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REVIEW

Hepatitis D (DELTA)

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SUMMARY

The hepatitis D virus (HDV) is unique in animal virology. It is the smallest of human pathogens, requires the HBsAg capsid of the hepatitis B virus (HBV) to assembly into infectious virions, and parasitizes the transcriptional machinery of the host. Hepatitis D is ubiquitous but prevalence varies throughout the world. It is the most severe form of chronic viral liver disorder. Vaccination against the HBV has decreased the circulation of HDV in industrialized countries but Hepatitis D remains a significant medical issue in many areas of the developing world.

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INTRODUCTION

The hepatitis D virus (HDV) was recognized in the late 1970s following the description of a new antigen, named delta, in the liver of patients with chronic hepatitis B virus (HBV) liver disease (Rizzetto *et al.,* 1977). The new antigen raised the interest of the National Institute of Health and in 1978 research moved from the clinical context in Italy to experimental studies in chimpanzees in the US. With the american resources, within two years the delta antigen was recognized to be the expression of a new infectious agent which was defective and behaved as an obligatory satellite of the HBV virus, requiring the HBsAg capsid for virion morphogenesis (Rizzetto *et al.,* 1980). The HDV was initially classified as the only genus of the deltaviridae family but more recently is has been reclassified in the family Kolmioviridae within the Ribozyviria realm of satellite nucleic acids, the family name coming from Finnish kolmio "triangle" in reference to the Greek letter "Δ" (delta) (Bender *et al.,* 2022)

The parenteral route is the way of transmission and intravenous drug addicts are the individuals at highest risk (Smedile *et al.,* 1994). Infection is present worldwide; however the global number of HDV patients remain uncertain. Three recent meta-analyses attempted to estimate the total number of HDV patients; two put the number between 48-60 million and 62-72 million in 2018 and 2019, respectively (Chen *et al.*, 2019; Miao *et al.,* 2020), while the third scaled the prevalence to 12 million people in 2020 (Stockdale *et al.,* 2020). The different figures emphasize the heterogeneity of reports on HDV due to disparate recruitment methodology and lack of sufficient quality data.

In the last 20 years vaccination against the HBV has decreased the circulation of HDV in high-income countries; nevertheless hepatitis D is returning to Western Europe through immigration from HDV endemic areas (Rizzetto *et al.,* 2021).

Key words:
Hepatitis D Virus, Chronic hepatitis D, HBsAg-positive infection, Liver disease.

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VIROLOGY

The virus

The HDV has a circular single-stranded minus RNA genome of only about 1700 nucleotides and is the smallest pathogenic virus in human virology (Taylor 2020) *Figure 1*.

The virion is an amorphous particle between 35-41 nm in diameter with no defined structure *Figure 2* composed of the HDV RNA genome and the HD antigen (HDAg), both enveloped by the HBsAg.

Eight genotypes with different geographic distributions have been identified by comparative phylogenetic analysis (Le Gal *et al.,* 2017), with 81-89% homology in nucleotide sequences within the same genotype and as much as 35% divergence between different genotypes.

Three major RNA species are found in humans (Rizzetto *et al.,* 2017). There are a 1.7 Kb genomic RNA contained in the virions, a complementary antigenomic RNA of positive polarity of the same size present in the liver, and a shorter 5'-capped and 3'-polyadenylated mRNA of 0.8 Kb of antigenomic

