis occurs after cessation of immune suppression, upon immune reconstitution. Allogeneic HSCT is associated with the highest rates of exacerbation in HBsAg-positive and reactivation in HBsAg-negative individuals due to powerful pretransplant immune-ablation regimens and subsequent transplant with cells from HBV-naive donors. In HBsAg-negative individuals, reverse seroconversion can occur years posttransplant. One 8-year follow-up study demonstrated that the cumulative probability of reactivation was 9.0%, 22%, and 43% at 1, 2, and 4 years after transplant (37). In autologous HSCT, lower rates of recurrence are observed due to less potent conditioning regimens and preexisting immunity. Rituximab, a mouse/human chimeric monoclonal antibody that targets anti-CD20 on normal and abnormal B lymphocytes, is associated with HBV reactivation rates on par with allogeneic

### TABLE 7 HBV-infected individuals at risk of HBV reactivation/exacerbation after pharmacologic immunosuppression and associated laboratory results during reactivation/exacerbation

<table>
<thead>
<tr>
<th>Serological and molecular profile</th>
<th>Indicator of HBV Reactivation/exacerbation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive CHB carrier</td>
<td>HBV DNA</td>
</tr>
<tr>
<td>Recovered from infection</td>
<td>HBsAg(-) or (+) serum/plasma</td>
</tr>
<tr>
<td>Isolated anti-HBc status</td>
<td>HBsAg(-) or (+) serum/plasma, hepatocytes</td>
</tr>
</tbody>
</table>

*aAdapted from reference 225.*
HSCT (Fig. 3). It causes prolonged lymphopenia in addition to B cell depletion. Total lymphocyte and B lymphocyte counts normalize approximately 6 months and 9 to 12 months after treatment, respectively (38). The mechanism underlying enhanced HBV reactivation risk of rituximab is unclear, although multiple indirect effects on CD8+ cytotoxic T cells have been postulated (39). Corticosteroid use (by itself or as a component of multi-drug chemotherapeutic regimens) is associated with rates of HBV recurrence that are substantial but lower than those observed with allogeneic HSCT and rituximab. Therapeutic regimens to prevent acute episodes of allograft rejection in solid organ transplant recipients, anti-TNF-alpha monoclonal antibody treatment, and conventional chemotherapies (not containing corticosteroids or rituximab) are associated with lower risks of HBV recurrence. Other anti-cytokine biopharmaceuticals have been cited as incurring a high risk of HBV reactivation (40); however, these drugs have been used for a briefer period and in a smaller number of patients compared to other immunosuppressive treatments, and therefore their effects have not been well characterized in the published literature.

A necroinflammatory response against resurgent HBV replication and consequent liver damage produces symptoms and laboratory evidence of acute viral hepatitis, including jaundice, elevated transaminases, and derangements of other serum markers denoting hepatic injury. Individuals who are HBsAg-positive can have hepatic flares during treatment with immunosuppressive drugs. In HBsAg-negative/anti-HBc-positive individuals, hepatitis can occur at any time, but it typically occurs when the immune system has reconstituted and recovered functionally, a median of 3 months after discontinuation of therapy (41). Rates of sequelae after HBV reactivation are significant and a major danger to these patients. Hepatitis, liver failure, and death due to fulminant liver failure have been observed in approximately 40%, 10% and 5% of HBsAg-positive individuals after chemotherapy for solid tumors, allogeneic HSCT, and anti-TNF alpha regimens (42). Rates of these complications after reverse seroconversion are lower but still considerable (hepatitis in ~5%, liver failure in 2%, death due to fulminant liver failure in 0.5%) (42–44). Recurrent hepatitis can result in delayed or indefinitely deferred treatment of individuals with underlying malignancies, which can diminish the efficacy of chemotherapy.

Individuals at risk of HBV exacerbation or reactivation should be identified prior to immunosuppression to implement appropriate management strategies that prevent adverse hepatitis B outcomes. To this end, public health and professional gastroenterology societies have recommended universal screening with initiation of antiviral prophylaxis prior to treatment with high-risk immunosuppressive regimens and consideration of prophylaxis or preemptive treatment for individuals who will undergo treatments associated with moderate risks of reactivation (8, 45, 46). Universal screening has been advocated due to the lack of efficacy of risk factor-based screening (47–51). In regions of low HBV prevalence, risk factors are underrecognized by care providers and patients. Moreover, the importance of identifying and treating individuals at risk of adverse outcomes related to hepatitis B exacerbation is underappreciated in high- and low-prevalence regions. Severe adverse consequences, including viral reactivation, hepatitis flares, interruption of chemotherapy, liver failure requiring liver transplant, hepatic decompensation, and death have been documented as a result of these lapses, underscoring the potential utility of mandating universal screening and preventive treatment broadly across treatment guidelines in all subspecialties.

As a preventive treatment strategy, prophylaxis has been favored over preemptive therapy, due to its efficacy. Among individuals undergoing treatment for lymphoma, prophylaxis is more effective than preemptive therapy in preventing hepatitis flares (52, 53), protracted elevations in HBV DNA (>2000 IU/ml) and reverse seroconversion (54). An additional advantage of prophylaxis is its simplicity. In comparison, preemptive therapy requires extensive, thoughtful follow-up, with continuous viral load monitoring and timely therapeutic intervention to prevent HBV-related complications.

