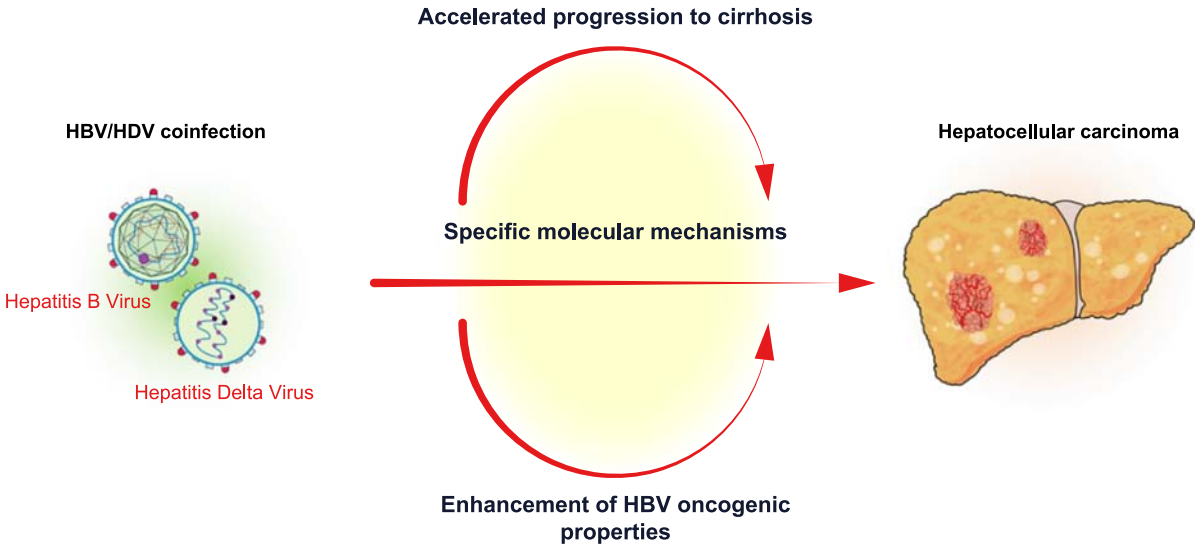
Review JHEP|Reports

# The oncogenic role of hepatitis delta virus in hepatocellular carcinoma

## Authors
Marc Puigvehí, Carlos Moctezuma-Velázquez, Augusto Villanueva, Josep M. Llovet
## Correspondence
augusto.villanueva@mssm.edu (A. Villanueva)josep.llovet@mountsinai.org (J.M. Llovet)

## Graphical abstract

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# The oncogenic role of hepatitis delta virus in hepatocellular carcinoma

Marc Puigvehí,[1,2,†] Carlos Moctezuma-Velázquez,[1,†] Augusto Villanueva,[1,3,6,*] Josep M. Llovet[1,4,5,6,*]

## Summary

**Hepatitis delta virus (HDV) is a small defective virus that needs hepatitis B virus (HBV) to replicate and propagate. HDV infection affects 20-40 million people worldwide and pegylated interferon (PegIFN) is the only recommended therapy. There is limited data on the contribution of HDV infection to HBV-related liver disease or liver cancer. Evidence from retrospective and cohort studies suggests that HBV/HDV coinfection accelerates progression to cirrhosis and is associated with an increased risk of hepatocellular carcinoma (HCC) development compared to HBV monoinfection. Although the life cycle of HDV is relatively well known, there is only ancillary information on the molecular mechanisms that can drive specific HDV-related oncogenesis. No thorough reports on the specific landscape of mutations or molecular classes of HDV-related HCC have been published. This information could be critical to better understand the uniqueness, if any, of HDV-related HCC and help identify novel targetable mutations. Herein, we review the evidence supporting an oncogenic role of HDV, the main reported mechanisms of HDV involvement and their impact on HCC development.**



Keywords: Hepatitis B virus; liver cancer; HCC, co-infection; molecular pathogenesis; defective; superinfection.

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[1]*Mount Sinai Liver Cancer Program, Division of Liver Diseases, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[2]*Hepatology Section, Gastroenterology Department, Hospital del Mar, IMIM, Universitat Autònoma de Barcelona, Barcelona, Catalonia, Spain*
[3]*Division of Hematology and Medical Oncology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, USA*
[4]*Translational Research in Hepatic Oncology, Liver Unit, IDIBAPS, CIBERehd, Hospital Clínic, University of Barcelona, Barcelona, Catalonia, Spain*
[5]*Institució Catalana de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain*
[6]*Denotes co-senior authorship.*
[†]*These authors contributed equally to this work.*

* Corresponding authors. Addresses: Mount Sinai Liver Cancer Program, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, 1425 Madison Avenue, Box 11-23, New York, NY 10029, USA; Tel.: +1 2126599503, fax: +1 212 849 2574, (JM. Llovet); or Division of Liver Diseases, Division of Hematology and Medical Oncology, Department of Medicine, Liver Cancer Program, Tisch Cancer Institute, Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029 USA (A. Villanueva).
*E-mail address:* augusto.villanueva@mssm.edu (A. Villanueva), josep.llovet@mountsinai.org (J.M. Llovet).

