

Hepatitis Delta Virus: Epidemiology, Natural Course and Treatment

Philippe Sultanik and Stanislas Pol*

Department of Hepatology, Paris Descartes University, France

*Corresponding author: Stanislas Pol, Department of Hepatology, Paris Descartes University, France, Tel: 33158413001; Fax: +33158413015; E-mail: stanislas.pol@aphp.fr

Received date: January 20, 2016; Accepted date: February 25, 2016; Published date: March 03, 2016

Copyright: © 2016 Sultanik P, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The hepatitis delta virus (HDV) is a defective hepatotropic virus that affects only patients with hepatitis B virus (HBV) infection. These 2 viruses share the same routes of transmission (parenteral, sexual, and mother to child). The hepatitis delta virus infection may result in acute hepatitis, including fulminant presentation or spontaneously resolving infection, and chronic infection. The prognosis depends on the chronology of the 2 infections, co-infection (higher risk of fulminant hepatitis) or super-infection (frequent evolution to chronicity). The harmful impact of HIV-associated infection is today debated even if HDV antibodies are present in 12% and 4% of HIV/HBV co-infected and HBV-mono-infected patients respectively. HDV may be treated by interferon, the unique treatment, but relapse is observed in 50% of cases after discontinuation of therapy: only 25% of treated patients achieved a sustained virologic response. Entry HBV/HDV inhibitors (Myrcludex) and prenylation inhibitors are awaited encouraging progress in treating HDV infection. The treatment is recommended in patients with significant fibrosis; viral suppression may result in fibrosis stabilization or reversal which may be also observed in the absence of complete viral eradication. The prevention of hepatitis delta virus infection is based on hepatitis B virus vaccination

Keywords: Hepatitis B virus; Hepatitis delta virus; Chronic hepatitis; Cirrhosis; Hepatocellular carcinoma; Interferon

Introduction

Hepatitis D virus (HDV) is a defective hepatotropic virus that affects only patients with hepatitis B virus (HBV) infection. These 2 viruses share the same routes of transmission (parenteral, sexual, and mother to child). HDV antigen was first detected in hepatocytes of HBV-infected patients with Hbc antigen expression but distinct from the latter [1]. The more severe prognosis of HDV infection in the setting of human immunodeficiency virus (HIV) coinfection seems questionable today, even if HDV infection is observed in 12% of HIV-coinfected patients compared to 4% in HBV-mono-infected patients, which come from high or moderate endemic areas for majority. Interferon treatment is less efficient in HDV infection than in HBV infection with only 25% of sustained virological response and more than 50% of relapse after discontinuation of therapy. The addition of nucleos(t)ide analogues to the Interferon regimen do not change the virologic response and have to be used only for HBV indication. New therapies are promising, especially the HBV/HDV entry inhibitors (Myrcludex) or prenylation inhibitors. Antiviral treatment is indicated for patients with significant fibrosis and can limit fibrosis progression, even without a complete viral suppression. The prevention of the HDV infection relies on vaccination against HBV.

Genomic structure

The hepatitis D virus, first discovered by Rizzeto in 1977, is a small (36 nm in diameter) negative single-stranded covalently closed circular ribonucleic acid (RNA) virus of 1672 to 1697 nucleotides, with high G-C content, that can form secondary double-stranded structures [2]. The RNA closely links to the small delta protein (p24) and the large delta protein (p27), defining the delta ribonucleoprotein which will be covered by the HBV surface proteins (HBs antigen) corresponding the

delta viral particle. Thus, the HDV is a defective virus, depending on another virus (HBV) also called auxiliary virus for replication and propagation.

The HDV genome contains three genomic and three antigenomic open reading frames, one of latter coding for the 24 kD delta antigen. The HDV has, like the HBV, a high genetic variability associated with a specific endemic distribution.

The recent strain genotyping of the virus identified that HDV circulates as quasi-species. New genotyping identifications have been added in the 2000's to the three major genotypes (I, II, III) identified in the 1990's with subtypes (A, B) [3,4]. The Deltavirus genre is nowadays classified into eight genotypes (HDV-1 to HDV-8) with a genomic variability of 15% and specific geographic distributions [5-7]. HDV-1 is ubiquitous, HDV-2 and -4 are present in Siberia, East Asia and South Asia, Japan and Miyako islands; HDV-3 is present in Amazon basin and HDV-5, -6, -7 and -8 are present in sub-Saharan Africa and were found in migrants living in France and contaminated in their country of birth [8,9].

Differences of pathogenicity between the genotypes are currently debated; HDV-3 being associated with severe acute hepatitis, and HDV-2 or -4 associated with less severe hepatitis [10].