Universal screening and prophylaxis have not been widely adopted, despite the soundness of their objectives. This gap may be due to the lack of incorporation of these recommendations into the practice guidelines of some subspecialty societies (31), physician concerns regarding the lack of data supporting the cost-effectiveness of universal screening, and a failure of individual practitioners to fully appreciate the hazards associated with HBV exacerbation.

Once antiviral treatment is initiated prophylactically, preemptively, or to treat a flare of hepatitis, it should be continued throughout immunosuppression and for a period thereafter, to prevent hepatitis flares that can arise with immune recovery and resurgence of the HBV immune response. The duration of antiviral therapy after immunosuppression is not uniformly agreed upon. Up to 12 months has been advocated, and an even longer duration (potentially lifelong) may be required in HSCT given immune naiveté after reconstitution (40).
Drugs that have been used to prevent HBV exacerbation in immunosuppressed patients include lamivudine, entecavir, and tenofovir. Lamivudine was first-line therapy for many years in this setting because it was the only direct-acting antiviral drug available for HBV. Numerous studies have demonstrated its efficacy in reducing HBV reactivation and its associated adverse outcomes (reviewed in reference 31). However, its low barrier to resistance is a significant disadvantage that limits its utility in patients who require prolonged treatment (greater than 6 months). Lamivudine is still recommended as an inexpensive option that is safe and effective when used for short periods during and after immunosuppression (8, 55, 56). Entecavir, with its high barrier to resistance, is now recommended for longer therapeutic courses and has been shown to have better efficacy compared to lamivudine in this setting. In a randomized prospective trial of individuals undergoing standard rituximab-containing therapy for diffuse large B cell lymphoma who were HBsAg-positive, who had low levels of HBV DNA (<2000 IU/ml), and who had no previous history of HBV treatment, lower rates of HBV reactivation, hepatitis, and HBV-related chemotherapy disruption occurred in those treated with entecavir compared to lamivudine (57). A retrospective study of patients undergoing therapy for solid tumors reported similar findings among those who were HBsAg-positive and had higher HBV DNA levels (>2000 IU/ml) (58). There are currently no studies reporting on tenofovir in this setting, despite its known efficacy in the treatment of chronic hepatitis B and guidelines recommending its potential use in preventive strategies.

Hepatitis B and HIV coinfection

Coinfections with HBV and HIV are common since these viruses share transmission routes. Globally, rates of HBsAg-positive chronic and occult (anti-HBV core antibody only) coinfections vary based on endemicity, with the highest rates in Africa and Asia (8 to 15%). Lower rates occur in the U.S. and Western Europe (<2%) (59).

HBV infection differs significantly in HBV/HIV-coinfected compared to HBV-monoinfected individuals. After acute HBV infection, a higher proportion of HIV-infected individuals become chronically infected compared to HIV-uninfected individuals (60, 61). HIV-infected individuals with CHB have higher levels of HBV replication (manifested as higher HBV viral loads) (62, 63), decreased rates of HBeAg clearance (62, 64), higher rates of reverse seroconversion (62, 65), higher rates of cirrhosis (63) and greater liver-related mortality (66, 67). Low CD4 counts in HBV/HIV-coinfected individuals are associated with higher incidence of cirrhosis, cirrhosis-related death, and HCC (68, 69). In contrast, HBV infection appears to have no effect on HIV disease progression or response to antiretroviral therapy (67, 70, 71).

To prevent liver-related complications of HBV/HIV coinfection, current guidelines recommend serologic assessment at entry into care for HIV and just prior to initiation of antiretroviral therapy (72, 73). Testing should include HBsAg, anti-HBc antibodies, and anti-HBs antibodies. Vaccination is recommended for individuals who lack serologic evidence of HBV infection. HIV-induced immune deficits result in decreased antibody responses to conventional HBV vaccine dosing (0, 3, 6 months) (74). Boosted regimens that consist of four doses (0, 1, 2, 6 months) of double-strength vaccine produce improved seroresponses (75). Additional doses should be administered to nonseroconverters. Anti-HBs antibody titers should be assessed annually in seroconverters; revaccination should be undertaken when antibody levels fall below protective levels (<10 IU/ml).

