Δυνητικές κλινικές εφαρμογές και σημασία του προσδιορισμού του cccDNA και του HBV-RNA στη χρόνια ηπατίτιδα B

Potential clinical implications and importance of cccDNA and HBV RNA determination in chronic hepatitis B

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• What is HBV cccDNA and HBV RNA?

• How are they detected?

• Are there any significant clinical correlations with these “markers”? 

• Are they potential biomarkers?
HBV structure and genome organization

Robert G. Gish et al. Antiviral Research, Volume 121, 2015, 47–58

eh.s.wsu.edu/labsafety/bloodbornediseases.html
HBV covalently closed circular DNA

cccDNA

Lucifora and Protzer, J Hepatol 2016

nucleus
cccDNA transcriptional activity is regulated by epigenetic mechanisms involving viral and host transcription factors.

Joseph Che-Yen Wang et al. PNAS 2014;111:11329-11334
- **cccDNA** is a template for the transcription of all HBV RNAs and of progeny virus RNA (some HBV RNAs but not pgRNA are transcribed from integrated virus as well)
- Difficult target for antiviral treatment and immune response
- Responsible for the chronicity of HBV infection
- Detected in **liver**: in the nucleus of infected hepatocytes
- Surrogate markers can be detected and measured in serum (e.g. HBcrAg, HBsAg, HBV DNA)

- **PgRNA** is a product of active transcription of cccDNA
- Located in the **liver**, in the nucleus and in the cytoplasm (encapsidated) of infected hepatocytes
- Encapsidated pgRNA can be released in the **serum** from infected hepatocytes (esp. in active transcription with RT block)
- The reverse transcription of pgRNA to (-) DNA is the target for antiviral treatment
- Surrogate marker for cccDNA
Detection of cccDNA and HBV RNA in clinical specimens is a challenge

- For cccDNA a liver biopsy is needed
- **Sensitivity**
  - low copy numbers (down to 0.01, mean 10 co/ hepatocyte)
  - Sample quality
  - Storage conditions
- **Specificity** big concern
  - Difficult discrimination cccDNA from liDNA or rcDNA
  - HBV RNA contamination with HBV DNA
- **Different protocols** and no consensus or standards
  - Real Time PCR

Lucifora and Protzer, J Hepatol 2016
Clinical studies on intrahepatic cccDNA and HBV RNA

• Natural History
• HCC
• Treatment
HBeAg (+) vs HBeAg (-)

Intrahepatic cccDNA and pgRNA are higher in HBeAg (+) patients

Laras et al. Hepatology, 2006
cccDNA During the phases of HBV Natural History

<table>
<thead>
<tr>
<th>IH cccDNA (log copies/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHB</td>
</tr>
<tr>
<td>CHB-IT</td>
</tr>
<tr>
<td>CHB-IC</td>
</tr>
<tr>
<td>CHB-LR</td>
</tr>
<tr>
<td>CHB-ENH</td>
</tr>
</tbody>
</table>

Med 0.18 4.80 3.81 0.22 0.97 copies/cell

*P = 0.002

*P = 0.003

Li W et al Plos One 2014
cccDNA During HBV Natural History

• Detection in HBsAg negative

Werle-Lapostolle et al. GASTROENTEROLOGY 2004;126:1750–1758
cccDNA and HCC

cccDNA is higher in non-tumorous tissue

Danny Ka-Ho Wong et al. J. Hepatol. 2006
Positive correlation between ih cccDNA and pgRNA with HBsAg levels in serum

HBsAg in HBV-HCC

**Tumor**

A. $R = 0.11; p = 0.35$

B. $R = 0.28; p = 0.01$

C. $R = 0.15; p = 0.28$

**Non-neoplastic liver tissue**

D. $R = 0.15; p = 0.17$

E. $R = 0.50; p < 0.001$

F. $R = 0.34; p = 0.01$

61 HCC patients

13 CHB

33 cryptogenic

6 HCV

9 Alc

Nested PCR detection of HBV DNA

<table>
<thead>
<tr>
<th></th>
<th>13 (100%)</th>
<th>24 (73%)</th>
<th>1 (17%)</th>
<th>5 (56%)</th>
</tr>
</thead>
</table>

