Quantitative HBV DNA measurements and the management of infected health care workers

A.A. van der Eijk

Department of Virology, Erasmus MC, Rotterdam, the Netherlands
Introduction

- Worldwide since 1970s, 45 HCWs with HBV transmission described resulting in 434 infected patients.
- ± 500,000 operations per year in the Netherlands
- Since 1970 3 HCWs transmitting HBV identified in NL, resulting in 42 infected patients.
Public Health Policy

- United States
  Exclusion from performing EPPs is based on the HBeAg status only.

- European consensus group
  Propose a cut-off level of $10^4$ HBV DNA copies/ml and recommends that all HBeAg-positive HCWs should be excluded.
Public Health Policy

- **The Netherlands**
  
  All HCWs with HBV DNA > $10^5$ geq/ml are excluded. This cut-off minimizes both transmission risk and loss of valuable medical personnel.

- **UK**
  
  All HBeAg-positive HCWs are excluded, as well as HBeAg negative HCWs with HBV DNA > $10^3$ geq/ml.

*IGZ Bulletin: Preventie Iatrogene Hepatitis B, 2002*

*NHS Executive. Hepatitis B infected health care workers, 2000*
Factors associated with transmission risk:

- Serum HBV-DNA level
- HBeAg positivity
- Duration of surgery
- Volume of blood transmitted
- Route of transmission: percutaneous vs. mucosal
- Skill and medical condition of HCW

Frequent Mutations in the HBV Genome

- YMDD genotyping
- PreCore HBeAg minus
- PreS1 PreS2
- S promoter deletion
- MHBs deletion
- Vaccine escape
- Protein encoding sequences (ORFs) subtype adw
- Stop 1896 → HBe
- Fulminant hepatitis
- T, B Cell escape
- HBx truncation core promoter mutation
HBV transmission and HBeAg status

- Most cases involved HBeAg positive HCWs
- First described case of transmission by HBeAg negative HCW in 1993
- All e-negative surgeons who transmitted HBV to patients have a precore mutant.
  - G-to-A transition at nucleotide 1896 introducing a stop in codon 28 is most common.
  - No production of HBeAg despite continuing replication.
- HBV DNA levels surgeons: $10^4 - 10^9$ geq/ml

Incident Investigation Teams, NEJM 1997; Corden, J Clin Vir 2002
Quantitative detection methods for HBV

- **Signal amplification systems:**
  - Hybrid Capture System (Digene)
  - Branched DNA assay (Bayer)

- **Target amplification systems**
  - COBAS Amplicor (Roche Diagnostics)
  - NASBA with molecular beacon detection
  - TaqMan or Real Time Detection Assay
Ranges of HBV DNA assays

- **In House TaqMan ultra sensitive**: > 50 – 10,000,000,000
- **In House TaqMan standard**: > 373 – 10,000,000,000
- **Roche COBAS Monitor**: > 1,000 – 1,000,000
- **Digene HCS version 2.0 ultra sensitive**: > 8,000 – 10,000,000
- **Digene HCS 2.0**: > 140,000 – 1,700,000,000
- **Bayer bDNA 1.0**: > 200,000 – 2,000,000,000

More information becomes available...

Diagnosis usually demonstrated:
- The presence of a specific virus
  but now additionally will involve:
- The quantity in which the virus is present
  Quantitative analysis
    ✔ to follow the course of chronic infections
    ✔ to detect disturbances in host-pathogen interactions
    ✔ to make soundly based therapeutic decisions
    ✔ to determine levels of infectivity

- A more precise description of the virus involved
  Qualitative analysis
    ✔ subtypes, genotypes, variants, mutants, genotypic resistance
    ✔ increasingly relevant for management of the infection
Disease Management

Pharmaceuticals

Laboratory

Diagnostics

“An information-based, integrated approach to managing a disease, to optimize clinical and economic outcomes.”
Percutaneous injury rates in surgery

- Surgeon’s percutaneous injury: 7% of operative procedures in general, gynecological, cardiac, orthopedic, or trauma surgery.

- Recontact to patient’s wound by sharp object causing surgeon’s injury: 32% of observed injuries to surgeon.

- Potential transmission of viral hepatitis: 2-3%.

Tokars, JAMA 1992
### Estimation of infectious particles transmitted by needlesticks with a HBV DNA level of \(10^5\) geq/ml

<table>
<thead>
<tr>
<th>Event</th>
<th>µl serum transmitted(^1)</th>
<th>Infectious particles transmitted(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suture needle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.33 mm needle, 5 mm penetration</td>
<td>0.03</td>
<td>&lt;1</td>
</tr>
<tr>
<td>1.12 mm needle, 5 mm penetration</td>
<td>0.23</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hollow needle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.07 mm needle, 2 mm penetration</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>1.07 mm needle, 5 mm penetration</td>
<td>0.44</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^1\) Calculation infectious particles: volume of serum in ml \(\times\) HBV DNA concentration in geq/ml \(\times\) 0.10 (a)

\(^*\) Number of infectious HBV particles \(\approx\) 10% of total number of HBV particles

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\(^1\) Bennett, J Am Coll Surgeons 1994; \(^2\) Heermann, J Clin Microbiol 1999
Infectious particles transmitted by maternal-fetal transfusion and risk of transmission.