## Introduction

More than 40 years after its discovery,[1] key features of hepatitis delta virus (HDV) infection remain unknown. This contrasts with other hepatotropic viruses, such as hepatitis B (HBV) and hepatitis C virus (HCV), which have been thoroughly investigated and for which there are effective treatments. The lower prevalence of HDV compared to HBV or HCV likely justified the limited research efforts dedicated to HDV, even though HDV infection is highly prevalent in certain countries.[2] HDV is a defective virus that co-exists with HBV, and is related to the most severe form of liver failure attributable to chronic viral hepatitis. HDV is understood to accelerate the progression to cirrhosis, and it is considered a main driver of the malignant hepatocyte transformation.[3] HBV/HDV-coinfected patients seem to be at an increased risk of HCC development compared to HBV-monoinfected individuals, although the evidence is still limited. Thus, HDV is not yet included on the list of oncogenic agents, whilst HBV and HCV are well defined carcinogens.[4]

Herein, we will review the evidence available regarding the oncogenic role and mechanisms of HDV-related carcinogenesis, as well as discussing the key unmet needs in HDV research.

## HDV epidemiology

The global prevalence of HDV is remarkably variable (Fig. 1).[5] As routine testing for HDV in HBV surface antigen (HBsAg)-positive individuals is not a standard procedure,[6] HDV prevalence is unknown in many countries, resulting in a global underestimation of HDV disease burden. To date, few studies have evaluated the global burden of HDV, and available data are mostly biased towards local reports from different regions in specific cohorts. The World Health Organization (WHO) estimates that there are at least 20 million people infected with HDV worldwide, which represents 5% of HBV carriers.[7] A recent meta-analysis including 182 articles from 61 countries estimated a pooled HDV prevalence of 10%, even after excluding intravenous drug users and individuals with high-risk sexual behaviour.[8] However, methodological errors regarding the definition of HDV infection, extrapolation of data from the different cohorts, and selection bias have been pointed out in this study. Thus, it is likely that such analysis represents an overestimation of the real prevalence.[9] In certain areas such as Nigeria, Gabon, Benin, Mauritania, Cameroon, Senegal, Iran, Peru, the Western Brazilian Amazon, the mountain regions of Colombia and Venezuela, Romania, Pakistan, and Tajikistan, the reported HDV prevalence exceeds 20%.[2,5,10,11] HDV is endemic in Punjab, Somalia, and Mongolia, with an estimated prevalence of up to 60–80% in HBV-infected patients.[12,13] Remarkably, these estimates of prevalence may be biased,

as they are usually extrapolated from high-risk cohorts. HDV prevalence also varies across risk factors, with prevalences of 37% and 17% in intravenous drug users and people with high-risk sexual behaviours, respectively.[8]

Eight different HDV genotypes have been identified,[14,15] with data suggesting different disease courses depending on genotype.[16] Genotype 1, the most common, has worldwide distribution but is predominant in Europe, the Mediterranean countries, Iran, Turkey, and North America. Genotypes 2 and 4 are found in Asia, genotype 3 has only been described in South America, and genotypes 5-8 are found almost exclusively in Africa.[17] Genotype 1 has been associated with worse outcomes than genotype 2, including progression to cirrhosis and higher rates of HCC.[18,19]

**Key points**

- Hepatitis delta virus (HDV) is a small defective virus that needs hepatitis B virus (HBV) to replicate and propagate
- Initial data suggest that HDV accelerates the progression to cirrhosis and increases the risk of hepatocellular carcinoma (HCC) in patients with HBV
- Proposed mechanisms for HDV enhance HBV-related oncogenesis include activation of pathways related to inflammation and fibrosis
- Unlike HBV, there is limited data to support a direct oncogenic role of HDV in human hepatocarcinogenesis

## HDV pathogenesis

### Life cycle

HDV is a circular single-stranded negative-sense RNA virus which encodes for a single protein, the delta protein or delta antigen (HDAg).[5] The delta antigen was discovered in Italy in 1977 by Rizzetto and collaborators while examining liver biopsies of chronic HBV patients with direct immunofluorescence. They noticed that some HBV-infected patients reacted and revealed a nuclear fluorescent pattern, which was initially thought to be a new HBV antigen.[1] It was not until 1980 when they realised that it was a new RNA virus.[20] The HDV genome spams between 1,672 and 1,697 base pairs depending on the genotype, making it the smallest virus infecting humans. HDV shares more characteristics with plant virusoids than with other human pathogens, such as the circular configuration of its RNA genome, its RNA self-cleavage (ribozyme activity) property and its RNA to RNA rolling circle replication.[21,22] HDV is a defective virus which needs the presence of HBV for infectivity and assembly purposes, as it lacks its own envelope and uses HBsAg instead.[1] The preS1 domain of the large HBsAg (L-HBsAg) is necessary to infect hepatocytes by binding to the sodium taurocholate cotransporting polypeptide (NTCP) receptor, and the small HBsAg (S-HBsAg) is essential for HDV assembly. Notably, HDV does not need active HBV DNA synthesis, which is inhibited in patients under effective anti-HBV treatment with nucleos(t)ide analogues (NUCs). HDV is able to replicate as long as the translation of these structural proteins continues. Furthermore, integrated HBV DNA can also provide the necessary envelope proteins for HDV virions, independently of HBV replication.[23,24] In addition to its dependence on HBV, HDV replication also needs a host (*i.e.* the hepatocyte), as the virus does not code for an RNA polymerase but uses the host's machinery.[2] The delta protein is expressed in 2 isoforms with complementary functions: the small form, called S-HDAg or p24 for its molecular weight of 24 kDa, regulates the nuclear import of HDV ribonucleoproteins and the replication process. The large form, called L-HDAg or p27, inhibits replication and participates in virion assembly.[25] During HDV replication, HDAg proteins form a ribonucleoprotein, which

