Chronic viral hepatitis: epidemiology, molecular biology, and antiviral therapy

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1. ABSTRACT

Viral hepatitis is a major cause of chronic liver disease, liver failure, and hepatocellular carcinoma worldwide, resulting in significant morbidity and mortality. New insights into the pathogenesis and molecular biology of hepatitis viruses have led to the discovery of novel antiviral agents. Likewise, a greater understanding of the natural history of chronic infection, predictors of disease progression, and predictors of virologic response to therapy has resulted in more effective treatment strategies. Recent data have increasingly demonstrated that the ability to achieve a successful response to antiviral therapy may significantly reduce the risk of progressive liver disease and hepatocellular carcinoma. Immunization practices and the use of potent antiviral therapy may have a major impact in reducing the burden of chronic liver disease and the incidence of hepatocellular carcinoma associated with chronic hepatitis B and chronic hepatitis D. Individualized treatment strategies and the development of direct acting antiviral agents may lead to further improvements in the ability to achieve a sustained virologic response to therapy in chronic hepatitis C. With new advances in the treatment of chronic hepatitis, efforts to optimize viral suppression while reducing the potential for antiviral drug resistance will become increasingly important.
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3. HEPATITIS B

3.1. Epidemiology

Approximately 2 billion people worldwide have been exposed to HBV, while up to 400 million people may have chronic infection (1, 9). The prevalence of chronic HBV infection varies geographically, as up to 45% of the world’s population resides in endemic areas in which the rate of HBsAg seropositivity is greater than 8%. Consequently, certain populations disproportionately make up most of the global population with chronic infection (10). In some hyperendemic areas, predominantly in Asia and sub-Saharan Africa, up to 20% of the population may be chronically infected with HBV, mostly acquired through vertical or horizontal transmission early in life (9, 10). In nonendemic areas, the geographic distribution of chronic HBV infection may reflect the immigration patterns of populations from endemic countries (11). Eight HBV genotypes (A through H) have been identified and their global distribution varies by geography and ethnicity (12). Genotypes A, F, G, and H are more common in western countries while genotypes B, C, and E predominate in Asia and Africa. Genotype D is widely distributed; however, it is more prevalent in the Mediterranean region.

3.2. Molecular biology

3.2.1. The hepatitis B virus and genome

HBV is an enveloped, partially double-stranded DNA virus of the Hepadnaviridae family (13). The HBV virion is approximately 42 nm in diameter, composed of an outer lipid envelope containing viral glycoproteins, including the HBsAg, and an inner nucleocapsid composed of hepatitis B core antigen (HBcAg). The nucleocapsid encloses a copy of the 3.2 kilobase (kb) double-stranded circular HBV DNA and DNA polymerase. HBV DNA is converted into four major RNA transcripts of 3.5 kb, 2.4 kb, 2.1 kb, and 0.7 kb size. The largest transcript (3.5 kb) functions as both messenger RNA and as pre-genomic RNA, which serves as a template for reverse transcription and synthesis of new HBV DNA during replication. The HBV genome is characterized by four overlapping open reading frames (ORF), C (core), P (polymerase), S (surface envelope), and X, in which RNA is translated into various viral proteins (Table 1). The C and S ORFs include in-frame initiation codons that allow for the translation of different proteins within the RNA transcripts. The C ORF encodes for either HBcAg or pre-core protein, the P ORF encodes the HBV DNA polymerase, and the S ORF encodes the surface envelope proteins (HBsAg). The precore protein ultimately undergoes proteolysis, becomes hepatitis B e antigen (HBeAg) in the endoplasmic reticulum, and is secreted into the extracellular space. The HBeAg may have an immunoregulatory role that facilitates development of chronic infection and is a key serologic marker of viral replication (14). The X ORF encodes the X protein (HBxAg) which may be involved in multiple functions including transcriptional activation, signal transduction, cell cycle regulation, and DNA repair (15).

3.2.2. Life cycle and viral replication

The HBV life cycle involves a sequence of events allowing for viral entry into hepatocytes and import of HBV DNA into the hepatocyte nucleus, followed by viral replication, assembly, and excretion of mature virions into the extracellular space (13). At the time of acute exposure, HBV binds to the surface of hepatocytes through interactions between cellular receptors and viral envelope proteins, of which the pre-S domain of the large HBsAg protein appears to play an important role (16). Binding of viral envelope proteins and cellular receptors leads to entry of the hepatitis B virion into the hepatocyte via endocytosis and the nucleocapsid is released into the cytoplasm.
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Uncoating of the nucleocapsid occurs at the nuclear membrane and relaxed circular HBV DNA (cccDNA) is released into the hepatocyte nucleus where it is converted into a covalently closed circular double-stranded DNA (cccDNA) molecule, a process that involves DNA repair and covalent ligation of both strands of circular DNA (17, 18). The cccDNA exists as a viral minichromosome within the hepatocyte nucleus, becomes a template for transcription of viral RNA, and can remain in the nucleus for the lifetime of the hepatocyte. As transcription of the HBV cccDNA occurs as an intranuclear process, some random integration of the HBV genome into the host chromosomes can also occur (19). Ultimately viral RNA derived from cccDNA is translated into HBV proteins (Table 1) and nucleocapsid assembly is initiated in the cytoplasm. Pregenomic RNA enclosed within nucleocapsids is then converted into viral rcDNA via reverse transcription by the HBV DNA polymerase. Further viral assembly occurs at the endoplasmic reticulum with subsequent budding of mature virions and vesicular transport into the extracellular space.

3.2.3. Impact of specific mutations

In the setting of chronic infection, HBV is characterized by a high rate of viral replication of approximately $10^{11}$ virions per day (20). The HBV polymerase lacks a proofreading mechanism, resulting in a very high rate of spontaneous mutations. Consequently, HBV may exist in the form of multiple quasispecies within a host and predominant viral strains arise through both endogenous and exogenous selection pressures, including host immunity, replication fitness of specific viral mutants, and antiviral therapy (21). Spontaneous mutations most commonly occur at the precore and core promoter regions, the frequencies of which vary depending on HBV genotype. The most frequent precore mutation (G186A) is more commonly associated with HBV genotype D infection, resulting in the formation of a stop codon within the precore region and loss of production of the precore protein as well as HBeAg. Mutations within the core promoter region are more frequently associated with genotype C and lead to a decrease in production of HBeAg (21). The presence of core promoter mutations appears to be associated with an increased risk of HCC based on several studies (22-31) and may account for the increased risk of HCC associated with genotype C infection (22-24). Mutations that arise in the setting of antiviral therapy may confer drug resistance and represent a major challenge, as the development of resistant HBV may result in treatment failure, clinical decompensation, and an increased risk of HCC.

3.3. Natural history

The development of chronic HBV infection following acute exposure varies by age and is dependent on both viral and host factors. Approximately 5% of adults who are acutely exposed to HBV develop chronic infection in contrast with individuals who are exposed through vertical transmission, in which the rate of chronicity is as high as 90% (32). Animal and human studies have revealed that a vigorous, multispecific cell-mediated immune response directed towards HBV is essential for resolution of acute infection. These studies have highlighted the importance of the CD8-positive cytotoxic T-cell response as well as production of noncytolytic cytokines, primarily interferon (IFN)-gamma, which promote clearance of infected hepatocytes and inhibition of HBV replication, respectively (33). Activation of the host innate immunity, in the form of Kupffer cells, natural killer (NK) cells, and NKT cells, may contribute to this process through direct cytoxicity and cytokine production. Ultimately, production of neutralizing antibodies directed toward HBeAg and HBsAg coincides with resolution and prevent reinfection. As intranuclear HBV cccDNA can remain within hepatocytes following recovery from acute infection, ongoing HBV-specific cell-mediated and humoral immunity are critical for long-term resolution and prevention of reactivation of HBV infection. In contrast, a failure to develop an inadequate adaptive HBV-specific cellular immune response may lead to viral persistence and chronic infection (33).

