Invited Speaker Abstracts

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THE DELTA VIRUS: FROM ITS DISCOVERY TO TODAY

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The key to the discovery of the Hepatitis D Virus (HDV) was the identification in Italy in the mid-seventies of the delta antigen in the liver of carriers of the HBsAg. The localization of Hepatitis B Virus (HBV) antigens in the liver in immunochemistry with home-made antisera was at the time diagnostic routine. Using in HBsAg carriers a newly prepared fluorescent-conjugated antiserum to the HB-core antigen (HBcAg), it was noted that the antiserum produced the typical intrahepatic HBcAg fluorescence, but also a different nuclear pattern; under the Electron Microscope the latter did not correspond to any structured HBV subunit and was defined as the HBsAg-associated delta antigen.

At the end of the 1970s the stage moved to the National Institute of Health in the USA where chimpanzees experiments revealed that the delta antigen was transmissible, but showed that it was not part of the HBV despite its obligatory association with the HBsAg; transmission of the delta antigen not only occurred in animals already infected with the HBV, but in these animals its expression was increased and more prolonged. The animals experiments raised the hypothesis that the delta antigen was related to an agent different from HBV yet dependent on HBV for thriving. This was ultimately confirmed by the finding in serum of the experimentally infected animals of a new RNA molecule representing the genome of a novel virus, thereafter called the Hepatitis D Virus.

Since then, much virological research has confirmed that HDV is a unique human virus; it is the smaller known animal virus, replicates by a peculiar rolling circle mechanism typical of plant-viroids, has no synthetic function of its own and is replicated by cellular RNA polymerases, contains in its RNA molecule a ribozyme, i.e. a segment of RNA acting as an enzyme and capable of cutting and ligating the viral RNA; the ribozyme is vital to the replication of HDV and is currently attracting interest as a RNA-silencing therapeutic tool in infectious, neoplastic and genetic diseases.
Serological assays to diagnose HDV infection were made available since the early 1980s and have led to the global mapping of hepatitis D in the 1980s.

The infection was found worldwide with prevalence rates varying in different countries. It was highly endemic in the Mediterranean basin, as well as in the Amazon Basin and many developing countries; clinical scrutiny showed that hepatitis D was the most severe and progressive disease among viral hepatitides.

In the last 20 years HDV infection has much declined in the developed world due to the control of HBV; it nevertheless remains a major health problem in poor areas of the world where no control of concomitant HBV has been yet achieved. However, though in Europe HDV has much diminished, it has not gone; infection is reconstituting a consistent reservoir among immigrants from endemic Eastern Europe and Northern Africa areas, and in these patients hepatitis D is recapitulating the clinical features of the florid disease described in the 1980s.

Therapy of hepatitis D remains a major unsolved problem. The nature of HDV makes therapeutic approaches difficult as the minute viral genome does not code for specific enzymatic functions that can be directly targeted by antivirals. Therapy still relies on Interferon, first introduced as an empiric treatment for chronic hepatitis D in the 1980s. Results with conventional IFN have been limited. Long-term Peg-IFN appears to be more efficacious. Currently research is targeted to the process of encapsidation of HDV-RNA into the HBsAg coat; efforts are ongoing in order to manipulate the HBsAg or the HD-Antigen in order to prevent virion assembly and thus prevent intrahepatic HDV spreading.

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DELTA VIRUS AND VIROIDS: SIMILARITIES AND DIFFERENCES

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Discovery of satellite viruses, and particularly of satellite RNAs, viroids and human hepatitis delta (HDV) RNA, overturned the more than 50-years-old paradigm regarding viruses as the lowest step of the biological scale, and revealed the existence of a subviral world populated by a quite diverse spectrum of small RNA replicons. Plant satellite viruses and satellite RNAs depend for their replication and transmission on co-infection of the host cell by a helper virus, but while the first ones encode a structural protein encapsidating their genome in differentiated particles, the second ones are encapsidated by the coat protein of the helper virus. On the other hand, HDV RNA encodes (in the antigenomic strand) a non-structural protein that modulates its autonomous replication in infected cells, but depends for transmission on hepatitis virus B. Finally, viroids are non-protein-coding and autonomously-replicating RNAs that infect plants without the concurrent presence of a helper virus. In this communication I will focus on the replication of viroids and a special class of satellite RNAs (dubbed viroid-like satellite RNAs) as representatives of the plant kingdom, and HDV RNA as the single representative of the animal kingdom. They all replicate through a rolling-circle mechanism involving circular templates in one or both polarity strands, but a comparative analysis reveals that there are multiple variations on this theme that include the nature of the templates, the enzymes and ribozymes involved, and even the subcellular site where replication takes place.

There are still many gaps in our knowledge of the properties of these unconventional replicons: i) Which are the molecular mechanisms responsible for their replication? Are these mechanisms operative in uninfected cells and, in such a case, what are their functions?, ii) What molecular signals do viroids and HDV RNA possess (and cellular RNAs lack) that incite certain DNA-dependent RNA polymerases to accept them as templates for the synthesis of complementary RNA strands?, iii) How these RNAs induce disease? Lacking protein-coding ability, viroids and viroid-like satellite RNAs must exert their pathogenic effects by direct interactions with host components; in this context, infections by representative viroids induce a strong RNA silencing response in their hosts that might be related to pathogenesis, iv) What determines the host range? Are viroids restricted to higher plants and HDV RNA to animal cells, or do they have counterparts in animals and plants, respectively?, and v) How did viroids, viroid-like satellite RNAs and HDV RNA originate? The presence of ribozymes in most of these RNAs strongly supports the view that they represent 'living fossils' of a precellular “RNA world” that assumed an intracellular mode of existence sometime after the evolution of cellular organisms.

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STRUCTURE AND REPLICATION OF HDV

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Three labs reported in 1986 that the genome of HDV is a small, 1,700-nt, single-stranded circular RNA with the ability to fold on itself, with ~74% base-pairing, to form an unbranched rodlike structure. HDV genome replication is unlike that of the helper DNA virus, HBV, and involves only RNA species. In addition to the genome, infected cells contain, in smaller amounts, an exact complementary RNA species, called the antigenome, and much smaller amounts of a 900-nt linear RNA with a 5'-cap and a 3'-poly(A) tail. This 900-nt RNA is the mRNA for the single virus encoded protein, the 195-aa small delta antigen, dAg-S, which is essential for the transcription and accumulation of newly processed HDV RNAs. During HDV replication a larger protein, the 214-aa dAg-L, is also produced. dAg-L is a dominant negative inhibitor of viral RNA accumulation, and has an essential role in the assembly of progeny virus. dAg-L arises as a consequence of a site-specific post-transcriptional RNA editing event which adds 19 aa to the 3'-end of the dAg-S open reading frame.

HDV RNA synthesis is believed to occur via a double rolling-circle mechanism, as proposed for certain viroids. In this model, transcription of circular genomic and antigenomic templates produces RNA transcripts that are greater than unit-length. An 85-nt site-specific ribozyme, found on each RNA strand, cleaves the nascent transcript to a unit-length RNA that is ligated to form a new circular RNA. It is unclear if ligation is carried out by the ribozyme or a cellular RNA ligase.

Maybe all HDV RNA-directed transcription is via redirection of the host RNA polymerase II. A controversial explanation is that the synthesis leading to new antigenomes is mediated by pol I. However, a unifying explanation is that the experimental situation can determine which polymerases are involved.

The HDV genome and antigenome associate with multiple copies of the dAg-S and dA-L. However, only ribonucleoproteins containing genomic RNA interact with HBV envelope proteins and are ultimately released as new virus particles. These particles are only slightly smaller than the infectious HBV. Within the HDV particle there are 70-200 molecules of a mixture of dAg-L and dAg-S. This ribonucleoprotein was initially thought to be a symmetrical nucleocapsid structure but is more likely to be genomic RNA that has associated with multimers of the dAg.

Although much has been learned, many questions regarding the HDV life cycle remain to be answered. For example, to what extent is the dAg-S directly involved in the RNA-directed transcription, and what are the consequences for the host cell, of the accumulation of HDV RNA species. There are also questions relating to how HDV exploits the envelope proteins of its helper virus, HBV. Both viruses depend upon hepatocytes as the susceptible host cell. Some data suggest that both viruses attach and enter hepatocytes by the same mechanism. And yet the mechanism remains unclear. Even the putative host receptor(s) remain to be identified. Our recent studies indicate that even after attachment, the entry for both viruses is a slow process and might occur via macropinocytosis, possibly through virus binding that leads to the activation of a cell-surface purinergic receptor.

Future studies should better clarify the extent of the relationship between the plant viroids and HDV, especially in terms of the ability of RNA species to bring about the redirection of host polymerases whose normal function is to transcribe DNA templates.

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HBV/HDV INTERACTION: GUIDE FOR A SUCCESSFUL MARRIAGE?