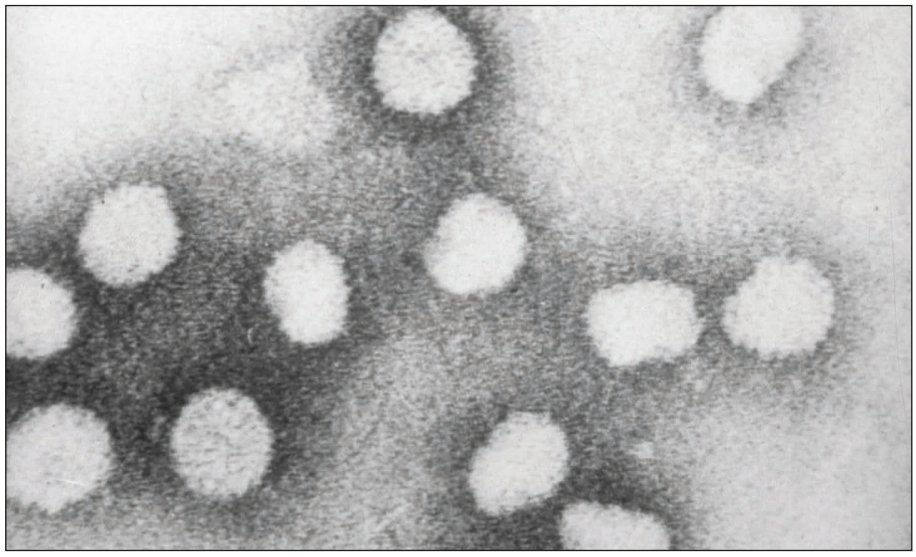

Figure 1 - *Schematic representation of the Hepatitis D virus.*

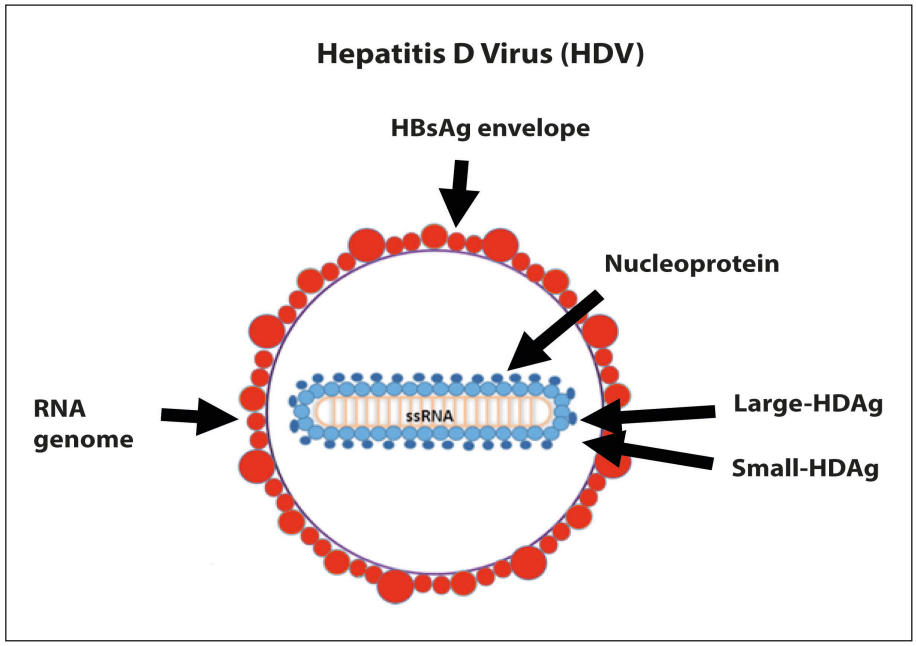

Figure 2 - *HDV virions.*

polarity found in the liver; this contains the open reading frame for translation of a small of protein, the HDAg. Both the genomic and antigenomic RNAs contain a single small domain of about 100 nucleotides that acts as a ribozyme, cleaving the viral RNA at specific sites without participation of protein enzymes (Been 2006) (*Table 1*).

Table 1 - *Unique features of the Hepatitis D Virus.*

Smallest infectious agent in man: 1700 nt
Circular, single stranded-neg.polarity RNA
Infectious at 10^{-11} serum dilutions in HBsAg +
Rolling circle mechanism of replication
Self-cleaving ribozyme
Transcription by cellular RNA polymerases

Life cycle

After entry of HDV into hepatocytes through the sodium taurocholate co-transporting polypeptide (NTCP) (Ny *et al.*, 2014), genomic HDV RNA is moved to the nucleus where it replicates through a rolling circle mechanism (Taylor 2020); this involves the transcription over the circular antigenomic RNA of multimeric linear strands of genomic sense which are cleaved by the ribozyme into monomeric linear strands; these are then ligated into the circular infectious RNA. Because of the small size and lack of cod-

ing capacity, the HDV has no replicative machinery of its own; the genome is copied by host DNA-dependent RNA polymerases redirected to reproduce the viral RNA as if it were a cellular DNA (Chang *et al.,* 2008). A common feature of HDV infection is the inhibition of HBV DNA synthesis while levels of HBsAg necessary to HDV assembly are not modified. During replication, two species of HDAg are generated, a small 24-kDa species (small HD Ag) essential for genome replication and a larger 27-kDa HDAg (large HDAg) species arising through post-trancriptional RNA editing, which contains a C-terminal region not present on the small antigen (Casey 2006). The L-HDAg promotes virion packaging while inhibiting replication (Taylor 2020). The linkage of the HDV ribonucleoprotein with the HBsAg in the cytoplasm in order to assemble the virion, requires a post-translational site-specific farnesylation of the C-terminus of the large HDAg (Glenn *et al.,* 1992).

