REVIEW ARTICLE

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Hepatitis D Virus Infection

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N Engl J Med 2023;389:58-70.
DOI: 10.1056/NEJMra2212151

CME at NEJM.org

Hepatitis D virus (HDV), also known as hepatitis delta virus, is a defective, hepatotropic pathogenic agent. The life cycle of HDV requires the hepatitis B surface antigen (HBsAg) provided by hepatitis B virus (HBV).[1] Like HBV, HDV is transmitted by the parenteral route through infectious body fluids; intravenous drug users are at highest risk for infection because of contaminated syringes.[2,3] Key features of the infection are its unique biologic characteristics and ominous medical effect. Clinical studies have shown that chronic hepatitis D is the most severe and progressive form of viral hepatitis in humans.[2] The infection is ubiquitous, yet 40 years after it was identified, the prevalence remains undetermined in many parts of the world. In three recent meta-analyses, the number of HDV-infected persons worldwide ranged from 12 million to 72 million, underscoring the heterogeneity of current epidemiologic data.[3] Valid therapies to cure hepatitis D are an urgent need, and new therapeutic strategies are in development.

A Unique Human Pathogen

HDV, the smallest viral pathogen infecting humans, has biologic features similar to those of the viroids of plants.[4] It belongs to the genus deltavirus within the family of Kolmioviridae.[5] Eight genotypes with 81 to 89% sequence homology and many subgenotypes have been identified.[6] Genotype 1, the most widely distributed, predominates in Europe and North America. Genotype 3, present only in the Amazon basin, has been associated with outbreaks of fulminant hepatitis D,[3] whereas genotype 5, present in Africa, is associated with a more benign disease and has a better response to interferon than does the ubiquitous genotype 1.[7]

The genome is a circular, single-stranded RNA of approximately 1700 nucleotides. It is too small to code for replicative enzymes or envelope proteins and codes only for a small, nonenzymatic protein, hepatitis delta antigen (HDAg).[8] Three HDV RNA species are found in humans: a 1.7-kb genomic RNA in the virions, a complementary antigenomic RNA of positive polarity and the same size in the liver, and a 0.8-kb messenger RNA (mRNA) of antigenomic polarity in the liver; the mRNA contains an open reading frame for translation of HDAg. The genomic and antigenomic RNAs contain a domain of approximately 100 nucleotides that acts as a ribozyme, cleaving the viral RNA at specific sites without the participation of viral encoded enzymes.[9] Although in natural infections, intrahepatic HDV RNA replicates without assistance from HBV, HDV relies on HBV for all other functions of its life cycle, including viral packaging, infectivity, transmission, and inhibition of host immunity.[2,8-12]

Virions are heterogeneous particles that do not have a rigid structure, with a diameter of approximately 35 nm (Fig. 1A), enclosing a ribonucleoprotein made up

of multiple HDAg copies and a copy of the genome. The particles are coated by HBsAg proteins derived from transcripts of covalently closed, circular HBV DNA, as well as from transcripts of HBV DNA integrated into the host chromosomal DNA.[10] No viral morphologic features were observed on electron-microscopical examination of HDAg-positive liver cells; immunoelectron-microscopical examination of nuclei containing HDAg showed immunoreactive aggregates of granular material with undefined outlines[11] (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Propagation of HDV occurs through the binding of large HBsAg in the HDV coat to the sodium taurocholate cotransporting polypeptide.[12] Large HBsAg contains an additional preS1 region with a myristoylated N terminal, which is essential for binding to the receptor on the liver surface. Hepadnaviruses other than HBV can support HDV infection; the virus has been transmitted to eastern woodchucks infected with the woodchuck hepatitis virus[14] and to bats carrying hepadnaviruses that are antigenically related to HBV.[15] However, the evolution of HDV has not been linked to hepadnaviruses. Many HDV-like viruses with no hepadnaviruses were recently discovered in distantly related species such as fishes, birds, amphibians, and invertebrates.[16] The life cycle and replication cycle of HDV are shown in Figure 1B and 1C.[8-13]

CURRENT EPIDEMIOLOGIC SCENARIO

The prevalence of hepatitis D has changed in the past 25 years, reflecting the control of HBV infection afforded by the worldwide implementation of HBV vaccination programs.[3] These programs are reducing the number of HBsAg carriers who are susceptible to HDV and, as a secondary effect, are contributing to the global decline of HDV infection. The current trend in the worldwide prevalence of HDV is shown in Figure 2.

HDV IN HIGH-INCOME COUNTRIES

Universal HBV vaccination that was initiated in the early 1990s in high-income countries has resulted in control of hepatitis B. As a consequence, younger persons (i.e., those most susceptible to HDV infection) are protected from hepatitis D, and the age-related prevalence of the infection is shifting to older persons.[3] Among native western Europeans, chronic HDV infections are now observed only in patients who have cirrhosis or advanced fibrosis.[17,18] Liver grafting is the only therapeutic option for such patients, and their treatment has an effect on liver transplantation programs, despite the low contemporary prevalence of HDV infection.[19]

Chronic hepatitis D remains prevalent in Romania, with hot spots throughout Russia.[3] HDV infection has diminished among persons who inject drugs,[20] but it remains prevalent among persons with human immunodeficiency virus (HIV) infection, and an estimated 12.5% of those who are coinfected with HIV and HBV worldwide have anti-HDV antibodies.[21]