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When introduced into human cells, in the absence of HBV, the HDV RNA can replicate efficiently, using an RNA-directed transcription process similar to the one used by plant viroids, and it associates with multiple copies of HDV-encoded proteins to form ribonucleoproteins (RNPs). However, RNPs are unable to exit the cell on their own. Cell exit occurs only in the presence of the HBV envelope proteins whose role is to provide the RNP export machinery. Packaging relies upon the RNP ability to interact with the HBV envelope proteins and upon the unique capacity of the latter to drive assembly and release of large amounts of lipoprotein vesicles. The resulting HDV virion therefore consists of an inner RNP coated with the HBV envelope proteins, whose role is also to provide a means to redirect the HDV genome to human hepatocytes, the target cells of the helper HBV, and thereby ensure propagation.
How the HDV RNP recruits the HBV envelope proteins is an interesting question considering that this process is crucial to the HDV life cycle. The interaction is sustained by the affinity of the large form of the HDV protein (L-HDAg) for the small HBV envelope protein (S-HBsAg). A tryptophan-rich motif in the C-terminus of S-HBsAg is essential to this interaction, and it thus represents a matrix domain for HDV assembly. This motif is strictly conserved among all HBV genotypes, suggesting that it plays an essential function in the HBV life cycle. Yet, lesions in the HDV matrix domain are permissive for both HBV virion assembly and infectivity, thus not essential to the HBV replication cycle. In fact, the tryptophan-rich motif is present in all *Orthohepadnavirus* envelope proteins and conserved because of the overlap between envelope protein (Env) and polymerase (Pol) genes, a characteristic of the HBV genetic organization and a consequence of the small size of the HBV genome.
More precisely, the DNA coding sequence for the matrix domain in Env also encodes the YMDD motif of the Pol catalytic domain (in the minus one frame). Since there is a strict requirement for YMDD in Pol, there is conservation of the matrix domain in Env. Thus, a conserved and essential motif in Pol (YMDD), imposes a conserved Trp-rich motif in S-HBsAg, which is useless to HBV but fortuitously used by HDV as a matrix domain for assembly.
Overall, one can see the interaction of HDV with its helper HBV as the consequence of: i) the small size of the HBV genome that indirectly creates a matrix domain in the envelope proteins, ii) the huge overproduction of envelope proteins that assemble transport vesicles and iii) the natural flexibility of the HBV envelope that can accommodate different types of particles: empty subviral particles of 22-nm in diameter and HBV virions of 42 nm in diameter.

Since HDV is directly dependent on HBV for propagation, it can be transmitted concomitantly with HBV to an individual who has no history of prior HBV infection, or it can be transmitted to an HBV chronic carrier. Such a superinfection often causes severe acute hepatitis and becomes chronic. They also lead to the inhibition of HBV replication during the acute phase of HDV infection. This phenomenon could result from a direct suppression of HBV replication exerted by the coexpressed HDV proteins, RNA or RNPs, or could be the consequence of an indirect interfering mechanism driven by inflammatory cytokines. The suppressive effect could also result from a hijacking of the envelope proteins by the RNPs in doubly infected host cells when HDV RNA replication reaches maximum levels upon superinfection. It is likely that the helper nucleocapsids be heavily outnumbered by the HDV RNPs in their access to the HBV budding machinery. In addition, faced with these huge amounts of RNPs to export, the budding system, though oversized for HBV, may reach saturation.

Clearly, the HBV-HDV interaction is not a harmonious association between two partners, but a relationship in which one partner (HDV) takes full advantage of the other (HBV) without nothing but damages in return for the latter.

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POST-TRANSLATIONAL MODIFICATION OF HEPATITIS D VIRUS NUCLEOCAPSID PROTEIN IN REGULATING VIRAL LIFE CYCLE

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Virus possesses a minimal genome which is positively or negatively regulated by genome-remodeling proteins, just like the complex eukaryotic genomes. Genome-remodeling proteins (histones) and many cellular replication or transcription factors work in concert to control the functional status of eukaryotic genomes. Other than this, several regulating post-translational modifications of these proteins play key roles for their message to the genomes. Regardign the minimal HDV genome, the most important modeling protein has been the viral nucleocapsid protein (NC), namely delta antigen, that participates in the viral genome replication, gene expression and the final virus assembly. HDV deltan protein conducts multiple and essential functions in different stages of viral life cycle, probably due to its post-translational modifications (PTM).

We studied the PTM of HDV small delta antigen and demonstrated the importance of phospphorylation of small delta antigen in viral antigenomic RNA replication. Moreover, the phosphorylation site of small delta antigen altered its interaction with different forms of cellular pol II, the RNA polymerase implicated in antigenomic RNA replication. The phosphorylated form of small delta antigen was found to preferentially interacting with hyperphosphorylated Pol II, suggesting the PTM to be important for viral RNA transcription prolongation step. We recently characterized the cellular proteins or nucleic acids associated with phosphor-delta proteins, and found out weakly associated Pin-1, but quite strong association with ribosomal RNA. The implications for these findings are now under investigation.

The small delta antigen is also acetylated. The acetylation on Lysine 72 has been studied before. It does not affect its replication but its subcellular localization and, even its association with HDV mRNA. Over-expression of one member of HDAC results in a suppression of HDV RNA replication level. These preliminary data suggested the PTM of HDV small delta antigens could involve in many steps of HDV transcription, replication and subcellular protein or RNA trafficking.

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LIFE CYCLE AND POTENTIAL THERAPEUTIC TARGETS

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Hepatitis delta virus (HDV) is an important cause of acute and chronic liver disease for which current therapies are inadequate. The HDV life cycle comprises a fascinating collection of biology, although several important details await further definition. Nevertheless, our understanding of the viral life cycle has revealed a variety of potential targets that could form the basis for novel therapeutic strategies. Here, the HDV life cycle will be reviewed, emphasizing how key aspects offer opportunities for developing new treatment strategies, some of which are on track to enter the clinic.

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ANIMAL AND *IN VITRO* MODELS OF DELTA HEPATITIS

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After the discovery of hepatitis delta virus (HDV) this agent has been successfully transmitted to chimpanzees in 1980 and woodchucks in 1984. Both animal models have given some inside on pathogenesis of HDV infection. Two forms of infection could clearly defined in chimpanzees: simultaneous HBV and HDV infection which is in most animals self-limited, and super-infection of HBV carriers which resulted in persistent HDV viremia in more than 90% of the animals infected. Transmission of HDV in woodchucks created a new virus with HDV nucleoprotein complex and woodchuck hepatitis virus (WHV) envelope. Transmission of HDV to mice indicate that HDV RNA replication is independent of the presence of HBV, however, cannot form infectious particles due to the absence of envelope proteins.

As HDV causes a severe disease several efforts have been undertaken to generate a specific vaccine to prevent HDV super-infection of HBV carriers. As antibodies to the HD-antigen are not neutralizing these vaccines have to induce a strong CD4+ and CD8+ T cell response which may not prevent infection, however, prevent spread of the virus. In the mouse HDV protein specific CD8+ cells have been found after DNA vaccination. The woodchuck model gained importance for this vaccine development. In these trials performed in the woodchuck synthetic peptides, the complete HDV protein p28, vaccinia recombinants expressing p28, and DNA vaccines expressing the p28 were used. All trials using only proteins failed to protect woodchucks from infection. Only vaccination with peptides and DNA vaccines showed some reduction of viremia after challenge, however, infection could also not be prevented.

More recent experiments indicate that DNA vaccination can prevent HDV replication in woodchucks which were infected simultaneously with HBV and HDV.

In vitro replication of HDV has been established in primary chimpanzee, woodchuck hepatocytes, and Huh7 human hepatoma cells transfected with HBV DNA and HDV cDNA. In this culture system it could be shown that antibodies to preS1 and preS2 domain of HBV can prevent the infection of primary hepatocytes of chimpanzees.

In conclusion so far an effective vaccine against HDV super-infection has not yet been established, however, there is need to develop such a vaccine as HDV infections are still endemic in areas which have high prevalence for chronic HBV infection.

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IMMUNOPATHOGENESIS OF DELTA HEPATITIS

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HBV and HCV-specific immune responses have been investigated extensively in numerous studies and have been associated with the control of acute and persistent infections. In contrast, only very limited knowledge exists on the pathogenesis of HDV-induced liver disease. HDV genotype 1 is not cytopathic and thus immune responses are likely to play an important role in the pathogenesis of HDV-induced liver disease. In one earlier study from Italy, patients with inactive but not with active-HDV disease displayed HDV-specific proliferative CD4 responses to HDAg suggesting that cellular immune responses most likely were able to control HDV infection. Our preliminary data suggest that the endogenous IFN-system is highly activated in delta hepatitis. In line with these findings perforin-expressing lymphoid cells including CD4+ T cells are more frequent in hepatitis D patients than in HBV or HCV monoinfection. However, antigen-specific T cell responses were rather weak in persistent HDV/HBV infection. Peripheral HDV-specific T ell responses declined during interferon-based antiviral therapy and the quality of T cell responses seemed to correlate with treatment response. Of note, HDV coinfection may also alter cellular immune responses against HBV as HBV-specific T cell responses were more frequent in HDV-coinfected than in HBV-monoinfected individuals. Overall, the immune network of innate and adaptive immune responses is complex in delta hepatitis and both HBV- and HDV-specific responses need to be considered. More studies are needed to determine immunological correlates of disease outcome possibly identifying novel target for immunointervention against HDV infection.