Individuals who are HBsAg-positive should be further evaluated to characterize chronic HBV infection. Testing should include HBeAg determination and HBV DNA quantification (plasma or serum). Isolated anti-HBc total (IgM+IgG) antibody occurs commonly (~20% in one study) (76). Typically, this is a benign finding; most (~85%) have persistent isolated anti-HBc, and approximately 10% have been shown to develop serologic profiles consistent with resolution of infection. However, evolution into chronic infection occurs in a small proportion (~1%), suggesting that follow-up with additional HBV testing (HBsAg, HBV DNA) is warranted for these patients. Individuals found to be HBV/HIV-coinfected should be treated with regimens that have high potency against HBV and minimal risk of resistance. Antivirals with activity against reverse transcriptase can be used to effectively treat HBV and HIV since this polymerase plays a key role in the replication of both viruses. Tenofovir is the current drug of choice in HBV/HIV-coinfected individuals due to high efficacy and low resistance rates even after 7 years of therapy (77, 78). It can be administered alone or in combination with emtricitabine (formulated as a single tablet) and is effective in individuals with lamivudine resistance. Monotherapy with lamivudine and adefovir are not recommended since rates of resistance to these drugs are high among HBV-monoinfected individuals and even higher in HBV/HIV coinfection. For example, resistance developed in 67% of HBV monoinfected individuals versus 90% of...
HBV/HIV-coinfected individuals treated with lamivudine for 5 years (79, 80). Entecavir monotherapy is also an option, but only in individuals without lamivudine resistance; high rates of entecavir resistance occur in the setting of preexisting lamivudine resistance (resistance to entecavir, 1% at 5 years in the absence of lamivudine resistance, versus ~50% with lamivudine resistance) (81). Sudden discontinuation of HBV treatment in antiretroviral therapy regimens can result in severe hepatitis flares and therefore should be avoided (82). Treatment efficacy should be assessed through HBV viral load determination, performed initially every 3 months. Within 6 to 12 months of treatment, HBV DNA should decline to undetectable levels using a sensitive nucleic acid amplification-based assay. Effective therapeutic management remains challenging in the developing world where these coinfections are common, due to drug availability, drug cost, limited availability of tests for therapeutic monitoring, and the lack of experienced subspecialists who can manage coinfected individuals (83).

HEPATITIS C
Chronic hepatitis C (CHC) is a progressive disease of pandemic proportions, with recent estimates reaching 185 million affected persons worldwide (84). Infections are transmitted readily from unaware, asymptomatic CHC carriers who, over decades, have an increased risk of hepatic and extrahepatic complications that cause morbidity and mortality (85). Approximately 20% of untreated individuals progress to cirrhosis, of which 15% develop HCC within 10 years. In the U.S., the prevalence of CHC (~2.7 to 5.2 million) is now more than double that of HIV (~1.3 million) (86, 87), with approximately 17,000 to 30,000 new HCV infections occurring each year.

Acute infections are cleared in approximately 20% of individuals; the remainder become chronically infected. Chronic infection is established by viral variants that can evade the host immune response and therefore have a selective survival advantage. These variants are generated during genome replication as a consequence of HCV’s RNA-dependent RNA polymerase that lacks proofreading functionality. Unlike other viruses such as HIV-1, which integrates permanently, or HBV, which retains cccDNA in the nucleus as minichromosomes, HCV does not integrate into the human genome or reside stably in host cells. HCV virions have a short half-life; therefore, rapid and continuous viral replenishment are required in order to maintain infections of hepatocytes (88, 89).

Screening, Diagnosis, and Prevention
Longstanding recommendations to screen for infection based solely on risk factors have been helpful but have failed to adequately identify the majority of asymptomatic carriers (90). Therefore, U.S. testing recommendations were amended to include one-time HCV screening for all persons born between 1945 and 1965, given the high prevalence of CHC in this population (approximately 75% of carriers) (85).

Serologic assays that detect HCV antibodies are used as screening tests to identify HCV-infected individuals. HCV NAATs are used to confirm reactive antibody results (Fig. 4, Table 8) (13, 85) and to differentiate individuals with spontaneous clearance (HCV-seropositive only) from those with CHC (HCV-seropositive and HCV RNA-positive) (91). Clinical practice guidelines recommend using a sensitive molecular method (LLOQ ≤15 IU/ml) for the diagnosis of acute and chronic hepatitis C infection (29, 92). Currently, two NAATs are FDA approved for diagnostic confirmation: a qualitative test based on transcription-mediated amplification, (a non-PCR, endpoint detection-based method) and a quantitative, real-time PCR-based HCV RNA test. Immunosuppression can cause false-negative anti-HCV antibody results. Approximately 5% of HIV-infected individuals have been documented to have CHC using HCV RNA detection tests but to have no detectable anti-HCV antibody using second- and third-generation HCV serology assays (76). Reduced sensitivity of serology tests has also been documented in chemotherapy-
TABLE 8  Testing for hepatitis C virus: types of assays (specimens), indications, result interpretations