Epidemiology

The routes of transmission for HDV are similar from those for HBV: parenteral (mainly by intra-venous drugs use and rarely after blood transfusion, with a disappearance in industrial countries where HBs antigen detection is the rule), sexual (mostly in men who have sex with men [MSM]), from mother to child (vertical transmission at delivery or post-partum infection in a HBs antigen carrier newborn) or sporadic [11,12]. The HDV infection affects around 5% of HBs antigen carriers, meaning around 15 to 20 million people worldwide with widely varying prevalence by areas. Its prevalence is highest in tropical

and sub-tropical areas and decreases in tempered areas. HDV is endemic in Mediterranean area (Southern Italy), in the Near East, in South America (Amazon Basin), in sub-Saharan or Central Africa and in East Europe. Despite a high HBV prevalence in South-East Asia, HDV infection is rare except in Vietnam and in the pacific islands: we cannot exclude that "Asian" HBV genomic variations of B or C HBV genotypes could limit their auxiliary function for HDV replication. In Africa, individuals are contaminated during childhood and teen age by vertical transmission from mother to child or horizontal transmission with tattoos, acupuncture, ritual scarifications, traditional medicine... In the Mediterranean area, people are mostly contaminated later during the fourth decade. In the United-States and in North Europe, low endemic areas, HDV has been introduced in the 1970's, mostly by the intra-venous drugs users (IVDU), with a rapid expansion -in France, 70% of the IVDU and 15% of the MSM who carried HBs antigen (HBsAg) were infected by HDV at the beginning of 1980's [13]. This prevalence dropped in the middle of the 1980's with prevention against HIV (condoms and single-use syringes). In the last EPIB study, HDV infection was observed in 12% of HIV-co-infected patients compared to 4% in HBV-mono-infected patients, mostly in people from high or moderate endemic areas.

Diagnosis

The diagnosis of HDV infection is made on the association of acute or chronic hepatitis (meaning transaminases level up to the normal range) and serologic markers. Viral replication is asserted by the presence of:

Delta antigen in liver and in sera but it is detected only in the first fourth weeks in the absence of immune-depression;

Anti-HDV Immunoglobulins (Ig) M antibodies in the sera, which can persist along with the chronic infection;

The viral HDV RNA in the sera (and in the liver), detected by RT-PCR [14] and of paramount importance for the diagnosis (replication of the virus) and follow-up (evaluation of treatment efficacy) [15-17].

There is no place for the HDV genotyping [18-19].

After eradication of the virus, the RNA become undetectable and only anti-HDV IgG antibodies remain detectable. The serologic markers for HBV infection depend on the timing of HDV infection and HBV infection. The HBV-DNA quantification allows HBV treatment initiation according the European recommendations. The HBsAg quantification could have an impact on prognosis and caring of co-infection B-delta [20].

Natural course of infection

The hepatitis B virus is required for development of hepatitis D virus infection. So, two different settings can be observed for epidemiology, prognosis and therapy: a co-infection by HBV and HDV or a surper-infection by HDV in a HBsAg carrier (Figure 1).

B/delta Co-infection

In the HBV/HDV co-infection, the auxiliary hepatitis B virus and HDV are both present in the inoculum. The hepatocytes are first infected by HBV; its replication leading to HDV expression in 1 to 2 weeks later. The virulence of HDV infection depends on HBV virulence. If HBV infection is weak and slow, it cannot provide enough help for HDV infection and the later becomes abortive. But, when

HBV infection is high and quick, HDV infection can occur. A clinical acute hepatitis appears after an incubation time from 2 to 6 weeks. The evolution can be bi-phasic with 2 flares of hepatic cytolysis, reflecting the first infection of hepatocytes by HBV and then the second infection by HDV. Fulminant acute hepatitis is more frequent in co-infection than in HBV alone (2 to 10% versus 1% respectively) [21] while rates of spontaneous death and relapse of HBV infection after liver transplantation are lower in acute fulminant HBV/HDV hepatitis than in HBV. Two to 20% of patients HBV/HDV co-infection will develop chronic hepatitis. Thus, if co-infection does not lead to death or liver transplantation, it is mostly resolved.

The serologic pattern is the following: HBV serum serological markers appear with HBsAg and anti-HBc antibodies; the delta antigen appears quickly and early in serum but often under the limit of detection. Detection of delta antigen in liver is more common. Anti-HDV IgM antibodies appear from 2 to 5 weeks in serum, then are substituted by anti-HDV IgG antibodies. Concomitantly, HBsAg disappears and is replaced by anti-HBs antibodies evidencing the resolution of HBV infection. Thus, the association of anti-HDV, anti-HBs and anti-HBc IgG antibodies is the serologic pattern of spontaneous resolution of HBV/HDV co-infection.

