Professor Florian van Boemmel
University of Leipzig, Germany
Novel hepatitis B biomarkers for profiling and guiding management

Florian van Bömmel
Universitätsklinik Leipzig
Sektion Hepatologie
Agenda

• HBV replication and circulating biomarkers
• Values of old and new biomarkers for determining the natural course of HBV infections
• Value HBV of biomarkers for monitoring treatment response
  – current treatments
  – future treatments
• Can immunological markers be helpful?
What should be expect from an ideal HBV biomarker?

- ability to stratify by disease stages and risk for complications (reactivation, cirrhosis, HCC)
- ability to predict functional cure (HBsAg loss)
- ability to predict definite cure (cccDNA eradication)
- helpful to identify treatment response before or early during treatment
Current and novel HBV biomarkers

Virus Entry → Nucleus

Uncoating → DNA-Recycling

DNA (+) → DNA (-) → (+)Strang-synthese

Uncoating → DNA-Recycling

DNA (+) → DNA (-) → (+)Strang-synthese

Transcription

pgRNA → cccDNA Formation → cccDNA → mRNA

Transcription + Reverse Transkription

Encapsidation

Translation

Secretion of viral Proteins

Secretion of HBV-RNA?

Secretion of Virions

Virions with HBV DNA

HBV RNA

HBcrAg

HBeAg

HBsAg

“HBcrAg” measures simultaneously HBeAg, HBcAg and p22cr

Serum HBV RNA is believed to exist in virus-like particles.

HBsAg consists of the components L, M and S

Correlation of serum HBcrAg levels with intrahepatic cccDNA

- HBcrAg levels are likely associated with transcription activity from intrahepatic cccDNA

- correlation between HBcrAg and HBV cccDNA (r= 0.692) demonstrated in serum and liver samples from 57 patients (p<0.001)

HBV-RNA in serum probably exists in virus like particles

Wang J et al., J Hep 2016
Serum HBV RNA is likely a mixture of intact (poly-A), spliced, and polyA-free pgRNA\textsuperscript{1}

Different serum HBV RNA species

\textbullet\ i) HBV pgRNA, pcRNA and viral mRNAs
\textbullet\ ii) splice variants
\textbullet\ iii) full length or truncated HBV RNAs

\textsuperscript{1}Liu S et al. Hepatology 2018; epub
## Methods for quantification of serum HBV RNA

<table>
<thead>
<tr>
<th>method</th>
<th>RT primer</th>
<th>Primer sites</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-qPCR#</td>
<td>RNA isolation (including DNase treatment) and subsequent PCR method with specific primers</td>
<td>HBV specific</td>
<td>X, C or S region</td>
</tr>
<tr>
<td>ddPCR#</td>
<td>Droplet digital PCR</td>
<td>HBV specific</td>
<td>X or C region</td>
</tr>
<tr>
<td>3’-RACE-based</td>
<td>oligo(dT) primer plus a unique artificial anchored sequence - to generate cDNA</td>
<td>Oligo(dT) Primer</td>
<td>Poly A tail</td>
</tr>
<tr>
<td>QuantiGene assays#</td>
<td>hybridization-based and use branched DNA (bDNA) signal amplification technology – measurement via Luminometer</td>
<td>n/a</td>
<td>X region</td>
</tr>
<tr>
<td>Indirect</td>
<td>Serum HBV RNA minus HBV DNA determined by real-time PCR</td>
<td>HBV specific</td>
<td>PreC and C</td>
</tr>
</tbody>
</table>

*In order to avoid DNA contamination during RT-qPCR, DNase I treatment of the nucleic acids extracted from serum is required*

Adapted from Liu S et al. Hepatology 2018; epub
Comparison of the 3’RACE method and specific RT-qPCR

Regression analysis of pgRNA results and concentrations measured using a RACE method shows agreement

Butler E et al. Hepatology 2018;68:2106-2117
Serum HBV pgRNA as serum marker for cccDNA activity

Humanized mouse model infected with HBeAg-positive wild-type HBV
Influence of HBeAg status and ALT levels on circulating HBV RNA

Natural history study in 574 treatment naïve patients

Correlation of circulating HBV RNA with HBV RNA, HBsAg and qHBeAg levels

Courses of HBV markers in the natural course of CHB

HBV bio markers show similar trends across disease stages, but differences may be important for their use

modified from: C. Höner zu Siederdissen et al. / Best Practice & Research Clinical Gastroenterology 31 (2017) 281–289
How long remain circulating HBV biomarkers detectable?