Real-time quantification of HBV DNA/RNA

<table>
<thead>
<tr>
<th>Number of patients with:</th>
<th>12 (92%)</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>detectable serum HBV DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>detectable intrahepatic total HBV DNA</td>
<td>13 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>detectable cccDNA</td>
<td>12 (92%)</td>
<td>6 (26%)*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>detectable pgRNA</td>
<td>12 (92%)</td>
<td>12 (52%)*</td>
<td>0</td>
<td>3 (60%)</td>
</tr>
</tbody>
</table>
• Tumor tissue contained lower cccDNA copy number compared with paired non-neoplastic liver
• Larger tumors (>3 cm) had less cccDNA compared with small tumors (less than or equal to 3 cm)
• High viral replicative activity in non-neoplastic liver was associated with higher HCC recurrence rate independent of Ishak fibrosis stage

The recurrence of HCC after surgery may be associated by persistent cccDNA, cccDNA transcription and integrated HBV sequences in the surrounding liver tissue.

Surgical specimens could be used for the detection of ih cccDNA and HBV RNA for the prediction of HCC recurrence.
Ultimate Goals of treatment

- **Complete cure** – cccDNA clearance
- **Functional cure** – obstruction of cccDNA transcriptional activity
**cccDNA in Treatment - HBe+**

**Table 1. Correlation of Changes in cccDNA Copy Number With Baseline and Posttreatment Parameters**

<table>
<thead>
<tr>
<th>Correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline viral load</td>
<td>0.15</td>
</tr>
<tr>
<td>Baseline HAI</td>
<td>0.97</td>
</tr>
<tr>
<td>Baseline ALT</td>
<td>0.86</td>
</tr>
<tr>
<td>Change in serum HBV DNA</td>
<td>0.008</td>
</tr>
<tr>
<td>Change in Total intracellular HBV DNA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in ALT</td>
<td>0.76</td>
</tr>
<tr>
<td>Change in HAI</td>
<td>0.709</td>
</tr>
<tr>
<td>Change in number of cells positive for nuclear HBcAg</td>
<td>0.48</td>
</tr>
<tr>
<td>Change in number of cells positive for cytoplasmic HBcAg</td>
<td>0.70</td>
</tr>
<tr>
<td>Change in number of HBsAg-positive cells</td>
<td>0.59</td>
</tr>
</tbody>
</table>

**cccDNA drop - ADV 48 w (22pts) = -0.80 log10 copies/cell (84%) vs Placebo (10pts): +0.32 log10 copies/cell, P = .002**

Patients who underwent **HBeAg seroconversion** had significantly **lower baseline levels of intracellular cccDNA** compared with patients who remained HBeAg +, **P 0.034**

Werle-Lapostolle et al. GASTROENTEROLOGY 2004;126:1750–1758
## Effects of Entecavir on cccDNA in HBeAg + Patients

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Baseline</th>
<th>Week 48</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrahepatic HBV total DNA</td>
<td>20</td>
<td>3.5×10⁷ (4.8×10⁶~6.7×10⁸)</td>
<td>4.5×10³ (7.2×10²~6.1×10⁴)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(Copies/10⁶ cells, Median, range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrahepatic HBV cccDNA</td>
<td>20</td>
<td>1.3×10⁶ (4.5×10⁴~2.1×10⁷)</td>
<td>3.6×10³ (6.2×10²~5.1×10⁴)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(Copies/10⁶ cells, Median, range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of HBV cccDNA (%)</td>
<td>20</td>
<td>3.4 (0.2–28.4)</td>
<td>85.0 (43.1~96.2)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Ming Shi et al. Plos One 2015
Treatment with different NAs

After 1 year of therapy, the magnitude of reduction of cccDNA is approximately one log lower than that of intrahepatic total HBV DNA.
HBeAg-positive mean cccDNA 1.11 vs 0.98 log10 copies/cell (P 0.439)

HBeAg-negative patients 1.13 vs 0.91 log10 copies/cell (P 0.408)

At year 1, ih HBV DNA was still detectable in all 117 patients (64 HBeAg-positive and 53 HBeAg-negative) and 112 (96%) had detectable but decreased cccDNA levels.
With prolonged treatment (median period 126 months), cccDNA was markedly reduced, with 49% of liver biopsies having undetectable cccDNA.

