Summary of the Viral Serum Biomarker Landscape

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Challenges in HBV cure

> 257 million carriers
887,000 death/year

90% of HCC are virally induced
Large majority due to HBV

Current antivirals: life-long therapies to maintain viral suppression

Obstacles to HBV elimination and gaps to cure

Intrahepatic viral reservoir: long-lived cccDNA
High viral antigen load
Defective innate and adaptive immune responses

Cancer death: ranks 3rd
Cancer incidence: ranks 6th
cccDNA is the key molecule of HBV lifecycle and is responsible of viral persistence

cccDNA is a minichromosome epigenetically regulated

HBV replicates via a RNA intermediate called pgRNA

Integrated HBV sequences do not account for viral replication, but may produce HBV RNA and proteins

Adapted from Cohen, Science 2018
HBV life cycle and NUC treatment

NUC treatments do not directly target cccDNA.

cccDNA transcription and viral antigens production are not blocked by NUC.
SOC therapy does not cure HBV: what do we want/need to achieve?

**THERAPY**

- Partial cure
- Functional cure
- Complete cure
- Sterilizing cure

**SERUM**

- HBsAg
- HBV DNA

**LIVER**

- cccDNA
- HBV DNA integration

Log10 c/mL

- Change from baseline

+/- anti-HBsAb

**Time**

Lok Hepatology/ J Hep joint publication; 2017
What should be expected from the ideal HBV biomarker?

- Non-invasive
- Helpful to identify treatment response before or early during treatment
- Ability to stratify by disease stages and risk for complications (reactivation, cirrhosis, HCC)
- Helpful in the management of patients
  - Who must be treated and when
  - If and when to stop treatment
  - Predict "good" vs "bad" flares
- Reflect intrahepatic cccDNA pool and activity
  - Predict functional cure (HBsAg loss)
  - Predict complete cure (cccDNA elimination)

« Gold standard »: intrahepatic assessment of
  - cccDNA amount
  - cccDNA activity (viral RNA production)

Hampered by the necessity of using invasive techniques (biopsy) and by technical limitations
Viral serum biomarkers: serum HBV DNA and HBeAg

- **Infectious Virions**
- **Cytoplasm**
  - pgRNA
  - Nucleocapsid
- **Nucleus**
  - rcDNA
  - cccDNA

**Subgenomics RNAs**
- Spliced
- Truncated

**Integrated Sequences**

- (Adapted from Testoni, Sem Liver Dis 2017)
Suppression of serum HBV DNA levels correlates with significant decrease in HCC risk

**ERADICATE-B study**

<table>
<thead>
<tr>
<th>HBV DNA (IU/mL)</th>
<th>Risk of HCC (per 100,000 person-year)</th>
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<tbody>
<tr>
<td>&gt;200,000</td>
<td>High</td>
</tr>
<tr>
<td>20,000–199,999</td>
<td>Medium</td>
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<tr>
<td>2,000–19,999</td>
<td>Medium</td>
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<tr>
<td>200–1,999</td>
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<td>&lt;2,000 and HBsAg ≤1,000</td>
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Risk of HCC correlates with serum HBV DNA levels. Chan, H L-Y. J Gastro 2012

NUC-induced serum HBV DNA suppression does not eliminate cccDNA

Serum HBV DNA suppression under NUC is not complete

**Boyd, JHep 2016**

Need for a more sensitive assay?

**Yuen, LBP30, ILC2020**
Serum biomarkers and CHB phases

CHB patients classification based on HBeAg, Serum HBV DNA, ALT levels and liver injury markers

Does not completely reflect the complexity of CHB patients

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 Modified from Charre, Antiviral Res 2019, and Fanning, Nat Rev Drug Discovery 2019

→ Harry Janssen talk
Viral serum biomarkers: HBsAg

HBsAg

Infectious Virions

SVPs

Extracellular / Serum

Cytoplasm

Nucleus

Nucleocapsid

pgRNA

rcDNA

cccDNA

Subgenomics RNAs

Integrated Sequences

HBsAg

HBeAg

Viral serum biomarkers:

- HBsAg
- HBeAg

Infectious Virions:

- Extracellular / Serum
- Cytoplasm
- Nucleus

Subgenomics RNAs:

- Spliced
- Truncated

Viral serum biomarkers:

- HBsAg
- HBeAg

Adapted from Testoni, Sem Liver Dis 2017
In patients with low serum HBV DNA, HBsAg levels contribute to HCC risk.