<table>
<thead>
<tr>
<th>Assay typea (specimen)</th>
<th>Indication</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV immunoassay for detection of anti-HCV IgM+IgG (serum or whole blood finger stick)</td>
<td>Screening for infectionb</td>
<td>Reactive: individual has been infected with HCV; additional HCV RNA detection or quantification test required. Nonreactive: individual has not been infected with HCV. False-negative results can occur in acute infection, in individuals undergoing hemodialysis and during immunosuppression (especially HIV infection). HCV RNA testing should be performed to definitively exclude infection when false-negative serology results are suspected.</td>
</tr>
<tr>
<td>HCV RNA detection by qualitative or quantitative NAATc (serum or plasma)</td>
<td>Confirmation of infection and differentiation between resolution of acute infection and chronic infection</td>
<td>HCV RNA detected: individual is infected with HCV. False-positive results can occur due to contamination; testing should be repeated if clinically indicated. HCV RNA not detected: individual is not infected with HCV. False-negative results can be obtained in the early phase of acute infection; therefore testing at 1, 3, 6, and 12 months after exposure is recommended.</td>
</tr>
<tr>
<td>HCV genotype determinationd (serum or plasma)</td>
<td>Selection of appropriate treatment regimen (see Table 8).</td>
<td>Genotypes 1–6 and subtypes of genotype 1 (gt 1a and gt 1b) are reported. Baseline and week-4 testing are performed to document response to therapy and to assess/encourage compliance. HCV RNA is often undetectable by week 4 of therapy with recommended DAAs. Undetectable HCV RNA 12 weeks after end of treatment indicates cure and is designated “sustained virologic response.”</td>
</tr>
<tr>
<td>HCV RNA quantification (serum or plasma)</td>
<td>Monitoring of therapeutic efficacy (baseline, week 4, and 12 weeks after end of treatment)</td>
<td>In gt1a infections, elbasvir should not be used in the presence of certain NS5A polymorphisms due to reduced efficacy. Variants at the following NS5A amino acid positions are reported: Amino acid 38 methionine → alanine, glycine, or threonine Amino acid 30 glutamine → aspartic acid, glutamic acid, histidine, glycine, lysine, leucine, or arginine Amino acid 31 leucine → phenylalanine, methionine, or valine Amino acid 93 tyrosine → cysteine, histidine, asparagine, or serine</td>
</tr>
<tr>
<td>Antiviral resistance-associated variant detection (plasma)</td>
<td>Selection of appropriate treatment regimen.</td>
<td></td>
</tr>
</tbody>
</table>

See reference 226 for specific tests, methods, assay performance characteristics, and additional interpretive issues in immunocompetent hosts.

- One-time screening based on 1945–1965 birth cohort or historical risk is recommended. Repeated screening is recommended for seronegative individuals with ongoing high-risk behaviors. Seropositive individuals with ongoing high-risk behaviors who have spontaneously cleared infection (anti-HCV antibody positive/HCV RNA not detected) should be screened for new infection using HCV RNA detection tests.

- Both qualitative and quantitative nucleic acid amplification tests are approved for confirming an antibody-positive test result. Confirmation of infection with quantitative HCV RNA tests has the benefit of simultaneously providing baseline viral load results. Additional baseline viral load testing is recommended if qualitative HCV RNA detection tests are used for confirmation.

- A single reverse transcription real-time PCR-based test has been approved for use in HCV genotype determination. Other commonly used laboratory-developed tests employ line probe hybridization of PCR products or direct Sanger sequencing.

- Testing at end of treatment and 24 weeks after end of treatment can also be considered to document response to therapy. Viral load determination at 6 weeks of therapy is currently recommended if HCV RNA is detectable at week 4. Treatment should be discontinued if HCV RNA has increased 10-fold or more at week 6 compared to week 4.

- No tests have received regulatory approval for this use; direct Sanger sequencing is most commonly employed.

induced immune suppression (76, 93). HCV NAATs are used to identify HCV infections when false-negative serology results are suspected (Table 8).

Once CHC has been diagnosed, molecular tests (HCV RNA quantification, genotyping, and resistance-associated variant detection assays) in combination with assessments of liver damage are used for patient management (Table 8). Quantitative HCV RNA tests are based primarily on real-time PCR (94–98), a chemistry that is simultaneously very sensitive and allows for quantification over a broad measurable range (~10 to 1 × 10^9 IU/ml). Currently, there are two commercial, FDA-approved real-time quantitative PCR tests that have been extensively reviewed (94, 95, 99–103). HCV core antigen quantification has been proposed as a surrogate for HCV RNA; however, its clinical utility is not well established (104, 105). It is likely to be unsuitable for therapeutic management since it is only reliable at levels >6,000 IU/ml, a concentration that is too high to be of use in determining treatment response; estimates suggest approximately half of lower-titer samples would be missed compared to real-time PCR (106). HCV genotype tests are used to determine optimal treatment. One real-time PCR-based test is FDA approved (107, 108); other assays utilize reverse hybridization linear array technology (109) or Sanger sequencing to detect genotypes 1 to 6 (107, 108, 110, 111).

An effective vaccine for the prevention of HCV infection has yet to be developed. Minimizing exposure risk is therefore the only available infection-prevention strategy.
method. Identification of carriers through screening can help to minimize exposure risk in household settings.

**Advances in CHC Therapy**

Pivotal advancements in therapy have greatly improved the standard of care, with significant benefits to patients with CHC (Fig. 5). PEG2α/RBV was the only therapeutic option for a decade before the first wave of direct-acting antiviral (DAA)-containing regimens containing the NS3/4A protease inhibitors boceprevir (Victrelis, Merck & Co. Inc., Whitehouse Station, NJ) and telaprevir (Incivek, Vertex Pharmaceuticals Inc., Cambridge, MA), in combination with PEG2α/RBV, were approved for treatment of genotype 1 infections in 2011 (Table 9). These DAAs quickly fell out of use due to intolerability. Subsequent DAA introductions have been far more successful.