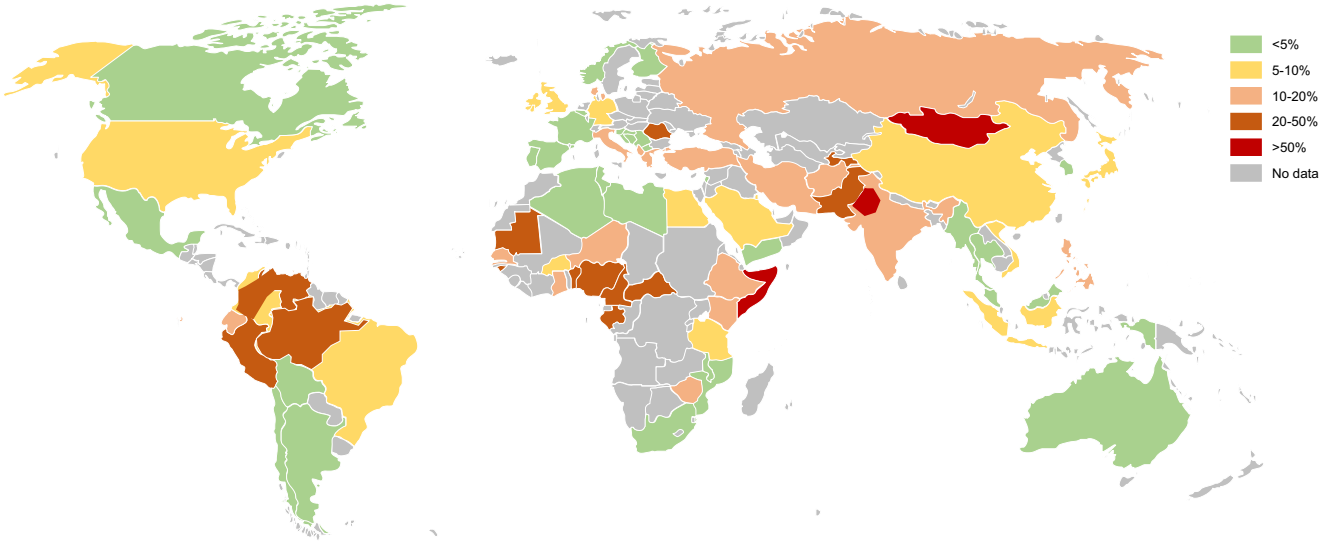


**Fig. 1. Global distribution of HDV infection among HBsAg carriers.** HDV prevalence is highly different among different countries. The most prevalent areas are Punjab, the Amazon basin, Somalia, and Mongolia. In European countries, the highest prevalences are seen in Romania and Albania. HBsAg, hepatitis B virus surface antigen; HDV, hepatitis delta virus.

will later get a coat in the endoplasmic reticulum consisting of the 3 HBV HBsAg proteins.[16] Thus, the outer envelope is the same for HBV and HDV, which has crucial implications in the interaction between the 2 viruses.[26]

## Potential HDV oncogenic mechanisms

There is no data on the molecular alterations present in patients with HDV-related HCC. This is particularly unfortunate for studies on genome-wide mutation profiling, which could identify druggable mutations particularly enriched in these patients. HDV does not integrate into the genome and lacks the machinery required to propagate in the absence of HBV. Thus, a direct oncogenic mechanism of HDV is unlikely. However, the interactions between HDV and HBV could also help promote HCC development.[27] Preliminary data have indicated potential mechanisms by which HDV can modify key signalling pathways related to fibrosis, including epigenetic changes, immune response modifications, specific dysregulation of long noncoding RNAs (lncRNAs), and proteomic changes (Fig. 2). Enhanced transforming growth factor-β (TGF-β) signalling has been proposed as a mechanism behind the accelerated liver disease in HBV/HDV-coinfected patients. TGF-β is involved in multiple cellular processes, including growth, differentiation, wound repair and apoptosis, with a major regulatory role in fibrosis and hepatocarcinogenesis.[28] L-HDAg can activate the TGF-β pathway, probably via the Smad3 protein, which could promote HCC development.[29] Since HBV can also upregulate TGF-β via the HBx protein,[30] this could be a mechanism by which HDV enhances HBV-related oncogenesis. L-HDAg has been shown to activate *c-Jun* and to antagonize the inhibitory effect of c-Jun over TGF-β.[29] This effect could be synergistic with that of the HBx protein, which also activates these 2 signalling cascades.[31] Another proposed mechanism involves nuclear factor kappa B (NF-κB), a transcription factor with crucial roles in inflammation, immunity, cell proliferation and apoptosis, and HCC development.[32] L-HDAg can induce NF-κB activation via tumor necrosis factor-α (TNF-α) stimulation[33] or oxidative stress.[34] L-HDAg has also been related to the activation of other known oncogenic pathways including the JAK-STAT pathway[35] (via activation of the signal transducer and activator of transcription 3 [STAT-3] downstream protein[34]) or *c-Fos* activation.[36] Another reported mechanism for HDV-related oncogenesis is downregulation of glutathione S-transferase P1 (*GSTP1*), a tumour suppressor gene. Transfection with S-HDAg in fetal hepatic cell lines inhibited *GSTP1* expression specifically by binding to its mRNA, which resulted in accumulation of reactive oxygen species and increased apoptosis.[37]

Inactivation of tumour suppressor genes through aberrant DNA methylation is frequent in HCC.[38] Some studies have evaluated the capacity of HDV to interfere with DNA methylation. For example, the chaperon protein clusterin, which is involved in cell death regulation and frequently overexpressed in HCC,[39] was found upregulated through histone acetylation in HDV-infected cell lines.[40] Also, a small study in an HCC cell line overexpressing HDV found a mild increase in the levels of methyltransferase 3b.[41] Other potential