Although hepatitis D had been vanishing in native populations of Europe, it is returning as a result of immigration from areas where HDV remains endemic.[3,22] In 2019, the overall prevalence of anti-HDV antibodies among HBsAg carriers in Italy was 9.9%, with a prevalence of 6.4% among native Italians but 26.4% among immigrants.[23] In the United States, the prevalence of HBsAg carriers in the period from 2011 through 2016 was only 0.36%, but 42% of the carriers had antibodies to HDV, and most of these carriers were persons who inject drugs or were from areas with a high prevalence of HDV.[24]

HDV IN THE REST OF THE WORLD

HBV vaccination has been implemented to various degrees in virtually all medium- and high-income countries, and in the future it is expected to substantially reduce the global prevalence of HDV infection. In the past 10 years, the prevalence has consistently been decreasing in northern Africa, Saudi Arabia, Israel, Turkey, Iran, India, and China.[3]

The burden of HDV infection remains high in Moldova and in many African and Asian countries where the percentage of HBsAg carriers in the population is high.[25] The clinical features of chronic hepatitis D are consistent with those of the florid inflammatory and progressive disease described in the 1980s in Europe.[26] Nevertheless, outbreaks of fulminant hepatitis D, such as those

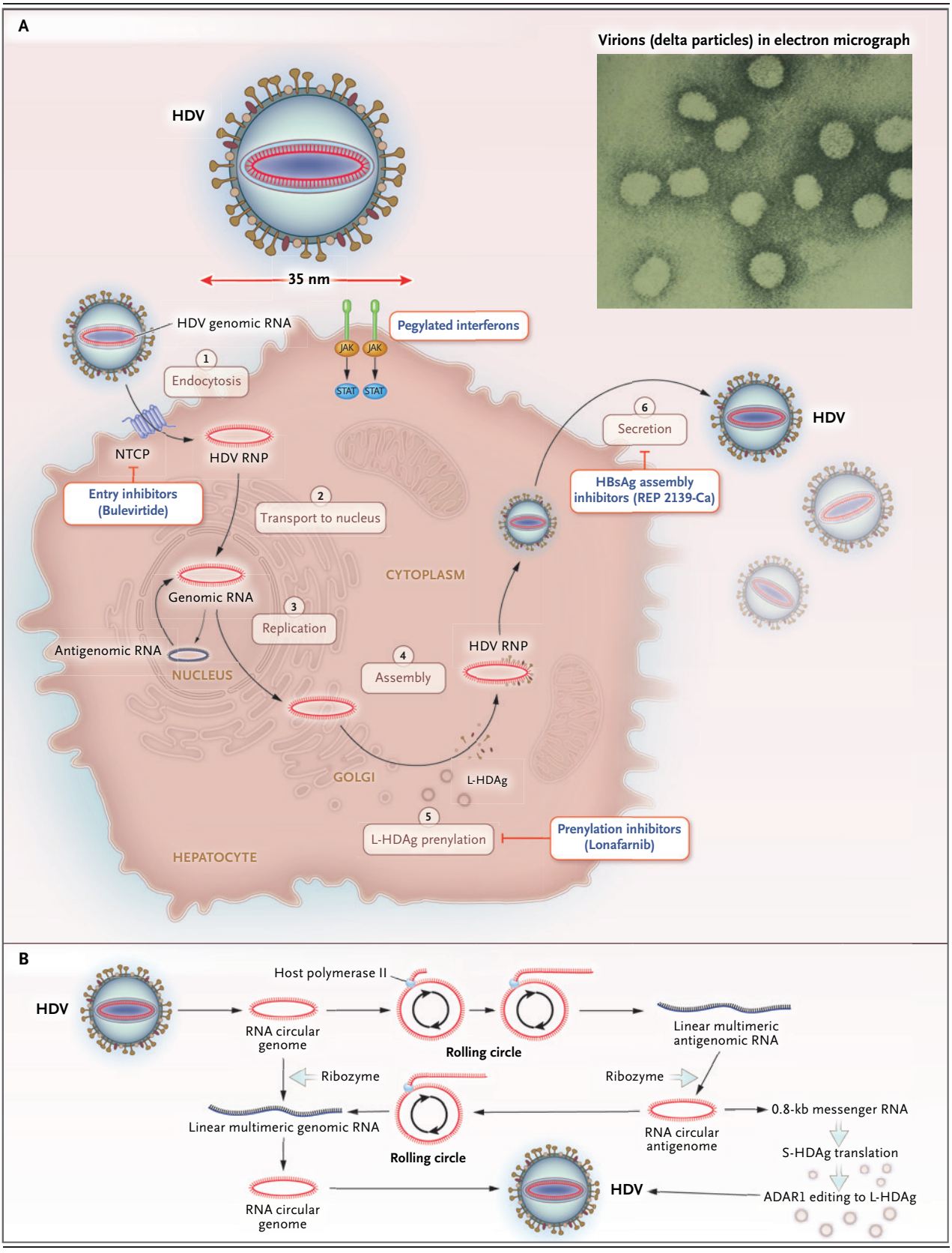

Figure 1 (facing page). Structure and Life Cycle of Hepatitis D Virus (HDV) and Targets of New Drugs for Hepatitis D.