Super-infection delta

In case of super-infection of a HBV carrier by hepatitis D virus, the HBV is already in hepatocytes. The patient may be an inactive HBV carrier (without or with mild B viral replication or liver injury) or have a chronic hepatitis with HBV replication. The delta super-infection often leads to a marked decrease or discontinuation of HBV replication, along with anti-HBe antibodies appearance, HBeAg clearance and HBV DNA undetectability, and even HBs Ag disappearance. Conversely, markers of delta replication (anti-delta IgM more than delta Ag, HDV RNA) become detectable [22]. The acute phase appears 2 to 6 weeks after contamination, mostly with a wide liver hepatocytes necrosis; and without biphasic form. In case of an unknown chronic HBV carrier, this episode could be interpreted as an acute hepatitis B or co-infection B/delta. When chronic HBsAg carriage is known, it could be interpreted as a HBV reactivation or a HBeAg seroconversion. This highlights the need to test delta serology in any HBV carrier. In delta super-infection, fulminant presentations are more common than in acute B hepatitis with an estimated risk around 15% and a high percentage of chronic delta hepatitis, around 80%. Chronic delta hepatitis has been suggested to induce more histopathological liver injury than HBV with a prompt evolution to cirrhosis and to hepatocellular carcinoma (HCC) [23,24].

Chronic delta hepatitis is more due to super-infection than co-infection B/delta. Like other hepatotropic virus (HBV and HCV), the risk of chronic delta infection is cirrhosis: several studies reported a high risk of cirrhosis, from 70 to 80% after 15 years of evolution, as compared to 15 to 30% in chronic hepatitis B and 10 to 25% in chronic hepatitis C. In an Italian cohort, 41% of patients with chronic HDV infection had cirrhosis and 13% were dead after a follow-up of 6 years [25]: the severity of chronic hepatitis D, the frequency of cirrhosis and their rapid evolution could explain this finding [25,26]. Another HDV specific pathogenesis mechanisms including the big delta protein for activation of transcriptional factors (STAT3, NF-KB) and the oxidative stress pathway through NADPH oxidase have been suggested to be part of the rapid evolution of the liver disease [27]. Nevertheless, we cannot exclude that confounding factors, such as duration of infection, might explain these differences; as it has been suggested by some

histological findings the absence of difference of severity between HBV or HVB/HDV carriers [28-31] and occurrence of hepatocellular carcinoma is the same (3 to 5%/year) than in B or C viral cirrhosis.

In the French Deltavir study [32], 28% of patients had a biopsy-proven cirrhosis at enrollment, 15% had at least one episode of cirrhosis decompensation and 2.7% had a HCC. After a median follow-up of more than 4 years, 20% of patients have developed cirrhosis, 10% had a decompensation of cirrhosis and 6.5% a HCC. In multivariate analysis, age, GGT level and detectable HDV RNA were predictor of cirrhosis decompensation and ASAT level was predictor of cirrhosis development.

The association of serum HBsAg, anti-HBc IgG antibodies positivity with anti-HBc IgM negativity makes the diagnosis of HDV super-infection. HBV DNA is usually undetectable or with very low levels. The delta antigen is present in the liver, with nuclear localization, sometimes cytoplasmic, except in fulminant presentations where it is mostly undetectable. HDV RNA is detectable in liver and serum. The constant positivity of anti-delta IgM antibodies (monomeric [7S]) with a titer of anti-delta IgG antibodies above 1/1000 signs chronic delta infection.

Special clinical features

Fulminant hepatitis: Fulminant presentations of acute delta hepatitis are more frequent than in acute B hepatitis but with a more favorable prognosis. Fulminant forms occur mostly in the setting of co-infection in Europe and United States and super-infection in tropical areas. Delta epidemics with fulminant forms have been reported in South America (especially in Amazonia), with a mortality rate from 10 to 20% in children; notably with histological features of microvesicular steatosis, similar to those found in case of drug-induced hepatitis or Reye syndrome. We cannot exclude that a special genotype could be part of this pathogenesis [5,10].

Special serology: Some HDV carriers have a negative HBs antigen. The episode is often interpreted as acute non-A non-B hepatitis. The HBV replication can be highly inhibited by HDV so that HBs antigen and HBV DNA become undetectable: in 10% of chronic HBsAg carriers with HDV super-infection, HBs antigen disappears after the acute phase of HDV infection. When the HBsAg effectively disappears, the cure of HDV infection can be seen. In some cases, despite the occurrence of anti-HBs antibodies neutralizing HBsAg, the HBsAg can arise again after the cure of HDV.