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TRIPLE AND QUADRIPLE INFECTIONS IN PATIENTS WITH DELTA HEPATITIS: HCV AND/OR HIV

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Hepatitis delta virus (HDV) is mainly transmitted trough sexual and parenteral routes (*Rizzetto, Dig Dis 2010; 28: 139-143*). Thus, coinfection with HIV and/or HCV is not uncommon. Data collected from a series of 106 individuals with chronic hepatitis delta, three quarters of them coinfected with HIV and/or HCV is the source of the current report.

Triply and quadriply delta coinfected patients must be well characterised virologically in order to plan the most appropriate treatment strategy (*Maida et al. AIDS Res Human Retroviruses 2008; 24: 679-83*). Periodic measurement of serum HBV-DNA, HDV-RNA and/or HCV-RNA must be performed. Likewise, periodic non-invasive assessment of liver fibrosis (i.e., using transient elastometry) is warranted in order to prioritize the need for any therapeutic intervention.

HIV-associated immunodeficiency is believe to be associated with a worsening in the natural history of chronic delta hepatitis, with evidence of increased replication markers (HDV-RNA levels and frequency of serum delta antigen recognition) and faster progression to end-stage liver disease. Early introduction of antiretroviral therapy might minimize these deleterious effects, by suppressing HIV replication and enhancing immune responses. Moreover, the use of potent anti-HBV agents with antiretroviral activity, such as tenofovir, has been associated with a steadily significant decline in serum HDV-RNA and amelioration of liver disease in a subset of HIV+ patients with delta hepatitis, an observation which requires further investigation (*Sheldon et al. Antiviral Ther 2008; 13: 97-102*). More recently, a small subset of HIV-HBV-HDV coinfected patients on long-term tenofovir-emtricitabine therapy evolved to serum HBsAg clearance, which in all instances was preceded by a steadily decline in serum HBsAg titers. Interestingly, serum HDV-RNA also became undetectable in these individuals. The intriguing question now is whether a cure for delta using oral nucleos(t)ide analogues was obtained.

Exposure to HCV preceding or following acquisition of delta hepatitis results in viral interference, which in most instances leads to replication of one virus and suppression of the other (*Martin-Carbonero et al. J Viral Hepat 2007; 14: 392-5*). It is HDV who in most instances suppress HCV, which in general results in sustained clearance of HCV. In a subset of patients, however, and particularly in the presence of immunosuppression (ie, in HIV coinfection with low CD4 counts), replication of all viruses (HBV, HCV, HDV) may be recognised at all times points or intermittently (*Schaper et al. J Hepatol 2010; 52: 658-664*).

Although treatment with pegylated interferon plus ribavirin may clear HCV in some of these triply or quadriply infected patients viremic for HCV, no sustained benefit is generally seen for delta hepatitis following treatment discontinuation (*Soriano et al. J Infect Dis 2007; 195: 1181-3).*

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THE EPIDEMIOLOGY OF HDV INFECTION IN TURKEY

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Introduction: HBV infection is the leading cause of chronic liver disease in Turkey. The overall rate of HBV carriers is 4% and the rate of HBsAg positivity is 40-70% in patients with chronic liver disease. The etiology of chronic liver disease shows difference between the regions of the country. In the western part of the country HCV infection is the second most frequent etiological factor meanwhile in the Eastern and Southeastern regions of the country HDV infection is the second and HCV infection is the third. HDV infection as well is frequent in our country parallel to the HBV frequency and Turkey lies in the zone of moderate frequency for HDV infection, as well as HBV infection.
Design: We evaluated and discussed the results of 62 studies on the HDV epidemiology that had been conducted by 20 different centers from different parts of the country .
Results:
1. When all studies were included in the analysis, HDV positivity was found as 20% and 32% among the 5961 cases with chronic hepatitis B (CHB) and 1264 cases with liver cirrhosis (LC) screened between 1980-2005, respectively.
2. Anti HDV positivity showed a significant tendency to decrease in time in both CHB and LC cases screened between 1980-2005. Anti HDV positivity in CHB was 31%, 19.4%, and 11% in time periods between 1980-1990, 1990-2000 and 2000-2005, respectively. Similarly, the rates decreased in cases with liver cirrhosis (43.3% in 1980-1990, 26.1% in 1990-2000 and 24% in 2000-2005)($p < 0.001$, X= 76.7 ve X= 30.85).
3. Significant differences in anti HDV positivity was observed in different parts of Turkey. It was lowest in the Western and highest in the Eastern and Southeastern regions of the country. In the Western regions, Anti HDV positivity was 15.3 % and 25.4% in CHB and liver cirrhotic cases, and in the Eastern and Southeastern regions this rate was 35.7% and 44.7%, respectively.
4. In the meta-analysis of 30 studies with large study groups, following universal HBV vaccination program 1995, HDV positivity rate showed a significant decrease in all regions of the country. In cases with CHB anti HDV positivity decreased from 29.1% to 12.1% in Central Anatolia and from 37.7 % to 27.1 % in Southeastern regions. In cases with LC, the rates decreased from 30.3 % to 20% in Western Anatolia and from 66.4 % to 46.4 % in Southeastern regions.
5. After 1995 HDV positivity rates in CHB cases were 4.8%, 12.1%, 23.5% and 27.1% for Western, Middle, East and Southeastern regions, respectively.
Conclusions: Certain factors may explain the high rate of HBV infection in Turkey: crowded family structure with close contact, unavailability of HBV vaccine to large populations before 1995, insufficiency of general health care service and sanitation. This situation resembles the condition some countries, as Italy and Greece, experienced years ago. In our country in the last 20 years, the increased awareness to HBV infection, the preventive measures, the protection provided by the vaccine, improved socioeconomic status and better life conditions led to a significant decrease in HBV and remotely in HDV infections. Indeed, HDV frequency is decreasing in all regions of the country and the mean age of cases with HDV infection is increasing, which leads to a decrease in new cases with HDV infection.As a conclusion,Between the years 1980-2005, anti HDV positivity was 20% and 32% in cases with CHB and LC throughout Turkey. Those rates are higher than European countries and closer to Middle East and Asian countries.A significant difference in anti HDV positivity is present among different regions of Turkey. Lowest rates are observed in western and highest rates are seen in the Southeastern regions of the country. In the last two decades, there was a significant decrease in Anti HDV positivity in all regions. According to the latest reports, Anti HDV positivity rates in western regions of the country are around 5%, similar to Mediterranean countries. In the Eastern and Southeastern regions of the country, the rates are still as high as 25%, which is similar to the rates which were seen in the western regions 2 decades ago.In recent years, there is a decrease in HBV and HDV infection. This trend will probably pursue in the years to come.

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EPIDEMIOLOGY OF HEPATITIS D VIRAL INFECTION: EUROPE

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Approximately 350 million people worldwide are HBsAg-positive and ~5% of them or 17,500,000 people, are also HDV-infected. Although the HDV infection depends on the HBsAg carriage, the worldwide distribution of both infections does not change in parallel. In Europe, the main burden of HDV infection lays in the former Soviet Union states and the northern Balkan countries.

A problem with HDV prevalence is the interpretation of reported rates. Only a few true population studies exist while in most reports the local HDV prevalence is restricted to patients with hepatitis B or cirrhosis, not including asymptomatic HBsAg carriers or in special populations, as prostitutes, homosexuals, intravenous drug addicts etc, i.e. populations not representative of the entire spectrum of HBV-infected people of the community.

Countries of Northern Europe as Denmark, Sweden and Norway have very low rates of HBV infection and the reported relatively high HDV prevalence concerns special high-risk groups, as intravenous drug users and not the general population. In the Western and Central Europe, the median HBsAg and HDV prevalence is also low (0.6% and 7.3%, respectively) and countries, as Belgium, Austria, the Czech Republic, Ireland and France have HDV prevalence below 5%. The HDV infection is reported relatively more prevalent in Poland (7.9%), Switzerland (8.2%), United Kingdom (8.5%), Germany (10.7%), Hungary (13.6%) and Portugal (17.3%). In some of these countries the higher rate of HDV infection may be related to mass immigration of people from areas of the world with high endemicity

South and Southeast Europe is an area of intermediate HBV prevalence (median 3%) but the HDV infection rate presents a wider variation. In Spain and Italy where the HBsAg prevalence reflects the picture seen in the western and central Europe (0.1% and 1.0%, respectively), the HDV prevalence is also relatively low (7.1% and 9.7%, respectively). In Balkan countries, however, the epidemiological picture is different. The current HBsAg prevalence in Greece (4.1%) and Bulgaria (5%) is intermediate and the HDV low (3.4% and 8.2%, respectively). In Greece, the community of Archangelos in Rhode Island has been well studied as a localised area of high HDV prevalence, while other nearby communities with similarly high HBsAg carrier rate, have low HDV prevalence. In other Balkan countries the reported HDV infection rate is higher (Albania 15%, Serbia 29.6%) and in Romania very high (37.9% in patients with hepatitis; 51.2% in cirrhosis; 56% in those with end-stage liver disease on transplantation lists).