Serological markers of HBV infection can be used to diagnose chronic infection versus resolution. The presence of antibodies directed against HBsAg (anti-HBs) in the absence of HBsAg indicates prior spontaneous resolution with long-term immunity against HBV. Alternatively, the persistence of detectable serum HBsAg is associated with chronic infection. In those who have undergone immunization, anti-HBs is typically present; however antibodies to the HBV core antigen (anti-HBc) are absent. Ultimately chronic infection may lead to progressive liver disease in a significant proportion of individuals and result in as many as 500,000 deaths annually worldwide (1). The age of acquisition of chronic infection may influence risks of disease progression. Up to 25% of those who acquire chronic HBV infection early in life ultimately develop cirrhosis or HCC (34). Risk factors for progression to cirrhosis include advanced age, elevated alanine aminotransferase (ALT) levels, and chronic increased alcohol intake. Additional viral factors such as the presence of HBeAg, increased HBV DNA level, and HBV genotype may also contribute to risks of disease progression (35, 36). HBV genotype C may be associated with an increased risk of HCC and cirrhosis, a prolonged interval to immune clearance, increased incidence of reactivation, and decreased efficacy of antiviral therapy with IFN alfa (37-39).

3.3.1. Phases of chronic infection

Once chronic HBV infection is established, the disease course may fall into one of four phases: immune tolerance, immune clearance, inactive HBsAg carrier state, or reactivation (40). The immune tolerant phase is associated with normal ALT levels and minimal histologic activity on liver biopsy. In this phase, HBeAg is detectable and serum HBV DNA levels are frequently very high. Immune tolerance is more commonly associated with younger individuals who likely acquired HBV infection perinatally. The immune clearance phase is characterized by elevated ALT, elevated HBV DNA levels, detectable HBeAg, and significant disease on liver biopsy. Inactive HBsAg carriers have persistently normal ALT levels, low or undetectable HBV DNA, and typically minimal disease.
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3.3.2. Hepatitis B and hepatocellular carcinoma

Chronic infection with HBV is the most common risk factor for HCC and accounts for up to 54% of HCC cases worldwide (4). Consequently, the geographic distribution of HCC incidence reflects the prevalence of HBV in high risk populations with the highest HCC-associated mortality described in eastern and southeastern Asia, sub-Saharan Africa, and the Pacific region (41). The increased incidence of HCC in these populations may be attributed to high rates of chronic HBV infection, typically acquired through vertical or horizontal transmission early in life (9, 10, 42). As chronic HBV infection is associated with up to a 20-fold increase in risk of developing HCC in the absence of cirrhosis, efforts to prevent or treat HBV-associated liver disease may have a major impact on the global incidence of HCC (4).

Several large prospective cohort studies have established serum HBV DNA and serologic markers of viral replication as key risk factors for development of HCC. The presence of HBeAg (43, 44) as well as HBeAg (45, 46) is associated with an increased risk of HCC and duration of antigenemia may contribute to an increase in risk. Likewise, spontaneous clearance of HBsAg is associated with a reduction in risk (47, 48). Serum HBV DNA is now well-established as a major risk factor for HCC, as demonstrated through prospective cohort studies of patients with chronic HBV infection (31, 36, 49-51). The Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) study prospectively assessed the association between HBV DNA levels and risk of HCC in a cohort of 3,653 untreated HBsAg-positive patients in Taiwan who were followed over a mean of 11.4 years. In this cohort of mostly HBeAg-negative patients, the risk of HCC increased with serum HBV DNA levels in a linear relationship independent of ALT levels, the presence of HBeAg, or cirrhosis (49). The greatest risk was reported in those with HBV DNA levels greater than 10^8 copies/mL; however further analyses within this cohort revealed that even lower HBV DNA levels may be associated with significant risk (52). The findings from this study highlighted the importance of HBV DNA in identifying individuals at risk of HCC and progressive liver disease, defining parameters for antiviral therapy, and introducing the potential role of antiviral therapy in the prevention of HCC through viral suppression.

Other viral factors which may potentially identify individuals at greater risk of HCC include HBV genotype and specific genomic alterations. Various studies in mostly Asian populations have reported an increased risk of HCC associated with HBV genotype C (22, 31, 53-57) and subgenotype Ce (51). In Alaska natives, a population characterized by a very high prevalence of chronic HBV infection, genotype C may be associated with a later onset of spontaneous HBeAg seroclearance and increased rates of vertical transmission, which may contribute to the risk of HCC associated with genotype C infection (38, 56). In addition, HBV genotype C is associated with a higher prevalence of mutations within the core promoter and pre-S regions of the HBV genome (22-24, 58-60), both of which have been associated with an increased risk of HCC (22-31, 58-62). The role of the HBsAg and X promoter mutations in HBV-associated HCC has yet to be fully elucidated (15, 63). Ultimately, a greater understanding of viral factors involved in HCC risk or carcinogenesis in the setting of HBV infection may greatly contribute to improved risk stratification, selection of treatment candidates, and identification of individuals who require more aggressive surveillance for HCC.

3.4. Antiviral therapy

3.4.1. Indications for therapy

The goal of antiviral therapy in chronic HBV infection is to achieve long-term viral suppression in order to prevent sequelae of chronic liver disease in those who are at greater risk of disease progression, HCC, and development of cirrhosis. Several guidelines have been published outlining parameters for initiation of antiviral therapy based on patient groups (Table 2). Clinical and laboratory parameters used to assess treatment candidacy include serum HBV DNA, ALT levels, and in some cases liver histology (64-66). As HBV DNA has now been established as a key risk factor for HCC and progressive liver disease through several prospective studies (31, 36, 49-51, 67-69), measurement of hepatitis B viremia is critical in the assessment of whether antiviral therapy is indicated. Since some data suggests that the risk of progressive disease exists even with low HBV DNA levels, particularly in the setting of HBeAg-negative infection, some groups have proposed broader criteria for initiation of antiviral therapy in order to include all at-risk groups (64, 66). Although immune tolerant individuals or those who are considered inactive HBsAg carriers must be followed longitudinally, no treatment is indicated; however, therapy should be considered in those with cirrhosis or HbsAg carriers undergoing immunosuppressive or cancer chemotherapy (Table 2).