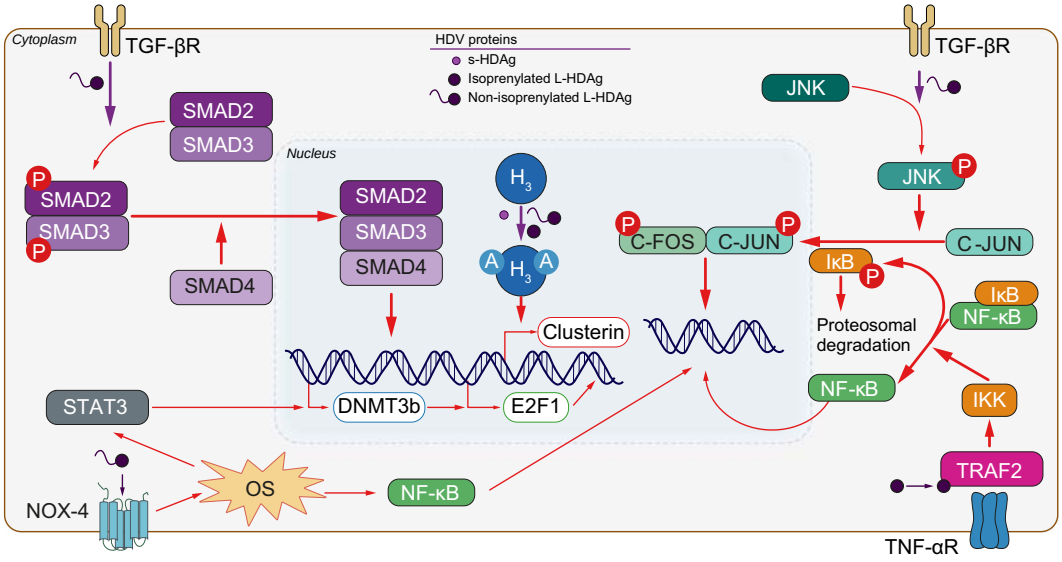


**Fig. 2. Potential mechanism of increased oncogenicity due to HDV.** L-HDAg potentiates TGF-β and c-Jun signalling cascades. It can also activate NOX-4 and promote oxidative stress, which enhances NF-kB and STAT3 signalling. STAT3 promotes transcription of DNMT3b, which induces expression of the E2F1 transcription factor. L-HDAg potentiates NF-αB activation via TNF-α receptor by interacting with TRAF2. Finally, both L-HDAg and S-HDAg increase clusterin expression by promoting acetylation of histone 3. HDV, hepatitis delta virus; L-HDAg, large delta antigen; OS, oxidative stress; S-HDAg, small delta antigen.

epigenetic mechanisms involved long non-coding RNAs (lncRNAs), including the deregulation of Y3 in HDV-related HCC.[42] Notably, dysregulation of lncRNAs is crucial in HDV replication.[43] In terms of immune dysregulation, there is data on humanised mice showing a higher number of interferon stimulated genes (ISGs) and cytokines such as TGF-β and interleukin-28 in HBV/HDV-coinfected human hepatocytes compared to HBV-monoinfected hepatocytes, suggesting enhanced inflammation in HDV.[44] Finally, changes in the cellular proteome have also been linked to HDV infection, including differential expression of 89 proteins, predominantly affecting DNA damage checkpoints and the cell cycle.[45] Most of this information comes from small studies, so a thorough evaluation of the key molecular features of HDV-related HCC is eagerly awaited.

## HDV infection and risk of HCC development

### HDV infection in patients with HBV

HBV is invariably present in all HDV-infected patients. The interaction between these 2 viruses is not completely understood but it seems to be reciprocal, and it may be crucial to the events that lead to disease progression. Intriguingly, HDV may persist for several months after liver transplantation without evidence of HBV replication.[46] Besides, it has recently been shown that HDV replication may persist even in the absence of HBV.[47] Two types of infection can be distinguished. The acquisition of both viruses at the same time (*i.e.* coinfection) is an important cause of severe hepatitis, but only 5% of patients will develop a chronic infection, with the vast majority evolving to spontaneous clearance of HDV.[23] However, when HDV infection is transmitted in the setting of an already established chronic HBV infection (*i.e.* superinfection), more than 90% of patients will evolve to chronic hepatitis, with a more severe course to cirrhosis.[23] Thus, to decipher the role of HDV in liver oncogenesis it is crucial to understand the interaction between both viruses. Longitudinal studies have shown that the long-term interplay between HDV and HBV is complex, with fluctuating levels of HDV RNA and HBV DNA viremia over time and oscillating predominance at different time points.[48,49] Importantly, in more than 50% of cases, HBV activity is detected at different time points, which has been associated with worse prognosis.[48]

Several mechanisms have been proposed behind HDV-related suppression of HBV replication, including a direct effect of HDAg and the induction of an antiviral immune response. Furthermore, it has been shown that both L-HDAg and S-HDAg can repress the HBV enhancers Enh1 and Enh2, and that L-HDAg can activate the transcription of the myxovirus resistance-A (MxA) protein, an interferon (IFN)-κ inducible peptide that inhibits viral replication and contributes to HBV suppression.[50] HBV can also downregulate IFN antiviral responses, and inhibit the transcription of the MxA gene, but in the presence of HDV this mechanism seems inefficient.[51] HDV replication may induce a strong type-I IFN signal, leading to an anti-HBV response.[52] This is further supported by a model that compared HBV-monoinfected and HBV/HDV-coinfected human liver chimeric mice, in which a stronger and more sustained antiviral response, including the activation of ISGs, was observed in coinfected mice.[44] Similarly, in a model of HepaRG cells, superinfection with HDV induced a strong type-I IFN response, with activation of ISGs (mainly RSAD2 and MxA), which was associated with suppression of HBV replication.[53]