Information of the HBV and HDV prevalence rates in Eastern European countries is scanty, but permits to define the area as one of intermediate-to-high prevalence for both infections (median rates 10.7% and 39%, respectively). In Moldova, a country neighbour to Romania, the HBsAg carrier rate is 10.7%, with 18.3% of the carriers being anti-HDV-positive. In the Moscow area 4.7% of the HBsAg-positive children are anti-HDV positive, while in the Samara region of Russia, 14.4% of the population are HBsAg-positive and 39% of them have HDV infection. In Siberia, the Asian part of the Russian Federation, and especially at its Arctic regions, both viral infections are very prevalent, with the HBsAg carrier rate at 11.8-13.4% of the local population

The epidemiology of CHD in Europe has changed during the last 3 decades. True longitudinal epidemiological studies are lacking and the best information comes from Italian and other cut-sectional studies performed at different time-intervals since the early 1980's. Among patients with HBsAg-positive chronic liver disease in Italy, the prevalence of anti-HDV was 24.7% in 1978-1981 and 28% in 1987, but declined to 14% in 1992 and to 8.3% in 1997. Decreasing prevalence of HDV infection has also been reported from Spain, Turkey, and Greece and it was thought that HDV infection is probably “a vanishing disease” in Europe. However, recent evidence from diverse European sites indicates that the decline of HDV infection has either stopped or it is on the rise. Apparently the HDV infection in Europe is maintained by a residual aging domestic pool of patients who survived the HDV epidemic in 1970-1980's and by a population of young patients with relatively recent HDV infection migrating to Europe from countries of high HDV endemicity. The latter group may be responsible for spreading the disease into metropolitan overcrowded ghettos.

To date 8 major genotypes or clades of the HDV have been identified, labelled HDV-1 to -8. The HDV-1 is the prevailing genotype in Europe. However the migration to Western Europe of people from other areas carrying different HDV genotype infection may change this epidemiological profile.

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HEPATITIS D IN THE AMERICAN CONTINENT: HEPATITIS AS A SERIOUS CONTINENTAL PROBLEM IN SOUTH AMERICA WITH THE PECULIAR HBV GENOTYPE F AND HDV GENOTYPE III

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The HDV infection is now a vanishing disease in US and Canadá and in most American countries, where it is almost restricted to intravenous drugs users. On the other hand, it is a spreading disease in in Amazonia. This region is composed by 07 Brazilian states and Venezuela, Suriname, Bolivia, Equador, Peru, Colombia and Guyana.

The distribution of the hepatitis B and D is very heterogeneous in Amazonia, changing according to geographic, social and ethnical aspects. The majority of HBV/HDV carriers live in areas, where it is very difficult to implement prevention and assistance actions.

Epidemiological data on Hepatitis B, D are still scarce, but populational studies demonstrated that HBV prevalence varies from 3 to 20 %. The HDV co-infects 20% of HBV carriers. The HBV genotypes F and A largely prevail, as well as HDV Genotype III. Hepatitis B and D have a high mortality rate in Brazilian Amazonia, contrasting with other parts of the country. Table I

Vaccination programs against HBV have been conducted in Brazil, however, it is still not widespread in all South American countries. In part, the lack of vaccination programs occurs due to cost issues and logistic problems such as transport, cold chain maintenance, availability of trained personnel, etc.) in remote areas.

The treatment of HBV/HDV is available in the Brazilian public health system, but it is not the reality in many other South American countries.

For both, HBV/HDV, the access to therapy is limited by drug costs (interferon, lamivudine, entecavir, adefovir for HBV; pegylated interferon), as well as the lack of access to virological tools for monitoring the treatment efficacy or viral drug resistance.

In addition, the Amazon is the only region where genotype III of HDV has been found. This genotype is the most divergent and seems to be more aggressive. Very few studies have been dedicated to this peculiar genotype III, specially with HBV Genotype F co-infection. co-infections . Both genotypes are the most divergent . Many cases of fulminant HBV/HDV hepatitis are annually reported in the Amazon Basin, mainly in young people who live in the forest or in areas surrounding small towns. In Brazil, as well as in other South American countries, insufficient data are available concerning viral hepatitis treatment and drug resistance. This is a worrisome situation, since treatment with antiviral drugs (Lamividin, and Adefovir) is available in the Brazilian public health system, but still without the possibility of screening for drug resistant viral strains.

A mysterious disease, which accompanies liver failure with hemorrhage and rapid death, is frequently reported among the population of the Amazon Region and has been observed since the 1930's. In the 80's a strong association between this syndrome and HBV/HCV co and super- was documented . The hallmarker of this disease is the presence of ballooned hepatocytes with a central nucleus surrounded by fat drops. Brazilian pathologists call this “morula - like” cells and French pathologists prefer the term “spongiocytes”.

Hepatocellular carcinome (HCC) is a growing actual problem in the Amazon, but there are few epidemiological studies about this topic. Most referral centers witness many cases of HCC, mostly in young people, even teenagers. In general, the diagnosis is confirmed at advanced HCC with very few, if any, therapeutic options.

This could be explained by HBV infection during childhood, but other possibilities must be studied such as the role of genotype F, which could have a greater oncogenic role. Here again, the role of HDV co-infection must be investigated.

In conclusion, HBV/HDV CO-INFECTION remain a serious health threat only in the Amazonia, where we recognize the highest prevalence of hepatitis B and also hepatitis D, in contrast with other non-Amazonic areas of Americas . The HBV genotype F, as well as HDV genotype III deserve further studies on its pathogenicity and oncogenesis

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EPIDEMIOLOGY OF HEPATITIS DELTA VIRUS INFECTION: ASIA

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Hepatitis delta virus (HDV) infection may induce fulminant hepatic failure and worsen the disease course of underlying chronic hepatitis B (CHB). In recent decades, the epidemiology of HDV infection changes a lot over the world including Asia.

The prevalence of HDV infection varies greatly in CHB patients with different disease severity from different countries of Asia and different areas of the same countries. The prevalence rates HDV infection in CHB ranged from 27% to 69.2% in south east Turkey, Saudi Arabia, Jordan, Lebanon, Pakistan, Mongolia, and Pacific Islands (such as Nauru and Kiribati), etc. In a report from Mongolia in 2006, up to 69.1% of chronic hepatitis B and 82.1% of HBV-related HCC were also anti-HDV positive. Anti-HDV seropositivity in patients with CHB and HBV-induced cirrhosis was lowest in the west and highest in the southeast of Turkey (5 vs. 27%, P< 0.0001 and 20 vs. 46%, P< 0.0001), respectively. The prevalence of HDV infection was low or very low (13.3% to < 0.5%) in general HBV carriers of Taiwan, Japan except in some islands of Okinawa (21.1% to 63.3%), Russia except in Yakutia (18-20%), China (12.92% in Shijiazhuang, 0.15% in Hong Kong), Iran, Korea, Indonesia, Malaysia, Thailand, Philippines (1.6%), Vietnam (1.3%) and Australia. The high prevalence of anti-HDV in the older HBV carriers in Okinawa seemed reflecting previous HDV infection in that cohort, rather than a current or imported infection of HDV. In contrast to that in general HBV carriers, HDV infection had been highly prevalent (20%-93%) in high risk groups (such as hemophiliacs, multiple transfusions, hemodialysis patients, prostitutes and intravenous drug abusers) in Asia.

There are 8 genotypes of HDV. Genotype 1 widely distributes around the world including Asia, except in Taiwan and Japan, where genotypes 2 and 4 HDV are more prevalent. In addition to interactions among populations (war, trading and travel, etc.) in human history, higher assembly efficiency of genotype 1 may contribute to its wide spread.

Decreasing HDV prevalence in Asia has been reported since 1990s. The HDV prevalence has decreased markedly both in general and high risk HBV carriers in Taiwan. New cases of acute HDV infection have been rarely found in hospitals of Taiwan after late 1990s. After 1995, the HDV prevalence in CHB and cirrhosis decreased from 29 to 12% and from 38 to 27% in central and southeast Turkey and from 38 to 20% and from 66 to 46% in west and southeast Turkey respectively. India also showed a similar decreasing trend. However, it was still found in 10.9% of overall chronic hepatitis B carriers, 20% of fulminant hepatic failure, 15% of cirrhosis and 33% of hepatocellular carcinoma in New Delhi. The HDV prevalence had decreased from 57% in Lebanon in 1987 to 1.2% in 2007; it had decreased from 32% to 8.6% in CHB patients of Saudi Arabia from 1987 to 2004. HDV infection remains low (0.44%) in patients with advanced HBV-related liver diseases underwent liver transplantation in Korea and many countries of Asia. Possible reasons contributing to the decline of HDV infection in Asia are: 1. The discovery of major transmission routes, persistent public education and increasing awareness result in the reduction of HDV infection even before the influence of mass HBV vaccination. 2. Mass HBV vaccination further decreases the incidence of acute HDV infection when most young generations get immunity to both HBV and HDV infections. 3. Promotion of using disposable needles in general practice and in high risk populations. 4. Improvement in socioeconomic conditions. However, a recent survey from Pakistan still showed a high prevalence of HDV infection in Sindh, up to 37% in rural area, and accounted for increased morbidity of liver disease in both rural and urban patients. It is more often found in HBeAg-negative than -positive chronic hepatitis B patients (65.4% vs. 34.6%) in Karachi. The prevalence of HDV infection remains high in Mongolia in 2005 and 2006. Of note, HDV infection still circulates in a significant portion of high risk populations (such as intravenous drug abusers) in Asia. Increasing prevalence of anti-HDV from 17.8% to 34% was observed in HBsAg positive intravenous drug abusers in Malaysia from 1986 to 1994.