Panel A shows the structure of HDV, which shares the hepatitis B surface antigen (HBsAg) envelope with hepatitis B virus. The virion contains a single-stranded, circular RNA genome of approximately 1700 nucleotides. HDV RNA codes for the small hepatitis D antigen (S-HDAg, 195 amino acids) required for replication. S-HDAg is edited to the large hepatitis D antigen (L-HDAg, 214 amino acids). L-HDAg inhibits replication of HDV and is required for viral assembly. The virions (delta particles), shown in the electron micrograph, vary morphologically and are approximately 35 nm in diameter. The targets of new therapies are indicated.[13] The first stage of the HDV life cycle is endocytosis: HDV binds to the sodium taurocholate cotransporting polypeptide receptor (NTCP), which is the specific liver-cell receptor for HBsAg. The second stage is transport to the nucleus. The third stage is replication of HDV genomic RNA into the HDV antigenome in the nucleus. The fourth stage is assembly of the neosynthetized HDV ribonucleoprotein (RNP) in cytoplasm. The fifth stage is farnesylation of the C terminal in L-HDAg; the post-translational modification of L-HDAg drives the combination of the RNP with HBsAg in order to assemble the virion. Finally, the HDV virion is secreted. Bulevirtide, an N-terminally myristoylated, HBV-large-envelope-protein–derived lipopeptide, inhibits NTCP irreversibly, blocking the access of the HBsAg-coated HDV to the hepatocyte. Pegylated interferon lambda, on binding with the specific receptor on the cell surface, initiates intracellular signaling cascades, leading to the expression of antiviral genes. Lonafarnib, an orally administered prenylation inhibitor, prevents viral morphogenesis by interfering with the farnesylation of L-HDAg. Lonafarnib is largely metabolized by cytochrome P450-3A4 (CYP3A4), and the CPY3A4 inhibitor ritonavir is added to diminish adverse effects while drug exposure is maintained. REP 2139-Ca is a nucleic acid polymer that inhibits the assembly and release of hepatitis B virus (HBV) subviral particles. Panel B shows viral replication. The HDV genome lacks coding capacity and has no replicative ability. Inside the nuclei, it is copied through a rolling circle mechanism that is not seen in mammalian cells but is typical of viroids of plants. The RNA is copied into the antigenome through multimeric linear transcripts of antigenomic sense that roll over the circular genomic RNA; the rolling is driven by host RNA polymerase II, redirected by HDV to reproduce its viral RNA. The multimeric transcript undergoes autocatalytic cleavage by a ribozyme into monomeric linear forms of RNA, which are then ligated by the ribozyme and probably by a host ligase to the final circular antigenomic RNA. A 0.8-kb messenger RNA transcribed from the antigenome is translated into the HDAg of 190 amino acids (S-HDAg), which is then changed into the large, 210-amino-acid HDAg (L-HDAg) through post-transcriptional RNA editing. S-HDAg promotes replication; L-HDAg inhibits replication and is required for packaging with HBsAg. ADAR1 denotes adenosine deaminase acting on RNA 1.

that occurred in the 1980s and 1990s,[27] are infrequently reported, which suggests that there is also some control of HDV infection in low-income countries. Local prevalence data are often erratic; a major bias is the disparate sampling of HDV cases.[25] In view of the pathogenic effect of HDV, data on the infection show a distinctly lower prevalence when screening is performed in asymptomatic HBsAg carriers who are at low risk for HDV infection and who are recruited for convenience at blood banks or community centers than in patients with HBsAg-positive liver disease who are recruited in medical centers.[3]

MEDICAL IMPLICATIONS

DIAGNOSIS

The diagnosis of hepatitis D involves specific serologic testing.[28] An algorithm for the clinical management of HDV infection is shown in Figure 3. The hallmark of exposure to HDV is the presence of antibodies to hepatitis D antigen (anti-HDV antibodies), which are found in all immunocompetent patients with the infection. Testing for the antibodies should be performed only in HBsAg-positive persons. HBsAg-positive persons with anti-HDV antibodies who do not have HDV RNA in serum may have had a previous HDV infection. Qualitative assays for anti-HDV antibodies are commercially available. However, no international standard regarding levels of anti-HDV antibodies has been developed. Sensitivity-controlled assays from a few major manufacturers are in use in high-income countries. Most assays from local producers elsewhere have no formal pedigree, and the quality of these assays has not been validated.[29] Recommendations from international associations differ regarding anti-HDV antibody screening. The European and Asian Pacific Associations for the Study of the Liver recommend testing for anti-HDV antibodies in all HBsAg-positive persons,[30,31] whereas the American Association for the Study of Liver Diseases recommends such testing only in high-risk patients,[32] including those who have immigrated from countries where HDV is endemic. Persons who are positive for anti-HDV antibodies should be tested for serum HDV RNA to determine whether an active infection is present.

In-house and commercial reverse-transcrip-

tase–polymerase-chain-reaction (RT-PCR) assays based on the international HDV RNA standard of the World Health Organization[33] are available for quantification of HDV RNA with the use of amplification targets in conserved regions of HDAg or the ribozyme.[34] The sensitivity of the RT-PCR assays for HDV RNA detection has increased over time, from a detection threshold of 1000 IU per milliliter to the current threshold of 6 IU per milliliter. However, an international quality-control study performed in 2016 showed a high degree of variation in detection and quantification among assays,[35] with consistent underestimations of the viral load, in particular with HDV genotypes 5 through 8, which are common in Africa.