HBV can also influence HDV, which would explain why patients with chronic HBV/HDV coinfection may have intermittent periods of HBV replication. The HBsAg, which is different between the HBV genotypes, is essential for HDV infectivity and assembly, and also regulates the nuclear export of HDV ribonucleoproteins. Hence, the number of hepatocytes that can be infected and the number of virions that successfully start replication after entry has been shown to be different between the variants, with genotypes B and D being the most supportive.[54] Likewise, the packaging signal located in the C-terminal region of L-HDAg, which differs across HDV genotypes, may mediate its affinity to the HBsAg, determining the difference in assembly efficiency between genotypes.[55] Hence, HDV genotype 1 can assemble and secrete more HDV virions than genotype 2,[56] which could explain why this genotype is associated with a more aggressive clinical course.[19]

### Impact HBV/HDV in progression to cirrhosis

Chronic hepatitis D promotes inflammatory cell infiltration and progressive fibrosis, leading to cirrhosis, as with other forms of chronic viral hepatitis.[16] Indeed, liver histology from HDV-infected patients is usually no different from that observed in other forms of viral hepatitis.[57] Although HDV has been reported to have direct cytolytic effects,[58] it seems that liver damage in patients with HDV infection is mainly driven by an immune-mediated process. HDV infection is associated with high levels of cytotoxic CD4+ T cells, as seen in HBV and HCV infections.[59] In contrast to HBV, HDV induces a strong innate immune response via IFN β/λ stimulation after being sensed by the pattern recognition receptor melanoma differentiation-associated protein 5 (MDA5), suggesting a different inflammatory profile.[60] Notably, persistently high levels of aspartate aminotransferase and alanine aminotransferase have been described in HBV/HDV-coinfected patients, probably reflecting a high degree of inflammation in the liver and enhanced fibrosis formation.[61,62]

Cirrhosis and HCC are the final stages of chronic liver inflammation, including HDV-related liver disease.[63] Cirrhosis underlies HCC in 70–80%[64] of cases, and thus, to decipher the oncogenic role of HDV it is necessary to evaluate its impact on cirrhosis development. In viral hepatitis, progression from chronic infection to cirrhosis depends on numerous co-factors such as alcohol consumption, diabetes, or the existence of viral coinfection.[65,66] HDV has repeatedly been included among these co-factors that enhance the risk of cirrhosis.[67] The natural history of HDV infection is shown in Fig. 3. The delta antigen was initially discovered in patients with HBV infection who presented with a particularly severe disease course,[68,70-72] including acute fulminant HBV hepatitis.[69] Different studies have evaluated the potential role of HDV as a catalyst of liver fibrosis in patients with HBV coinfection. A summary of these studies is shown in Table 1. Additionally, a few studies have compared the risk of cirrhosis in HBV/HDV-coinfected patients to that in HBV-monoinfected patients. In a small study from Italy, progression to cirrhosis was observed in 77% of HDV-infected patients compared to 30% of HBV-monoinfected patients over a follow-up period of 1–15 years. Strikingly, in 70% of these patients progression to cirrhosis occurred in the first 2 years of follow-up.[73] In another Japanese study with a median follow-up of 10 years, only 12% of 69 HBV/HDV-coinfected patients developed cirrhosis, but this was significantly higher than the 4% observed in HBV-monoinfected individuals.[74] Conversely, in 2 studies performed in Taiwan, including 90 and 64 patients with HDV, there was no increase in the risk of cirrhosis development in HBV/HDV-coinfected patients compared to HBV-monoinfected patients.[75,76] Cirrhosis has been reported in 51–75% of histological samples in 2 large series of HBV/HDV-coinfected patients from Italy.[77,78] Furthermore, a retrospective multicentric European study including 200 HBV-cirrhotic patients showed that HDV-infected patients were younger (34 *vs.* 48 years) than those with HBV monoinfection.[79] Recent data consistently show that patients with HBV/HDV coinfection have higher odds of developing cirrhosis than individuals with HBV monoinfection. For instance, a prospective study in Greece showed a higher rate of liver-related events during follow-up among HBV/HDV-coinfected than HBV-monoinfected individuals (20% *vs.* 8% at 4 years); the vast majority of events being cirrhosis development.[81] Another report from Australia found a higher risk of cirrhosis progression in patients with HDV,[82] while a very recent study in Vietnam showed that HDV-infected patients were more likely to be Child-Pugh B or C than Child-Pugh A, indicating a predisposition towards advanced liver disease in patients with HDV.[62]

Altogether, these studies suggest that HDV is associated with a more aggressive liver disease, leading to accelerated progression and an increased risk of cirrhosis. However, the mechanisms by which HDV hastens the progression to end-stage liver disease have not been elucidated. Accordingly, it is plausible that HDV confers a higher risk of HCC development than HBV monoinfection. Whether this is a result of enhanced inflammation/fibrosis mediated by HDV or a direct oncogenic effect remains unknown.