HDV without HBV

In experimental models HDV replication can proceed in host cells in the absence of HBV proteins, as shown by transfection into cultured cells with cDNA constructs of HDV and with DNA copies of the HDV-RNA and the RNA itself (Dandri *et al.,* 2014; Giersch *et al.,* 2014). Mammalian cells can also autonomously support efficient HDV replication. In NTCP-transduced Hep G2 cells and dividing primary human hepatocytes, HDV replication was maintained after serial in vitro-passages despite blocking of the extracellular spreading of HBV with Myrcludex (Giersh *et al.,* 2019), suggesting that the virus can survive liver regeneration and be amplified through human cell divisions both in vitro and *in vivo*.

HDV may be even more independent than currently appreciated. Human hepatocellular carcinoma cells line Hep3B and PLC/PR5/5 (Alexander cells), containing HBV integrants but no marker of HBV replication, were transfected with a HDV construct. Both lines supported HDV replication suggesting that it might be rescued to productive infection by HBV integrants in the absence of the full HBsAg envelope protein (Freitas *et al.,* 2014).

In humans, HDV replication independent from HBV was reported only in liver transplants; the HDAg remained detectable weeks to months without HBV markers in the liver of transplanted patients who were protected with immunoglobulins against the HBsAg (Ottobrelli *et al.,* 1991; Mederacke *et al.,* 2012).

Origin of HDV

It was assumed that HDV evolved from viroids, infectious agents of plants that have also circular genomes and replicate by a rolling circle mechanism using the catalytic activity of ribozymes (Flores *et al.,* 2012). However HDV-like viruses without HBV or hepadnavirus infection were recently discovered in distantly related species as fishes, birds, amphibians, and invertebrates (Perez Vargas *et al.,* 2021). This finding and the evidence that human HDV can use for transmission envelope glycoproteins from viruses of diverse genera such as the Hepatitis C Virus and the Dengue Virus, suggest that HDV-like viruses have been associated with animals long before their first appearance in humans and throughout the entire evolutionary history of the metazoan (Perez Vargas *et al.,* 2021).

THE CLINICAL PARADIGM

All clinical studies in endemic areas have shown that HDV is highly pathogenic, most often causing progressive liver disease (Smedile *et al.,* 1991; Wranke *et al.,* 2018). Progression is more rapid than in other types of viral hepatitides. Liver cirrhosis develops in approximately 70% of cases within 5 to 10 years of infection, with a threefold higher risk in HDV/HBsAg co-infected than in HBV mono-infected patients (Fattovich *et al.,* 2000); patients are on average ten years younger than those with HBV.

The role of HDV in development of hepatocellular carcinoma (HCC) remains uncertain. However, in recent studies HDV was independently associated with a higher risk of HCC than HBV monoinfection (Alfaiate *et al.,* 2020; Farci *et al.,* 2020).

The efficiency of HDV transmission and the outcome of infection are determined by the HBsAg state of the exposed individual (Rizzetto 2019). In acute HBV/HDV coinfection of HBV-naïve individuals, HDV is acquired simultaneously with HBV. Most usually coinfection results in an acute self-limited hepatitis with clearance of the HBV and of the HDV. Coinfection has become very rare in Italy; the incidence has diminished from 3.2 cases per 1 million in 1987 to 0.04 cases in 2019 (Stroffolini *et al.,* 2022).