In experimental models, HDV was transmitted through the envelope of viruses that differed

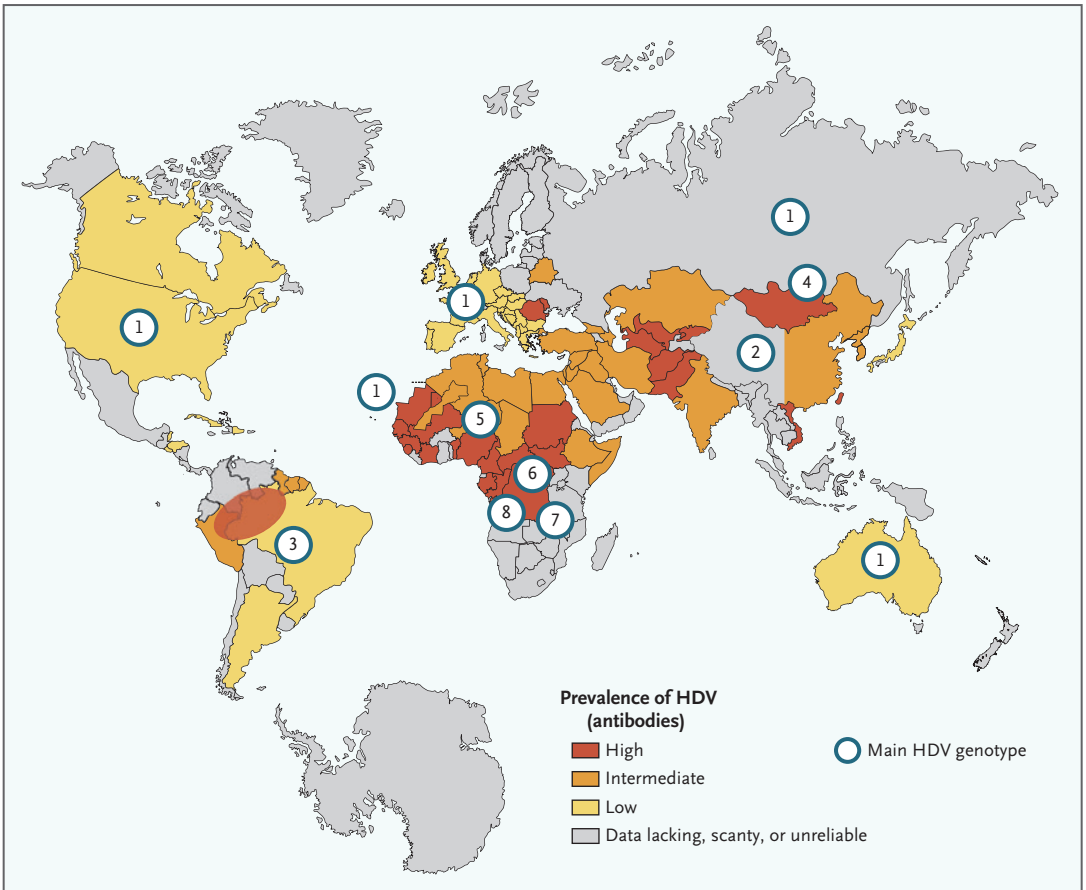

Figure 2. Prevalence of Antibodies to HDV in the World in the Past 10 Years.

The prevalence of antibodies to HDV in the past 10 years in areas for which data are not lacking, scanty, or unreliable is shown. The highest prevalence of these antibodies has been in Africa and Asia.[3] African countries with the highest prevalence are Cameroon (with a prevalence of approximately 47% among HBsAg-positive hospitalized patients), Gabon (28% among HBsAg carriers), Central African Republic (50% among patients with chronic hepatitis B and hepatocellular carcinoma), and the Democratic Republic of Congo (26% among HBsAg-positive patients with jaundice). Asian countries with the highest prevalence are Mongolia (with a prevalence of 50 to 60% in the general HBsAg-positive population), Uzbekistan, Tajikistan, and Kyrgyzstan (82%, 15%, and 42%, respectively, among HBsAg-positive patients with cirrhosis), and Pakistan (30 to 50% among HBsAg-positive patients in the so-called delta belt of the country). In the countries with intermediate prevalence, HDV infection is declining. Eight HDV genotypes in various regions of the world have been identified. The locations of these genotypes (circled numbers) are shown.

from hepadnaviruses, including hepatitis C virus (HCV).[36] Although intriguing, this finding does not appear to be relevant in the real world, since none of 323 HCV RNA–positive, HBsAg-negative patients were positive for HDV RNA in a recent study.[37]

HDV screening remains low in both developed and developing countries. Of 157,333 patients with chronic hepatitis B in the United States between 2010 and 2020, only 6.7% underwent HDV testing.[38] One strategy to increase HDV screening is reflex testing for anti-HDV antibodies in all persons who test positive for HBsAg. This would prevent health care providers who are not familiar with hepatitis D from overlooking a diagnosis of HDV infection. Implementation of reflex testing recently led to an increase by a factor of 5 in the number of HDV diagnoses in Spain.[39]