**TABLE 9** FDA-approved DAA-containing therapies for HCV

<table>
<thead>
<tr>
<th>Drug name/year of FDA approval</th>
<th>DAA</th>
<th>Nucleotid(s)e class inhibitor</th>
<th>Recommendation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Victrelis (Merck &amp; Co., Inc.)/2011</td>
<td>PEG-INF/RBV+boceprevir</td>
<td>NS3, NS4A serine protease</td>
<td>Currently NOT recommended (gt 1)</td>
</tr>
<tr>
<td>Incivek (Vertex Pharmaceuticals, Inc.)/2011</td>
<td>PEG-INF/RBV+telaprevir</td>
<td>NS3, NS4A serine protease</td>
<td>Currently NOT recommended (gt 1)</td>
</tr>
<tr>
<td>Olysio (Janssen Therapeutics)/2013</td>
<td>Sofosbuvir</td>
<td>NS5B</td>
<td>Recommended (with RBV, or PEG-INF/RBV)</td>
</tr>
<tr>
<td>Sovaldi (Gilead Sciences, Inc.)/2013</td>
<td>Ledipasvir+sofosbuvir combination</td>
<td>NS5A/NS5B</td>
<td>Recommended (gt 1, 4, 5)</td>
</tr>
<tr>
<td>Harvoni (Gilead Sciences, Inc.)/2014</td>
<td>Ombitasvir/paritaprevir/ritonavir+dasabuvir (+/-RBV)</td>
<td>NS5A/NS5B</td>
<td>Recommended (gt 1)</td>
</tr>
<tr>
<td>Viekira pak (Abbvie, Inc.)/2014</td>
<td>Ombitasvir, paritaprevir, (+ritonavir)</td>
<td>NS5A/NS5B</td>
<td>Recommended (gt 1, 2, 3)</td>
</tr>
<tr>
<td>Technivie (Abbvie, Inc.)/2015</td>
<td>Elbasvir/grazoprevir</td>
<td>NS5A/NS5B</td>
<td>Approved recommendations pending next guideline update (gt 1, 2, 3, 4, 5, 6)</td>
</tr>
<tr>
<td>Zepatier (Merck)/2015</td>
<td>Daclatasvir+sofosbuvir (+/-RBV)</td>
<td>NS5A/NS5B</td>
<td>Recommended (gt 1, 4)</td>
</tr>
<tr>
<td>Daklinza (Bristol-Myers Squibb Company)/2015</td>
<td>Sofosbuvir+velpatasvir</td>
<td>NS5B/NS5A</td>
<td>Recommended (for genotype 4)</td>
</tr>
</tbody>
</table>

*as per American Association for the Study of Liver Diseases and Infectious Diseases Society of America.
Simeprevir (Olysio, Janssen Therapeutics, Titusville, NJ), a NS3/4A protease inhibitor, and sofosbuvir (Sovaldi, Gilead Sciences, Inc., Foster City, CA), a potent HCV nucleotide analog NS5B polymerase inhibitor, were approved in 2013 and demonstrated >95% cure rates in clinical trials and rates almost as high in routine clinical practice (84 to 88%) (112, 113). Solvaldi was the first all-oral, interferon-free DAA-containing regimen (combined with ribavirin [RBV]) and the first DAA regimen approved for treatment of HCV genotype 2 or 3 infections (114). Boceprevir- and telaprevir-containing regimens are no longer recommended for use, given the potent therapeutic efficacy and favorable tolerability of subsequent DAAs (Table 7). Currently recommended regimens are simplified (one pill daily for some), with fixed durations (12 or 24 weeks) and extremely high (>95%) response rates (115–117).

Recommendations for testing, managing, and treating HCV were updated in 2014 and again in 2015 in response to the rapidly changing landscape of HCV treatment (118). Further changes are likely given the number of regimens in development. Substantive changes to these recommendations are likely to be made now that the new NS5A inhibitor, velpatasvir, in combination with sofosbuvir has received regulatory approval. Recent reports demonstrate true pangenotypic efficacy, with response rates of 99% across genotypes 1 to 6 (119–121). Further, a 12-week regimen containing these two drugs plus RBV was shown to cure approximately 95% of patients with decompensated cirrhosis (Child-Turcotte-Pugh classification) (122).

Until pangenotypic regimens such as velpatasvir/sofosbuvir are broadly utilized, HCV genotyping will still be required for therapeutic management (Fig. 6). For