## Treatment of HDV infection

Treatment for HDV is currently limited to PegIFN alpha 2a-2b for 48 weeks. Antiviral efficacy is modest, with virologic response rates ranging between 17% and 47%. Furthermore, HDV relapse after treatment occurs in more than 50% of responders,[83] highlighting the need for new treatments. For that purpose, surrogate markers that can be used to develop clinical endpoints have recently been proposed.[84] Additionally, new drugs, such as inhibitors of viral entry into the hepatocyte,

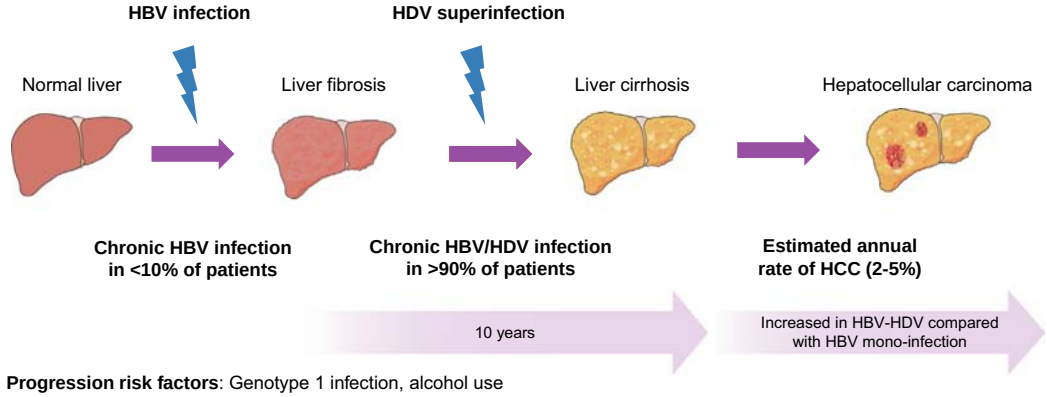


**Fig. 3. Natural history of HDV infection.** Commonly, HDV infects hepatocytes already infected by HBV (*i.e.* superinfection). After that, 90% of patients will develop a chronic HBV/HDV infection with a faster evolution to cirrhosis in 10 years. Some risk factors, such as alcohol consumption or genotype 1 infection may accelerate liver disease development. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus.

**Table 1. Studies assessing the clinical course of patients with HDV infection**

| Study | Design | Location | HBV, n<br>HDV, n | Age, mean ± SD or median (range), yr | Follow-up, mean ± SD or median (range), yr | Baseline cirrhosis, n (%) | Disease progression, n (%) | Limitations |
|---|---|---|---|---|---|---|---|---|
| **Studies comparing HBV-monoinfected with HBV/HDV-coinfected patients** | | | | | | | | |
| Coghill, 2018[82] | Retrospective | Australia | 370<br>179 | 40 (16–86)<br>41 (16–69) | n.a.<br>n.a. | 83 (22)<br>67 (37) | [a]54/287 (19)<br>55/112 (49)* | Retrospective. Only 79 patients were tested for HDV RNA. Information about alcohol consumption not collected. No operational definition of cirrhosis. |
| Manesis, 2013[81] | Ambispective | Greece | 1,997<br>81 | 47.5 (35.1–57.9)<br>43.1 (31.4–53.1) | 3.6 (3.3–3.9)<br>4.2 (2.9–5.3) | 145 (7)<br>16 (20)* | [a]142/1,852 (8)<br>12/65 (18) | Ambispective. Only a subset of patients with HBV were tested for HDV, probably based on risk factors. HDV RNA was not tested. |
| Cross, 2008[80] | Cross–sectional | England | 882<br>82 | 35 (29–46)<br>36 (30–47) | n.a. | 109 (12)<br>22 (27)* | n.a.<br>n.a. | Retrospective and cross-sectional. No HDV RNA was tested. Susceptible to selection bias, being King´s College a tertiary referral center. |
| Liaw, 2004[76] | Retrospective | Taiwan | 64<br>64 | n.a.<br>n.a. | 12.3 ± 6.4 (1–20)<br>8.2 ± 5.2 (1–21) | 0<br>0 | [a]11/64 (17)<br>12/64 (19) | Retrospective. Sample size. |
| Huo, 2000[75] | Retrospective | Taiwan | 426<br>90 | 42 ± 15 | 5.7 ± 3.4 (1–17) | 0<br>0 | [a]56/426 (13)<br>15/90 (17) | Cirrhosis diagnosis was based on imaging features. Patients with acute decompensation that lead to death were excluded. Patients with a follow-up of less than 1 year were excluded. |
| Fattovich, 2000[79] | Retrospective | Western Europe | 161<br>39 | 48 (11–78)<br>34 (13–28) | 6.7 (0.5–16.5) | 161 (100)<br>31 (100) | [b]31/161 (19)<br>12/39 (30) | Competing outcomes were censored. No HDV RNA was determined. HDV status was forced into the multivariate analysis. |
| Tamura, 1993[74] | Prospective | Japan | 1,058<br>69 | n.a.<br>n.a. | 10.1 (3–18) | n.a.<br>n.a. | [a]43/1,058 (4)<br>8/69 (12)* | HDV RNA was not assessed. No multivariate analysis was performed. |
| Fattovich, 1987[73] | Retrospective | Italy | 128<br>18 | 36 ± 13<br>26 ± 8 | 4.7 ± 2.7<br>5 ± 3.6 | 29<br>5 | [a]19/69 (28)<br>10/13 (77)* | No HDV RNA was determined. Some patients received corticosteroids during follow-up. Inclusion criteria included presence of transaminitis for 12 months. Patients with a follow-up of less than 1 year were excluded. |
| Colombo, 1983[77] | Retrospective | Italy | 142<br>50 | 37<br>(8–72) | n.a. (1–5) | 31 (22)<br>21 (42)* | [a]13/56 (23)<br>4/23 (17) | HDV diagnosis was based on liver biopsies. Some patients received steroids during follow-up. No multivariate analysis. |
| **Studies in HBV/HDV coinfected patients** | | | | | | | | |
| Rosina, 1999[78] | Retrospective. | Italy | 159 | 34 ± 11 | 6.5 ± 4.9 | 73 (46) | n.a. | No control group available. Patients with follow-up of less than 6 months were excluded. |
| Govindarajan, 1986[72] | Not clear | USA | 23 | n.a. | 2.5 (0.5–11) | 7 (35) | 6/16 (38) | No other factors for progression were evaluated. Assessment for coinfection with HCV was not possible. |
| Rizzetto, 1983[70] | Retrospective | Italy | 137 | 34 (1–70) | n.a. (2–6) | 32 (23) | 31/75 (41) | Assessment for co–infection with HCV was not possible. |
| Rizzetto, 1979[68] | Retrospective | Italy<br>Japan<br>USA | 63 | 35 (20–66) | n.a. (1–4) | n.a. | 9/63 (14) | Included cases with both acute and chronic presentations. Assessment for coinfection with HCV was not possible.<br>No other cofactors of progression of liver disease were assessed. |