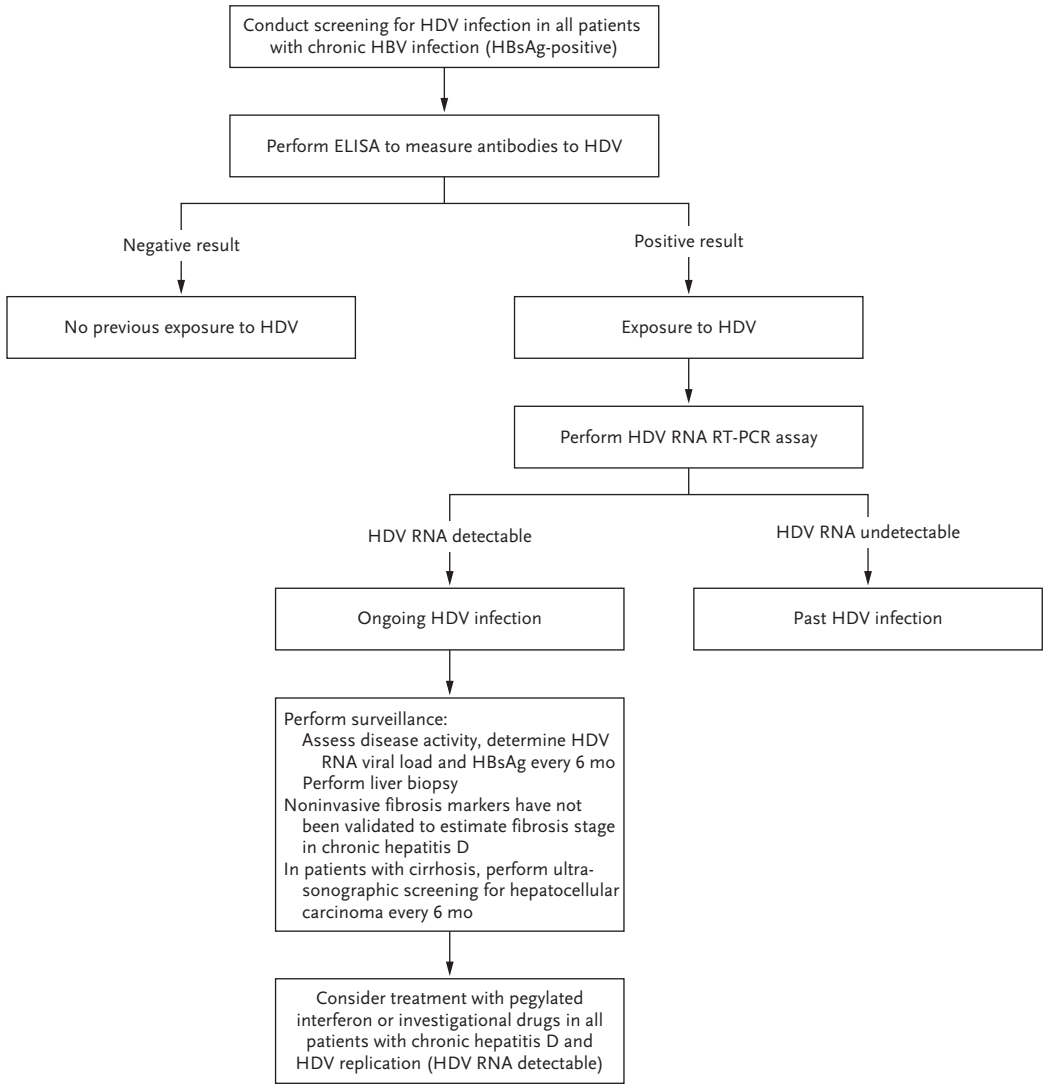

Figure 3. Algorithm for the Diagnosis and Management of HDV Infection and Hepatitis D.
ELISA denotes enzyme-linked immunosorbent assay, and RT-PCR reverse-transcriptase polymerase chain reaction.

NATURAL HISTORY

The two forms of HDV infection are simultaneous coinfection with HBV and HDV in persons with no previous HBV exposure and HDV superinfection in those with chronic HBV infection.[40] The need for HBsAg is met differently in the two types of infection. In coinfection, HDV is rescued by the HBsAg from the concomitant de novo HBV infection; in superinfection, HDV is rescued by the preexisting HBsAg in the superinfected HBV carrier. The clinical course of coinfection is most often self-limited, with acute hepatitis. Superinfections account for approximately 90% of cases of chronic HDV infection and chronic hepatitis D (Fig. S2).

CLINICAL COURSE OF CHRONIC HDV DISEASE

Studies have shown that chronic hepatitis D is more severe and progressive than hepatitis B.[27,40] During 1 to 15 years of follow-up in one study, histologic deterioration was documented in 77% of Italian patients who were positive for anti-HDV antibodies, as compared with 30% of HBsAg carriers who did not have anti-HDV antibodies.[41] In another study, HDV infection increased the risk of hepatocellular carcinoma by a factor of 3.[42]

Persistent, severe HDV viremia is the most important risk factor for progression.[43,44] The clinical and virologic features and natural history of chronic hepatitis D are shown in Figures 4 and 5.[13,17,40-49] Chronic hepatitis D was initially perceived as being almost invariably severe. In a study involving 284 patients with chronic hepatitis D in Italy at the end of the past century, 93% of the patients had active hepatitis or cirrhosis, and only 7% had milder disease.[50] This perception, and the limited availability of diagnostic assays, led in past decades to the practice of investigating HDV infections predominantly in HBsAg-positive patients with symptomatic, active liver disorders who were seen at tertiary medical centers. Minor, asymptomatic forms of HDV disease were probably overlooked because testing was not performed. Recent data obtained from secondary medical centers indicate that the proportion of patients with milder HDV disease may be more substantial than previously recognized.[13,51]

THERAPY FOR CHRONIC HEPATITIS D

There is no approved therapy for chronic hepatitis D in the United States. Although pegylated interferon alfa has been used off label, in accordance with the recommendations of major liver societies,[30-32] data on the results of this use are limited.[52] New therapeutic strategies are aimed at depriving the virus of the collateral HBsAg functions that are critical for its life cycle, activating the host immune response, or both.[12] Drugs in clinical development are lonafarnib, nucleic acid polymers, pegylated interferon lambda, and bulevirtide.[12,52-55] The mechanisms of action of these drugs are shown in Figure 1B.[12,52-55]

THERAPEUTIC END POINTS

The optimal end point for therapy would be the loss of HBsAg, but this has rarely been achieved with interferon. Virologic responses have been evaluated on the basis of a sustained viral response (i.e., undetectable HDV RNA in serum for 6 months after discontinuation of therapy). A sustained viral response has thus far been considered the hard end point of therapy. However, only up to 30% of patients treated with interferon have had a sustained viral response, and long-term relapses have been common because of the very high infectivity of residual, undetectable HDV in patients with persistent HBsAg (end titration of HDV infectivity in HBsAg-positive chimpanzees, 10^{-11} serum dilution[56]).