**HBsAg seroclearance** = standard definition of «**functional cure**», goal for new therapeutic strategies

In patients with low serum HBV DNA, HBsAg levels contribute to HCC risk

**HBsAg loss allows safe discontinuation of antiviral therapy** (immune control of the virus?)

**Do we really need HBsAg loss?**
STOP-NUC study *(vanBommel LB06; ILC 2020)*

Need for a surrogate marker of HBsAg loss to be used in early phase clinical trials

Loss or decline? How much decline? How fast?
Viral serum biomarkers: HBsAg components

HBsAg components better discriminate CHB phase 3 patients (Inactive Carriers)

Different dynamics of HBsAg components under treatment

- Results summary -

Pfefferkorn, JHep 2020
Viral serum biomarkers: HBcrAg

(Adapted from Testoni, Sem Liver Dis 2017)
Viral serum biomarkers: HBcrAg

Correlates with intrahepatic cccDNA transcriptional activity

Might predict HCC incidence and discriminate patients with different levels of liver disease

Differs among CHB phases and might discriminate «active» from «inactive» HBeAg(-) patients
Viral serum biomarkers: HBcrAg

Correlates with intrahepatic cccDNA transcriptional activity


Might predict HCC incidence and discriminate patients with different levels of liver disease

(Honda, JID 2016; Testoni, JHep 2019)

Differs among CHB phases and might discriminate « active » from « inactive » HBeAg(-) patients

(Seto, CMID 2014; Maasoumy, CMID 2015; Riveiro-Barciela, CMID 2017; Testoni, JHep 2019)

PCA analysis of non-treated CHB patients

Limited use in HBeAg(+) patients

Need for better sensitivity?

High cccDNA transcriptional activity
High fibrosis, necroinfl. Activity
Could not be identified by serum HBV DNA and HBsAg alone
Viral serum biomarkers: circulating HBV RNA

(Aadapted from Testoni, Sem Liver Dis 2017)
Viral serum biomarkers: which RNA? Where?

Correlate with intrahepatic cccDNA transcriptional activity (Hu-Hep mice)  
(Giersch, JHep 2017)

Differ among CHB phases  
(Wang, JVH 2018)

May help predict response to IFN and IFN/NUC and HBe loss in HBeAg(+) patients  
(Jansen, 2016; vanBömmel 2018)
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<td>• Wang J, Jhepatol, 2016</td>
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<td>• Jansen L, J InfectDis 2016</td>
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<td>• Prakash K, Virol J 2018</td>
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<td>• Lam AM, Antimicrob Agents Chemother 2017</td>
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<td>• Hacker HJ, Ann N Y Acad Sci 2004</td>
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<td>HBx RNA</td>
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<td>Naked Capsids</td>
<td>• Bai L, J Virol 2018</td>
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<td>• Bai L, J Virol 2018</td>
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Both cccDNA and integrated sequences may contribute to serum HBV RNA population.

According to the different quantification strategies, the biological/clinical significance may be significantly different.

Adapted from Liu, Hepatology 2018
Viral serum biomarkers: target engagement vs endpoint prediction

(Adapted from Testoni, Sem Liver Dis 2017)
Viral serum biomarkers: target engagement vs endpoint prediction

CAMs

Infectious Virions

Circulating RNA

HBsAg

HBcrAg

Extracellular / Serum

Nucleocapsid

Subgenomics RNAs

Cytoplasm

pgRNA

Integrated Sequences

Nucleus

rcDNA

cccDNA

HBeAg

3,5 kb

Spliced

Truncated

0,7 kb

2,1 / 2,4 kb

(Aadapted from Testoni, Sem Liver Dis 2017)
Viral serum biomarkers: target engagement vs endpoint prediction

No one fits all!!!
Need of an « integration assay »?

(Adapted from Testoni, Sem Liver Dis 2017)