In superinfection of HBsAg carriers exposed to HDV, the preexisting HBsAg state "rescues" the HDV, prompting the rapid establishment of its infection. (Smedile *et al.*, 1991). The HBsAg state acts as a powerful magnet for HDV; as little virus as that contained in 10^{-11} dilutions of the serum of a HBV/HDV-coinfected chimpanzee was sufficient to transmit the infection to a chimpanzee carrying the HBsAg (Ponzetto *et al.,* 1987). The superinfection process mantains the endemicity of HDV, supporting the continuous trasmission of the virus from infected to non-infected HBsAg carriers; as in this setting the HBV infection persists over time, HDV most often becomes chronic, inducing chronic hepatitis D (CHD) in healthy carriers of HBsAg or additional HDV disease in carriers with previous HBV disease. The prototype of the patient with chronic hepatitis D is a HBsAg positive subject with anti-HD and HDV-RNA in blood, with no or only low amounts of HBV-DNA in serum and a liver disease characterized by marked inflammatory activity *Table 2*.

Table 2 - *Features of chronic hepatitis D.*

Rapid progression to cirrhosis
Anti-HBe+; IgM anti-HBc –
HBV-DNA low or absent
No specific histologic features
Occasionally splenomegaly +++

The serological hallmark of exposure to the HDV is the antibody to the HD-antigen (anti-HD) (Olivero and Smedile 2012). Anti-HD should be tested according to the reflex testing algorithm (Terrault and Ghany 2021), i.e. automatically determined in every individual positive for the HBsAg, independently from his clinical status; healthcare providers may not be experienced with hepatitis D, and reflex testing prevents a diagnosis of hepatitis D being overlooked.

Antibody positive carriers should be tested for the HDV RNA in serum, in order to diagnose an active infection *Table 3.* Several Real Time PCR techniques are now available to measure the viral genome with a sensitivity of 10 IU HDV RNA/ml (Olivero and Smedile 2012, Ricco *et al.,* 2018, Olivero *et al.,* in press). Results may vary, whether HDV RNA is extracted by manual or authomatic techniques; the analytical sensitivity of manual extraction is 1-2 long higher than automatic extraction, which is nevertheless preferred by routine laboratories because it is faster and more practical (Stelzl *et al.,* 2021).

The HDV RNA, IgG anti-HD, and IgM anti-HD develop in primary HDV infection but their pattern is different in coinfection and superinfection (Smedile *et al.,* 1991, Rizzetto *et al.,* 2017). In acute coinfections, antibody markers are expressed only transiently and HDV RNA may no longer be detectable at onset of disease. They decrease rapidly and disappear after the clearance of the HBsAg. All HDV markers persist in superinfection, with a rapid increase to high titers of both IgG anti-HD and IgM anti-HD. The inflammatory activity of the liver disease correlates with the titer of HDV RNA (Caviglia *et al.,* 2021, Palom *et al.,* 2021) and of IgM anti-HD (Poggio *et al.,* 2011).

Table 3 - *Diagnostics of HDV infection.*

HDV Serum markers	
Total IgG antibodies against HDAg (anti-HD)	General marker of exposure to HDV Screening test Marker of HDV infection/disease
IgM antibodies against HDAg (IgM anti-HD)	Marker of HDV-related liver disease
HDV-RNA	Marker of HDV replication and active infection

EPIDEMIOLOGICAL CHANGES

The epidemiology of HDV has consistently changed in the last decade following the advent of vaccination against the HBV (Rizzetto *et al.,* 2021, Brancaccio *et al.,* 2019). As the critical factor determining the epidemiology of hepatitis D is the prevalence of HBsAg carriers in the population; reducing their numbers vaccination deprives the defective HDV of the HBV network necessary to propagate its infection. The decline of HDV has been dramatic in high income countries which started HBV vaccination programs in the 1990s and have by now reached optimal control of hepatitis B; likewise, formerly intermediate endemicity countries with vaccination programs of more recent inception have been now downgraded to low endemicity areas. The prevalence of HDV remains high in many countries of Africa and in Central Asia that have exceedingly high rates of HBV (Rizzetto 2019).

In Italy was endemic in the 1980s but few cases of new hepatitis D were reported in local-born Italians in the last decade; in 2017 only 3,3% of native-Italians younger than 30 years were positive for anti-HD (Stroffolini *et al.,* 2017). Along with the control of HBV, the clinical features of hepatitis D have distinctly changed in the country. In native Italians chronic HDV disease still outlives only in a cohort of ageing patients who acquired HDV infection long ago: in 2021 their mean age was 58, all had advanced liver fibrosis, the proportion of those with overt cirrhosis reached 71.1% and of those with HDV viremia diminished to 66.1%. (Caviglia *et al.,* 2021). This residual tail of HDV infections is bound to spontaneously extinguish in a generation time, yet it still maintains the demand for liver transplantation and will remain a medical issue for years to come. Though in the industrialized world HDV infection is vanishing, HDV infection is returning in Italy and high income-countries through new infections introduced by immigrants from areas where HDV remains endemic (Sagnelli *et al.,* 2021). This new input is resulting world-wide in a pattern of decreasing domestic and increasing migrant HDV infections; in Italy the proportion of migrants distinctly increased in recent years and in 2019 out of an overall 9.9% percentage of anti-HD in HBsAg carriers with liver disease, the rate was 6.45% among Italian natives but 26.4% in immigrants (Stroffolini *et al.,* 2020).