In summary, prevalence and incidence of HDV infection show a decreasing trend in Asia, except in a few countries. It is expected that HDV infection will be further reduced when the HBV-vaccinated cohorts in Asia gradually replace the older generations. Before that, it is still important to identify risk factors of HDV transmission in different countries and take appropriate preventive measures.

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DIAGNOSIS OF HDV INFECTION: NEED FOR STANDARDIZATION

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The diagnosis of HDV infection is made in HBs antigen carriers, in a context of coinfection or superinfection. During acute HDV infection, HD antigen and anti-HD IgM may be transiently detected. However, in most cases, only total anti-HD antibodies will be present. The diagnosis of infection is based on the detection of HDV RNA by means of real-time PCR. HDV RNA can be quantified and HDV RNA monitoring is useful to monitor antiviral therapy. Recent quality controls have shown good performance of serological assays for anti-HD antibodies. In contrast, they have revealed major issues as to HDV RNA quantification. The establishment of an international standard and a universal quantification unit and standardization of real-time PCR assays is now mandatory and should be taken as a priority by the field as the incidence and prevalence of HDV infection increase in certain populations.

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HISTOPATHOLOGY OF DELTA HEPATITIS

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Delta hepatitis in the liver displays histopathology of classic viral hepatitis in acute self limited or chronic course. There is no pathognomonic histologic feature but in most cases the degree of necroinflammatory lesions is higher than in hepatitis B alone. Histopathology suggests immune mediated mechanism of liver damage rather than a direct cytopathic effect of the virus.

We performed histopathology on 82 liver biopsies from patients with chronic delta hepatitis, assessed histologic activity and the degree of fibrosis i.e. the stage of the disease according to the score established by Ishak et al and compared it to the presence of HBs Ag, HBcAg and Delta-Ag. Histopathology of chronic delta hepatitis displays the pattern of chronic viral disease in the liver. Portal tracts are enlarged and infiltrated by mononuclear lymphocytes and monocytes. Bile ducts may be involved with reactive cholangitis but are not destroyed. Correlated to the activity of the disease there may occur destruction of the limiting plate resulting in interface hepatitis. The amount of fibrosis is variable but may lead to bridging fibrosis or cirrhosis in the late stage. Lobular hepatitis shows no zonal distribution but displays a spotty necrosis seemingly bound to virus containing hepatocytes. Confluent necrosis may occur. The distribution of collagen formation is restricted to portal and septal fibrosis. When assessed according to the activity index 42 patients showed a mild disease (score 0-7), whereas 40 patients were found to have a moderate to severe disease (score 8-18). With regard to the stage and degree of fibrosis 64 patients had mild to moderate fibrosis (portal fibrosis and occasional septae) wheras 18 showed stage 3 to 4 with briding fibrosis and transition to cirrhosis.

When compared to histopathology no direct association was found between viral replication for HBV and HDV supporting the hypothesis the histopathological pattern that liver damage in chronic delta hepatitis is an immune-mediated disease and that the virus itself has no relevant cytopathic effect on hepatocytes.

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HEPATITIS DELTA VIRUS GENUS IS COMPOSED OF 8 'CLADES' AND SEVERAL 'SUBCLADES' AS ASSESSED BY EXTENSIVE PHYLOGENETIC ANALYSES OF 1000 HDV PARTIAL OR COMPLETE SEQUENCES

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Background and aims: Hepatitis Delta virus (HDV) is a satellite of Hepatitis B virus (HBV) that requires HBV envelope for transmission and propagation and infects 20 million people worldwide. The HDV genome is a 1.6 to 1.8-kb single stranded negative RNA genome characterised by an extensive intramolecular complementarity and a mechanism of rolling circle for that enables its replication, using host polymerases. Deltavirus genus is composed of 8 monophyletic groups or clades, designated HDV-1 to -8, that show a characteristic geographical distribution. HDV-1 is ubiquitous. HDV-2 and -4; and HDV-3 are restricted respectively, to South and East Asia and to the North of South America. HDV-5, -6, -7 and -8 have been described in infected patients from Subsaharan Africa and recent studies performed in Africa begin to confirm these data. Since 2001, more than 1,000 partial or complete HDV sequences have been characterized in our laboratory. In this study, we provide further information about the genetic variability of the Deltavirus genus.

Patients: The strains were isolated from 1,114 patients living in different parts of the world, including France, Turkey, Mauritania, Niger, Algeria, Senegal, Yakoutia and Amazonian region. A total of 1,000 patients were diagnosed in France. Among them, 589 were Africans, 227 and 105 were respectively from Western or Eastern Europe; 67 came from Asia and the 12 remaining from other regions.

Methods: The R0 region of the HDV genome, encompassing nucleotides 889 to 1289 (sequence numbering according to Wang, et al., 1986) was amplified and sequenced bi-directionally for all isolates. Among them 150 strains were fully sequenced. Sequences were aligned together with 28 published sequences, and extensive phylogenetic analyses were carried out.

Results: We characterised 838 HDV-1 strains (75.2%), 7 HDV-2 (1%), 7 HDV-3 (2.2%), 161 HDV-5 (15.1%), 17 HDV-6 (1.5%), 35 HDV-7 (3%) and 16 HDV-8 (1.4%). The phylogenetic analyses definitively confirm that HDV genus is composed of 8 clades, with an interclade divergence over the complete genome sequence of more than 16%. Interestingly, our results strongly suggest that HDV clades could be further divided into several subclades: HDV-1(1a, -1b and 1c); HDV-2 (2a, 2b); HDV-3 (3a, 3b), and HDV-5 (5a, 5b and 5c), with a 9% to 16% inter-subclade divergence. HDV clades and subclades show and confirm the typical geographical distribution previously described.

Conclusions: This study confirms the very high genetic variability of Deltavirus genus. This genetic diversity must be taken in account in the diagnosis and monitoring of the infected patients. Indeed, several quantification techniques of HDV viral load are now available, and the choice of primers and probe may be critical in the performance of those techniques. Nonetheless it remains to be proved whether HDV clades are related to different clinical patterns. Indeed, several other factors, such as the time and duration of the infection, the viral load, the HBV helper strain and the patient's genetic background may be involved in setting off the pathogenicity.

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THE NATURAL HISTORY OF DELTA HEPATITIS

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Chronic delta hepatitis (CDH) represents a severe form of chronic viral hepatitis, induced by the hepatitis D virus (HDV) in conjunction with the hepatitis B virus (HBV). Delta hepatitis may lead to disease in humans through co-or superinfection. The former leads to acute hepatitis which clinically can range from mild hepatitis to fulminant hepatitis and death. Severe or fulminant hepatitis is more often observed with HBV-HDV co-infection compared to HBV mono-infection. Chronic infection after acute hepatitis B + D co-infection is infrequent. CDH develops in 70-90% of patients with superinfection. CDH runs a more progressive course than chronic hepatitis B and may lead to cirrhosis within two years in 10-15% of patients. However, as with any immune mediated disease, different patterns of progression, ranging from mild to severe progressive disease, is observed. Active replication of both HBV and HDV may be associate with a more progressive disease pattern. However, in series from the Far East a more protracted course of CDH has been reported. Different HDV and HBV genotypes may contribute to various disease outcomes. In a longitudinal follow-up study from Taiwan of a cohort of CDH patients with mostly genotypes B and C HBV and genotypes 1 and 2 HDV, age, genotype C HBV and genotype 1 HDV were found as independent factors for adverse outcomes by multivariate analysis. More recent studies on the natural history of CDH from Italy also challenge the impression of CDH being a disease with an ominous prognosis. It appears that, CDH runs a severe course towards early development of cirrhosis in parallel with possibly high HDV replication after which the pace of HDV replication may decrease associated with retarded further progression of disease. The contribution of CDH to hepatocellular carcinoma (HCC) development is controversial. A European wide study had reported a 3.2 fold risk of HCC development in patients with CDH compared to HBV monoinfected patients. Recent studies from Italy, Taiwan and England, however, failed to show an increased risk for HCC development in CDH. Further studies on CDH-induced liver related complications are awaited for clarification.