3.4.2. Nucleoside and nucleotide analogues

Nucleoside and nucleotide analogues are the most commonly used antiviral agents in the treatment of chronic HBV infection as a result of their combined high efficacy and good tolerability. Five oral agents are currently approved as treatment for chronic hepatitis B: lamivudine, telbivudine, entecavir, adefovir, and tenofovir. Nucleoside and nucleotide analogues specifically target HBV polymerase activity and inhibit HBV DNA synthesis; however, rates of antiviral efficacy and the incidence of
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### Table 2. Recommendations for initiation of antiviral therapy in chronic hepatitis B infection

<table>
<thead>
<tr>
<th>Condition</th>
<th>EASL Guidelines (64)</th>
<th>AASLD Guidelines (65)</th>
<th>U.S. Algorithm (66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg-Positive</td>
<td>HBV DNA &gt; 2,000 IU/mL and/or ALT &gt; ULN</td>
<td>HBV DNA &gt; 20,000 IU/mL and ALT &gt; 2 x ULN</td>
<td>HBV DNA ≥ 20,000 IU/mL and ALT &gt; ULN</td>
</tr>
<tr>
<td></td>
<td>• Consider liver biopsy if ALT &gt; ULN or HBV DNA &gt; 2,000 IU/mL; initiate therapy if moderate to severe histologic disease present</td>
<td>• Observe 3-6 months, initiate therapy if no spontaneous HBsAg loss</td>
<td>• Consider liver biopsy if HBV DNA ≥ 20,000 IU/mL, ALT ≤ 2 x ULN, and age &gt; 40 or family history of HCC</td>
</tr>
<tr>
<td></td>
<td>HBV DNA &gt; 2,000 IU/mL; initiate therapy if moderate to severe histologic disease present</td>
<td>• Consider therapy if histologic disease and ALT ≤ 2 x ULN</td>
<td>• Consider therapy if known histologic disease, regardless of ALT</td>
</tr>
<tr>
<td>HBsAg-Negative</td>
<td>HBV DNA &gt; 2,000 IU/mL and/or ALT &gt; ULN</td>
<td>HBV DNA &gt; 20,000 IU/mL and ALT &gt; 2 x ULN</td>
<td>HBV DNA ≥ 2,000 IU/mL and ALT &gt; ULN</td>
</tr>
<tr>
<td></td>
<td>• Consider liver biopsy if ALT &gt; ULN or HBV DNA &gt; 2,000 IU/mL; initiate therapy if moderate to severe histologic disease present</td>
<td>• Consider liver biopsy if HBV DNA &gt; 2,000 IU/mL and ALT &gt; ULN; initiate therapy if histologic disease present</td>
<td>• Consider liver biopsy if HBV DNA ≥ 2,000 IU/mL, normal ALT, and age &gt; 35</td>
</tr>
<tr>
<td></td>
<td>HBV DNA &gt; 2,000 IU/mL; initiate therapy if moderate to severe histologic disease present</td>
<td></td>
<td>• Consider therapy if known histologic disease, regardless of ALT</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>HBV DNA &gt; 2,000 IU/mL and/or ALT &gt; ULN</td>
<td>HBV DNA &gt; 2,000 IU/mL and ALT &gt; ULN</td>
<td>HBV DNA ≥ 2,000 IU/mL and ALT &gt; ULN</td>
</tr>
<tr>
<td></td>
<td>• Consider liver biopsy if ALT &gt; ULN or HBV DNA &gt; 2,000 IU/mL; initiate therapy if moderate to severe histologic disease present</td>
<td>• Consider liver biopsy if HBV DNA &gt; 2,000 IU/mL and ALT &gt; ULN; initiate therapy if histologic disease present</td>
<td>• Consider liver biopsy if HBV DNA ≥ 2,000 IU/mL, normal ALT, and age &gt; 35</td>
</tr>
<tr>
<td></td>
<td>HBV DNA &gt; 2,000 IU/mL; initiate therapy if moderate to severe histologic disease present</td>
<td>• Consider therapy if known histologic disease, regardless of ALT</td>
<td>• Consider therapy if known histologic disease, regardless of ALT</td>
</tr>
<tr>
<td>Immunosuppression or Chemotherapy</td>
<td>HBsAg-positive</td>
<td>HBsAg-positive</td>
<td>HBsAg-positive</td>
</tr>
<tr>
<td></td>
<td>• Initiate oral antiviral therapy prior to onset of chemotherapy and continue for 12 months following completion</td>
<td>• Initiate oral antiviral therapy at onset of chemotherapy and continue for 6 months following completion; longer duration of therapy if HBV DNA &gt; 2,000 IU/mL</td>
<td>• Initiate oral antiviral therapy several weeks prior to onset of chemotherapy and continue for 6 months following completion; longer duration of therapy if HBV DNA ≥ 2,000 IU/mL</td>
</tr>
<tr>
<td></td>
<td>HBsAg-negative, anti-HBe-positive</td>
<td>reactivation by HBV DNA positivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Monitor ALT and HBV DNA; initiate oral antiviral therapy if confirmed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Adapted from Lok and McMahon (65), Keeffe et al. (66), and European Association for the Study of the Liver (64). 2 ULN of ALT in the U.S. Algorithm defined by 30 IU/mL in men and 19 IU/mL in women. Abbreviations: EASL: European Association for the Study of the Liver; HBV: hepatitis B virus; anti-HBc: antibody to hepatitis B core antigen; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen; ALT: alanine aminotransferase; ULN: upper limit of normal; HCC: hepatocellular carcinoma.

Drug resistance varies greatly between agents (70). Lamivudine and telbivudine are L-nucleoside analogues which inhibit DNA synthesis by promoting premature DNA chain termination. Entecavir is a guanosine nucleoside analogue that disrupts HBV replication through inhibition of several steps including base priming, HBV DNA polymerase reverse transcriptase activity, and DNA synthesis. Adefovir and tenofovir are acyclic nucleotide analogues that inhibit HBV polymerase activity and promote premature DNA chain termination. Telbivudine, entecavir, and tenofovir have demonstrated the greatest potency in achieving viral suppression relative to other nucleoside and nucleotide analogues (Figures 1A and 1B) (70). Entecavir and tenofovir appear to be particularly effective, achieving viral clearance in 67% to 76% of HBeAg-positive and 90% to 93% of HBeAg-negative patients at one year of therapy (Figures 1A and 1B) (71-73). Antiviral therapy with entecavir or tenofovir may also result in loss of HBsAg within the first year of treatment, which has not been reported in clinical trials involving other oral agents (71, 73). Emtricitabine and clevudine are two additional L-nucleoside analogues with efficacy against chronic HBV infection; however, they are not approved for therapy at this time in most countries. Although emtricitabine appears to have significant potency against HBV, preliminary studies have demonstrated a potential for development of drug resistance (74). Clevudine has also demonstrated efficacy against HBV (75, 76), but use of this drug is limited due to reports of myopathy noted in a phase III clinical trial.

### 3.4.3. Interferon alpha

In addition to a vigorous, multispecific cell-mediated immune response against HBV, endogenous IFN production is critical to achieving inhibition of HBV replication, viral suppression, and resolution of acute infection (33). Likewise, the use of alpha IFNs in the treatment of chronic hepatitis B may result in viral suppression in a significant proportion of patients. Both standard IFN alfa-2b and long-acting peginterferon (PegIFN) alfa-2a are currently approved as antiviral agents used in the treatment of chronic hepatitis B in the U.S. and many countries. Although not formally approved for treatment of chronic hepatitis B in the U.S., PegIFN alfa-2b has demonstrated similar efficacy to PegIFN alfa-2a (77, 78). A 48-week course of PegIFN alfa-2a may result in complete viral suppression in a significant proportion of patients, a higher rate of HBeAg seroconversion relative to oral nucleoside or nucleotide analogues, and the potential for HBsAg loss (Figures 1A and 1B) (79, 80). Although direct comparisons are limited, prolonged therapy with oral agents may potentially result in similar rates of HBeAg seroconversion and HBsAg loss compared with PegIFN. IFN-based therapy has several advantages over the oral nucleoside and nucleotide analogues including similar antiviral efficacy, a fixed duration of therapy, and no known association with drug resistance; however, IFN and PegIFN are associated with significant side effects that may limit tolerability.