**Figure 1**: Scheme depicting the design of the retrospective study.

- Patients with HBeAg neg. CHB (n=96)
- qHBV DNA
- qHBsAg
- qHBV RNA
- qHBcrAg

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>LMV or ADV or LdT</th>
<th>TDF or ETV</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.5±30 (range, 1-101) months</td>
<td>52±11 (range, 7-81) months</td>
<td></td>
</tr>
</tbody>
</table>
Circulating HBV biomarkers decrease during long term NA treatment

HBV RNA:

HBcrAg: detectable in all (> 3 log) but undetectable in all but 4 samples from year 4 on

van Bömmel F, ... Lampertico P. EASL 2019
• Value of old and new biomarkers for determining the natural course of HBV infections
Distinct HBcrAg levels across phases of chronic hepatitis B
HBcrAg levels may predict risk of liver fibrosis in HBeAg - patients

An HBcrAg cut-off value of 4 LogU/ml was able to distinguish between HBeAg HBcrAg+ patients (n = 21) with mild vs. minimal liver disease (described as fibrosis and/or necroinflammatory activity scores >2 or <1, respectively) (PPV 0.44; NPV 0.92; p <0.0001; 95% confidence interval 0.06 to 0.55).

Association between serum and intrahepatic HBV RNA levels and liver histopathology in patients under entecavir treatment

A serum HBV-RNA level cut-off of $2.45 \log_{10}$ copies/mL had the highest accuracy for distinguishing mild (score <2) from severe liver histopathology

Undetectable HBV RNA can distinguish inactive carriers from active stages

- HBV RNA undetectable in 34 ICs with follow up over up to 5 years

Krauel A., van Bömmel, F. AASLD 2015
HBsAg levels can determine the stage of HBV infections
Different pattern of HBsAg composition during acute HBV infection

Strong decrease of PreS1 (LHBs) and PreS2 (MHBs) during acute HBV

Gerken et al. Gastroenterology 1987
LHBs and MHBs show stronger association with IC stage than total HBsAg levels

Pfefferkorn M, ...van Bömmel F. GUT 2018
Circulating HBsAg may have different sources

LHBs and MHBs are believed to be only derived from cccDNA

from: Hadziyannis et al, Genes 2018
• Value of HBV biomarkers for monitoring treatment response
  – current treatments
HBcrAg kinetics are associated with response to NA mono or combination treatment

Early differences in HBcrAg levels in HBeAg neg. patients with or without respond to PegIFN

HBV RNA in serum is an early marker for HBeAg seroconversion during treatment with NUCs

van Bömmel F. et al., Hepatology 2015
Serum HBV DNA and HBV RNA before and after start of entecavir in patients (N=11)

Wang J et al. J Hepatol 2018; 65:700
Performance of HBV biomarkers in predicting HBeAg and HBsAg loss during NA treatment
Serum HBV RNA in predicting treatment response to Peg-IFNa-2a in HBeAg-positive patients

Early prediction of HBeAg seroconversion

Serum HBV RNA levels

Peg-IFNa-2a monotherapy

Peg-IFNa-2a + lamivudine combination

van Bömmel F et al. JID 2018:218
Serum HBV RNA in predicting treatment response to Peg-IFNa-2a in HBeAg-positive patients

van Bömmel F et al. JID 2018:218
Association of HBV RNA (pgRNA virion levels) and viral rebound after discontinuation of NUCs

<table>
<thead>
<tr>
<th>HBV RNA</th>
<th>Viral rebound (n)</th>
<th>No viral rebound (n)</th>
<th>Total (n)</th>
<th>*p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>21</td>
<td>0</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Below the LoQ</td>
<td>3</td>
<td>9</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Total (n)</td>
<td>24</td>
<td>9</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-Square test; n, number of CHB patients.
Preliminary data from FINITE study show an association of re-treatment with kinetics of HBV RNA and HBcrAg after NAdisc

w/o re-treatment at week 144 with re-treatment at week 144

van Bömmel F et al. AASLD 2018; poster
Serum HBV RNA as a predictor of HBsAg seroreversion

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Composite Reversion (n = 12)</th>
<th>No Composite Reversion (n = 20)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiviral drugs:</td>
<td>9 (75)</td>
<td>13 (65)</td>
<td>0.703</td>
</tr>
<tr>
<td>Peg-IFN combined with NAs, n (%)</td>
<td>3 (25)</td>
<td>7 (35)</td>
<td>0.703</td>
</tr>
<tr>
<td>Peg-IFN alone, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of HBsAg loss before drug withdrawal (weeks), $^9$ median (range)</td>
<td>42.5 (24-84)</td>
<td>43 (0-100)</td>
<td>0.585</td>
</tr>
<tr>
<td>Follow-up time after drug withdrawal (weeks), $^9$ median (range)</td>
<td>48 (20-92)</td>
<td>171 (48-273)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HBV RNA positive, n (%)</td>
<td>7 (58.33)</td>
<td>0 (0)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The positive and negative predictive value of end of treatment serum HBV RNA levels was 100% and 80%, respectively.

