Diagnostic Tools for Occult Hepatitis B

Man-Fung Yuen

DSc, MD, PhD

Chair Professor
Li Shu Fan Medical Foundation Professor in Medicine

The University of Hong Kong
Outline

• Diagnosis of OBI
  – HBV DNA
    – Methods
    – Challenges
  – Anti-HBc
    – Prevalence

• Novel markers/ tests for OBI
  – Linearized HBsAg
  – Hepatitis B core-related antigen (HBcrAg)
    • HBV reactivation
OBI: Definition and Gold Standard of Diagnosis

- Definition: Detection of HBV DNA in blood or liver of HBsAg negative persons

- Detection:
  - Gold standard: Episomal HBV DNA in the liver
  - Commonly used: HBV DNA in the blood
  - Surrogate often used: anti-HBc in the blood

*Raimondo G et al., J Hepatol 2019 (in press)*
Diagnosis of OBI

- **Detection:**
  - Gold standard: Episomal HBV DNA in the liver
  - Commonly used: HBV DNA in the blood
  - Surrogate often used: anti-HBc in the blood

- **Nested PCR:**
  - More definite diagnosis of OBI
  - Positive for $\geq 2$ different genomic regions e.g. S, Precore-core, Polymerase, X regions

*Raimondo G et al., J Hepatol 2007;46:160-170*
HBV DNA Detection by nested PCR

Patient 1  2  3

No. of bands +ve  4  4  3  2  0  1

OBi  ✔  ✔  ✗

Wong DKH…Yuen MF. Hepatology 2011;54:829-36
Detection Rate using Different Genomic Regions

<table>
<thead>
<tr>
<th>No. of samples with detectable PCR</th>
<th>NT (n = 29)</th>
<th>P*</th>
<th>T (n = 30)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>27 (93%)</td>
<td></td>
<td>22 (73%)</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>18 (62%)</td>
<td>0.013</td>
<td>10 (33%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Core</td>
<td>13 (45%)</td>
<td>&lt;0.001</td>
<td>14 (47%)</td>
<td>0.020</td>
</tr>
<tr>
<td>Pol</td>
<td>19 (66%)</td>
<td>0.026</td>
<td>11 (37%)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

* Compared to X region

*Wong DKH…Yuen MF. Hepatology 2011;54:829-36*
Detection of HBV DNA in Blood

- Liver biopsy for HBV DNA measurement: gold standard
  - Invasive
  - Inconvenient

- Detection of HBV DNA in blood
  - In blood screening setting
    - Mini-pool NAT
    - Confirmatory test
  
  - Individual setting
    - Sensitive commercial assays
Challenge I: Detecting Blood HBV DNA

- Blood screening
  - NAT (lower limit of detection 2-4 IU/mL for individual sample)
  - Mini pool testing: dilution factor (usually 6 – 20 donations/ pool): decrease sensitivity significantly

- Individual testing
  - Approved standardized HBV DNA assays: LLOD 10 – 20 IU/mL
  - Challenges
    - Serum HBV DNA usually present in low concentrations and fluctuates with time
    - Intermittently detectable in same patients
    - Sensitivity of assays
  - Improve detection rate
    - Larger volume of sample for DNA extraction e.g. > 1 ml of serum/ plasma
Challenge II: Low Intrahepatic HBV Viral Load in OBI

- Viral load extremely low even in the liver
  - Technical difficulty: requires highly sensitive assays
  - Detection of HBV DNA in blood even more difficult

- **OBI detection rate: always underestimation**

*Wong DKH…Yuen MF. Clin Microbiol Infect 2016;22:e1-3*
Challenge III: HBV DNA Detectability Decreases with after Time of HBsAg Seroclearance (OBI)

- Measurement of HBV DNA (LLOD 1.1 IU/mL)
  - Detectability rate upon follow-up after HBsAg seroclearance
    - Within 1 year: 19 out of 142 patients (13.4%)
    - 5 – 10 years: 6 out of 99 patients (6.1%)
    - > 10 years: 1 out of 27 patients (3.7%)

Droplet Digital PCR Assays for OBI Detection?