### 3.4.4. Predictors of response

Several baseline factors and on-treatment parameters have been associated with achievement of a...
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Figure 1. One-year efficacy of approved antiviral agents for chronic hepatitis B infection. Virologic response in HBeAg-positive (A) and HBeAg-negative (B) chronic HBV infection following one year of therapy (70-73, 77-80). Parameters of response include negative serum HBV DNA, HBeAg seroconversion, and loss of HBsAg. The data summarized is based on various clinical trials and not direct comparisons. Abbreviations: HBeAg: hepatitis B e antigen, HBV: hepatitis B virus, HBsAg: hepatitis B surface antigen, PegIFN: peginterferon.

successful response to therapy. Recent prospective data from the GLOBE trial, which assessed the efficacy of telbivudine in HBeAg-positive and -negative patients, has identified several predictors of HBV clearance that may be associated with oral nucleoside and nucleotide analogue therapy (81). Low baseline HBV DNA (< 9 log_{10} copies/mL) and elevated ALT levels (≥ 2x the upper limit of normal [ULN]) were found to be strong predictors of HBV clearance and HBeAg seroconversion following two years of telbivudine therapy in HBeAg-positive patients, while low body mass index (BMI) (< 22.5) was predictive of viral clearance in both HBeAg-positive and HBeAg-negative infection. During the course of antiviral therapy, complete viral suppression at 24 weeks was identified as a key predictor of sustained HBV DNA clearance at two years, low incidence of antiviral drug resistance, and HBeAg seroconversion. Likewise, a rapid early decline in quantitative HBsAg levels during therapy may be predictive of HBsAg loss (82). Additional prospective studies will be required to determine whether these findings are reproducible and applicable with the use of other oral antiviral agents. In the setting of PegIFN therapy, elevated baseline ALT, low HBV DNA, and low quantitative HBeAg levels may be predictive of HBeAg seroconversion in HBeAg-positive infection (83, 84). Similar baseline characteristics including elevated ALT, low HBV DNA, younger age, and female gender may predict a successful response to PegIFN in HBeAg-negative individuals (85). HBV genotype appears to also influence response to IFN-based therapy, as genotypes A or B may be associated with a more favorable outcome, particularly in HBeAg-positive infection (77, 80, 86-88). In light of concerns regarding tolerability, identifying favorable baseline features such as these may be an important consideration in the selection of candidates for IFN-based therapy.

3.4.5. Antiviral drug resistance

Although nucleoside and nucleotide analogues with increased tolerability and high potency against HBV have become available, a significant proportion of patients require long-term therapy. A primary consideration of particular importance in the setting of long-term therapy is the development of antiviral drug resistance. The emergence of mutations associated with resistance during a course of therapy may result in HBV persistence and a greater risk of progressive liver disease, clinical decompensation, and HCC (89, 90). Once antiviral therapy with an oral nucleoside or nucleotide analogue is initiated, patients should be monitored closely for evidence of drug resistance and the implementation of strategies to minimize the potential for developing resistance are essential. Several factors may contribute to the emergence of mutations conferring drug resistance including the frequency of mutation, the presence of persistent viral replication, selective pressure exerted by an antiviral agent, and the replication fitness or viability of new mutations that occur (18).

The incidence of resistance varies greatly among antiviral agents and may increase with duration of therapy (Figure 2). The development of resistance associated with any particular agent is influenced by its genetic barrier, defined by the probability of viral mutation in response to the selective pressure exerted by the agent (91). Genotypic resistance refers to the presence of mutations in the HBV polymerase that are known to be associated with treatment failure (92). Lamivudine therapy is associated with a relatively high resistance rate of up to 65% at five years (89). Lamivudine resistance primarily involves the rtM204V/I mutation, which occurs within the YMDD locus of the HBV polymerase enzyme (93). Adefovir, telbivudine, and emtricitabine all appear to be associated with significant rates of resistance. After five years of therapy, 29% of HBeAg-negative patients treated with adefovir developed genotypic resistance (94). Rates of resistance associated with telbivudine and emtricitabine have been reported to be 25% at two years and 13% at one year, respectively (74, 95, 96). In contrast, entecavir and tenofovir have high genetic barriers to resistance with low reported rates of resistance. Recent data have described a very low cumulative probability of genotypic resistance to entecavir at 1.2% after six years of therapy in nucleoside-naive patients (97). However, in patients with pre-existing resistance to lamivudine characterized by the rtM204V/I mutation, resistance to entecavir can develop in as many as one-half of patients after five years of therapy (98).
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Figure 2. Incidence of genotypic resistance to antiviral therapy in chronic hepatitis B infection. The incidence of genotypic resistance during antiviral therapy for chronic HBV infection, defined by the presence of known mutations resulting in treatment failure, is shown (74, 89, 94-96, 98, 100, 101). Lamivudine, adefovir, telbivudine, and emtricitabine have higher reported rates of resistance relative to entecavir and tenofovir, in which the incidence of antiviral drug resistance remains very low. Abbreviations: HBV: hepatitis B virus.

Consequently, the use of entecavir monotherapy in patients with known genotypic resistance to lamivudine is not recommended (66, 99). Thus far, no resistance mutations associated with tenofovir therapy have been identified after three years of treatment (100, 101). Due to their favorable long-term resistance profiles as well as high potency and tolerability, entecavir and tenofovir have been proposed as first-line agents in the treatment of chronic HBV (64, 66).

3.4.6. Monitoring virologic response

During the course of antiviral therapy for chronic hepatitis B, monitoring to ensure adequate virologic response may have a major impact on achieving optimal rates of long-term viral suppression and minimizing the potential for antiviral drug resistance. In addition, the ability to suppress HBV DNA through antiviral therapy may slow the progression of disease and reduce the incidence of HCC, as suggested in a key prospective study of patients with advanced chronic HBV infection (102). Thus, monitoring of serum HBV DNA levels during therapy with nucleoside or nucleotide analogues has been proposed (91). HBV DNA levels obtained at 12 weeks of therapy may identify primary treatment failure, defined by the inability to reduce HBV DNA by $\geq 1 \log_{10}$ IU/mL from baseline. Subsequent assessment of HBV DNA at 24 weeks and then every 3 to 6 months thereafter may identify inadequate virologic responses at later time points during therapy. If primary treatment failure or an inadequate virologic response occurs, the patient should be assessed for adherence, otherwise further intervention may be required in order to achieve complete viral suppression. Virologic breakthrough, defined as a $\geq 1 \log_{10}$ IU/mL increase in serum HBV DNA levels above the nadir during therapy, may indicate the presence of phenotypic resistance (92). Once antiviral resistance is confirmed or suspected based on inadequate viral suppression, an agent of another class with a higher potency may be added in order to avoid cross-resistance, which occurs more commonly between drugs within the same class (Table 3) (65, 66, 93, 103). The role of combination therapy in treatment-naïve patients has not been well established and is not recommended, although this may become increasingly important in the future (104).

The primary endpoints of antiviral therapy for chronic hepatitis B are based on virologic response measured by HBV DNA levels and serological markers. Although a finite course of 48 weeks is recommended for therapy using PegIFN, the duration of therapy with nucleoside or nucleotide analogues is individualized, and in some cases, indefinite. HBeAg seroconversion, defined by the loss of HBeAg and emergence of anti-HBe, is an important endpoint that represents a successful response to therapy; however the ability to detect HBeAg only applies to HBeAg-positive infection. Once this endpoint is achieved, oral antiviral therapy should be continued for at least six months following the appearance of anti-HBe (65), or preferably beyond the point of seroconversion until 12 months following HBV DNA negativity (64, 66). In HBeAg-negative infection, antiviral therapy is required long-term. Discontinuation of treatment may be considered
if HBsAg clearance is achieved; however, this is a rare occurrence and the optimal duration of therapy is uncertain (65). Ultimately, the presence of intranuclear HBV cccDNA presents a challenge in HBV therapy since complete eradication of HBV is not easily attainable and the potential for reactivation is present, particularly in the setting of immunosuppression. If treatment is discontinued, patients must be monitored long-term for evidence of HBV reactivation or disease progression.