Liver transplantation and hepatitis D virus: Relapse of HDV infection after liver transplantation for B/delta cirrhosis is frequent (70% of cases) despite immuno-prophylaxis and anti-HBs immunoglobulin perfusions. Prognosis seems less severe than delta infection in native liver. Markers of HBV infection are very frequent. Conversely, risks of relapse of HDV or HBV infection after liver transplantation performed in emergency for B/delta co-infection are lower than the risks of graft infection by HBV after fulminant B hepatitis [33,34].

Hepatitis D virus and human immunodeficiency virus: The HBV replication is usually low or absent in chronic delta hepatitis. Nevertheless, some cases with both HBV and HDV replication exist, especially in HIV-co-infected patients, and seem to have a more severe prognosis. Histological features are close to those found in chronic HBV with more inflammation and more lobular injury and with eosinophilia degranulation in hepatocytes [35]. Majority of patients infected with HBV, HDV and HIV have died at the end of 1980's from

severe liver diseases [13]. Nowadays, the prevalence of HDV markers is higher among HIV-positive than HIV-negative patients (12 vs. 4%) but HIV status does not impact the severity of the liver disease, probably reflecting the positive effect of the immune restoration and the HBV suppression associated with the dual efficacy of antiviral drugs against HIV and HBV [36].

In conclusion, patients with HDV infection are exposed to fulminant hepatitis (co-infection, super-infection) or potentially severe chronic infection. This provides evidence for developing preventive policy including universal vaccination against HBV and with "comportemental" prevention in chronic HBV carriers, even with vaccination against HDV (which is under development).

Treatment of delta infection

Acute B-delta hepatitis

The typical acute presentation does not need any specific treatment; only general recommendations for acute hepatitis: complete rest and special food are not necessary, but corticosteroid should be avoided (risk to enhance evolution to chronicity), as well as alcohol consumption and oestrogenic therapy (both during 3 to 6 months).

The specific treatment of fulminant hepatitis, related to HDV or other causes, is the liver transplantation that have to be done when encephalopathy or liver insufficiency (level of factor V below 30%) appear. The 5-year survival rate is around 70% after emergency liver transplantation for fulminant HDV hepatitis. The main risk is the relapse of the disease after transplantation, which can be avoid by anti-HBs immunoglobulin perfusions; for all life long and to get a level of anti-HBs antibodies above 100 mU/ml. This risk is quite rare, as compared to the high risk of relapse (70%) after B/delta transplantation for liver cirrhosis. The prognosis of HDV infection relapse on the liver graft seems less severe than on the native liver. The negativity of chronic HBV infection markers is usual. Thus, B/delta co-infections usual lead to complete viral eradication, and death or emergency liver transplantation is rare. We remind that the best treatment of HDV infection is the preventive vaccination against HBV infection.

Chronic B-delta hepatitis

The aim of the treatment is viral eradication. Indications for therapy were biopsy-proven chronic HDV infection with HDV replication markers [37] but noninvasive markers are probably accurate enough like for HBV and HCV to limit the indication of liver biopsy. For HDV, replication is defined with transaminases level above the normal range, anti-delta IgM and HDV RNA detection in serum (sometimes delta antigen in liver) [37,38].

Most of drugs that have been tested as HDV therapies are inefficient (prednisolone, azathioprine, levamisole, arabinoside adenine or recent nucleoside analogues such as ribavirin, famciclovir or lamivudine) [39].

Interferon: The only therapy that has been reported to be efficient against chronic HDV infection is alpha-interferon, currently pegylated alpha-interferon, given for 12 to 18 months. The efficacy should be assessed by negativity of anti-HDV IgM and negativity of HDV RNA tested by quantitative Polymerase Chain Reaction (PCR).

Pilot studies reported a decrease of liver inflammation and viremia under alpha-interferon; given at the doses of 2.5 to 7.5 million international unit (mIU)/m² three times a week during 2 to 16 weeks [40-43]. The benefit was short, as quite all patients relapsed after withdrawal of treatment. The use of long duration therapy (5 mIU/m² three times a week for 4 months, then 3mIU/m² three times a week for 8 weeks) provided, in a controlled study, the normalization or the decrease (more than 50%) of transaminases in 42% and 26% of patients treated for 4 and 12 months, respectively, but in only 3% of patients 1 year after end of treatment against 7%, 7% and 0% in the control group. Despite the lack of antiviral benefit, this therapy had an effect on liver inflammation [41]. These results led to enhance the therapy with the use of 9 mIU/m² 3 times a week during 12 months, with normalization of transaminases in 71% of patients and negativity of HDV replication in 50% of patients 6 months after end of treatment along with a histological benefit [41]. The issue was the relapse after withdrawal of treatment: only half of patients with biological response maintained a normal biology during follow-up. The recommended dose was then 9 mIU/m² 3 times a week during 48 weeks [41], with lot of secondary adverse effects that can limit treatment feasibility.