Anti-HDV, antibodies against HDV; APRI, aspartate aminotransferase-to-platelet-ratio index; CAH, chronic active hepatitis; EIA, enzyme immunoassay; HBV, hepatitis B virus; HDAg, delta antigen; HDV, hepatitis delta virus; IF, immunofluorescence; n.a., not available; OR, odds ratio; RIA, radioimmunoassay; RR, relative risk.

*Statistically significant difference between HBV-monoinfected and HBV/HDV-coinfected patients

[a]Progression to cirrhosis;

[b]Decompensation.

inhibitors of HBsAg secretion, and virus assembly inhibitors are currently under investigation.[85]

### Preliminary evidence of HDV as a risk factor for HCC development

The studies that evaluated the association between HDV and HCC are generally small, retrospective and have suboptimal designs. Thus, the extent of HDV involvement in HCC development is controversial. The unfailing presence of HBV in all HDV-infected hepatocytes leads one to question how HDV modifies the carcinogenic risk already imposed by HBV. Patients with HBV can develop HCC in the absence of cirrhosis. This is due to direct oncogenic viral effects such as DNA integration into the host genome, prolonged expression of the HBx regulatory protein or epigenetic changes.[30] Compared to other risk factors, HBV-related tumours have a different genetic profile, with a higher rate of chromosomal alterations and *TP53* mutations.[30] Apart from enhancing fibrosis and inflammation, there is no evidence to suggest a direct oncogenic mechanism of HDV.

Studies comparing HCC incidence between HBV/HDV-coinfected and HBV-monoinfected patients provide the best evidence from which to determine the oncogenic potential of HDV (Table 2). Evidence from these retrospective and cohort studies

**Table 2. Studies evaluating association between HDV and hepatocellular carcinoma.**

| Authors & reference | Study type | Location | HBV, n<br>HDV, n (%) | Follow–up (range) | HCC incidence in HBV<br>HCC incidence in HBV/HDV | Main limitations |
|---|---|---|---|---|---|---|
| **Studies comparing HBV/HDV-coinfected *vs.* HBV-monoinfected** | | | | | | |
| Coghill, 2018[82] | Case–control | Australia | 4,407<br>179 (3%) | None | 5.4%<br>7.8% (RR 1.1; 95% CI 0.9–2.2; $p = 0.17$) | Case-control design. |
| Béguelin, 2017[87] | Prospective | Switzerland | 771<br>119 (15%) | 8.7 (IQR 5–13.8) years | –<br>RR 9.3 (95% CI 3–28.6; $p$ <0.001) | HIV cohort. Only 73 (61%) patients presented HDV RNA+ |
| Kushner, 2015[88] | Retrospective | United States | 2,175<br>73 (3%) | Not stated | –<br>Adjusted OR 2.1 (95% CI 1.1–3.9; $p = 0.025$) | Low HDV prevalence and very low HDV testing in the cohort (8%) |
| Ji, 2012[86] | Retrospective | Sweden | 9,162<br>323/327 (4%) | None | –<br>Acute OR 6.11 (2.77– 11.65) / Chronic OR 3.90 (1.61–7.22) | Cross-sectional. Not clear definition of acute *vs.* chronic HDV infection. |
| Cross, 2008[80] | Cross–sectional | England (London) | 962<br>82 (9%) | None | 7.8%<br>9.7% (OR 1.34; 95% C.I. 0.62–2.91; $p$ = ns) | Cross–sectional (no follow-up, high risk of bias) |
| Fattovich, 2000[79] | Retrospective | Europe | 200<br>39 (20%) | 80 (6–198) months | 2–4%<br>13% (RR 3.2; 95% CI 1–10; $p = 0.0523$) | Retrospective. Shows a trend without statistical significance |
| Tamura, 1993[74] | Prospective | Japan | 1,127<br>69 (6%) | 121 (36–216) months | 3%<br>9% ($p$ <0.01) | Old study performed in a small Japanese region. |
| **Studies evaluating HCC incidence in HBV/HDV-coinfected** | | | | | | |
| Wranke, 2018[91] | Retrospective | Worldwide | –<br>1,576 | None | –<br>1.9% | Study design, no comparison |
| Amougou, 2016[95] | Case–control | Cameroon | –<br>24 | None | –<br>OR 29.3 (95% CI 4.1–1231) compared to controls | Study design, healthy controls |
| Romeo, 2014[89] | Retrospective | Italy | –<br>193 | 9.5 years | –<br>OR 1.88 (95% CI 1.11–3.19; $p = 0.019$) in HDV–RNA+ | Study design, no comparison |
| Buti, 2011[94] | Retrospective | Spain | –<br>158 | 6 years | –<br>3% | Study design, no comparison |
| Niro, 2010[92] | Retrospective | Italy | –<br>126 | 8 years | –<br>1% HCC annual rate | Study design, no comparison |
| Romeo, 2009[90] | Retrospective | Italy | –<br>299 | 28 years | –<br>2.8% HCC annual rate | Study design, no comparison |
| Gheorghe, 2005[93] | Retrospective | Romania | –<br>166 | 10 years | –<br>12% | Study design, no comparison |