• Value of HBV biomarkers for monitoring treatment response
  – future treatments
The main targets & drug discovery efforts

- Entry inhibitors
- Targeting cccDNA
- Inhibitors of HBsAg release
- RNA interference
- NUCs “Polymerase inhibitors”
- CpAMs “Capsid inhibitors”
- Immune modulation
  - Toll-like receptors agonists
  - Anti-PD-1 mAb
  - Vaccine therapy
  - Redirection of T cells
- CD8+ T cell
- Dysfunctional T-cell response
- Insufficient B-cell response

Treatment with siRNA directed against S-gen HBV mRNA produces changes in HBcrAg levels
Influence of the capsid assembly modulator NVR 3-778 on viral replication

NVR 3-778 inhibited the production of secreted HBV RNA and intracellular HBV RNA encapsidation in HepG2.2.15

In contrast, the nucleotide analog tenofovir (TFV) inhibited HBV DNA production and did not inhibit but actually increased the levels of both intracellular encapsidated HBV RNA and secreted HBV RNA in a dose-dependent manner.
Influence of the capsid assembly modulator NVR 3-778 on viral replication in HBeAg+ patients with chronic HBV infection

NVR 3-778 influences the decrease on serum HBV RNA after the end of treatment

Patients received tenofovir disoproxil fumarate (TDF) on study Day 30, two days after completing study treatment with NVR 3-778 400mg QD or pegIFN

## Influence of new antiviral treatments on serum HBV RNA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition mechanism</th>
<th>Effect on HBV RNA levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAMs</td>
<td>Inhibition of viral replication (rcDNA formation), production of secreted HBVDNA virions and intracellular HBV RNA encapsidation</td>
<td>↓ Serum HBV RNA levels</td>
</tr>
<tr>
<td>RNAi</td>
<td>siRNAs in ARC-520 were designed to target all HBV mRNAs</td>
<td>↓ HBV RNA levels in liver tissue</td>
</tr>
<tr>
<td>3p-siRNA</td>
<td>bi-functional 3p-siRNAs combining RIG-I–mediated immune activation with target gene silencing</td>
<td>↓ HBV RNA levels in liver tissue</td>
</tr>
<tr>
<td>Dihydroquinolizinone (DHQ)</td>
<td>Inhibition via knockdown of PAPD5 and PAPD7</td>
<td>↓ HBV RNA levels (due to double knock out of PAPD5 and PAPD7)</td>
</tr>
<tr>
<td>NAPs</td>
<td>Blocking the release of subviral particles from infected or „integrated“ hepatocytes</td>
<td>No direct influence on the HBV RNA levels</td>
</tr>
</tbody>
</table>
HBsAg-loss during long term treatment with tenofovir

Marcellin P, et al. APASL 2012
HBsAg composition changes early in patients achieving HBsAg loss during treatment with tenofovir

HBsAg components as response markers for new and more effective treatments?

Pfefferkorn M, et al. Submitted 2019
Mechanisms of viral persistence

cccDNA reservoir
Antigenic load
Liver tolerance
HBV persistence

Defective CD8+ response
Defective B cell response
Inefficient innate response
Defective immune responses

Anti-HBc shows reverse kinetics than viral BM in the natural course

Lin CL, Kao JH. CMH 2016
Risk of relapse after NAdisc is associated with anti-HBc levels

Heng C et al. Clin Gastroenterol Hepatol 2019
Restoration of antiviral immunity

Distinct T-cell populations associated with flare after NA discontinuation

Rivino L, Kennedy P, et al. JCI 2018
Repression of intrahepatic expression of innate immunity genes in CHB patients

Factors associated to a SHARPER repression:
- HBeAg(-) status
- high qHBsAg
- Low disease activity (VL<2000 & ALT<N)

67 GENES BELONGING TO:
- PRRs/TLRs
- antiviral effectors & ISGs
- type I/III IFN pathways
- APOBECs
- liver disease-related pathways

Lebossé, Testoni et al, J Hepatol 2017
HBV biomarkers: Summary

• HBsAg ultimate marker for functional cure (HBsAg loss)
  – HBsAg components may allow early identification of responders!
• other end points (e.g., sustained response) are not yet well defined, but are accurately predictable by HBV RNA or HBcrAg
• on treatment kinetic of HBV RNA and HBcrAg may predict HBeAg seroconversion or sustained response
  – value for novel treatments to be explored
• studies comparing multiple BMs are warranted
• Immunologic BMs may represent an additional benefit to viral markers and need to be explored
Thank you for your attention!