**FIGURE 6** Regimens currently recommended for treatment of CHC (7).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 12</th>
<th>Week 16</th>
<th>Week 24</th>
<th>SVR12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4, 5, 6</td>
<td>VEL+SOF</td>
<td></td>
<td></td>
<td>(gt 1a, alternative if no cirrhosis + baseline high fold-change NSSA RAV for ELBJ or gt 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 6</td>
<td>ELB+GRA **-RBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 6</td>
<td>DAC+SOF **-RBV</td>
<td></td>
<td></td>
<td>(gt 2 + cirrhosis, RBV ineligible, up to 24 weeks)</td>
<td>(gt 1, alternative if cirrhosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 6</td>
<td>LED+SOF **-RBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>PAR<strong>TV/ OMB+DAS</strong>-RBV</td>
<td></td>
<td></td>
<td>(gt 1a, alternative if cirrhosis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PAR<strong>TV/ OMB</strong>-RBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 4</td>
<td>SOF+SPV **-RBV</td>
<td>(gt 1a, if cirrhosis without Q80K polymorphism; 1b alternative)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2, 3</td>
<td>SOF **-RBV</td>
<td>(gt 2, + cirrhosis)</td>
<td></td>
<td>(gt 3 + DAC or IFN ineligible or gt 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 4, 5, 6</td>
<td>SOF **-RBV+ PEG-IFN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** SVR = sustained virologic response; VEL = Velpatasvir; ELB = Elbasivir; GRA = Grazoprevir; SOF = Sofosbuvir; DAC = Daclatasvir; LED = Ledipasvir; PAR = Paritaprevir; OMB = Ombratavir; DAS = Dasabuvir; RTV = Ritonavir; SPV = Simeprevir; RBV = Ribavirin; gt = genotype; PEG-IFN = pegylated interferon; RAV = resistance-associated variants

* Italic indicates alternative regimen recommendation; † gt 1a requires NS5A resistance testing, 1a/b +/- cirrhosis; ‡ gt 2 if RBV ineligible; § For gt 4, 5, or 6 alternative if IFN ineligible; *gt1b -RBV only if cirrhosis, 12 wks; ** -RBV only for gt 4

example, response rates are lower for genotype 3 than for genotype 1 or 2. Genotype 3 infections therefore require an altered regimen comprising an additional 12 weeks of therapy (123). In addition to genotype determination, subtyping of genotype 1 has also become important in the DAA era. Genotype 1a is more difficult to treat than genotype 1b and requires alteration in therapy (prolonged treatment and/or addition of RBV) for optimal response. Subtype (1a versus 1b) is therefore currently useful for predicting response and facilitating selection of the most effective treatment regimen. There is currently no clinical utility for the determination of other subtypes (genotype 1 or non-1).

For the most part, DAA regimens have a high barrier to HCV resistance, and antiviral failure due to resistance-associated variants occurs uncommonly. Resistance-associated variants have been identified only in a small proportion of treatment failures (116). Testing for resistance-associated variants prior to treatment initiation may not be useful (120, 124), except in select patients (for example, those with cirrhosis who have already failed combination DAA therapy). This small, yet emerging, population represents the new “difficult-to-treat” population and requires careful consideration from testing and treatment perspectives.

The Role of HCV Viral Load Monitoring in Therapeutic Management

HCV RNA is measured at baseline, during treatment, at the end of treatment, and for detecting relapse after treatment discontinuation. Undetectable HCV RNA in serum or plasma is termed “virologic response.” Undetectable HCV RNA at 12 or 24 weeks following the end of treatment is termed “sustained virologic response” (SVR, further qualified by the time of measurement as SVR12 and SVR24) (92, 118). “Undetectable” and “unquantifiable” are important, clinically distinct terms that are often confused (100, 125) (Table 10, Table 11). An understanding of analytical performance characteristics of quantitative assays is helpful to clarify the difference between them (Table 8).

In the era prior to combination DAAs, HCV RNA quantification during therapy was used to tailor length of treatment and to predict SVR. Currently, it is used primarily to assess adherence to treatment (118, 126). Combination DAA therapy is so effective that patients are expected to have undetectable HCV RNA after 4 weeks of therapy. If HCV RNA is quantifiable at week 4, viral load testing is recommended again at week 6 of therapy; treatment should be discontinued if viral load has increased 10-fold or more at this time point (Fig. 7). Finally, an “undetectable” result is considered synonymous with either “HCV RNA detected, ≤ LLOQ” or a “target not detected” for all assessments, including end of treatment and SVR (12 or 24 weeks following end of treatment).

### Hepatitis C and HIV Coinfections

HIV and HCV are transmitted via similar routes, making coinfections common; therefore, at-risk HIV-infected individuals should be screened for HCV. The highest rates of coinfection are observed in intravenous drug users (IDU) (127). Furthermore, HCV reinfection can occur in IDU successfully treated for HCV, at rates as high as 30% (128). A rise in HIV–HCV coinfections has been observed recently in men who have sex with men who did not report IDU as a risk factor (129). Annual screening for HCV is now recommended for HIV-infected IDU and men who have sex with men (particularly those who engage in unprotected sex), given these coinfection risks (130–135).

HCV must be identified and treated early in coinfection because HIV-associated immune impairment significantly accelerates liver disease progression compared to HCV-monoinfected individuals (136–139). In a large, prospective study, approximately 45% of 282 paired biopsies from coinfected patients had progression to liver fibrosis, with roughly one-third progressing by one Metavir stage within a median of 2.5 years (140–142). Accelerated liver disease progression results in greater all-cause liver-related morbidity and mortality as well as dysfunction of other organs (139, 143).