To improve clinical results, the initial efficacy assessment of lonafarnib, pegylated interferon lambda, and bulevirtide has been adjusted to the less restrictive virologic threshold of a decrease of 2 $\log_{10}$ IU per milliliter[10] in the HDV RNA level from the original viremic titer.[52] The Food and Drug Administration endorsed this surrogate as a likely predictor of a clinical benefit, with the understanding that it is not per se evidence of a benefit but instead requires subsequent confirmation with the use of clinical end points such as progression to cirrhosis, decompensation, hepatocellular carcinoma, and death.[57] However, the surrogate of a decrease of 2 $\log_{10}$ IU per milliliter has become a virologic end point of therapy rather than a marker of initial treatment efficacy. It seems incongruous that

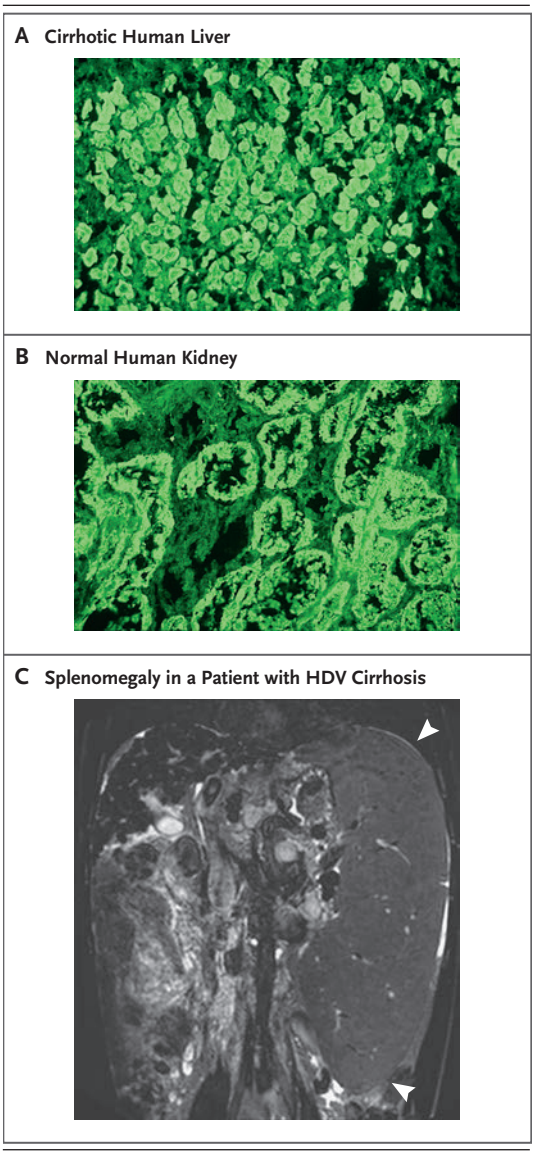

Figure 4. Features of Chronic Hepatitis D.

The clinical, biochemical, and histologic features of hepatitis D are not specific. In the majority of patients, serum HBV DNA is undetectable or at the borderline of detectability because of the repression of HBV viremia induced by HDV through interferon-dependent and interferon-independent mechanisms. The majority of patients (>70%) have serum antibodies to hepatitis B e antigen; the antigen is detectable in a minority of patients. Approximately 10% of patients with early florid disease have autoantibodies directed against uridine diphosphate glucuronosyltransferase in the microsomes of the liver and of the kidney (liver–kidney–microsomal autoantibodies type 3 [LKM-3]); patients with LKM-3 are not at risk for autoimmune hepatitis. In approximately one quarter of the patients, the serum HDV RNA level decreases or becomes undetectable during the natural course of the disease. Panels A and B show a cirrhotic liver specimen and a normal kidney specimen, respectively, stained in indirect immunofluorescence with serum from a patient with chronic hepatitis D. Patients with cirrhosis occasionally have severe splenomegaly unrelated to the degree of portal hypertension (Panel C, arrowheads).

the therapeutic end point for HDV therapy can be both clearance of the virus and its persistence at variable titers after declining by 2 $\log_{10}$ IU per milliliter from the original pretherapy titer. Clinical data from patients in whom HDV RNA has been eliminated and those in whom viremia is maintained do not support the idea that these two outcomes correspond to the same goal of resolution of infection or disease and provide an equivalent clinical prognostic perspective.

Short of undetectable viremia, no threshold for a serum HDV RNA level corresponding to a clinical benefit has yet been defined. In clinical studies, the mere presence of HDV viremia has been associated with an increased risk of various adverse clinical events, and studies of interferon have shown that the absence of HDV RNA is associated with better clinical outcomes than is a decrease of 2 $\log_{10}$ IU per milliliter in the HDV RNA level.[58,59] It therefore seems appropriate to consider the characteristics of patients in whom HDV is cleared with therapy separately from the characteristics of those who have a partial therapeutic response, since these two groups are distinct patient populations with prognoses that are likely to differ.