Adverse effects under alpha-interferon treatment are frequent. The main side effects are flu-like syndrome, digestive disorders and neuro-cognitive disorders, which are present in one third of patients. They can lead to stop treatment. The other side effect is asthenia, sometimes crippling, which can be due to treatment or to the underlying liver disease. Biological effects are leucopenia with neutropenia or thrombopenia, especially in cirrhotic patients. These side effects are reversible and majority of patients will complete the treatment. Some severe side effects (cardiovascular, psychiatric, ophthalmological, thyroid disorders or exacerbation of autoimmune disease) have to be screened during treatment to stop therapy early [44]. All these adverse effects justify the pre-treatment screen for autoimmune disease or hematological disorder.

The anti-HDV treatment is relatively disappointing even if:

A potential histo-pathological benefit in patients with severe liver disease is expected;

A potential eradication of both HDV and HBV with anti-HBs seroconversion can be expected after a several-year treatment.

Encouraging results have also been reported in patients with co-infection with HIV [45].

Despite these results, we can say that only high doses of alpha-interferon were able to provide improvement of liver fibrosis. A clinical case of a patient who underwent an alpha-interferon therapy with 5 mIU/m² during 12 years showed a regression of cirrhosis with a seroconversion of HBsAg. In the controlled study of P. Farzi where 33 of 42 patients were followed during 12 years, the liver biopsy, performed 122 months after treatment, showed inflammation and fibrosis improvement, including regression of cirrhosis in two patients, with disappearance of anti-HDV IgM only in patients treated with high doses; one death or one liver transplantation were observed respectively in 15, 45 and 67% of patients treated with high doses (9 mIU/m²), low doses (3 mIU/m²) and in controls, respectively [41,46].

In addition, pegylated interferon, known to be better than standard interferon in treatment of HBV infection (at least for tolerance), seems to provide hopeful but insufficient results [5]. The doses are 1.5 µg/kg or 180 µg a week for alpha-2b-interferon and alpha-2a-interferon, respectively. The reported results are in fact similar to those with

standard interferon and recommended durations of treatment are 12 to 18 weeks.

Interferon and analogues: To improve the effect of pegylated interferon (PEG), some studies tested the combination of PEG with nucleos(t)ides analogues (adefovir [ADV], tenofovir [TDF] or entecavir).

The HIDIT-1 [47] study that compared PEG+placebo vs. PEG-ADV vs. ADV in patients with co-infection B-delta showed no difference for the undetectable HDV RNA rate between the groups (around 30 and 25% in each group, the ADV group confirming the absence of efficacy of the analogues alone). In a retrospective and prospective study with follow-up of patients enrolled in HIDIT study, 58 of 90 patients had a median follow-up time of 4 years: 6 patients developed a complication (liver transplantation, hepatocellular carcinoma, liver decompensation, death) and the complication rate was similar in each group of treatment. The incidence of complications in cirrhotic was 4.9% per year. Importantly, 56% of patients who achieved a sustained virological response (undetectable HDV RNA 6 months after the end of treatment) had a late relapse. This study shows that sustained virological response 24 weeks after the end of treatment is not the good virological endpoint in HBV/HDV co-infection.

Another study (HIDIT-2) compared PEG vs. PEG+TDF during 96 weeks. Results are disappointing (34% and 42% of undetectable HDV RNA at 48 weeks, respectively and 47% and 33% at the end of treatment, respectively) with high relapse rate after end of treatment [16,48].

These studies evidence that interferon, despite disappointing results, still remains the only therapy in HDV infection.

Future therapies: The developments of two new antiviral strategies are hopeful: the inhibitors of HDV entry (myrcludex) [49] and the inhibitors of prenylation, a key step in virion assembly [50], which are ongoing in phase II clinical trials.

Myrcludex inhibits the functional membrane receptor of HBV and HDV (sodium taurocholate cotransporting polypeptide) [51]. Promising trials in animal were followed by promising human clinical trials.

The lonafarnib (100 mg twice a day) is an oral inhibitor of farnesyl transferase that inhibits farnesylation or prenylation of delta antigen. It blocks the assembly and the package during the HDV replication and had been reported to decrease HDV replication [52]. A dose-study to evaluate the virological impact, with a boost with ritonavir (100 mg) or with PEG-alpha2a at 1 and 2 months has been reported [53]. Results were compared to the proof-of-concept study results (lonafarnib alone) [50] and to the HIDIT-2 [48] PEG+TDF results. The boost with ritonavir 100 mg or the combination with PEG-alpha-2a 180 µg/week improved the antiviral efficacy with a viral load decrease of 2 log and reduced digestive side effects [53].