<table>
<thead>
<tr>
<th>HBV DNA level (geq/ml)</th>
<th>Transmission risk</th>
<th>Infectious particles transmitted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; $10^8$</td>
<td>22%</td>
<td>6400</td>
</tr>
<tr>
<td>$10^7$</td>
<td>4%</td>
<td>640</td>
</tr>
<tr>
<td>$10^6$</td>
<td>1%</td>
<td>64</td>
</tr>
<tr>
<td>$10^5$</td>
<td>$\approx 0%$</td>
<td>6</td>
</tr>
</tbody>
</table>

0.64 µl serum transmitted during delivery by maternal-fetal transfusion

* Calculation infectious particles: volume of serum in ml x HBV DNA concentration in geq/ml x 0.10 (a)

a) Number of infectious HBV particles $\approx 10\%$ of total number of HBV particles

### Cases of doctor-to-patient of HBV

<table>
<thead>
<tr>
<th>Author</th>
<th>HCW’s profession</th>
<th>HBV DNA (geq/ml)</th>
<th>Quantification technique</th>
<th>Time sample taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harpaz et al., 1996</td>
<td>Thoracic surgeon</td>
<td>$1.0 \times 10^9$</td>
<td>Semiquantitative PCR dot-blot hybridization, with serum containing $10^8$ chimpanzee-infectious particles as comparison.</td>
<td>4 months after transmission</td>
</tr>
<tr>
<td>The Incident Investigation Teams, 1997</td>
<td>(1) General surgeon (2) Gynaecologist (3) Gynaecologist (4) General surgeon</td>
<td>$1.0 \times 10^7$ $4.4 \times 10^6$ $5.5 \times 10^6$ $2.5 \times 10^5$</td>
<td>Liquid Hybridization and enzyme-linked oligonucleotide assay</td>
<td>12 weeks after transmission</td>
</tr>
<tr>
<td>Molyneaux et al., 2000</td>
<td>Surgeon</td>
<td>$1.03 \times 10^6$</td>
<td>Lightcycler PCR</td>
<td>Unknown</td>
</tr>
<tr>
<td>Spijkerman et al., 2002</td>
<td>Surgeon</td>
<td>$5.0 \times 10^9$</td>
<td>Limited dilution PCR</td>
<td>1 year after identification first infected patient.</td>
</tr>
<tr>
<td>Corden et al., 2003</td>
<td>(1) Surgeon (2) Surgeon (3) Surgeon (4) Surgeon (5) Surgeon (6) Surgeon</td>
<td>$1.12 \times 10^8$ $2.55 \times 10^5$ $6.72 \times 10^5$ $6.35 \times 10^4$ $4.20 \times 10^8$ $9.47 \times 10^8$</td>
<td>Chiron Quantiplex Branched DNA assay and Roche Amplicor HBV DNA monitor assay</td>
<td>At least 3 months after transmission in all surgeons.</td>
</tr>
</tbody>
</table>

EHJC Buster, AA van der Eijk et al, Antiviral Research 2003;60(2):79-85
Implications of choosing a HBV DNA level as a cut-off level

- Variability in time of HBV DNA levels in HBV carriers
- Reliability and reproducibility of the molecular diagnostic tests.
HBV DNA levels in HBeAg negative patients

- Martinot-Peignoux et al. J Hepatol (2002);36:543-46
  - Quantitative HBV DNA levels in inactive HBsAg carriers
  - Mean HBV DNA concentration 1300 copies/ml
  - 98% of sera of inactive HBeAg negative carriers contained HBV DNA levels below $10^5$ copies/ml

  - Evidence for fluctuations in HBV DNA levels in HBeAg negative HBV carriers
Single sampling vs repeated testing

- Frequency of testing in relation to maximum HBV DNA level and the safety margin needed to account for fluctuations in HBV DNA level and variability of assay used for quantifying HBV DNA.
Reliability and reproducibility

- The need for internationally defined reference standards

- Since December 7, 2003 the use of an internal calibration standard is mandatory to standardise the commercial as well as commercial kits

- A standard is developed for HBV. However, this standard is developed only for genotype A
Geographic distribution of HBV genotypes
Reliability and reproducibility

- Qualitative and quantitative assays must yield reproducible results

- inter- and intra-assay variability is more profound in samples with low HBV DNA level

- The use of an internal control is imperative to monitor the quality of extraction and amplification
Discussion

1. Should HBV DNA be measured instead of HBeAg?

- Active replication of HBV is associated with the presence of HBeAg
- Knowledge of HBV DNA levels in HBeAg-negative persons makes exclusion of HCW solely based on HBeAg status only obsolete
Discussion

2. Which level of HBV DNA is acceptable to prevent transmission of HBV from HCW to patient during EPPs?

- Choosing a low level ($10^3$ copies/ml) it must be realized that
  - inter- and intra-assay variability is more profound in samples with low HBV DNA level
  - repeated testing will lead to a greater proportion of exclusion
- Regular monitoring of HBV DNA levels can narrow safety margin
- Introduction of internationally defined reference standards for all genotypes as well as participation in international quality control programs is required
Discussion

3. To what extend is the loss of valuable HCWs acceptable?

- Vaccination against HBV is safe and should be mandatory
- Each HCWs who carries HBV must be referred to a hepatologist
- Antiviral therapy may reduce the viral load and thus may prevent unnecessary exclusion of valuable medical personnel
Discussion