PRELIMINARY RESULTS OF THERAPY WITH EXPERIMENTAL DRUGS

In view of the progressive nature of HDV disease, treatment should be considered in all patients with chronic hepatitis D and active HDV replication (detectable HDV RNA) (Fig. 3). So far, only patients without cirrhosis or with compensated cirrhosis have been treated, pending the verification of side effects of therapies in patients with decompensated cirrhosis. Long-term studies of drugs for HDV are ongoing. Tenofovir or entecavir has been added to therapy for HDV in some studies in order to prevent possible reactivation of HBV after the inhibition of HDV.

Targeting HBV with antiviral agents (lamivudine, adefovir, entecavir, or tenofovir) is of no avail in controlling HDV infection. These agents inhibit the replication of HBV DNA but do not interfere with the synthesis of HBsAg, which is necessary for HDV.[3] However, new antiviral agents are in development that aim at a functional cure of HBV infection, which is defined by both the absence of circulating HBsAg and undetectable HBV DNA in serum. These agents could be of interest in providing synergism for the cure of HDV infection.[58] The clinical status of current and investigational drugs for chronic hepatitis D is shown in Figure S3.

Lonafarnib

In a small series of patients, a decrease in the HDV RNA level of at least 2 $\log_{10}$ IU per milliliter from baseline or the lower limit of quantification was reached at the end of treatment in 8 of 9 patients (89%) who received the combination of oral lonafarnib (25 or 50 mg twice a day) with ritonavir and pegylated interferon alfa.[60] The most common side effects were gastrointestinal, with diarrhea, nausea, and loss of appetite and weight.

Clinical trials have investigated different doses of lonafarnib; combination therapy of lonafarnib with pegylated interferon alfa, pegylated interferon lambda, or ritonavir; and various treatment durations. D-LIVR, a phase 3, international, multicenter, randomized, controlled trial (ClinicalTrials.gov number, NCT03719313), has recruited 400 patients. The aim of this trial is to evaluate 50 mg of lonafarnib plus ritonavir, given twice per day with or without pegylated interferon alfa, as compared with placebo or pegylated interferon alfa alone for 48 weeks in patients receiving maintenance therapy with anti-HBV nucleotides (entecavir or tenofovir).

Nucleic Acid Polymer REP 2139-Ca

In one trial, the use of intravenous REP 2139-Ca in combination with pegylated interferon alfa led to off-treatment HBsAg loss and undetectable HDV RNA in 6 of 12 patients.[61] Clinically signifi-

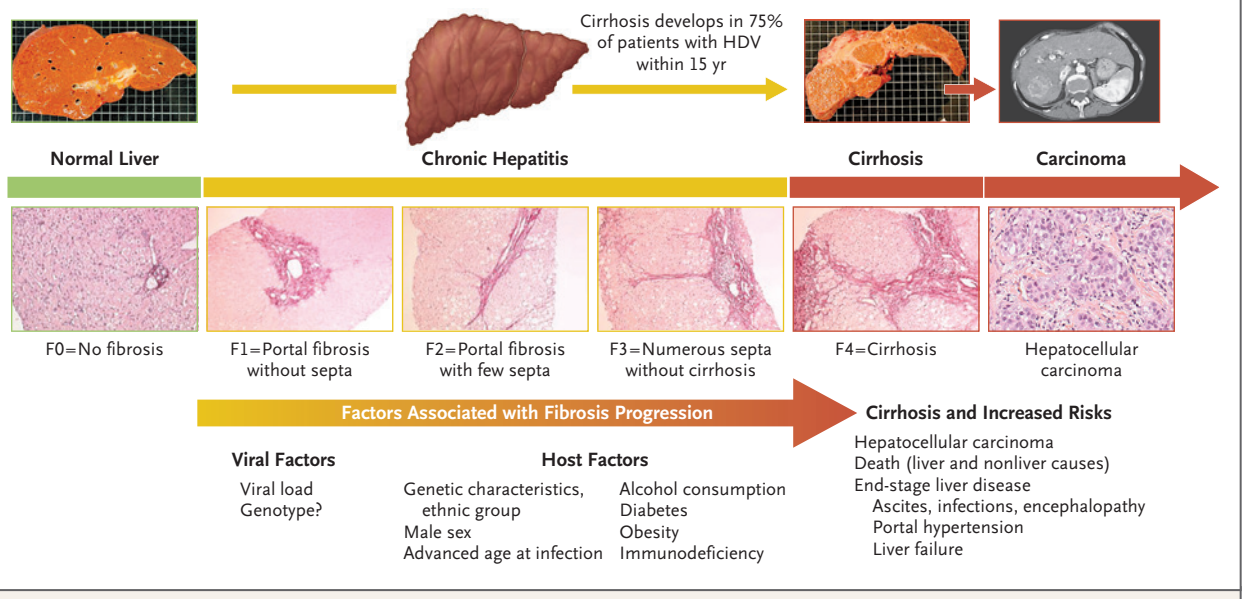

Figure 5. Natural History of Chronic Hepatitis D.

Chronic hepatitis D is associated with a more rapid progression to cirrhosis and a higher risk of hepatocellular carcinoma than HBV monoinfection. Identifying patients in whom fibrosis progresses rapidly would be crucial for determining the prognosis, but mechanisms of fibrotic progression are poorly understood. Histologic findings (hematoxylin and eosin stain) are shown. Liver fibrosis is assessed with the use of Metavir staging, which ranges from F0 (no fibrosis) to F4 (cirrhosis). Some HDV genotypes may influence disease activity. Persistent HDV viremia is a factor in disease progression and a predictor of death. In liver disease in general, factors associated with fibrosis are male sex, older age at infection, excessive alcohol consumption, obesity, diabetes, and immunodeficiency.

cant flares in alanine aminotransferase (ALT) levels occurred during therapy. Efficacy was sustained 3.5 years after the discontinuation of therapy[62]; these good results await confirmation in larger series.

