Whats new on HBsAg and other markers for HBV infection?

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Why diagnostic markers are important

• They are the basis for clinical decision makings
  – treatment or no treatment?
  – progress or no progress?
  – HCC or no HCC?
  – cured or not cured?
Viral markers associated with "hard" outcomes

HBV DNA

HBsAg, HBeAg

Yang et al. NEJM 2002
REVEAL study: Chen et al., JAMA 2006
HBeAg positive patients

HBsAg > 100,000 U/ml indicative for “immune tolerance” (33)
HBsAg > 25,000 U/ml has >90% PPV for liver fibrosis <F1 (32).

HBsAg levels <3.85log IU/ml are associated with moderate to severe fibrosis (36) (100%-sensitivity, 86%-specificity and 100%-negative predictive value (NPV) in genotype B and C patients (higher in GT A/D))

The challenge is to determine fibrosis or hepatic necroinflammation!
HBeAg negative patients

HBsAg < 1,000 IU/ml and HBV DNA <2,000 IU/ml has high PPV (83%-87.9%) in for inactive carrier phase and reduced risk for HCC (42, 44).

Low HBsAg titer (<200 IU/ml, <100 IU/ml) are also predictive for subsequent HBsAg loss (47–49).

Challenge: 10%–20% individuals may experience reactivation and 4-20% may revert back to HBeAg positive hepatitis.
On treatment marker – PEG-IFN

HBeAg pos.  
HBsAg <1,500 IU/ml at week 12 corresponds to 57% PPV for anti-HBe seroconversion and 17.6% HBsAg clearance. 
No decline of HBsAg until week 12 showed a NPV of 97%-100% for Genotype A and D. 
HBsAg at week 12 >20,000 IU/mL showed a NPV of 92%-98% for Genotype B and C. 
HBsAg > 20,000 IU/ml at week 24 associated with 100% NPV for anti-HBe seroconversion.

HBeAg neg.  
No HBsAg decline (any decline) and <2 log decline of HBV DNA showed a NPV of 100% for nonresponse in genotype D patients.
On treatment marker- NA

- In patients treated with tenofovir, a reduction in HBsAg level of at least 1 log by week 12 or 24 were **predictive for HBsAg loss** with a positive predictive value of up to 45% and a NPV of up to 97%.
- HBsAg levels <100 IU/ml after 2 years of NA treatment may help to predict **stable anti-HBe serconversion** and stable virological and biochemical response in HBeAg positive patients.
- Consolidation therapy of > 3 years and suppressed HBV DNA > 2 years increase the chance of stable off-therapy response in HBeAg negative patients who discontinue treatment. Low level of HBsAg (i.e. <100 IU/ml) are associated with **off-treatment response**.

How can HBsAg testing be improved?

HBsAg

preS1          preS2          S

Large HBs protein
Middle HBs protein
Small HBs protein
The ratio of LHBs, MHBs and SHBs is distinct for specific infection phases

<table>
<thead>
<tr>
<th></th>
<th>(a) ICs</th>
<th>(c) HBeAg-negative CHB</th>
<th>(a) vs (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHBs (log$_{10}$ ng/mL)*</td>
<td>1.9±0.5 (−1.9–2.92; 1 nd)</td>
<td>2.5±0.6 (0.7–3.6)</td>
<td>3.2×10$^{-7}$</td>
</tr>
<tr>
<td>MHBs (log$_{10}$ ng/mL)*</td>
<td>1.8±0.6 (0.8–2.9; 10 nd)</td>
<td>2.1±0.8 (0.1–3.5; 1nd)</td>
<td>0.0003</td>
</tr>
<tr>
<td>SHBs (log$_{10}$ ng/mL)*</td>
<td>3.1±1.1 (0.5–4.5)</td>
<td>3.6±0.5 (2.1–4.8)</td>
<td>0.0777</td>
</tr>
<tr>
<td>total HBsAg (log$_{10}$ ng/mL)*</td>
<td>3.1±1.1 (0.5–4.5)</td>
<td>3.7±0.6 (2.1–4.8)</td>
<td>0.4809</td>
</tr>
<tr>
<td>LHBs (%)*</td>
<td>2.3±1.6 (0.0–7.5)</td>
<td>6.0±3.3 (0.5–22.0)</td>
<td>3.1×10$^{-12}$</td>
</tr>
<tr>
<td>MHBs (%)*</td>
<td>1.8±1.9 (0.0–7.7)</td>
<td>4.4±4.3 (0.0–22.0)</td>
<td>8.3×10$^{-4}$</td>
</tr>
<tr>
<td>SHBs (%)*</td>
<td>95.9–2.6 (89.6–100.0)</td>
<td>89.6±5.8 (65.7–99.0)</td>
<td>4.1×10$^{-11}$</td>
</tr>
</tbody>
</table>

*Mean±SD (range).