Finally, promising results have been reported with the use of REP 2139-Ca, a polymer nucleic acid that provides HBsAg clearance in duck hepatitis model and in human: around ten B/delta co-infected patients received 500 mg IV of REP 2139-Ca once a week during 15 weeks then a combination of 500 mg IV of REP 2139-Ca + PEG-alpha-2a 180 µg once a week during 48 weeks: all patients had, from the 5th week of treatment a decrease of HBsAg titer, a reduction of HDV RNA and appearance of anti-HBs antibodies [54]. We are currently waiting for the results after the end of treatment.

Other therapies: In the setting of decompensated cirrhosis, liver transplantation can be proposed. The HDV infection relapse on the liver graft is frequent (70%) but with a better prognosis than the infection on the native liver or than the relapse with HBV alone. An immune-prophylaxis, as already cited, is justified to decrease the risk of HBV relapse after transplantation. The 5-year survival rate is around 90% [18]. When hepatocellular carcinoma is established, the best therapeutic strategy may be the liver transplantation that cures the tumor and the cirrhosis, with a low risk of tumor recurrence at 2 years if the tumor size is small (<3 cm). The surgical resection for small tumor provides a similar 2-year survival rate but tumor recurrence is frequent (around 60%), suggesting a lower efficacy on long survival than liver transplantation. The best treatment of delta hepatitis virus infection remains prevention with vaccination against HBV; efficiently (antiHBs-positive) vaccinated patients cannot be infected with HDV. The risks of delta co-infection (fulminant presentation) and chronic delta infection (with cirrhosis and hepatocellular carcinoma) encourage the incentive approach for vaccination. Finally, development of a specific vaccine against HDV could avoid super-infection in chronic HBV carriers.