4. HEPATITIS D

4.1. Epidemiology

Hepatitis D virus (HDV) is a defective virus that requires the presence of HBV, notably in the form of HBsAg, for assembly of virions and infectivity. Consequently, HDV infection is limited to HBsAg carriers. HDV has a global distribution and can be found in up to 5% of all HBsAg carriers, leading to chronic infection in approximately 15 million people worldwide (3). Although HDV infection is dependent on coexisting HBV, the distribution of HDV varies greatly and does not entirely reflect the epidemiology of chronic HBV infection. Regions associated with a high prevalence of HDV infection include areas of South America, the Middle East and Mediterranean, sub-Saharan Africa, and some Pacific Islands; although the prevalence of HDV infection appears to have decreased in some areas of the Mediterranean, possibly as a result of HBV immunization strategies and increased awareness of transmission risks (105). In contrast, the prevalence of HDV infection is relatively low in Southeast Asia and China (7). Modes of HDV transmission are similar to those that occur in association with HBV, including sexual contact, close contact within families, and rarely through perinatal contact (3, 7, 106). Parenteral exposure is also a major source of HDV transmission as injection drug use appears to be a dominant route of new infection, particularly in nonendemic areas (3, 7).

4.2. Molecular biology

4.2.1 The hepatitis D virus genome and hepatitis D antigen

HDV is an enveloped, single-stranded RNA virus within the genus Deltavirus (3). The hepatitis D virion is approximately 36 nm to 43 nm in size and includes a nucleocapsid composed of large (L), middle (M), and small (S) HBsAg proteins. Within the nucleocapsid are a circular, covalently closed RNA genome and the hepatitis delta antigen (HDAg). The circular HDV genome is the smallest known to occur in animals with only approximately 1,700 nucleotides and assumes a rod shape under physiologic conditions. Utilizing the host enzyme, RNA polymerase II, the single-stranded circular HDV RNA genome of negative polarity serves as a template for producing a positive strand of circular antigenomic RNA. The antigenomic HDV RNA then functions as both a 0.8 kb mRNA translated into the HDAg through a single ORF and as a template for replication of full genomic HDV RNA. The single ORF encodes both short (HDAg-S) and long (HDAg-L) forms of HDAg, which facilitate viral replication and virion assembly, respectively (109). Additional characteristics of HDAg based on several specific functional domains include a nuclear targeting signal allowing transport of HDAg to the hepatocyte nucleus, an RNA binding domain, and the ability to form multimers (3, 109). The HDAg protein itself may promote HDV RNA transcription through stimulation of the host RNA polymerase II (110).

4.2.2 Life cycle and dependence on hepatitis B

As HDV only carries sufficient genomic material to produce the HDAg, it must utilize both host and HBV functions in order to carry out RNA transcription, production of viral proteins, virion assembly, and maintaining infectivity. The HDV virion attaches to hepatocytes through interactions with its HBsAg nucleocapsid. The HDV genome and HDAg are then released into the hepatocyte and are directed to the nucleus where host RNA polymerase II is used in the replication and transcription of the circular genomic HDV RNA. The HDAg-S and HDAg-L proteins appear to undergo post-translational modifications, at which time they have an important role in directing the HDV genome through various cellular compartments (109, 111). Ultimately HDAg and genomic HDV RNA are enclosed by the HBsAg nucleocapsid during virion assembly and mature hepatitis D virions are released into the extracellular space.

4.3. Natural history

Infection with HDV may occur in the form of acute co-infection with simultaneous HBV infection or as a superinfection in the setting of pre-existing chronic HBV. Since HDV is entirely dependent on HBV for replication and virion assembly, infection with HDV is not possible in the absence of HBsAg. The diagnosis of HDV infection can be made by serological studies including positivity for serum HDAg and antibodies against HDAg (anti-HD). Both acute co-infection and superinfection may be associated with an increased risk of fulminant hepatitis. However, less than 5% of cases progress to chronicity following acute HBV and HDV co-infection (3). In patients with underlying chronic HBV infection, superinfection

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Table 3. Management of antiviral drug-resistance in chronic hepatitis B infection

<table>
<thead>
<tr>
<th>Antiviral agent</th>
<th>Treatment options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamivudine</td>
<td>Continue lamivudine and add adefovir or tenofovir</td>
</tr>
<tr>
<td></td>
<td>Switch to emtricitabine/tenofovir</td>
</tr>
<tr>
<td>Adefovir</td>
<td>Continue adefovir and add lamivudine or telbivudine</td>
</tr>
<tr>
<td></td>
<td>Switch to or add entecavir (only if no prior lamivudine resistance)</td>
</tr>
<tr>
<td></td>
<td>Switch to emtricitabine/tenofovir</td>
</tr>
<tr>
<td>Entecavir</td>
<td>Switch to or add adefovir or tenofovir</td>
</tr>
<tr>
<td></td>
<td>Switch to emtricitabine/tenofovir</td>
</tr>
</tbody>
</table>

1 Adapted from Lok and McMahon (65), and Keeffe et al. (66). 2 Combination emtricitabine/tenofovir has not been formally approved for the treatment of chronic hepatitis B infection.
Chronic viral hepatitis

Figure 3. Interferon therapy for chronic hepatitis D infection. The rates of SVR following IFN-based therapy in the treatment of chronic HDV infection, based on randomized clinical trials and prospective observational studies. SVR in these studies was defined by undetectable HDV RNA by PCR at least 6 months following completion of therapy. Duration of therapy varied among studies, with 48 (115, 117, 122, 123), 72 (119), or 96 weeks (118, 120, 121) of IFN alfa therapy. Black colored bars represent IFN-alfa monotherapy; dark gray, combination IFN-alfa and ribavirin; and light gray, combination IFN-alfa and lamivudine. Abbreviations: SVR: sustained virologic response, HDV: hepatitis D virus, IFN: interferon, PCR: polymerase chain reaction, PEG-IFN: peginterferon alfa. Studies including PEG-IFN.

4.4. Antiviral therapy

Although HDV is dependent on HBV to maintain chronic infection, nucleoside and nucleotide analogues appear to be ineffective in achieving viral suppression in chronic HDV. Despite clearance of HBV associated with oral antiviral therapy, the presence of HBsAg persists in most cases, allowing for continued HDV replication. At this time, effective treatment of chronic HDV infection is primarily limited to IFN-based therapy. To date, lamivudine is the only oral nucleoside analogue that has been studied prospectively in the treatment of HDV infection and has not demonstrated efficacy as monotherapy (115, 116) or led to an increased rate of HDV clearance in combination with IFN (115, 117). Several small randomized clinical trials (115, 117-119) and prospective observational studies (120-123) have assessed the efficacy of IFN-based therapy in achieving a sustained virologic response (SVR), defined by negative serum HDV RNA six months after completion of therapy. Reports of SVR vary widely among studies, ranging from low rates of 9% to as high as 50% (Figure 3). Treatment discontinuation rates of over 25% may in part explain lower response rates in some clinical trials (117, 119, 121). Early clinical studies of IFN therapy for HDV infection noted that higher doses of IFN were associated with improved rates of viral clearance (124, 125). Likewise, the use of long-acting IFN therapy, in the form of PegIFN alfa-2a or -2b, in treatment naive individuals or prior treatment nonresponders may be associated with improved efficacy, but prospective data are limited (119, 123).