Limited information is available on the demographic and clinical features of HDV migrants. In series in France (Roulot *et al.,* 2020) and in Sweden (Kamal *et al.,* 2020), their age was younger and around seventy % of the patients had an active HDV disease. Cirrhosis was significantly less frequent in African than in European immigrants regardless of HDV genotype. Persistent replication of HDV was associated with decompensation, HCC occurrence and death.

THERAPY

Therapy of CHD has relied on interferon alpha (IFNα) which was empirically introduced in clinical practice more than 30 years. Similar to functional HBV cure, the ideal endpoint of CHD treatment would be the loss of the HBsAg; however, this is rarely achieved with therapy and a more pragmatic endpoint is the clearance of HDV RNA.

The overall efficacy of IFNa is poor and the addition of antivirals against the partner HBV, such as Adefovir (ADV), Entecavir (ETV) and Tenofovir (TDF), is of no avail (Rizzetto 2018).

Rates of sustained viral response (SVR) with pegylated IFNα (pegIFNα) were generally ~25-30% (Wedemeyer *et al.,* 2011) and late relapses are common. In a 10-year follow-up of the HIDIT-II trial, 8 of 14 (57%) CHD patients who had achieved SVR, experienced a virologic relapse up to nine years after completing therapy (Bremer *et al.,* 2020). The high rate of HDV relapse is not surprising, considering the limited sensitivity of current diagnostic assays for serum HDV RNA with lower detection limit around 15 copies/mL, corresponding to approximately 930 IU/mL; thus even a SVR may be an inadequate endpoint to identify patients who remain in permanent remission.

As the HDV relies for its replication on the synthetic machinery of the infected hepatocyte and has no enzymatic activities to be targeted by conventional antivirals, new therapeutic strategies are directed to deprive the virus of functions necessary to complete its life cycle that are provided by the hepatitis B virus (HBV) and by the host. Current options are:

1) The block by the synthetic peptide Bulevertide of the HBsAg entry into cells through the inhibition of the NTCP receptor (Tu and Urban 2018): the drug was afforded by the European Medicines Agency a conditional marketing authorization on July 31, 2020 under the trade name Hepcludex (European Medicines Agency. Hepcludex. https://www.ema.europa.eu/en/medicines/human/EPAR/hepcludex. Accessed April 15, 2021). The recommended dose was 2 mg. The optimal treatment duration was stated as unknown and the recommendation was to continue treatment as long as it is associated with clinical benefit though it did not specify how clinical benefit should be measured.
2) The inhibition with Lonafarnib of the farnesylation of the large HD antigen, required for virion assembly (Koh *et al.,* 2015).
3) The presumed reduction by the nucleic acid polymer REP 2139 of the release of the HBsAg and subviral HBV particles necessary for HD virion morphogenesis (Vaillant 2016).

Preliminary data, published so far only in abstract form (Wedemeyer *et al.*, 2019, Wedemeyer *et al.*, 2020, Yurdaydin *et al.,* 2018) indicate that Bulevertide and Lonafarnib in monotherapy reduce serum HDV-RNA and improve liver biochemistry but do not reduce the HBsAg and HD viremia rebounds after therapy; however, they appear to provide consistent additional efficacy to Peg IFNα therapy (Elazar 2020). In a pilot study, REP 2139 in combination with Peg-IFNα induced the clearance of serum HDV RNA and of the HBsAg in about half of 12 treated patients but confirmation of these excellent data is still awaited. (Bazinet *et al.,* 2017).

Prolonged treatments raise the problem of safety, in particular in association with the poorly tolerated Peg-IFNα. Peg-IFN lambda might provide an alternative, as this cytokine is credited with inducing fewer side effects than Peg-IFNα; trials are in progress to determine its efficacy and tolerance (Etzion *et al.,* 2019).