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HDV INFECTION AND HEPATOCELLULAR CARCINOMA

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Compared to the infection with hepatitis B virus only, infection with hepatitis D virus is associated to a more rapid progression of liver fibrosis, earlier appearance of hepatic decompensation, as well as increased risk of hepatocellular carcinoma (HCC) development. The association between HDV and HCC has been demonstrated in both the West and the East. In a prospectively collected cohort of 200 European patients with compensated cirrhosis type B, 39 were found to be chronically infected by HDV, with a significantly increased risk for HCC compared to HDV uninfected patients (36% vs 13%). The link between HDV and HCC was demonstrated also by the retrospective analysis of 299 patients with chronic HDV infection in Milan, who were followed up over a mean period of 233 months. In that study 146 patients had cirrhosis and 46 developed HCC, at annual rates of 2.8%. HCC developed after a mean follow up of 83 months since diagnosis of cirrhosis, at a mean age of 55 years (range 35-68). Patients with a HCC were predominantly males (76%) with an unknown source of infection (87%) and serum markers of persistent HBV and HDV replication still detectable in 23 (50%) and 34 (74%) patients, respectively. The cumulative probability of developing HCC was influenced by previous treatment with interferon (OR, 2.8; CI: 1.10-4.32) and persistent hepatitis B virus (HBV) replication (OR, 5.05; CI: 2.57-10.32). By backward deletion, HBV replication, interferon and corticosteroids were positively associated to increased risk of HCC development. Twenty-five patients (54%) were still alive after 12-324 months from HCC diagnosis: 6 underwent liver transplantation and the remaining 19 were treated with percutaneous ablation techniques. At variance with HBV-related cirrhosis, clinical decompensation and not HCC was the first dominant complication to occur in our series of HDV infected patients. The same was observed in the previously mentioned 39 western European patients with HDV-related cirrhosis. Interestingly, in a retrospective analysis of 962 HBV patients in London, HCC rates were similar in the 82 HDV-infected patients and the 880 HDV-non infected patients, thus attenuating the pathogenic importance of HDV in HBV carriers. Instead, our findings of previous treatment with interferon and HBV replication being associated with an increased risk of HCC suggest that severity of HDV infection is an important predictor of HCC, since it reflects selection of patients with more aggressive liver disease. In Taiwan, a longitudinal study of 194 patients infected with HDV showed a prevalence of adverse outcomes, including HCC 28% vs 8% in patients infected by genotype I of HDV, compared to those infected by genotype II of HDV. In our study of the 35 patients who had a sustained response to therapy, i.e. persistently negative HDV-RNA, nevertheless 20 progressed to HCC. We speculate that treated patients had a more severe course than untreated thereby being at greater risk of HCC, and that HBV was pathogenetically important in these patients. Our findings somehow link to an old study describing a collection of 79 HBsAg seropositive patients with a HCC treated between 1977 and 1983, in Italy, where low titer anti-HDV was detected in 8 (19%) in the absence of tissue or serum markers of active HDV replication, further suggesting the pathogenic role of HBV in liver carcinogenesis. In Italy, only a minority of all patients undergoing liver transplantation for HBV-related end stage liver disease were infected by HDV, with a substantial increase of the prevalence compared in the last decade to the eighties (10% vs 3%).

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TREATMENT OF DELTA HEPATITIS WITH INTERFERONS

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Chronic hepatitis D is caused by persistent infection with hepatitis D virus (HDV), a defective RNA virus that incorporates the hepatitis B virus surface antigen (HBsAg) as its envelope protein. The unconventional replicative cycle of HDV and its high pathogenic potential make this virus a difficult target for antiviral therapy. HDV lacks its own polymerase and specific HDV inhibitors have not yet been developed. Moreover, despite the vital link of HDV with HBV, potent and specific inhibitors of HBV replication have little or no effects on HDV replication or liver disease activity.

The only option currently available for the treatment of chronic hepatitis D is interferon-α (IFN), which is the single licensed drug for this disease. Its efficacy is related to the dose and duration of treatment. Overall, a one-year course of high doses of IFN is associated with only a 10 to 20% chance of sustained HDV clearance and a 10% chance of HBsAg clearance. Limited data are available on the long-term effects of IFN on the natural history of hepatitis D. A prospective controlled long-term follow-up study for up to 20 years showed that high doses of IFN for one year significantly improved the long-term clinical outcome and survival of patients with chronic hepatitis D. Reversion of advanced liver fibrosis occurred in some patients who initially had active cirrhosis. Several strategies have been explored to improve the efficacy of IFN, most notably a longer duration of treatment, but most patients still fail to clear the virus. Moreover, these alternatives are poorly tolerated.

The efficacy and safety of pegylated interferon (Peg-IFN) in chronic hepatitis D was recently investigated in three small-size studies in which Peg-IFN-α2b was given 1.5µg/kg weekly for 12 months. Both in IFN-naïve patients and in previous nonresponders to standard IFN, the results were overall better than with standard IFN. A sustained virological response was achieved in 43%, 25% and 19% of the patients. In the HIDIT-1 trial, 90 patients were randomized to receive either 180µg of Peg-IFNα-2a weekly plus 10 mg of adefovir daily, 180µg of Peg-IFNα-2a weekly plus placebo or adefovir alone for 48 weeks. Sustained HDV RNA clearance was observed in 25% of patients who received Peg-IFN but in none of those receiving adefovir alone. Data on the efficacy of combination therapy with Peg-IFN are very limited. As with standard IFN, the addition of ribavirin or lamivudin or adefovir to Peg-IFN showed no significant advantages over Peg-IFN monotherapy.

At present, there are no baseline biochemical or virological variables that are predictive of a sustained virological response. However, as with standard IFN, patients without cirrhosis are the most likely to respond, highlighting the importance of early diagnosis and treatment in chronic hepatitis D. Differences in disease duration and liver histology on entry into the trials may have contributed to the observed discrepancies in the rates of virological response. Albeit limited in size, these studies have underscored the importance of quantitative assays for monitoring the response to therapy. Viral kinetics analysis provided evidence that a negative HDV RNA after 6 months of therapy is the best predictor of sustained virological response to Peg-IFN, although in a minority it failed to distinguish responders from relapsers. Quantification of HDV viremia might also identify slow virological responders who might benefit from a more prolonged course of IFN therapy.

Larger studies are needed to better define the predictive value of a negative HDV RNA testing during the first 6 months of therapy. The major problem is the lack of commercial assays for the qualitative and quantitative assessment of HDV viremia. Quantification of serum HBsAg levels provides an additional tool to improve treatment monitoring.

The superior results obtained with Peg-IFN suggest the use of this drug as a first-choice treatment for chronic hepatitis D. However, large controlled trials are still needed to determine the greater efficacy of pegylated versus standard IFN and to identify the best treatment schedule. Treatment with Peg-IFN should be offered to all patients with well-compensated HDV chronic liver disease as soon the diagnosis is made, whereas it is contraindicated in those with advanced or decompensated liver disease for whom liver transplantation is the only therapeutic choice. Continuing medical and psychiatric monitoring is mandatory. Although the results with Peg-IFN are better than with standard IFN, the rate of relapse remains high and a high proportion of patients still do not respond. Treatment of chronic hepatitis D remains a major challenge, emphasizing the need for the development of novel therapeutic approaches that target the life cycle of HDV.

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COMBINATION TREATMENT IN DELTA HEPATITIS

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Despite being rare, because of the lack of an effective treatment and the severity of the liver disease, chronic hepatitis delta (CHD) is a serious health problem.

Because of the paucity of the patients, most of the studies related to the treatment of CHD are inconclusive. However, interferon has been shown to be the only effective drug for the treatment of CHD, yet its effectiveness is very low. Interferons and pegylated interferons may provide a 15 to 45% sustained virologic response (1).

The replication strategy of delta virus is completely different from that of hepatitis B virus. Therefore, the nucloes(t)ide analogs, which are effectively used in the treatment of chronic hepatitis B, have no place in the treatment of CHD. These drugs may be beneficial only if they success a reduction in the level of HBsAg, which is required for the formation of the delta virion.

Lamivudine, which is the most commonly used drug in the treatment of CHB, does not decrease the level of cccDNA or HBsAg. Therefore, it has no effect in the treatment of CHD, either as a monotherapy or as a part of interferon combination (2). Surprisingly, in a randomized study, improvement in necroinflammatory activity was found to be more significant in the combination group (-5.25±1.08) comparing to interferon-treated patients (-1.44±1.59) (3). However, no significant improvement was observed in regards to fibrosis. The rate of sustained virologic response was statistically similar in combination therapy (4/14) and interferon treated patients (2/12).

In recent years, the most powerful antivirals have been shown to decrease the HBsAg titer and to achieve HBsAg loss. Then, some promising results are expected from the combination therapies of interferons with entecavir or tenofovir disoproxil fumarate (TDF). A case report described an HBsAg seroconversion after 10 month treatment of peginterferon alfa-2a plus TDF plus emtricitabine. Although there are ongoing studies investigating the effectiveness of interferons and TDF or entecavir in delta hepatitis, there has been no published clinical study so far.

Based on the data of the reducing effect of adefovir dipivoxil on cccDNA and HBsAg titer, its combination with peginterferon alpha-2a was tried (4). This study demonstrated that there was no difference between peginterferon alpha-2a (PegIFNa2a) and PegIFNa2a+adefovir combination, in terms of HDV RNA negativity. However, HBsAg reduction was more pronounced in combination arm comparing to monotherapies. Forty percent of the patients in the combination arm achieved at least 1 log reduction of HBsAg level, while this figure is 5% in the PegIFNa2a-treated patients.