Despite efforts to optimize adherence, a significant proportion of patients with chronic HDV infection will ultimately fail antiviral therapy with IFN. Recent in vitro studies suggest that HDV may indeed resist the antiviral effects of IFN-based therapy through
inhibition of intracellular signaling mediated by IFN-alpha (126). Pretreatment factors predictive of response to therapy are limited, although the presence of cirrhosis may be associated with an increased likelihood of treatment failure (118). Modification of treatment regimens including extending the duration of IFN therapy does not appear to significantly improve SVR rates based on available prospective data; however, case reports suggest prolonged therapy may improve virologic response in some individuals (127). During the course of antiviral therapy, HDV RNA levels at the six-month time point may be predictive of treatment outcome, as HDV RNA negativity appears to predict SVR (123) and failure to reduce HDV RNA levels by 3 log_{10} may be associated with a 100% negative predictive value of achieving an SVR (122). Unfortunately, the measurement of serum HDV RNA levels by PCR is generally limited to clinical studies, as commercially available standardized assays are currently not available. Once sustained viral suppression is achieved with IFN, some data suggest patients may benefit from histological improvement and increased survival (115, 122, 128, 129). Patients who respond to antiviral therapy should be monitored closely as late relapse of hepatitis D viremia has been described (124).

At this time, no antiviral agent specifically targeted against HDV exists and IFN-based therapy remains the only treatment option available for patients with chronic HDV infection (109). PegIFN should be considered the preferred treatment choice in order to optimize virologic response and adherence to therapy with once-weekly dosing (130). The addition of alternative antiviral agents such as ribavirin (RBV) to an IFN-based regimen does not appear to improve rates of SVR in the setting of chronic HDV infection (118, 119). Potential targets of new antiviral therapies may include disruption of post-translational modifications associated with the HDAg-S and HDAg-L proteins that are required for interaction with the HBsAg and hepatitis D virion assembly (109). Studies investigating the ability to inhibit prenylation of the HDAg-L protein have demonstrated effective suppression of HDV in animal models, although human studies involving potential new therapies for HDV are limited (131, 132).

5. HEPATITIS C

5.1. Epidemiology

Chronic infection with HCV is a major cause of chronic liver disease worldwide, affecting as many as 170 million people with a global prevalence of approximately 3% (2). HCV is endemic in most regions of the world, although its distribution varies geographically with the highest prevalence in some regions of Northern Africa and the Mediterranean. In western countries, chronic HCV has increasingly become a major cause of ESLD. HCV is currently the most common chronic blood borne infection in the U.S. with a prevalence of 1.3%, accounting for up to two-thirds of newly diagnosed chronic liver disease and half of all liver transplants (5, 6, 133, 134). HCV is classified into six major genotypes, which not only differ in geographic distribution but also by genetic composition, such that genotypes may vary by up to 35% in nucleotide sequence (135). HCV genotype 1, which is divided into subtypes 1a and 1b, is more prevalent in North America, South America and Western Europe. Genotype 2 is more commonly encountered in the Mediterranean and some regions of Asia, genotype 3 in Southeast Asia and India, genotype 4 in the Middle East and Africa, genotype 5 in South Africa, and genotype 6 in Eastern and Southeastern Asia (135, 136).

The transmission of HCV occurs primarily through parenteral exposure. Individuals at the highest risk of exposure to HCV include injection drug users, hemophiliacs, and recipients of blood transfusions prior to 1992 or coagulation factor transfusions prior to 1987 (137). Other populations at risk of HCV infection included hemodialysis patients, with an incidence of HCV ranging from 10% to 65% (138), and individuals infected with human immunodeficiency virus (HIV), in whom as many as one-third are co-infected with HCV (139). Injection drug use has now become the primary mode of HCV transmission in both the U.S. and in Europe (137, 140). Recent reports have revealed that the seroprevalence of HCV may increase to over 70% within three to five years of habitual injection drug use (141).

5.2. Molecular biology

5.2.1. The hepatitis C virus and genome

HCV is an enveloped, single-stranded RNA virus of the Flaviviridae family (142). The HCV virion is thought to be approximately 40 nm to 70 nm in size and composed of a nucleocapsid made up of the HCV core protein surrounded by a lipid envelope containing the two envelope glycoproteins, E1 and E2 (142, 143). The HCV nucleocapsid encloses the single-stranded 9.6 kb RNA genome. The HCV RNA genome has a single ORF and encodes a polyprotein of approximately 3,000 amino acids in length, which is subsequently cleaved into several structural and nonstructural HCV proteins following translation (Table 4). The HCV structural proteins, which include the core and envelope proteins, are essential structural components of the hepatitis C virion. The nonstructural proteins are involved in several key aspects of the HCV life cycle, including post-translational processing of the HCV polyprotein, formation of the HCV replication complex, RNA synthesis, virion assembly, and virion release into the extracellular space (143).

5.2.2. Life cycle and viral replication

Although many advances have been made in elucidating the mechanisms involved in HCV replication, particularly with the development of cell culture replication systems, several components of the HCV life cycle have yet to be defined in detail. HCV is initially bound to hepatocytes, likely through interactions between HCV envelope proteins and cell surface receptors. The hepatitis C virion is then internalized into the cytoplasm via endocytosis and uncoating of the nucleocapsid occurs, releasing the single-stranded HCV RNA genome into the cytoplasm. HCV RNA is subsequently translated into the HCV polyprotein, a process mediated through binding of the host 40 S ribosomal subunit and an internal ribosome
Table 4. Hepatitis C viral proteins and functions (143)

<table>
<thead>
<tr>
<th>Hepatitis C protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>Viral nucleocapsid</td>
</tr>
<tr>
<td>Envelope glycoproteins (E1 and E2)</td>
<td>Component of viral envelope, mediates receptor binding and fusion between virion and hepatocyte</td>
</tr>
<tr>
<td>P7</td>
<td>Promotes release of viral particles</td>
</tr>
<tr>
<td>NS2-3 protease</td>
<td>Involved in processing and cleavage of HCV polyprotein to form non-structural proteins</td>
</tr>
<tr>
<td>NS3 serine protease</td>
<td>Involved in processing and cleavage of HCV polyprotein to form non-structural proteins</td>
</tr>
<tr>
<td>NS3 helicase/NTPase</td>
<td>Unwinding of double-stranded RNA or single-stranded RNA with secondary structures</td>
</tr>
<tr>
<td>NS4A</td>
<td>Cotransporter for NS3 serine protease</td>
</tr>
<tr>
<td>NS4B</td>
<td>Promotes formation of HCV replication complex</td>
</tr>
<tr>
<td>NS5A</td>
<td>Possibly associated with the formation of HCV replication complex</td>
</tr>
<tr>
<td>NS5B RNA-dependent RNA polymerase</td>
<td>Mediates HCV RNA synthesis</td>
</tr>
</tbody>
</table>

entry site (IRES) located at the 5′ non-translated region (NTR) of the HCV RNA genome (143). The HCV polyprotein is then cleaved into the various structural and nonstructural proteins through the activity of host signal peptidase, NS2-3 protease, and NS3 serine protease (Table 4). The formation of a membrane-associated replication complex, also described as a membranous web, occurs at the endoplasmic reticulum (144, 145). HCV RNA replication then proceeds via activity of the HCV RNA-dependent RNA polymerase. Ultimately viral particles and mature virions are released into the extracellular space. In contrast to HBV, the HCV life cycle occurs entirely within the hepatocyte cytoplasm and does not include an intranuclear component such as HBV cccDNA.

