Molecular characterization of hepatitis B virus strains infecting blood donors with high HBsAg and undetectable HBV DNA levels: implications for blood safety and screening policy

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## Relative efficacy of HBV screening assays

<table>
<thead>
<tr>
<th>HBV infection features</th>
<th>Detected by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBsAg</td>
</tr>
<tr>
<td>Window period</td>
<td>No</td>
</tr>
<tr>
<td>Primary OBI</td>
<td>No</td>
</tr>
<tr>
<td>2nd window period</td>
<td>No</td>
</tr>
<tr>
<td>Chronic infection</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-HBc+ OBI</td>
<td>No</td>
</tr>
<tr>
<td>Anti-HBs only OBI</td>
<td>No</td>
</tr>
<tr>
<td>Anti-HBc only</td>
<td>No</td>
</tr>
<tr>
<td>HBsAg only</td>
<td>Yes</td>
</tr>
</tbody>
</table>
**HBV screening in French blood donations**

- **1970**
  - HBsAg (ABBOTT PRISM® HBsAg)
- **1980**
  - Anti-HBc (ABBOTT PRISM™ HBcAb)
- **1990**
  - HBV DNA (Procleix-Ultrio™)
- **2000**
- **2010**

- High sensitivity and adequate specificity
- Pre-seroconversion window period & occult infections
  - Estimated HBV residual risk: 1 in 4 millions donations
- **But:**
  - High cost
  - Redundancy of HBsAg and HBV DNA direct markers
Maintaining HBsAg testing?

- Cost reduction of blood testing
- Complementarity of anti-HBc and HBV DNA testing (Enjalbert et al. Transfusion 2014;54:2485-95)
- Anti-HBc testing issues on blood availability in high endemic settings
- Potential impact on blood safety?
Distribution of HBV markers in French blood donors

- Period: 2010-2013
- Excluding overseas territories
- 10 186 279 donations tested → 806 HBV reactive (≈ 1/10,000)
### HBsAg & HBV DNA discrepant levels in 740 samples confirmed HBsAg+

<table>
<thead>
<tr>
<th>Sample screening</th>
<th>Number (%)</th>
<th>HBV DNA load (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Undetected</td>
</tr>
<tr>
<td><em><em>NAT</em> neg.</em>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 (5%)</td>
<td>13 (32%)</td>
</tr>
<tr>
<td><strong>NAT pos.</strong></td>
<td>699 (95%)</td>
<td></td>
</tr>
<tr>
<td>• HBsAg &lt; 100 IU/mL</td>
<td>58 (8%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>• HBsAg &gt; 100 IU/mL</td>
<td>641 (87%)</td>
<td>13 (2%)</td>
</tr>
</tbody>
</table>

*NAT: Procleix-Ultrio (LOD 12 IU/mL)
Hypotheses

- **Ratio**: 1 viral particle / 1,000-10,000 HBsAg
  - Natural course of infection
  - HBV genotypes

- **Hypotheses**:
  - NAT failure
  - Impaired viral replication

- **Infectivity?**
Objectives

- Prevalence of HBsAg+/ NAT non-reactive or non-repeatable reactive donations
- Detect and/or confirm HBV DNA presence
- Evaluate and compare performance of NAT assays to detect these samples
- Perform genetic characterization of the viral strains associated with this phenotype
- Evaluate viral replicative properties \textit{in vitro} as a surrogate marker of infectivity
Study design

HBV+ donations

INTS/DATS
Viral load, genotyping

Group 1 (n=13)
HBsAg +/DNA NR

DNA extraction (2-5mL plasma)

3 nested PCRs
whole genome
BCP/PC
Pre-S/S

Sequencing

Genotyping
Genetic variability analysis

Group 2 (n=16)
HBsAg +/DNA R or NRR & non-quantifiable

3 nested PCRs
whole genome
BCP/PC
Pre-S/S

Ultracentrifugation
(10-12 mL plasma)

Pos

Neg
HBV DNA amplification performance

Successful amplification rate (%)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCP/PC (296 bp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-S/S (1,434 bp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole genome (3,160 bp)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Preliminary results

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 13)</td>
<td>(n = 16)</td>
<td>(n = 29)</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>34</td>
<td>35.5</td>
<td>34.8</td>
</tr>
<tr>
<td>(mean; range)</td>
<td>(19 – 59)</td>
<td>(18 – 61)</td>
<td>(18 – 61)</td>
</tr>
<tr>
<td><strong>HBsAg (ng/mL)</strong></td>
<td>1,355</td>
<td>2,113</td>
<td>1,881</td>
</tr>
<tr>
<td>(median; range)</td>
<td>(110 – 39,500)</td>
<td>(150 – 19,030)</td>
<td>(110 – 39,500)</td>
</tr>
<tr>
<td><strong>HBV DNA confirmed</strong></td>
<td>12 (92%)</td>
<td>15 (94%)</td>
<td>27 (93%)</td>
</tr>
<tr>
<td><strong>HBV genotypes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>9</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>-</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>1</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>2</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>3</td>
<td>4 (15%)</td>
</tr>
</tbody>
</table>
Sequences analysis

EcoR1 3222

pol

Pre-S/S  G381A

Pre-core/core  HBx  G2020 T/C x6  C1935G X9  A2978T/C x3

Insert. 2 nt.  Delet. 5nt.

Pre-S/S  Pre-S1  Pre-S2 x3
Construction of HBV replicons

**Method 1**

1st PCR amplification with HBV-specific primers

- HBV DNA
- HBV primer 1
- Adapter
- SapI

2nd PCR amplification using adapters

- HBV genome
- Huh7 transfection & re-circularization with SapI

HBV genome expression & replication

**Method 2**

2 distinct PCR amplifications

- HBV DNA
- HBV primer 1
- Rest. Enz.
- EcoRI
- HBV primer 2

Cloning of 1.2 HBV construct

- HBV primer 3
- HBV primer 4

- Plasmid
- EcoRI

Huh7 transfection

HBV genome expression & replication
Preliminary conclusions & perspectives

● Conclusions:
  - Extremely low level of HBV DNA confirm in >90% of ID-NAT non-reactive blood donations with concomitant high HBsAg levels
  - Phenotype not associated with donor age or HBV genotype
  - Impaired viral replication rather than NAT failure is suggested
  - Mutations potentially affecting viral replication identified

● Perspectives:
  - Increase the number of samples and controls of various genotypes
  - Collaborative study (Croatia, Poland, Switzerland, South Africa, Malaysia,...)
  - Develop an in vitro HBV replication system
    • functional characterization of HBV variants
    • evaluation of infectious risk
    • increase knowledge about distinct molecular control of viral replication & HBsAg production → potential clinical implications
  - Funding

HBsAg ???
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