• Technically, improves assay sensitivity

• Example
  – Intrahepatic cccDNA LLOD
    • Digital PCR vs. real time PCR
      – 10 – 100-fold more sensitive

*Caviglai CP et al. J Hepatol 2018;69:301-7*
Anti-HCV as Surrogate for OBI

• Real OBI vs. past infection
  – Low rate of HBV DNA detectability in anti-HBc positive samples
  – Usually < 1%

  – In blood screening setting
    • Cannot exclude seronegative OBI and “window period” HBV infection
    • Exclusion of blood products in areas with high prevalence of anti-HBc e.g. > 10%
      – Pressure on blood product availability
      – ? Infectivity
Prevalence of HBsAg –ve/ anti-HBc +ve in Different Countries

Seto WK, Yuen MF. In Immunosuppressives: Advances, Applications and Analyses (2017)
Anti-HBc Positivity Rate Increases with Age

Liu K…Yuen MF. J Infect Dis 2019 (in press)
Novel Tests to Diagnose OBI
Circulating Viral Particles in HBV

Yuen MF et al. Nat Rev Dis Primers 2018;4:18035
Conventional HBsAg Test (LLOD: 0.05 IU/mL)

Seto WK…Yuen MF. Hepatol Int 2013;7:98-105
Linearized HBsAg (LLOD: 0.005 IU/mL) & HBcrAg

Seto WK… Yuen MF. Hepatol Int 2013;7:98-105
Characteristics of HBcrAg: HB core-related Antigen Reagent

- HBeAg, HBCAg, and p22cr are HB core-related proteins having homologous region.
- Antibodies for homologous region are used to detect the three proteins.
- **Solid phase:** Three kinds of anti HBcr MoAbs
- **Conjugate:** Two kinds of anti HBcr MoAbs

Detection limit: 2 log U/mL
Measurement range: 3 – 7 Log U/mL
HBcrAg in CHB Patients and Patients with HBsAg Seroclearance (OBI)

Median HBcrAg levels in natural history of treatment-naïve CHB

- Seto et al. *16
- Maasoumy et al. *26

- Lower limit of detection: 2 log U/mL

Mak LY…Yuen MF. Aliment Pharmacol Ther 2018;47:43-54
Linearized HBsAg & HBcrAg in Patients with HBsAg Seroclearance (OBI)

Seto WK… Yuen MF. Clin Microbiol Infect 2014;20: 1173-80
Detectable Linearized HBsAg & HBcrAg in HBsAg Seroclearance (OBI)

55 patients with HBsAg seroclearance
- 16 (29.1%) had detectable linearized HBsAg
- 12 (21.8%) had detectable HBcrAg
  - 22 (40%) had detectable in either linearized HBsAg or HBcrAg
- only 1 (1.8%) patient had detectable HBV DNA

Detectable Linearized HBsAg & HBcrAg in HBsAg Seroclearance (OBI)

329 patients with HBsAg seroclearance

- 85 (25.8%) detectable linearized HBsAg
- 69 (21%) detectable HBcrAg
  - 133 (40.4%) detectable of either one
  - 21 (6.4%) detectable both
- Only 7 (2.1%) detectable HBV DNA

Seto WK… Yuen MF. Hepatol Int 2013;7:98-105
HBV Reactivation in HBsAg –ve, Anti-HBc +ve Patients Receiving Immunosuppressive Therapy

Patients receiving anti-CD 20

Cumulative 41.5% in 2 years

Patients receiving immunosuppressants after HSCT

Cumulative 40.8% in 2 years

Seto WK...Yuen MF J Clin Oncol 2014;32933:3736-43

Seto WK...Yuen MF. Hepatology 2017;65:1451-61
Detectable HBcAg at Baseline Associated with the Risk of for HBV Reactivation in HBsAg-/ anti-HBc + patients Immunosuppressive Therapy

Seto WK…Yuen MF. Am J Gastroenterol 2016;111:1788-95
Conclusions

- **Diagnosis of OBI**
  - Always a challenging task because of
    - Working on extremely low HBV DNA levels
    - HBV DNA levels fluctuate: ? Need to repeat tests
    - No good and reliable surrogates to replace HBV DNA
  
  - Prevalence always underestimated

- **Improvement on detection**
  - ? Digital PCR
  - Other serologic markers
    - Novel: HBcrAg
    - Improvement on the old: HBsAg (linearized)
Thank you