HIV/HCV-coinfected individuals were categorized as a “difficult to treat” population in the era of pegylated interferon alfa plus ribavirin (peg-IFN/RBV) therapy. Successful treatment resulted in lower risks of liver disease progression, morbidity and mortality (144, 145);

### TABLE 10 Definitions used in assessing quantitative HCV RNA test results

<table>
<thead>
<tr>
<th>Result</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target not detected</td>
<td>HCV RNA is not detected, no observable PCR amplification or detection</td>
</tr>
<tr>
<td>Lower limit of quantification (LLOQ)</td>
<td>Lowest HCV RNA titer within the test’s dynamic range that is quantifiable and accurate</td>
</tr>
<tr>
<td>Limit of detection (LOD)</td>
<td>Lowest amount of analyte in a sample that can be detected (e.g., detection of HCV RNA ≥95%)</td>
</tr>
<tr>
<td>Upper limit of quantification (ULOQ)</td>
<td>The highest HCV RNA titer result within the test’s dynamic range that is quantifiable and accurate</td>
</tr>
</tbody>
</table>
however, response rates were much lower (20%, genotype 1) compared to monoinfections (40 to 60%) (146–148). Additionally, therapy was extremely complicated (147, 149, 150), with high rates of adverse events such as severe cytopenias, and with complex antiretroviral drug interactions. Coinfected individuals were consequently seldom treated. Improved response rates were observed with boceprevir and telaprevir; however, these drugs were never approved for use in coinfected patients because of their adverse event profiles (e.g., severe rashes) and their pharmacokinetic interactions with antiretroviral drugs (151, 152).

In the era of combination DAAs, HIV/HCV-coinfected individuals are no longer considered difficult to treat. Response rates are equivalent to HCV-monoinfected individuals, side effect profiles are manageable, and patients on antiretroviral therapy can be safely treated if compatible DAAs are used. Ledipasvir plus sofosbuvir for 12 weeks produced similarly high response rates (155). Both regimens had a favorable safety profile. Delayed treatment is disadvantageous in these patients. Higher liver-related mortality has been observed once Metavir fibrosis stages F3 and F4 are reached (156). Accordingly, guidelines now recommend early DAA treatment for HIV/HCV-coinfected patients to prevent the development of significant fibrosis and progression of disease (157–159). Possible interactions between antivirals should be evaluated prior to initiating therapy (http://www.hep-druginteractions.org/). More details on the use of specific combinations of DAAs and antiretroviral drugs are outlined in published guidelines (7).

**CHC-Related Liver Transplantation**

Individuals who have undergone liver transplantation as a consequence of CHC-induced liver failure are a special population of patients. In the absence of effective therapy, liver infection recurs within 6 months after transplantation in approximately 75% of recipients; cirrhosis and allograft loss occurs within 5 years in 30% (160–

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### TABLE 11 Interprets of quantitative HCV RNA test results

<table>
<thead>
<tr>
<th>Titer result (IU/ml)</th>
<th>Reported results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Target not detected” or “Not detected”</td>
<td>“HCV RNA not detected.”</td>
<td>Crossing threshold value exceeds the limit for the assay or no crossing threshold value is obtained.</td>
</tr>
<tr>
<td>&lt; LLOQ</td>
<td>“HCV RNA detected, less than [LLOQ] IU/ml HCV RNA.”</td>
<td>Calculated result is below the measurable range.</td>
</tr>
<tr>
<td>Within measurable range</td>
<td>Results are reported as “[number] IU/ml, HCV RNA detected.”</td>
<td>Calculated results are quantifiable within the measurable range (i.e. &gt;LLOQ and &lt;ULOQ).</td>
</tr>
<tr>
<td>&gt; ULOQ</td>
<td>Results are reported as “greater than [ULOQ] IU/ml HCV RNA.”</td>
<td>Calculated results are above the measurable range.</td>
</tr>
</tbody>
</table>

---

**FIGURE 7** Current recommendations for viral load monitoring during treatment for CHC (7).
A small proportion of patients (roughly 4% to 7%) develop posttransplant cholestatic hepatitis C, characterized by very high levels of viremia and an accelerated course of liver injury that is associated with a high risk of rapid allograft failure.

In patients with severe liver disease, effective antiviral therapy pretransplantation is an important clinical intervention to prevent HCV recurrence posttransplantation. The outlook prior to DAA therapy was poor since these patients were difficult to treat and even more difficult to cure with peg-IFN/RBV. However, DAAs have been shown to be highly effective even in patients who were hardest to treat (genotype 1-infected, with early cirrhosis or compensated liver disease). Many can be cured prior to liver transplantation, thereby preventing recurrent infection of the graft (163–165). A regimen of sofosbuvir plus weight-based RBV for up to 48 weeks was well tolerated and effective, resulting in a posttransplant SVR of 69% and reduced allograft loss when HCV RNA was suppressed for at least 28 days prior to transplantation (166). Newer DAA combinations have been even more impactful, producing pretransplant SVR rates of 90 to 97% (118, 167–170). Remarkably, long-term follow-up has demonstrated that some of these successfully treated patients have significant histologic improvement and can be de-listed for transplantation (171). Elimination of the need for transplantation is arguably one of the most critical therapeutic benefits of curing late-stage CHC and is a singular example of why DAAs are such an important medical advance. Given the risk of progression to liver failure associated with advanced fibrosis (Metavir stage F3) and compensated cirrhosis (Metavir stage F4) and given the efficacy of DAA therapy in these patients, guidelines recommend they be categorized among those with the highest priority for immediate treatment.