5.3. Natural history

In contrast with HBV, up to 85% of adults progress to develop chronic infection following acute exposure to HCV (146). In hepatocytes that are initially infected by HCV, a series of intracellular events result in the development of an antiviral state within each cell and in the surrounding tissue which plays a critical role in the host’s ability to limit viral production and ultimately activate the cellular immunity required to achieve clearance of infected cells (147). Activation of cellular transcription factors such as interferon regulatory factor (IRF)-3 and nuclear factor (NF)-kappa B leads to secretion of chemokines and proinflammatory cytokines including IFN-alpha and IFN-beta. The adaptive immune response, primarily in the form of activated anti-HCV CD4-positive helper T cells as well as CD8-positive cytotoxic T cells, are particularly important in the eradication of HCV both in the acute setting and in the treatment of chronic infection. During acute HCV infection, CD8-positive secretion of IFN-gamma coinciding with a durable CD4-positive proliferative response is associated with viral clearance. In contrast, acute HCV infection progressing to chronicity is marked by the absence of these responses (148). Likewise, HCV clearance following IFN-based therapy correlates with vigorous, durable, and multispecific CD4-positive proliferative responses and an enhanced IFN-gamma secretory response (149-151).

As evidenced by the large proportion of patients who develop chronic infection, HCV has developed means of evading host immunity. Various mechanisms of HCV immune evasion have been studied including dysfunctional homing of activated T cells to the liver, impaired antigen presentation, viral mutational escape, increased activity of regulatory T cells, or inhibitory effects by viral particles (152). HCV is also associated with a high rate of replication of up to $10^{12}$ virions per day (153), in which the HCV RNA-dependent RNA polymerase does not have proof-reading capability and mutations arise rapidly. Consequently, HCV exists in the form of multiple quasispecies within a host (135).

As the majority of adults acutely exposed to HCV develop chronic infection and up to 20% may progress to ESLD, chronic HCV infection has become a major source of liver-related morbidity and mortality (146). Up to 10,000 deaths per year in the U.S. occur as a result of HCV-associated chronic liver disease. Multiple variables have been identified as risk factors for progressive HCV-related liver disease including male gender, advanced age, obesity, chronic increased alcohol intake, coexisting hepatic steatosis, and HIV co-infection with low CD4 cell counts (154). HCV genotype has a major impact on response to antiviral therapy, but does not appear to influence the natural history of disease. Chronic HCV infection may also be associated with HCC, particularly in the setting of advanced liver disease with cirrhosis. Although HBV has the greatest association with risk of HCC worldwide, HCV infection is the most frequent cause of HCC in the U.S. and the incidence of HCC related to chronic HCV is increasing (155, 156).

5.4. Antiviral therapy

5.4.1. Combination peginterferon and ribavirin

Antiviral therapy with PegIFN alfa-2a or PegIFN alfa-2b in combination with RBV remains the gold standard for the treatment of chronic HCV infection (137, 157, 158). Despite advances in IFN-based therapy over the last 12 years, including the use of long-acting PegIFN and improved strategies to optimize response to therapy, the ability to achieve long-term viral suppression occurs in only one-half of patients (159, 160). Initial clinical trials of combination PegIFN and RBV reported SVR rates of 52% in genotype 1 infection following a 48-week course and SVR rates up to 84% in genotype 2 or 3 patients with only 24 weeks of therapy (159-161), with SVR defined by undetectable serum HCV RNA at 6 months following completion of therapy. In addition, these initial studies established that high-dose or weight-based RBV did not improve efficacy in genotype 2 or 3 infection (161). In contrast, weight-based RBV is superior to fixed-dose in the treatment of genotype 1 infection, as demonstrated in a large prospective clinical trial (162). Although achievement of SVR can be a challenge, particularly in difficult to treat populations such as genotype 1 infection, a sustained clearance of HCV may have a major impact on the natural history of chronic HCV infection. Achievement of SVR may potentially halt fibrosis progression, prolong survival, and decrease the risk of HCC, ESLD, or decompensating events associated with progressive liver failure (163-172).
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In addition, long-term cohort studies have established that achieving an SVR is durable; indicating that eradication of HCV is possible with successful antiviral therapy (163, 170).

5.4.1.1. Predictors of response

The ability to more accurately predict a successful response both before and during antiviral therapy with combination PegIFN and RBV has led to the development of individualized treatment strategies for patients with chronic HCV infection. HCV genotype is the strongest predictor of response and determines the length of antiviral therapy required. As patients with HCV genotype 1 infection have a much lower likelihood of achieving SVR and require a longer treatment course, a liver biopsy may be performed prior to undergoing therapy to assess whether histologically significant HCV-associated liver disease is present (137). Other factors associated with a poor response to therapy include increased age, obesity, African-American ethnicity, high HCV RNA levels, and the presence of advanced fibrosis or ESLD (159-161, 173). The identification of specific genetic polymorphisms associated with response to therapy, such as one recently discovered near the IL28B gene, may become increasingly important as the technology to detect genomic differences between individuals advances (174).

The most important predictive factor during a course of antiviral therapy is serum HCV RNA level. Achievement of a rapid virologic response (RVR), defined by complete HCV RNA suppression at four weeks of therapy, has a positive predictive value for SVR in genotype 1 patients of around 90% (175). An early virologic response (EVR), defined by at least a 2-log10 decrease in HCV RNA at week 12, has a negative predictive value for SVR of approximately 100% and serves as a stopping point for patients who fail to meet this parameter (160, 176). An additional stopping point may be considered at 24 weeks if HCV RNA is detectable given the very low likelihood of SVR in these patients (175). These criteria highlight the importance of achieving clearance of HCV at an early point in time during therapy. A recent review of genotype 1 patients enrolled in six different trials of combination PegIFN and RBV found that more rapid clearance of serum HCV RNA at various time points was associated with higher rates of SVR. In this study, rates of SVR for patients with RVR, complete EVR with undetectable HCV RNA at week 12, EVR with detectable HCV RNA, and no EVR were 87%, 68%, 27%, and 5%, respectively (177).

Early discontinuation of therapy has been proposed in patients with rapid viral clearance, although reports are mixed and this is generally not recommended in all genotypes who achieve RVR (178). Studies suggest that a shortened course of 12 to 16 weeks in genotype 2 or 3 infection (179-185) and 24 weeks in genotype 1 infection (186-188) may be as effective as a full course of therapy; however, this may only apply to certain patients groups such as those with low baseline serum HCV RNA levels. Alternatively, a prolonged course of therapy has been proposed for patients who do not rapidly clear HCV early during therapy. Extending a treatment course to 72 weeks in “slow responders”, defined by positive HCV RNA at week 12 followed by clearance at week 24, may increase SVR rates in these patients (189-191); although one recent prospective study did not report a significant improvement with extended treatment duration (192).

5.4.1.2. Impact of adherence

As rapid suppression of HCV and maintenance of viral clearance over a treatment course are essential to achieving optimal rates of SVR with PegIFN and RBV, efforts to ensure adherence to therapy are critically important in prevention of virologic breakthrough and relapse. Adherence to PegIFN and RBV has a direct association with likelihood of SVR based on clinical trial data, in which 80% adherence to therapy based on dosage and duration of therapy was associated with significantly improved response rates (193). RBV dosing is also particularly important, as decreases of the cumulative RBV dose below 60% may be associated with a poor outcome (194). Patients undergoing antiviral therapy with PegIFN and RBV should be monitored closely for known side effects including flu-like symptoms, fatigue, depression, and hematologic changes. If hematologic side effects such as neutropenia, thrombocytopenia, or hemolytic anemia occur, dose reductions may be required (137, 157) and the use of growth factors may be considered in selected cases (178).