Pegylated Interferon Lambda

In the phase 2 Lambda Interferon Monotherapy trial,[63] subcutaneous pegylated interferon lambda was administered weekly at a dose of 180 μg in 14 patients and at a dose of 120 μg in 19 patients for 48 weeks. In an intention-to-treat analysis, the virologic response 24 weeks after the end of therapy was 36% and 16%, respectively. Influenza-like symptoms and elevated ALT and aspartate aminotransferase levels occurred during treatment. In 8 patients (24%), hyperbilirubinemia led to drug discontinuation.

Bulevirtide

In phase 2 studies, bulevirtide monotherapy reduced serum HDV RNA levels and normalized ALT levels in a consistent proportion of patients, findings that prompted the European Medicines Agency in July 2020 to grant marketing authorization for the 2-mg subcutaneous daily dose of the drug in patients with chronic, compensated hepatitis D.[64] Several short-term, real-life studies with small numbers of patients have replicated these results.[65]

In the MYR202 trial, which compared a combination of daily subcutaneous bulevirtide (at a dose of 2, 5, or 10 mg) and tenofovir with tenofovir alone for 24 weeks in 120 patients with chronic hepatitis, a decrease in the HDV RNA level of 2 $\log_{10}$ IU per milliliter or more occurred in 50 to 77% of the patients who received bulevirtide with tenofovir, as compared with only 4% of those who received tenofovir alone.[66] The combination treatment was safe and had an acceptable side-effect profile. ALT levels normalized in more than half the patients; however, bulevirtide monotherapy had a minimal effect on HBsAg levels, and after discontinuation of therapy, serum HDV RNA levels almost invariably rebounded. This finding suggests that long-term treatment is warranted. Whether it will be possible to discontinue the drug after long-term treatment and whether the virologic efficacy will be maintained are not known. Another question is the safety of bulevirtide administered as long-term therapy, especially in patients with cirrhosis, given the prolonged, treatment-induced increase in bile acid levels.

Data from a 48-week interim analysis in a phase 3, open-label, randomized trial (MYR 301) of bulevirtide monotherapy in patients with chronic hepatitis D are reported elsewhere in this issue of the *Journal*.[67] Patients were randomly assigned in a 1:1:1 ratio to receive subcutaneous bulevirtide at a dose of 2 mg per day (49 patients) or 10 mg per day (50 patients) for a total of 144 weeks or to receive no bulevirtide for 48 weeks followed by bulevirtide subcutaneously at a dose of 10 mg per day for 96 weeks (control group, 51 patients). A combined response of an undetectable HDV RNA level or a decrease by at least 2 $\log_{10}$ IU per milliliter in the HDV RNA level and normalization of the ALT level (the primary end point) occurred in 45% of the patients in the 2-mg group (22 of 49 patients), 48% of those in the 10-mg group (24 of 50 patients), and 2% of those in the control group (1 of 51 patients); HDV RNA was undetectable in 12% and 20% of the patients receiving the lower and higher doses of bulevirtide, respectively. Regardless of the virologic response, ALT levels normalized in 51% and 56% of the patients in the 2-mg and 10-mg groups, respectively, versus 12% of those in the control group. The level of HBsAg did not become undetectable or decrease by at least 1 $\log_{10}$ IU per milliliter in the 2-mg or 10-mg groups by week 48.

In a second long-term trial (MYR204), the primary outcome measure is a sustained viral response in patients treated with pegylated interferon alfa in combination with bulevirtide, pegylated interferon alfa alone, or bulevirtide alone. Patients receive treatment for 48 weeks, followed by 48 weeks of maintenance therapy with bulevirtide monotherapy. In an interim analysis of data at 24 weeks of treatment,[68] HDV RNA was undetectable in 13% of the patients who received pegylated interferon alfa monotherapy, 24% of those who received 2 mg of bulevirtide plus pegylated interferon alfa, 34% of those who received 10 mg of bulevirtide plus pegylated interferon alfa, and 4% of those who received 10 mg of bulevirtide alone.

In both the MYR204 study and real-life stud-

ies,[65,69] short-term combination treatment with pegylated interferon alfa and bulevirtide met the difficult goal of undetectable HDV RNA more frequently than did bulevirtide alone. Recent evidence has shown that spreading of HDV occurs not only through de novo HBsAg-dependent virion propagation but also through HBsAg-independent division of infected cells. In cell-culture models, interferon did not inhibit the HBsAg-independent division but did inhibit the spread of HBsAg and HDV to dividing cells.[70] Thus, the therapeutic synergism may be due to bulevirtide-induced inhibition of extracellular spread and interferon-induced inhibition of cell division–mediated HDV spread.

CONCLUSIONS

The overall prevalence of HDV infection is unknown; however, HBV vaccination is reducing the incidence of HDV infection worldwide. The risk of cirrhosis and hepatocellular carcinoma is higher among patients with HDV infection than among those with HBV monoinfection. Screening for antibodies to HDV is generally available; the sensitivity of many assays has not been validated. HDV RNA testing is not widely available. Pegylated interferon alfa has been used in accordance with published guidelines, with limited efficacy. Preliminary data indicate that bulevirtide can control the disease in more than half of patients. Bulevirtide was conditionally approved by the European Medicines Agency in July 2020; it is not yet available in the United States. Other new drugs for the treatment of HDV infection are currently under investigation. Combination therapy with these agents, possibly administered with newly developed inhibitors of HBsAg synthesis, holds promise as a cure for chronic hepatitis D.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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