Intrahepatic cccDNA distribution

Baseline
First year
4-5 years

copies/cell

0,01
0,1
1
10
100
Intrahepatic cccDNA and pgRNA

• In clinical practice measuring cccDNA or pgRNA in liver almost impossible
  – Liver biopsy
  – Method variability and limitations

• Residual cccDNA can re-initiate viral replication and full-blown infection after immunosuppression or stop of treatment

• Reliable surrogate marker in serum
HBV RNA in Serum

Hepatocyte

Nucleus

cccDNA

RT

RNA

DNA

X

HBV RNA

SVP

HBsAg

NAs

HBV RNA
Hepatitis B virus RNA is measurable in serum and can be a new marker for monitoring lamivudine therapy

A. Rokuhara et al, J Gastroenterol 2006; 41:785–790

- 24 patients
  - 16 HBeAg +
- BL and 2 and 6 months after initiation of treatment
- Serum HBV RNA drop
- Serum HBV RNA correlation with HBcrAg and HBV DNA
Serum HBV RNA at week 12 of treatment predicts early virological response in patients receiving nucleoside analogue therapy

Huang et al. Antivir Ther. 2015;20(4):369-75

- HBV RNA was Detectable at Baseline (mean = 5.2 log copies/ml) in 40% of patients (n=21)
  - 52 pts - 44% HBeAg-positive

- Serum HBV RNA at week 12 of treatment (LAM-ETV) predicted a shorter interval to undetectable HBV DNA (P=0.035)
  - interval from detectable to undetectable serum HBV DNA
    - <16 weeks HBV RNA = 3.8 ±3.8 log copies/ml vs ≥16 weeks HBV RNA= 6.6 ±3.5, P=0.013
Serum HBV RNA as predictor of HBeAg seroconversion during NA treatment

Van Bommel al. Hepatology. 2015

15 pts with HBeAg seroconversion had higher HBV RNA decline compared to 35 without (P < 0.001 for months 3 and 6)
Peg-IFN–based treatment induced a stronger decrease in the HBV RNA load than NA monotherapy, and this decline was more pronounced in responders than in nonresponders.
In HBV e antigen–negative patients, a lower baseline HBV RNA level was independently associated with response to Peg-IFN and adefovir (odds ratio, .44; P = .019).

Jansen et al, JID 2016:213
Follow up of 33 CHB patients - standard NAs treatment (3y) - HBV DNA not detectable at EoT

Serum HBV RNA was positive in 21 (63.64%) patients at EoT
Viral rebound (HBV DNA re-appearance at 24 weeks post) occurred in 21 (100%) patients whose HBV RNA was positive at EoT, whereas, only in 3 (25%) of the 12 patients whose HBV RNA levels were below the LoD.

In our lab

• Ongoing study supported by Asklipios grant (Gilead)
• Optimization of method for HBV RNA quantification in serum
• EoT sera of patients who stopped long term NA treatment
Example of one patient with early viral and biochemical relapse after treatment termination.

- HbsAg
- ALT
- HBV DNA

EOT HBV RNA +

- HBV RNA 24.776 c/mL
- HBV RNA 516.800 c/mL
- HBV RNA 368.600 c/mL
- HBV RNA 186.200.000 c/mL
**Serum HBV RNA**

- Serum HBV RNA reflects cccDNA transcriptional activity
- Serum HBV RNA could prove to be a reliable prognostic marker for significant viral rebound after stopping NA therapy
- Measuring serum HBV RNA might also help the identification of patients with OBI and carriers in risk for HBV reactivation and prove to be an important marker for new treatment strategies and compounds
THANK YOU