References

1. Rizzetto M, Canese MG, Aricò S, Crivelli O, Trepo C, et al. (1977) Immunofluorescence detection of new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. *Gut* 18: 997-1003.
2. Wang KS, Choo QL, Weiner AJ, Ou JH, Najarian RC, et al. (1986) Structure, sequence and expression of the hepatitis delta (delta) viral genome. *Nature* 323: 508-514.
3. Casey JL, Brown TL, Colan EJ, Wignall FS, Gerin JL (1993) A genotype of hepatitis D virus that occurs in northern South America. *Proc Natl Acad Sci U S A* 90: 9016-9020.
4. Chao YC, Chang MF, Gust I, Lai MM (1990) Sequence conservation and divergence of hepatitis delta virus RNA. *Virology* 178: 384-392.
5. Casey JL, Niro GA, Engle RE, Vega A, Gomez H, et al. (1996) Hepatitis B virus (HBV)/hepatitis D virus (HDV) coinfection in outbreaks of acute hepatitis in the Peruvian Amazon basin: the roles of HDV genotype III and HBV genotype F. *J Infect Dis* 174: 920-926.
6. Dény P, Zignego AL, Rascalou N, Ponzetto A, Tiollais P, et al. (1991) Nucleotide sequence analysis of three different hepatitis delta viruses isolated from a woodchuck and humans. *J Gen Virol* 72: 735-739.
7. Dény P (2006) Hepatitis delta virus genetic variability: from genotypes I, II, III to eight major clades? *Curr Top Microbiol Immunol* 307: 151-171.
8. Le Gal F, Gault E, Ripault MP, Serpaggi J, Trinchet JC, et al. (2006) Eighth major clade for hepatitis delta virus. *Emerg Infect Dis* 12: 1447-1450.
9. Radjef N, Gordien E, Ivaniushina V, Gault E, Anais P, et al. (2004) Molecular phylogenetic analyses indicate a wide and ancient radiation of African hepatitis delta virus, suggesting a Deltavirus genus of at least seven major clades. *J Virol* 78: 2537-2544.
10. Wu JC, Choo KB, Chen CM, Chen TZ, Huo TI, et al. (1995) Genotyping of hepatitis D virus by restriction-fragment length polymorphism and relation to outcome of hepatitis D. *Lancet* 346: 939-941.
11. Rizzetto M, Purcell RH, Gerin JL (1980) Epidemiology of HBV-associated delta agent: geographical distribution of anti-delta and prevalence in polytransfused HBsAg carriers. *Lancet* 1: 1215-1218.
12. Raimondo G, Smedile A, Gallo L, Balbo A, Ponzetto A, et al. (1982) Multicentre study of prevalence of HBV-associated delta infection and liver disease in drug-addicts. *Lancet* 1: 249-251.
13. Housset C, Pol S, Carnot F, Dubois F, Nalpas B, et al. (1992) Interactions between infections caused by human immunodeficiency virus 1, hepatitis delta virus in 260 chronic carriers of hepatitis B virus. *Hepatology* 15: 578-583.
14. Zignego AL, Deny P, Feray C, Ponzetto A, Gentilini P, et al. (1990) Amplification of hepatitis delta virus RNA sequences by polymerase chain reaction: a tool for viral detection and cloning. *Mol Cell Probes* 4: 43-51.
15. Castelnau C, Le Gal F, Ripault MP, Gordien E, Martinot-Peignoux M, et al. (2006) Efficacy of peginterferon alpha-2b in chronic hepatitis delta: relevance of quantitative RT-PCR for follow-up. *Hepatology* 44: 728-735.
16. Wranke A, Heidrich B, Ernst S, Calle Serrano B, Caruntu FA, et al. (2014) Anti-HDV IgM as a marker of disease activity in hepatitis delta. *PLoS One* 9: e101002.
17. Le Gal F, Gordien E, Affolabi D, Hanslik T, Alloui C, et al. (2005) Quantification of hepatitis delta virus RNA in serum by consensus real-time PCR indicates different patterns of virological response to interferon therapy in chronically infected patients. *J Clin Microbiol* 43: 2363-2369.
18. Brichler S, Le Gal F, Butt A, Chevret S, Gordien E (2013) Commercial real-time reverse transcriptase PCR assays can underestimate or fail to quantify hepatitis delta virus viremia. *Clin Gastroenterol Hepatol* 11: 734-740.
19. Brichler S, Le Gal F, Neri-Pinto F, Mansour W, Roulot D, et al. (2014) Serological and molecular diagnosis of hepatitis delta virus infection: results of a French national quality control study. *J Clin Microbiol* 52: 1694-1697.
20. Brichler S, Setshedi M, Renou C (2013) Resolution of chronic hepatitis delta infection after five years of peginterferon-adeфовir: lessons from a case report. *Clin Res Hepatol Gastroenterol* 37: e81-84.
21. Govindarajan S, De Cock KM, Redeker AG (1986) Natural course of delta superinfection in chronic hepatitis B virus infected patients-histopathological studies with multiple liver biopsies. *Hepatology* 6: 640-644.
22. Krogsgaard K, Kryger P, Aldershvile J, Andersson P, Sørensen TI, et al. (1987) Delta-infection and suppression of hepatitis B virus replication in chronic HBsAg carriers. *Hepatology* 7: 42-45.
23. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, et al. (2000) Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). *Gut* 46: 420-426.
24. Manesis EK, Vourli G, Dalekos G, Vasiliadis T, Manolaki N, et al. (2013) Prevalence and clinical course of hepatitis delta infection in Greece: a 13-year prospective study. *J Hepatol* 59: 949-956.
25. Colombo M, Rumi MG, Donato MF (1983) Delta infection in the course of chronic hepatitis B. In: Verme G, Bonino F, Rizzetto M. *Viral hepatitis and delta infection* New York: Alan R Liss 203-208.
26. Fattovich G, Boscaro S, Noventa F, Pornaro E, Stenico D, et al. (1987) Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. *J Infect Dis* 155: 931-935.
27. Williams V, Brichler S, Khan E, Chami M, Dény P, et al. (2012) Large hepatitis delta antigen activates STAT-3 and NF- κ B via oxidative stress. *J Viral Hepat* 19: 744-753.
28. Romeo R, Sangiovanni A, Rumi M, Colombo M (2005) Low probability of major liver related events in chronic hepatitis delta. *Hepatology* 42: 723A.
29. Nouredin M, Gish R (2014) Hepatitis delta: epidemiology, diagnosis and management 36 years after discovery. *Curr Gastroenterol Rep* 16: 365.
30. Wedemeyer H, Manns MP (2010) Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead. *Nat Rev Gastroenterol Hepatol* 7: 31-40.
31. Hughes SA, Wedemeyer H, Harrison PM (2011) Hepatitis delta virus. *Lancet* 378: 73-85.
32. Roulot D, Brichler S, Layese R, Samuel D, Marcellin P, et al. (2015) the Deltavir Study Group. Predictive factors of cirrhosis, hepatic decompensation and hepatocellular carcinoma in patients with chronic hepatitis delta. *J Hepatol* 62: S541-542.
33. Ottobrelli A, Marzano A, Smedile A, Recchia S, Salizzoni M, et al. (1991) Patterns of hepatitis delta virus reinfection and disease in liver transplantation. *Gastroenterology* 101: 1649-1655.