Long-term randomized studies will hopefully determine whether the new therapies can further increase eradication of HDV within a reasonable time of treatment compatible with the tolerance and safety of the patient, and whether they may be adjusted to maintain latent, clinically inactive HDV infections with continued therapy (Lok *et al.,* 2021).

References

Alfaiate D., Clément S., Gomes D., Goossens N., Negro F. (2020). *Chronic hepatitis D and hepatocellular carcinoma: A systematic review and meta-analysis of observational studies. J Hepatol.* **73**, 533-539.

Bazinet M., Pantea V., Cebotarescu V., Cojuhari L., Jimbei P., et al. (2017). Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and REP 301-LTF): a non-randomised, open- label, phase 2 trial. *Lancet Gastroenterol Hepatol.* **2**, 877-889.

Been M.D. (2006). HDV ribozymes. *Curr Top Microbiol Immunol.* **307**, 47-65.

Bender D., Mirco Glitscher M., Hildt E. (2021). Viral hepatitis A to E: prevalence, pathogen characteristics, and pathogenesis 2022. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. **65** (2), 139-148.

Brancaccio G., Nardi A., Madonia S., Fasano M., Verucchi G., et al. 2019 -The present profile of chronic hepatitis B virus infection highlights future challenges: An analysis of the Multicenter Italian MASTER-B cohort. *Dig Liver Dis.* **51**, 438-442.

Bremer B, Anastasiou OE, Hardtke S, Caruntu FA, Curescu MG, et al. (2020). Residual low HDV viraemia is associated with HDV RNA relapse after PEG-IFNa-based antiviral treatment of hepatitis delta: results from the HIDIT-II study Liver Int. **41**, 295-299.

Casey J.L. (2006). RNA editing in hepatitis delta virus. *Curr Top Microbiol Immunol.* **307**, 67-89.

Caviglia G.P., Martini S., Ciancio A., Niro G.A., Olivero A., et al. (2021). The hepatitis D virus in Italy. A vanishing infection, not yet a vanished disease. *J Adv Res.* **33**, 183-187.

Chang J., Nie X., Chang H.E., Han Z., Taylor J. (2008). Transcription of hepatitis delta virus RNA by RNA polymerase II. *J Virol.* **82**, 1118-1127.

Chen H.Y., Shen D.T., Ji D.Z., Han P.C., Zhang W.M., et al. (2019). Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. *Gut*. **68**, 512-521.

Dandri M., Lütgehetmann M. (2014). Mouse models of hepatitis B and delta virus infection. *J Immunol Methods*. **410**, 39-49.

Elazar M., Glenn J.S. (2020) Combination of Novel Therapies for HDV. *Viruses*. **14**, 268. doi: 10.3390/v14020268.

Etzion O., Hamid S.S., Lurie Y., Gane E., Bader N., et al. (2019). End of study results from LIMT HDV study: 36% durable virologic response at 24 weeks post-treatment with pegylated inter-