Another nucleoside analogue, ribavirin, is effective against hepatitis C virus, and it has been shown experimentally to inhibit HDV genome replication in hepatocyte cultures. However, in a pilot study including patients with CHD, ribavirin monotherapy did not result in biochemical, virological, or histological improvements (5). Yet, ribavirin was tried with the combination of interferons in the treatment of CHD. In these trials, ribavirin combinations were not superior to interferon monotherapies. Two-year duration of interferon plus ribavirin treatments (6, 7) and a 24-week peginterferon plus ribavirin combination (8) achieved almost 20% sustained virologic response in patients with CHD.

In conclusion, interferon and nucleos(t)ide combination therapies do not provide an additive or synergistic effect in the treatment of CHD. However, the combinations with newer drugs are awaited with interest.

References:

1. Farci P. Treatment of chronic hepatitis D: New advances, old challenges. Hepatology 2006;44:536-9.

2. Yurdaydin C, Bozkaya H, Onder FO, et al. Treatment of chronic delta hepatitis with lamivudine vs lamivudine + interferon vs interferon. J Viral Hepat 2008;15:314-21.

3. Canbakan B, Senturk H, Tabak F, et al. Efficacy of interferon alpha-2b and lamivudine combination treatment in comparison to interferon alpha-2b alone in chronic delta hepatitis: a randomized trial. J Gastroenterol Hepatol 2006;21:657-63.

4. Wedemeyer H, Yurdaydin C, Zachou K, et al. Pegylated interferon alfa-2a plus adefovir combination therapy is

superior to pegylated interferon alfa-2a alone or adefovir monotherapy in reducing HBsAg levels in HDV-coinfected patients with low HBV viremia. Hepatology 2006;44, Suppl 1: 553A.

5. Garripoli A, Di Marco V, Cozzolongo R, et al. Ribavirin treatment for chronic hepatitis D: a pilot study. Liver 1994;14:154-157.

6. Kaymakoglu S, Karaca C, Demir K, et al. Alpha interferon and ribavirin combination therapy of chronic hepatitis D. Antimicrob Agents Chemother 2005;49:1135-8.

7. Gunsar F, Akarca US, Ersoz G, et al. Two-year interferon therapy with or without ribavirin in chronic delta hepatitis. Antivir Ther 2005;10:721-6.

8. Niro GA, Ciancio A, Gaeta GB, et al. Pegylated interferon alpha-2b as monotherapy or in combination with ribavirin in chronic hepatitis delta. Hepatology 2006;44:713-20.

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A ROLE FOR NUCLEOS(T)IDE ANALOGS IN HDV TREATMENT?

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Knowledge supporting NUC: Although HBV is not directly involved in the replicative cycle of HDV, the Hepatitis B surface Antigen (HBsAg) represents the envelope of HDV without which HDV cannot be secreted out of hepatocyte. Moreover active HBV replication accelerates progression of liver disease in patients with chronic HDV infection. These reasons, associated to the unsatisfactory treatment with interferon, have provided the rationale for testing Nucleos(t)ide Analogs in hepatitis D. Contrary clinical data: In a first pilot study (Lau, 1999) five pts received Lamivudine 100 mg daily for 12 months . Despite a general reduction of HBV-DNA so that viremia was undetectable in four pts and decreased by 5 logs in the other, ALT remained abnormal, HDV-RNA detectable and HBsAg positive in all. Liver biopsy showed no consistent improvement in inflammatory or fibrotic score. A more extensive study (Niro, 2005) compared a 12 months versus a 24 months-course of Lamivudine. A total of 31 pts were randomized to treatment, 11 received placebo and 20 Lamivudine for 12 months; thereafter all were given Lamivudine for 12 months and followed-up for 16 weeks. At the end of treatment HDV-RNA was negative and ALT normal in 3 patients; however, only 2 patients remained virus-free at the end of follow-up Nineteen paired biopsies were available pre and post-treatment; 5 patients had a > 2 point decrease in the Ishak score and were defined as responders.

No significant differences in necro-inflammation and fibrosis scores were observed in the two groups. One patient exhibited a complete response and became HBsAg negative (normal ALT, negative HDV-RNA, histological improvement). Twelve or 24 months lamivudine treatment did not significantly affect biochemical, virologic or histological parameters of chronic delta hepatitis in the others. In a third study Manesis (2007) confirmed that after Lamivudine the only significant change was in serum HBV-DNA; levels of HDV-RNA, HBsAg or ALT evaluated by quantitative analysis were not affected. In a pilot study 15 patients were treated with Famciclovir for 6 months (Yurdaydin, 2002). HBV-DNA decreased in 9 of the 15 pts but rose again after treatment. Famciclovir had no effect on ALT, HBsAg levels or serum HDV-RNA; there was no improvement on liver histology.Combination therapy of Interferon with either Lamivudine (Wolters, 2000) or Ribavirin (Niro, 2006) given for 12 months was of no additional value if compared to Interferon monotherapy.High expectation was deserved to clevudine a potent inhibitor of hepadnaviruses, which suppressed HDV in chronically infected woodchucks. Suppression was correlated with the marked reduction of hepatitis virus surface antigen in individual animals (Casey, 2005), consistent with the concept that repression of surface antigen expression could be a useful antiviral strategy for HDV. Severe myopathy associated with long term treatment and characterized by depletion of mitochondrial DNA limited the use of clevudine.Favourable Clinical Data:

Case reports referred remission of chronic hepatitis delta after lamivudine or adefovir dipivoxil therapy. More recently a beneficial effect of potent nucleos(t)ide analogues was shown in HIV-positive patients with chronic delta hepatitis (Sheldon, 2008). Overall in 13 patients (81%) longitudinally treated (median time of 6.1 years), a reduction in HDV viremia and ALT levels was observed and three patients achieved undetectable HDV-RNA and normal ALT levels. NUC Resistance: Nucleos(t)ide analogues target the reverse transcriptase of hepatitis B virus. The major HBV lamivudine-resistant mutations in the polymerase gene within the reverse transcriptase (rt) region at rtM204V or rtM204I are associated with changes in the overlapping envelope gene products, in particular, the gene encoding small envelope protein at position s195 and s196. Vieether (2005) demonstrated that the lamivudine resistance mutations corresponding to sW196L/S inhibited secretion of HDV particles. The HBsAg variant was deficient for HDV assembly as it could no longer interact with the large delta antigen. How this effect on HDV packaging and release influence cytopathology is unknown.

Conclusion: HDV-HBV coinfection is associated with diverse pattern of inhibition of viral replication and viral dominances may change over time. At present the use of HBV polymerase inhibitors depends on serum levels of HBV and presence or absence of cirrhosis. Both the duration of treatment and the clinical beneficial in presence of HDV viremia remain undefined.Long-term therapy with high genetic barrier nucleos(t)ide analogues is not sufficiently explored, but the lack of data on long-term safety and efficacy make recommendations difficult and an accurate follow-up of on-treatment and treated patients necessary.

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LIVER TRANSPLANTATION FOR DELTA INFECTION

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Patients chronically infected with HBV and HDV are less at risk of HBsAg reappearance than patients infected with HBV alone. The rate of HBsAg reappearance in patients with HBV-HDV cirrhosis was 50-60% in patients who did not receive long-term HBIG, and 17% in those receiving long-term HBIG in a European series. Combination HBIG and nucleos(t)ide analogue further reduce the risk of recurrence to less than 10%. The overall lower HBV recurrence rate in these patients is probably due to the fact that most of these patients have low HBV DNA level at time of liver transplantation and that HDV has an inhibitory effect on HBV replication.

In contrast, HDV reinfection is frequent and was observed in 80% of cases in the first post-transplant months. The course of HDV reinfection is different if HBsAg reappears or not. In the few cases where HBsAg reappeared, it was associated with a combined HBV-HDV replication, the development of an acute, then chronic hepatitis. HBV-HDV recurrence is in general less severe than HBV recurrence alone. In the patients who remained HBsAg negative after transplantation, the amount of HDAg in the liver graft was low and the liver graft remained histologically normal. At long-term, HDV markers disappeared from liver and serum. The hypotheses for explaining the presence of HDV replication in HBsAg negative patients are: a) HBV markers could be present, but not detectable; b) HDV is present in the hepatocytes in the absence of HBsAg but cannot replicate; c) the level of HDV RNA in liver is much lower in patients without than with HBsAg, which may explain the absence of liver graft lesions.

In conclusion, the risk of HBsAg reappearance after liver transplantation in HBV-HDV cirrhotic patients who received long-term HBIG is low. It seem further reduced in patients receiving combination HBIG + Nucleos(t)ide analogue. In this setting the absence of HBsAg is essential to avoid HDV replication. Overall survival after liver transplantation is over 80% at 5 years

References:

Samuel D, Muller R, Alexander G, Fassati L, Ducot B, Benhamou JP et al. Liver transplantation in European patients with the hepatitis B surface antigen. New Engl J Med 1993; 329:1842-1847.

Shouval D, Samuel D. Hepatitis B immune globulin to prevent HBV graft reinfection following liver transplantation: a concise review. Hepatology 2000;32:1189-1195.

Roche B, Feray C, Gigou M, Roque-Afonso AM, Arulnaden JL, Delvart V et al. HBV DNA persistence 10 years after liver transplantation despite successful anti-HBs passive immunoprophylaxis. Hepatology 2003;38:86-95.