5.4.2. Retreatment of failed therapy

Many patients with chronic hepatitis C who undergo IFN-based therapy ultimately develop viral persistence despite attempts to achieve a sustained response. Failure of therapy may occur as a result of breakthrough viremia following a period of undetectable HCV RNA during therapy, relapse during the six months following completion of therapy, or some patients may be considered “null” responders characterized by persistent viremia throughout their course. Prospective studies investigating the retreatment of patients who failed prior IFN-based therapy have reported the highest response rates in combination IFN alfa/RBV relapers and IFN alfa nonresponders, with rates of SVR up to 58% and 28% after combination PegIFN and RBV, respectively (Figure 4) (195-202). Nonresponders to prior combination IFN/RBV have a much lower reported SVR with retreatment, ranging from 8% to 15% and as high as 20% in studies involving patients with lower baseline HCV RNA levels (195-199, 201-203). Based on these studies, patients with low baseline HCV RNA levels or mild histologic liver disease may have a higher likelihood of a successful response to retreatment (195, 196, 198, 201-203). Patients who fail to respond to PegIFN have the lowest SVR rates with retreatment; consequently, repeated courses of PegIFN do not appear to have a major impact on clinical outcome (202).

Alternative treatment strategies have been proposed for patients demonstrating a null response to PegIFN therapy. To date, three large prospective studies have evaluated the role of maintenance therapy with PegIFN in prior treatment failures (202, 204, 205). The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) and the Colchicine versus Peg-Intron
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Figure 4. Efficacy of interferon-based therapy for prior treatment failures in chronic hepatitis C infection. Virologic response defined by SVR in prior treatment failures is shown associated with combination PegIFN/RBV, CIFN/RBV, and albinterferon/RBV therapy, respectively (195-203, 206-210). The data summarized is based on various clinical trials and not direct comparisons. Abbreviations: CIFN: consensus interferon, HBV: hepatitis B virus, IFN: interferon, NR: nonresponders, PegIFN: peginterferon, RBV: ribavirin.

Long-term (COPILOT) trials did not reveal any significant survival benefit in patients who received PegIFN after up to four years of follow up (204, 205), while the Evaluation of Peg-Intron in Control of Hepatitis C Cirrhosis (EPIC) trial has yet to present final results from maintenance treatment arms (202). Other formulations of IFN-based therapy such as consensus interferon (CIFN) and albinterferon alfa-2b have emerged as alternative treatment options for PegIFN nonresponders. CIFN is derived from a consensus sequence of the most common amino acids found in naturally occurring alpha interferon subtypes and has demonstrated modest SVR rates in the retreatment of IFN/RBV and PegIFN/RBV nonresponders (Figure 4) (206-209). Albinterferon alfa-2b is composed of IFN alfa-2b fused to recombinant human albumin, requires dosing only every 2 weeks, and has also demonstrated efficacy in the treatment of PegIFN/RBV nonresponders as well as treatment-naïve patients (210-212). Although alternative IFN-based therapies may have a role in the retreatment of PegIFN nonresponders, SVR rates remain relatively low and the introduction of specifically targeted agents against HCV will likely become a more viable treatment option in the future for this challenging patient population.

5.4.3. Direct Acting Antiviral (DAA) agents

New investigational agents that selectively target various key steps in the HCV life cycle and HCV replication, known as direct acting antiviral (DAA) agents, have emerged as viable treatment options in the future for patients with chronic hepatitis C. These new antiviral agents include protease and polymerase inhibitors, immune modulators, and other molecules, some of which have now completed early-phase clinical trials. Reports from prospective clinical trials involving these new agents have demonstrated significant improvements over the current gold standard therapy, combination PegIFN and RBV (Figure 5). More recent data have also revealed that rapid early clearance of HCV may be associated with SVR, suggesting that virologic parameters during a course of combination therapy involving DAA agents will be important in predicting response and individualizing therapy.

Protease inhibitors that specifically target the HCV NS3/4A serine protease include telaprevir, boceprevir, and ciluprevir (BILN 2061). At this time, telaprevir and boceprevir are the most well-studied, as phase III clinical trials involving both agents are currently ongoing. Results from phase II randomized clinical trials of telaprevir in naïve HCV genotype 1 patients, the Protease Inhibition for Viral Evaluation (PROVE) 1 and PROVE 2 trials, have reported SVR rates as high as 69% with a 12-week course of triple therapy followed by 12 weeks of combination PegIFN and RBV (Figure 5) (213, 214). Recent data from a European open-label phase II study was notable for SVR in up to 85% of naïve genotype 1 patients following 24 weeks of a similar treatment protocol, with no differences noted between treatment arms including PegIFN alfa-2a or -2b. A key finding from this study was that all patients who achieved an RVR subsequently developed an SVR (215). Telaprevir has also been evaluated in the retreatment of genotype 1 patients who previously failed combination therapy with PegIFN and RBV as described in the PROVE 3 trial. In this trial, rates of SVR were as high as 39% in nonresponders and 76% in
Figure 5. Efficacy of new antiviral therapies in the treatment of chronic hepatitis C infection with genotypes 1 or 4. The rates of SVR in the treatment of genotype 1 chronic HCV following standard of care combination PegIFN and RBV for 48 weeks in comparison with telaprevir, boceprevir, and nitazoxanide based on randomized clinical trials. Results shown from randomized clinical trials of nitazoxanide included only patients with HCV genotype 4 infection. Other antiviral agents are shown as treatment of HCV genotype 1. Rates of SVR are not based on direct comparison between agents. Abbreviations: BOC: boceprevir, HCV: hepatitis C virus, NTZ: nitazoxanide, PegIFN: peginterferon, RBV: ribavirin, SVR: sustained virologic response, TVR: telaprevir. Results from clinical trials of nitazoxanide involved patients with genotype 4 infection.
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virologic response and guiding individualized therapy. Additional prospective studies will be required to more clearly define appropriate dosing regimens, treatment strategies, combinations with other STAT-C agents, as well as assess safety and the potential for antiviral drug resistance.

6. PERSPECTIVE

As our understanding of the molecular biology, life cycle, and key elements that promote viral persistence in hepatitis viruses continues to expand, we may further develop novel antiviral agents with high potency and specificity, improve treatment strategies to optimize long-term outcomes, and potentially decrease the global health burden associated with liver disease arising from chronic viral infection. The development of new oral antiviral agents with high potency and genetic barrier to resistance has made a significant impact on the ability to achieve long-term viral suppression in chronic HBV infection. Treatment strategies designed to optimize HBV clearance and minimize the potential for drug resistance may continue to be important. Although the prevalence of chronic infection with HDV is low, its clinical significance is great in light of an increased risk of fulminant hepatitis, progressive disease, and low response rates with antiviral therapy. As oral nucleoside and nucleotide analogues are ineffective against HDV, IFN-based therapy remains the only treatment option. Further efforts towards development of antiviral agents targeted against key post-translational events in the HDV life cycle may lead to improvements in our ability to achieve viral suppression. Treatment of chronic HCV infection may continue to rely on the antiviral efficacy of PegIFN and RBV; however new therapies targeted against HCV in the form of DAA agents will greatly improve virologic response in treatment-naïve individuals and offer a treatment alternative in those who previously failed IFN-based therapy. Identifying predictive factors and monitoring virologic parameters during the course of HCV therapy will continue to play an important role as strategies evolve towards individualized therapy in an effort to optimize treatment outcomes and adherence. Ultimately the ability to achieve long-term viral suppression in the setting of chronic infection with hepatitis B, C, or D may have a significant impact on reducing morbidity and mortality associated with progressive hepatic fibrosis, and ESLD.

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**Key Words:** Hepatology, Liver, Hepatocyte, Virus, hepatitis B, Hepatitis C, hepatitis D, Hepatocellular Carcinoma, Cirrhosis, Nucleoside, Nucleotide, Interferon, Peginterferon, Therapy, Resistance, Review

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