feron lambda monotherapy in patients with chronic hepatitis delta virus infection. *J Hepatol.* **70** (Suppl.), e32.
Farci P., Niro G.A., Zamboni F., Diaz G. (2020). Hepatitis D virus and hepatocellular carcinoma. *Viruses*. **13** (5), 830. doi: 10.3390/v13050830.
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. Gut.* **46**, 420-426.
Flores R., Ruiz-Ruiz S., Serra P. (2012). Viroids and hepatitis delta virus. *Semin Liver Dis.* **32**, 201-210.
Freitas N., Cunha C., Menne S., Gudima S.O. (2014). Envelope proteins derived from naturally integrated hepatitis B virus DNA support assembly and release of infectious hepatitis delta virus particles. *J Virol.* **88**, 57425754.
Giersch K., Helbig M., Volz T., Allweiss L., Mancke L.V., et al. (2014). Persistent hepatitis D virus mono-infection in humanized mice is efficiently converted by hepatitis B virus to a productive co-infection. *J Hepatol.* **60**, 538-544.
Giersch K., Bhadra O.D., Volz T., Allweiss L., Riecken K., et al. (2019). Hepatitis delta virus persists during liver regeneration and is amplified through cell division both in vitro and in vivo. *Gut*. **68**, 150-157.
Glenn J.S., Watson J.A., Havel C.M., White J.M. (1992). Identification of a prenylation site in delta virus large antigen. *Science*. **256**, 1331-1333.
Kamal H., Westman G., Falconer K., Duberg A.S., Weiland O., et al. (2020). Long-term study of hepatitis D infection at secondary care centers: the impact of viremia on liver-related outcomes. *Hepatology.* Mar 7.
Koh C., Canini L., Dahari H., Zhao X., Uprichard S.L., 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.
Le Gal F., Brichler S., Drugan T. Alloui C., Roulot D., et al. (2017). Genetic diversity and worldwide distribution of the deltavirus genus: a study of 2,152 clinical strains. *Hepatology.* **66**, 1826-1841.
Lok A.S., Negro F., Asselah T., Farci P., Rizzetto M. (2021). Endpoints and New Options for Treatment of Chronic Hepatitis D. *Hepatology.* **74**, 3479-3485.
Mederacke I., Filmann N., Yurdaydin C., Bremer B., Puls F., et al. (2012). Rapid early HDV RNA decline in the peripheral blood but prolonged intrahepatic hepatitis delta antigen persistence after liver transplantation. *J Hepatol.* **56**, 115-221.
Miao Z., Zhang S., Ou X., Li S., Ma Z., et al. (2020). Estimating the global prevalence, disease progression and clinical outcome of hepatitis delta virus infection. *J Infect Dis.* **221**, 1677-1687.
Ni Y., Lempp F.A., Mehrle S., Nkongolo S., Kaufman C., et al. (2014). Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology.* **146**, 1070e83.
Olivero A., Smedile A. (2012). Hepatitis Delta Virus Diagnosis. *Seminars Liver Dis.* **32**, 220-227.
Olivero A., Rosso C., Ciancio A., Abate M.L., Nicolosi A., et al. Clinical application of Droplet Digital PCR for Hepatitis Delta Virus quantification. *Biomedicines*. 1613307, in press.
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.
Palom A, Sopena S, Riveiro-Barciela M, Carvalho-Gomes A, Madejón A, et al, 2021 -One-quarter of chronic hepatitis D patients reach HDV-RNA decline or undetectability during the natural course of the disease. *Aliment Pharmacol Ther.* **54**, 462-469.
Pérez-Vargas J., Pereira de Oliveira R., Jacquet S., Pontier D., Cosset F., et al. (2021). HDV-Like Viruses. *Viruses*. **13**, 1207. doi: 10.3390/v13071207.
Poggio P.D., Colombo S., Zaccanelli M., Rosti A. (2011). Immunoglobulin M anti-hepatitis D virus in monitoring chronic hepatitis delta. *Liver Int*. **31** (10), 1598.
Ponzetto A., Hoyer B.H., Popper H., Engle R., Purcell R.H., et al. (1987). Titration of the infectivity of hepatitis D virus in chimpanzees. *J Infect Dis.* **155**, 72-78.
Ricco G., Popa D.C., Cavallone D., Iacob S., Salvati A., et al. (2018). Quantification of serum markers of hepatitis B (HBV) and Delta virus (HDV) infections in patients with chronic HDV infection. *J Viral Hepat.* **25**, 911-919.
Rizzetto M., Canese M.G., Aricò S., Crivelli O., Trepo C., et al. (1977). Immunofluorescence detection of a new antigen-antibody system (delta/anti-delta) associated with hepatitis B virus in liver and in serum of HBsAg carriers. *Gut.* **18**, 997-1003.