LHBs may be a better marker than HBsAg to verify HBeAg negative infection (inactive carriers)

Major challenge – the HBV life cycle

HBeAg positive patients

HBeAg negative patients

We need to measure activity of cccDNA

Hepatitis B core-related antigen (HBcrAg)

HBcAg
HBeAg
p22cr

HBcrAg = composite marker, reflecting the activity of cccDNA
HBcrAg correlates well with HBV DNA and intrahepatic cccDNA

### HBV DNA

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Correlation coefficient</th>
<th>P value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>190</td>
<td>.79 (genotype B)</td>
<td>&lt;.001</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>.87 (genotype C)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Overall: .807</td>
<td>&lt;.001</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>HBeAg-positive: .847</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBeAg-negative: .632</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>.820^a</td>
<td>&lt;.001</td>
<td>21</td>
</tr>
<tr>
<td>138</td>
<td>Overall: .69</td>
<td>&lt;.0001</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>HBeAg-positive: .66</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBeAg-negative: .59</td>
<td>&lt;.0001</td>
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</table>

### Intrahepatic cccDNA

<table>
<thead>
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<th>No. of subjects</th>
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</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>.664</td>
<td>&lt;.0001</td>
<td>21</td>
</tr>
<tr>
<td>138</td>
<td>.70</td>
<td>&lt;.00001</td>
<td>22</td>
</tr>
<tr>
<td>31</td>
<td>.482</td>
<td>&lt;.0006</td>
<td>24</td>
</tr>
</tbody>
</table>

HBcrAg detectable in 78% of patients with undetectable HBV DNA due to antiviral therapy

Mak et al. Aliment Pharmacol Ther. 2018;47:43–54
EOT- HBcrAg as a marker to predict relapse after stop of NA treatment

HBcAg as a marker in HBeAg negative infection

Riveiro-Barciela et al Clin Microbiol Infect 2017. 4
HBcrAg as a marker in HBeAg negative infection

HBcrAg ≤3 log U/ml plus HBV DNA ≤2,000 IU/mL was highly accurate for identifying inactive carriers
Several applications of HBcrAg

**Natural History**

- **Spontaneous HBeAg seroconversion**
  - Baseline level < 4.9 log IU/mL, or ≥ 2 logs drop at week 28 \(^{36}\)

- **Spontaneous**
  - 79% had undetectable HBsAg.
  - In the 21% with detectable HBsAg, median level was 2.7 log IU/mL.

**HCC**

- **HCC development**
  - Baseline level > 4.67 log IU/mL
  - On treatment (NA level) > 3.89 log IU/mL \(^{56}\)

- **Recurrence of HCC after curative surgery**
  - HBcrAg at HCC diagnosis > 4.8 log IU/mL \(^{73}\)

**Antiviral therapy**

- **Treatment-induced HBeAg seroconversion**
  - Baseline level < 4.5 log IU/mL when given combination PEG-IFN + NA \(^{46}\)

- **HBsAg loss**
  - Baseline level < 3.7 log IU/mL when given PEG-IFN +/- NA \(^{47}\)

- **Liver function test improvement**
  - ALT and AST decrease when NA level > 3.7 log IU/mL \(^{52}\)

**Immunosuppression**

- **Occult HBV flare**
  - Baseline detectable HBcrAg \(^{80}\)

**Specificity, sensitivity, false positivity, false negativity, etc. for a certain cut-off value patients are lacking. Studies in caucasian**

Mak et al. Aliment Pharmacol Ther. 2018;47:43–54
HBV RNA – central for the HBV life cycle

Wang et al. Journal of Hepatology 2016 vol. 65 j 700–710
Serum HBV-RNA resembles intrahepatic viral RNA but not cccDNA

Quasispecies of serum HBV-RNA and intrahepatic HBV-RNA and cccDNA.

Wang et al. Journal of Hepatology 2018 vol. 68 j 16–24
Production of viral RNA is ongoing despite suppressed HBV DNA

Wang et al. Journal of Hepatology 2018 vol. 68 j 16–24
HBV-RNA correlates with necroinflammation and fibrosis

Serum HBV-RNA levels have the highest accuracy for distinguishing mild (score <2) from severe liver histopathology at 2.45 log10 copies/ml (PPV 80% NPV 89%)

Wang et al. Journal of Hepatology 2018 vol. 68 j 16–24
HDV RNA levels are influenced by several factors.
Summary

• HBV DNA, HBeAg and HBsAg remain the most important diagnostic marker
  – L-HBs may be a better marker than total HbsAg

• Current markers do not reflect the acitivity of cccDNA

• HBV RNA and HBcrAg emerge as valuable markers, which need further validation