PROPOSAL FOR THE DEVELOPMENT OF AN INTERNATIONAL REFERENCE PREPARATION FOR HEPATITIS D VIRUS RNA

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Hepatitis D Virus (HDV)

- Member of the genus *Deltavirus*
- 8 major clades (HDV-1 to -8)
- Genetic variability ranges from 20 to 35%
- 36-43 nm defective viral particle; needs HBV to replicate
- Circular ss (-) RNA genome of ~ 1.7 kb which is encapsidated by 60 molecules HDAg
- Virion is enveloped by HBsAg
- Transmitted via infected blood or blood products; sex contact
- Individuals at risk are HBV carriers, IDUs, hemodialysis patients and highly promiscuous groups
- Worldwide ~ 5% of HBV carriers are anti-HDV positive (10 - 15 million people)
- Mortality rate lies between 2 and 20% (ten times higher than for HBV)
Geographic Distribution of HDV Infection

HDV Prevalence:
- High
- Intermediate
- Low
- Very Low
- Very Low
- No Data

Hepatitis Slides CDC, mod.
HBV-HDV Coinfection

- Simultaneous infection with HBV and HDV
- Severe acute disease and fulminant hepatitis
- Low risk of chronic HDV infection (1 - 3%)
- Early markers HBV DNA, HBsAg and HDV RNA
- Anti-HDV antibodies develop late and after infection usually decline to subdetectable levels
- HDAg is normally present only transiently on a very low level
HBV-HDV Superinfection

- HDV infection of chronic HBV carriers
- Causes acute hepatitis with short incubation time
- High risk of severe chronic liver disease with cirrhosis (70 – 80%)
- HDV viremia appears in serum during preacute phase
- Titre of HBsAg declines when HDAg appears
- Patients with chronic hepatitis D show persisting HDV RNA, anti-HDV antibodies and HDAg
HDV Infection - Prevention and Treatment

- No specific vaccine or post-exposure prophylaxis
- HBV-HDV coinfection can be prevented with HBV vaccine or HBIG
- Prevention of HBV-HDV superinfection depends primarily on education to reduce high-risk behaviour
- No effective antiviral therapy is currently available
  - Nucleoside analogues and other antiviral agents are inefficient
- Approved treatment for hepatitis D is αIFN (PEG-αIFN)
- In cases of end-stage chronic HDV infection liver transplantation could be an option
Diagnosis of HDV Infection – Serology (1)

- Anti-HDV: hallmark of diagnosis of HDV infection.
- Both IgG and IgM class are detectable but IgG is most widely used
- In acute HDV infections, serum IgG anti-HDV may be initially undetectable or present in very low titer
- In contrast, serum IgM anti-HDV in relatively high titers appear early in the course of acute HDV infections, but it tends to disappear within weeks if the infection resolves
Diagnosis of HDV Infection – Serology (2)

- Serum HDAg appears in high titers very early in the course of acute HDV infections
- HDAg rapidly disappears if the acute infection resolves
- Very low titers of HDAg may also be detected in chronic HDV infections only by WB analysis, which is not widely available.
- HDAg can also detected in liver tissue (in nuclei of hepatocytes) by immunostaining HDAg detection in liver tissue
Diagnosis of HDV Infection – NAT

- Serological tests often lack on sensitivity
- The most sensitive method is NAT
- Detection of HDV RNA using real-time RT-PCR (LOD: 10 – 100 cps/mL)
- Despite the high sequence diversity, primers and probe(s) of NAT assays can be selected from highly conserved regions to cover all 8 HDV clades
- HDV RNA appears very early in the course of acute HDV infections and remains persistently detectable only in cases who progress to chronicity
- Thus, undetectable serum HDV RNA by RT-PCR and positive anti-HDV practically mean past HDV infection
- Monitoring of treatment
- In-house developed, as no commercial tests are available
Need for an International HDV Reference Material

- No standardized assays for the assessment of HDV viremia are currently available
- HDV reference material is particularly important for:
  - the development and calibration of diagnostic assays
  - the calibration of secondary references and working standards
  - the evaluation of standardized preparations used in QC and QA
  - assay comparison
- Standardized assays for the detection and quantification of HDV viremia are needed:
  - to monitor the response to therapy of chronic HDV patients, so that effective treatment can be initiated early before the development of advanced liver disease
  - to screen for HDV infections in endemic areas to get more epidemiological data
Proposal for the 1st International Standard for HDV RNA (1)

- Proposal to ECBS of WHO in October 2009
- Collaboration with the Institute of Hepatology, Ankara University, Turkey (Prof. Bozdayi)
- Several HDV-positive plasma samples (250 – 300 mL) are available
- Samples represent clade HDV-1
- Viral load $10^5 – 10^7$ cps/mL
- Feasibility study (cooperation with other labs is planned; e.g. Institute for Medical Virology, J.L University Giessen, Germany)
- Standard preparation will consist of 2000 – 4000 vials containing $10^5$ cps/vial
- Filling (0.5 – 1 mL) and lyophilization
Proposal for the 1\textsuperscript{st} International Standard for HDV RNA (2)

- Collaborative study to evaluate the candidate reference materials
- Submission of the report to WHO in 2011
- If the characterization of the samples reveals suitable antibody titres it would also be possible to establish an international anti-HDV antibody reference material
WHO Biological Standardization Programme

- WHO is mandated by its Member States to "...develop, establish and promote international standards for biological products."

- In practice, biological products cover
  - Vaccines
  - Blood and blood products
  - Biological therapeutics
  - *In vitro* diagnostic devices

- WHO Biological Reference Preparations
WHO Biological Reference Preparations

- International Standard [expressed in IU]
- Reference Reagent
- International Reference Panel

- Endorsed and adopted by Expert Committee on Biological Standardization (decision making body)
- Catalogue on the website www.who.int/biologicals
WHO Biological Reference Preparations – PEI Projects

- 1st IS Anti-HBc (ECBS 2008)
- 1st International Reference Panel HBV Genotypes
  - NAT assays (ECBS 2009)
  - HBsAg tests (ECBS 2010)
- 1st International Reference Panel B19V Genotypes (Collaboration with CBER/FDA)
- New proposals ECBS October 2009
  - 1st IS HDV RNA
  - 1st IS HEV RNA
  - 1st IS HCV core
Thank you for your attention!