HDV, hepatitis delta virus.

suggests that HBV/HDV coinfection accelerates progression to cirrhosis and is associated with an increased risk of HCC development compared to HBV monoinfection.[79,80,82,86–88] This increased risk is difficult to estimate due to the nature of the data. A thorough European study including 200 patients with HBV-related cirrhosis showed that those with HBV/HDV coinfection had an estimated relative risk (RR) of HCC development of 3.2 compared to those with HBV monoinfection over a follow-up of 6.6 years.[79] Similar studies reported an increased incidence of HCC from 3% to 9% in HBV/HDV-coinfected individuals compared to HBV-monoinfected individuals.[74] The largest cohort published to date is a population-based study performed in Sweden, including 9,160 patients with HBV, 327 with chronic HDV infection and 323 with acute HDV-infection.[86] After adjusting for age, the authors found a significantly higher risk of HCC in patients with acute (RR 6.1; 95% CI 2.8–11.7) or chronic (RR 3.9; 95% CI 1.6–7.2) HDV-infection.[76] Another large study including 2,175 American veterans with HBV found a 2.9-fold higher incidence of HCC in individuals with HDV, after adjusting for cirrhosis and HCV infection.[88] Conversely, in another study including 962 consecutive HBV-infected patients, those with HBV/HDV coinfection (n = 82) had a similar prevalence of HCC (9.7% *vs.* 7.8%) as those with HBV monoinfection[80] A recent prospective study in a HIV cohort identified HDV as a strong predictor of HCC, with an RR of 9.3.[87] Although results were described for all patients with HDV, only 73/116 (63%) were RNA positive, which can impact on disease progression.[89] Finally, a case-control study in Australia reported no association between HDV infection and HCC in a cohort of 179 patients with HDV.[82]

There are also studies on HDV-related HCC that do not compare incidence rates in patients with HBV monoinfection. In a retrospective study from Italy, involving 299 HDV-infected patients over a follow-up of 28 years, 46 patients developed HCC, which represents an annual rate of 2.8%.[90] HDV RNA status was not associated with HCC development in this study. Years later, the same authors reported an association between HDV RNA >600,000 copies/ml and progression to cirrhosis and HCC.[89] The biggest descriptive cohort of HDV-infected patients published so far included 1,576 patients from 19 countries and reported a low HCC annual incidence of 1.9%.[91] Smaller studies reported variable rates of HDV-related HCC ranging between 13%[92,93] and 3%[94] after a median follow-up of 5 to 10 years. Even considering the suboptimal quality of the evidence, it is reasonable to assume an association between HDV and HCC development, likely due to the more aggressive underlying liver disease imposed by HDV.

**Box 1. Unmet needs in HDV-related HCC research.**

- To catalogue the worldwide epidemiological distribution of HDV.
- To standardize methods to detect and monitor HDV infection, such as the ones proposed by the WHO.
- To understand the clinical course of HDV/HBV co-infection as opposed to HBV monoinfected patients, particularly in terms of their HCC risk.
- To dissect the contribution of HDV to HCC development in well-designed, prospective studies adjusting for well-known HCC risk factors (*e.g.*, sex, family history, smoking, HBV genotype, viral load, presence of basal core promoter and precore mutations, stage of liver disease, HIV or HCV co-infections, amongst others).
- To perform a molecular characterization of HDV-related HCC, covering mutational landscape and signatures, DNA copy number alterations, gene expression de-regulation, immune profiling and epigenetic aberrations.
- To develop new effective therapies to HDV.

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus; WHO, World Health Organization.

## Conclusions and future directions

Many questions remain unanswered regarding the oncogenic role of HDV. It has been suggested that HDV accelerates the disease course, leading to cirrhosis and likely enhancing HCC development, compared to HBV monoinfection. However, studies evaluating HCC incidence in HBV/HDV-infected patients are discordant and they mostly provide low levels of evidence. Furthermore, the potential mechanisms underlying HDV-specific oncogenesis are poorly understood. Overall, well-designed prospective studies comparing cohorts of HBV/HDV-coinfected and HBV-monoinfected individuals will be key to determine the oncogenic capacity of HDV. A summary of the actual unmet needs in HDV research are shown in Box 1. Remarkably, in this context of unknown pathophysiological mechanisms, molecular studies evaluating HDV-infected HCC samples will be a great resource to understand the singularities of this condition and identify novel targets for therapies in HDV-infected patients.

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## Conflicts of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

All authors made substantial contributions to each stage of the preparation of this manuscript for publication.

## Supplementary data

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