Ottobrelli A, Marzano A, Smedile A, Recchia S, Salizzoni M, Cornu C et al. Patterns of hepatitis delta virus reinfection and disease in liver transplantation. Gastroenterology 1991;101,1649-1655. Krogsgaard K, Kryger P, Aldershvile J, Andersson P, Sorensen TL, Nielsen JO. Delta infection and suppression of hepatitis B virus replication in chronic HBsAg carriers. Hepatology 1987;7:42-45. Samuel D, Zignego AL, Reynes M, Feray C, Arulnaden JL, David MF et al. Long-term clinical and virological outcome after liver transplantation for cirrhosis due to chronic delta hepatitis. Hepatology 1995;21:333-339.

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NOVEL TREATMENT OPTIONS FOR DELTA HEPATITIS

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According to the WHO, 10-20 million chronic HBV-carriers are co-infected with HDV. As a satellite virus using HBV as its helper, the spread of HDV depends on the expression of the HBV-envelope proteins in host hepatocytes. However, due to its entirely different and mostly host protein-dependent life cycle, HDV replication is insensitive against the reverse transcriptase inhibitors used for the treatment of chronic hepatitis B. This peculiarity has profound consequences for the available and possible future medications for HDV-infected patients. Moreover, since there are no drugs at hand that address HDV RNA or the hepatitis delta antigen (HDAg) directly, therapeutic options are so far limited to the reduction of HBV-surface antigen expression or the inhibition of cellular factors required for HDV-replication and spread.

Currently, the only option for treating hepatitis delta is IFNα/pegIFNα, resulting in moderate sustained virological response rates at long term and high dose application. Experimentally it has been shown that inhibitors of farnesyltransferase inhibit HDV-secretion in mice by blocking farnesylation of the large HDAg. Some other specific approaches like the inhibition of the HDV-ribozyme or siRNA are feasible but far from entering the clinical stages. Thus there is a strong medical need to develop drugs that interfere with specific steps of HDV replication.

We have recently shown that HBV L-protein derived lipopetides efficiently block HBV entry into hepatocytes *in vitro* and *in vivo*. Due to their similar entry pathways the peptides are also potent inhibitors of HDV infection. Myrcludex B, the lead substance of these peptides has been synthesized according to GMP standards and has been successfully tested with respect to its pharmacokinetics and its toxicity. The IC50s against HBV and HDV *in vitro* is ~200 pM. Myrcludex B targets hepatocytes of even non HBV-susceptible animals with extraordinary efficacy, suggesting the presence of a species-independent but hepatocyte-specific receptor. Taken into account that protection of non-infected hepatocytes during natural turnover of infected hepatocytes may support the immunological response and clearance of infection, Myrcludex B might be a promising candidate for the control of HDV infection in chronic HDV/HBV infected patients in the near future. In addition, the strong liver-tropism of HBV L-protein derived peptides provides the basis for the design of novel drugs such as liver-specific IFNα or hepatotropic liposomes that deliver HDV-specific siRNA to hepatocytes.

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UNMET NEEDS IN DELTA HEPATITIS

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Hepatitis (D) delta (HDV) is the most dangerous of all viral hepatitis, treatment options are limited. Comprehensive data on the global epidemiology are missing. The word wide prevalence of chronic hepatitis D is estimated to be between 20 - 50 million. However, this may be an underestimation since epidemiology data from many areas of the world including those regions where hepatitis B is highly prevalent are missing. This EASL monothematic conference 2010 on delta hepatitis has revealed enourmous new information on the epidemiology and morbidity due to HDV infection from parts of the world from which data on HDV infection have been missing sofar. The international community of hepatologists should undertake a particular effort to collect comprehensive epidemiology data on the global spread of HDV infection. WHO as well as EASL, AASLD and in particular the African, the Asian-Pacific and the Latin American associatons for the study of liver diseases should become more active, increase awareness and support this important area of research. Concerning basic science and virology we need to better understand the life cycle of HDV, mechanisms of HDV virus and host interactions, pathogenesis and in particular the interaction between HBV and HDV infection in order to identify new therapeutic targets that are urgently needed to make significant therapeutic progress in the future. Concerning pathogenesis we need to understand the specific role of the HDV virus in hepatocarcinogensis. In the diagnostic arena we need to promote anti HDV antibody testing for all HBV carrier around the world and need to provide universal availability of standardized HDV RNA testing, quantitative as well as qualitative. The role of HDV genotypes and consequently genotyping needs also to be explored further concerning natural history, pathogenesis, and response to therapy. Finally: therapy. Since HDV is a rather rare disease we need international consortia that work together with the pharmaceutical industry, public and private funding organizations as well as governments to perform multicenter international trials using known drugs like pegylated interferons and newer oral HBV nucleoside inhibitors like tenofovir and entecavir. Here the results of the HIDIT 1 trial - the largest prospective interventional trial in the therapy of HDV to date - are promising. This study also paved the way for HIDIT 2 which is ongoing and combines pegylated interferon alfa 2a with tenofovir for a prolonged treatment period of 96 weeks. While we still do not know whether clearance of HDV infection is a realistic goal and will be achievable HBV and HDV viral kinetics in HBV/HDV coninfected patients may lead us the way to future therapies. For the time being intelligent combinations, e.g. interferons or other alternative immune modulators plus direct antivirals like HBV nucleos(t)ide analogues are at present the only realistic weapons. The development of these therapies needs to be guided by viral kinetics of both viruses, quantitative HBsAg levels and other immunological parameters of the innate and adaptive immune system. HBsAg / anti HBs seroconversion associated with a sustained loss of HDV RNA is the final goal and endpoint of HDV therapy. Hopefully direct antiviral agents specifically targeting the HDV virus life cycle will be added to our weapons against HDV in the not too far distant future. The pharmaceutical industry needs to be convinced that it is worthwhile and ethically necessary to invest in HDV therapies. Pegylated Interferons alfa may be more beneficial for HDV than in HBV monoinfections. On the other hand foundations like the Melinda and Bill Gates foundation as well as WHO, United Nations and all the individual governments need to be convinced that HDV infection and it's consequences is a major global health burden. The epidemiology is changing in the Western world driven by the aging HDV populations in Southern Europe and the increase in global migration. Finally, HDV infection is a particular health problem in the developing world where the burden of HDV infection is still poorly understood.

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TOWARDS STANDARDIZATION OF HDV RNA MEASUREMENT

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The hepatitis D virus (HDV) is a defective 1678 nucleotide single-stranded RNA virus that requires the helper function of hepatitis B virus to replicate. HDV genotype 1 (HDV-1) is the most predominant one worldwide, and is associated with a broad spectrum of chronic HDV disease. Co- and superinfections with HBV-dependent HDV can lead to serious complications, such as fulminant acute hepatitis or severe chronic active hepatitis, often progressing in cirrhosis. Chronic HDV infection may also lead to the development of hepatocellular carcinoma. Since no effective antiviral therapy is currently available for treatment, liver transplantation may be considered for fulminant acute cases and end-stage chronic HDV. Disease conditions could be occasionally improved with administration of alpha-IFN. Nowadays, the methods of choice for the diagnosis of ongoing HDV infection and treatment monitoring are nucleic acid amplification technique (NAT) assays. Monitoring of viremic course of HDV after treatment with pegylated IFN by quantitative real-time PCR is state of the art. At the time being only few commercial HDV NAT assays are available on the market. Most used NAT tests are home-brewed. All these assays are not well standardized and the quantitative values are difficult to compare. This can cause problems in the treatment of chronic hepatitis D. An international reference material is an urgent requirement to standardize the NAT tests. Furthermore, the comparison of standardized NAT results will facilitate new strategies for a successful treatment.

The Paul-Ehrlich-Institut, as one of the WHO Collaborating Centres for Biological Standards and Standardization, proposed the project of the development of the first international standard for HDV RNA (genotype 1). The WHO Expert Committee on Biological Standardization (ECBS) endorsed the proposal in October 2009. The project will be done in close cooperation with the Institute of Hepatology, Ankara , Turkey and with the Institute for Medical Virology, Justus von Liebig University in Giessen, Germany. The development of the standard preparation will follow the WHO recommendation for the Universitypreparation, characterization and establishment of international and other biological reference standards (see below). The type of standard proposed (i.e. HDV diluted in human plasma, analogous to the other WHO NAT standards for blood borne viruses) would be suitable for all current NAT methods. HDV RNA-high titre plasma samples (HDV-1) with a sufficient volume, provided by the Institute of Hepatology of the Ankara University, will be characterized in a feasibility study to find out suitable candidates for the preparation of the standard. The studies will include different NAT systems as well the parallel testing with a well characterized amored HDV RNA sample. The feasibility study will be performed by laboratories with expertise in molecular diagnostics of HDV. The potential cancidate materials have a vial load from 10^5 - 10^7 copies/mL. The proposed standard preparation will consist of 2000 - 4000 vials containing about 10^5 copies/vial. The filling volume will be between 0.5 - 1 mL per vial. A pilot study will ensure that the lyophilisation will have no influence on the integrity of HDV RNA. The worldwide collaborative study will be conducted to evaluate the candidate reference materials. The final report will be submitted to the ECBS of WHO in July 2013 for establishment of the first International Standard for HDV RNA.