In transplanted patients who are at risk for HCV recurrence, newer combination-DAA regimens have been shown to be effective, safe, and tolerable. A multicenter, open-label study of sofosbuvir plus RBV for 24 weeks demonstrated SVR12 of 70% (172); adding daclatasvir (24 weeks) further improved SVR12 rates to 95% and obviated the need for retransplant in the majority of patients (173). An additional benefit of DAAs has been improved survival. Attainment of SVR reduces liver-related mortality by approximately 90%. Given the significant risks of recurrent CHC after liver transplantation and the therapeutic efficacy of DAA regimens in this setting, recent guidelines recommend that liver transplant recipients be classified as patients with the highest priority for immediate treatment.

HEPATITIS D
Clinical Significance
Hepatitis D virus (HDV) can transmit, package, and become pathogenic in humans only if the individual is infected with HBV (specifically if HBsAg is detectable). HDV is the smallest infectious agent known to infect humans (174), having a defective, single-stranded HDV RNA virus with two types of viral antigens, small (sHDAg or p24) and large (IHDAg or p27) protein.

HDV transmission most commonly occurs in intravenous drug users that are CHB carriers; therefore prevention of HDV infections should target this at-risk population. The overall prevalence of HDV is relatively low in the U.S. (175), primarily due to the low prevalence of HBV. However, prevalences as high as 70% have been observed in individuals with CHB in regions where HBV is endemic such as the Middle East and South America (176, 177). In the U.S., the prevalence of HDV in an urban cohort of active drug users with CHB was high (~50%) (178) compared to 8% in HBsAg carriers in northern California (179), suggesting that repeated exposure increases risk of transmission. There is no vaccine for HDV; measures to prevent needle sharing in intravenous users are among the most effective means of reducing transmission rates.

HDV/HBV coinfection leads to rapid disease progression, with increased morbidity and mortality rates. Superinfection (hepatitis B carrier subsequently infected with HDV) is responsible for the majority of HDV infections (~90%) and leads to a rapid onset of cirrhosis in ~80% of cases that develops within 2 years of exposure in 10 to 15% of patients (180, 181). Viral transmission of both HBV and HDV together occurs less frequently and has a more severe acute hepatitis rate and greater mortality rates compared to HDV acquisition after HBV infection.

The survivability of HDV depends on the host immune system and clearance of infection. In the absence of HBV clearance, active replication by either virus as detected by NAAT of blood or plasma indicates a significant risk of liver damage. HDV inhibits HBV DNA levels; therefore, HBV DNA tends to be lower in both HBeAg-negative and HBeAg-positive patients (182) irrespective of the phase of the CHB infection. Despite these decreased viral loads, HDV-infected CHB carriers move rapidly into later phases of disease. Hepatic decompensation is typically the clinical endpoint; however, the increased risk of HCC (versus HBV mono-infection) is now fairly well supported, and a variety of potential mechanisms have been reviewed recently (183).
Among immunocompromised individuals, HDV infection is observed in HIV/HBV-coinfected individuals. In Europe, a coinfection rate of approximately 15% was documented based on HDV seroprevalence (184). Infection was more common in intravenous drug users than in men having sex with men. Liver disease is worse, with more rapid hepatic decompensation, cirrhosis, and death in HDV/HBV/HIV compared to HBV/HIV coinfection (185).

**Diagnosis of HDV Infection**

Serological assays are used to determine exposure to HDV in CHB carriers (186). These assays detect anti-HDV IgG, a marker that can persist in a chronic coinfection or decline with viral clearance. An acute infection can be diagnosed through the detection of anti-HDV IgM, which can persist in chronic coinfections and serves as a surrogate marker for HDV replication (175). Additionally, diagnostic serological and molecular markers can be helpful when assessing transmission of a coinfection versus superinfection. HDV RNA viral loads are not standardized, nor are there any FDA-approved tests; therefore, the clinical utility of routine monitoring is not well established. Routine serologic testing for HDV has been advocated for those with HBV/HIV coinfection, given greater disease severity in triple infections (185). The need for such screening likely depends on the geographic prevalence of HDV. HDV serology and NAAT are typically obtained simultaneously when HDV infection is suspected. False-negative HDV serology rates among HBV/HIV-infected individuals are not well documented.

**Treatment Strategies**

HDV infects only in the setting of CHB; therefore, the goal of therapy is to eradicate both viruses by curing HBV. The mainstay of treatment is peginterferon-alpha for 48 weeks (minimally). HBV DNA viral load monitoring in EDTA plasma or serum is sufficient to monitor for treatment efficacy. Nucleos(t)ide analogs by themselves are not useful in suppressing HDV RNA since the virus uses the host enzymes for replication.

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**REFERENCES**


Chronic Hepatitis B, C, and D