34. Samuel D, Muller R, Alexander G, Fassati L, Ducot B, et al. (1993) Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med* 329: 1842-1847.
35. Buti M, Jardi R, Allende H, Cotrina M, Rodriguez F, et al. (1996) Chronic delta hepatitis: is the prognosis worse when associated with hepatitis C virus and human immunodeficiency virus infections? *J Med Virol* 49: 66-69.
36. Piroth L, Pol S, Lacombe K, Mialhes P, Rami A, et al. (2010) Management and treatment of chronic hepatitis B virus infection in HIV positive and negative patients: the EPIB 2008 study. *J Hepatol* 53: 1006-1012.
37. Aragona M, Macagno S, Caredda F, Crivelli O, Lavarini C, et al. (1987) Serological response to the hepatitis delta virus in hepatitis D. *Lancet* 1: 478-480.
38. European Association For The Study Of The Liver (2012) EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 57: 167-185.
39. Rizzetto M, Verme G, Recchia S, Bonino F, Farci P, Arico S, et al. Chronic HBs Ag hepatitis with intrahepatic expression of delta antigen: an active and progressive disease unresponsive to immunosuppressive treatment. *Ann Intern Med*. 1983; 98: 437-441.
40. Rosina F, Pintus C, Meschievitz C, Rizzetto M (1991) A randomized controlled trial of a 12-month course of recombinant human interferon-alpha in chronic delta (type D) hepatitis: a multicenter Italian study. *Hepatology* 13: 1052-1056.
41. Farci P, Mandas A, Coiana A, Lai ME, Desmet V, et al. (1994) Treatment of chronic hepatitis D with interferon alfa-2a. *N Engl J Med* 330: 88-94.
42. Niro GA, Rosina F, Rizzetto M (2005) Treatment of hepatitis D. *J Viral Hepat* 12: 2-9.
43. Farci P (2003) Delta hepatitis: an update. *J Hepatol* 39 Suppl 1: S212-219.
44. Fontaine H, Pol S (2001) Side effects of interferon-alpha in treating hepatitis C virus infection. *Transplant Proc* 33: 2327-2329.
45. Buti M, Esteban R, Jardi R, Rodriguez-Frias F, Allende H, et al. (1992) Treatment of chronic type D hepatitis and concomitant human immunodeficiency infection with alpha-interferon. *J Hepatol* 14: 412-413.
46. Farci P, Roskams T, Chessa L, Peddis G, Mazzoleni AP, et al. (2004) Long-term benefit of interferon alpha therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. *Gastroenterology* 126: 1740-1749.
47. Wedemeyer H, Yurdayd C, Dalekos GN, Erhardt A, Akaloğlu Y, et al. (2011) Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 364: 322-331.
48. Wobse M, Hardtke S, Ernst S, Benjamin H, Bremer B, et al. (2015) On-treatment HBsAg kinetics to predict long-term HDV RNA response to PEG-IFNa treatment of hepatitis delta. *J Hepatol* 62 :S-572.
49. Yan H, Zhong G, Xu G, He W, Jing Z, et al. (2012) Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* Nov 13; 3.
50. Bordier BB1, Ohkanda J, Liu P, Lee SY, Salazar FH, et al. (2003) In vivo antiviral efficacy of prenylation inhibitors against hepatitis delta virus. *J Clin Invest* 112: 407-414.
51. Urban S, Bartenschlager R, Kubitz R, Zoulim F (2014) Strategies to inhibit entry of HBV and HDV into hepatocytes. *Gastroenterology* 147: 48-64.
52. Koh C, Canini L, Dahari H, Zhao X, Uprichard SL, Haynes-Williams V, et al. (2015) Oral prenylation inhibition with lonafarnib in chronic hepatitis D infection: a proof-of-concept randomised, double-blind, placebo-controlled phase 2A trial. *Lancet Infect Dis* 15: 1167-1174.
53. Yurdayin C, Idilman R, Choong I, Kalkan C, Keskin O, et al. (2015) Optimizing the prenylation inhibitor Lonafarnib using ritonavir boosting in patients with chronic delta hepatitis. *EASL 2015 Abst O118*) *J Hepatol* 62: S-252.
54. Bazinet M, Pantea V, Cebotarescu V, Cojuhari L, Jimbei P, et al. (2015) Significant reduction of HBsAg and HDV RNA by the nucleic acid polymer REP 2139 in caucasian patients with chronic HBV/HDV co-infection. *J Hepatol* 62: S-257.