Rizzetto M., Hoyer B., Canese M.G., Shih J.W.K., Purcell R.H., et al. (1980). Delta antigen: the association of delta antigen with hepatitis B surface antigen and ribonucleic acid in the serum of delta infected chimpanzees. *Proc Natl Acad Sci* New York. **77**, 6124-6128.
Rizzetto M., et al. (2017). Hepatitis D. In Clinical Virology IV Edition Eds. D. D. Richman, R.J. Whitley, F.G. Hayden. *ASM* Press, Washington DC. 1409-1423.
Rizzetto M. (2018). Targeting Hepatitis D. *Semin Liver Dis.* **38**, 66-72.
Rizzetto M. (2019). Hepatitis D Virus In: Clinical Epidemiology of Chronic Liver Disease. Eds Robert J. Wong, Robert G. Gish Springer. 135-148.
Rizzetto M., Hamid S., Negro F. (2021). The changing context of hepatitis D. *J Hepatol.* **74**, 1200-1211.
Roulot D., Brichler S., Layese R., BenAbdesselam Z., Zoulim F., et al. (2020). Origin, HDV genotype and persistent viremia determine outcome and treatment response in patients with chronic hepatitis Delta. *J Hepatol.* **73**, 1046-1062.
Sagnelli C., Sagnelli E., Russo A. (2021). HBV/HDV Co-Infection: Epidemiological and Clinical Changes, Recent Knowledge and Future Challenges. *Life* (Basel). **11** (2), 169.
Smedile A., Rizzetto M., Gerin J.L. (1994). Advances in hepatitis D virus biology and disease. In: Boyer T, Ockner RK, editors. Progress in liver disease, vol. XII. Philadelphia, PA. *Saunders.* 157-175.
Stelzl E., Ciesek S., Cornberg M., Maasoumy B., Heim A., et al. (2021). Reliable quantification of plasma HDV RNA is of paramount importance for treatment monitoring: A European multicenter study. *J Clin Virol.* **142**, 104932.
Stockdale A.J., Kreuels B., Henrion M.Y.R., Giorgi E., Kyomuhangi I., et al. (2020). The global prevalence of hepatitis D virus infection: systematic review and meta-analysis. *J Hepatol.* **73**, 523-532.
Stroffolini T., Sagnelli E., Sagnelli C., Russello M., De Luca M., et al. (2017). Hepatitis delta infection in Italian patients: towards the end of the story? *Infection*. **45**, 277-281.
Stroffolini T., Ciancio A., Furlan C., Vinci M., Fontana R., et al. (2020). Migratory flow and hepatitis Delta infection in Italy: a new challenge at the beginning of the third millennium. *J Viral Hepat* Apr 27.
Stroffolini T., Morisco F., Ferrigno L., Pontillo G., Iantosca G., et al. (2022). Acute Delta Hepatitis in Italy spanning three decades (1991-2019): Evidence for the effectiveness of the hepatitis B vaccination campaign. *J Viral Hepat*. **29**, 78-86.
Taylor J.M. (2020) Infection by hepatitis Delta virus Viruses. **12** (6), 648. doi: 10.3390/v12060648
Terrault N.A., Ghany M.G. (2021). Enhanced Screening for Hepatitis D in the USA: Overcoming the Delta Blues. *Dig Dis Sci.* **66**, 2483-2485.
Tu T., Urban S. (2018). Virus entry and its inhibition to prevent and treat hepatitis B and hepatitis D virus infections. *Curr Opin Virol.* **30**, 68-79.
Vaillant A. (2016). Nucleic acid polymers: broad spectrum antiviral activity, antiviral mechanisms and optimization for the treatment of hepatitis B and hepatitis D infection. *Antiviral Res.* **133**, 32-40.
Wedemeyer H., Yurdaydìn C., Dalekos G.N., Erhardt A., Çakaloglu Y., et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med.* **364**, 322-331.
Wedemeyer H., Schoneweis K., Bogomolov P.O., Voronka V., Chulanov V., et al. (2019). Interim results of a multicentre, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of Myrcludex B in combination with peg-interferon alpha 2a in patients with chronic HBV/ HDV co-infection. *J Hepatol.* **70** (Suppl.), e81.
Wedemeyer H., Schöneweis K., Pavel O., Bogomolov P.O., Chulanov V., et al. (2020). 48 weeks of high dose (10 mg) bulevirtide as mono- therapy or with peginterferon alfa-2a in patients with chronic HBV/HDV coinfection. *J Hepatol.* **73**, S52.
Wranke A., Pinheiro Borzacov L.M., Parana R., Lobato C.S., et al. (2018). Clinical and virological heterogeneity of hepatitis delta in different regions world-wide: The Hepatitis Delta International Network (HDIN). *Liver Int.* **38**, 842-850.
Yurdaydin C., Idilman R., Keskin O., Kalkan Ç, Karakaya F.M., et al. (2018) A phase 2 dose-optimization study of lonafarnib with ritonavir for the treatment of chronic delta hepatitis-analysis from the LOWR HDV-2 study using the Robogene real-time qPCR HDV RNA assay. *J Viral Hepat*. **25** (Suppl. 2), 10.