Biomarkers of HBV cccDNA expression

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The cccDNA:
the key molecule in HBV life-cycle and persistence in CHB

- cccDNA is first generated from incoming virions
- cccDNA “appears” as a stable minichromosome in nondividing hepatocytes
- HBV replicate via an RNA intermediate (pgRNA)
- cccDNA is responsible for the production of all HBV RNAs, proteins and virions
- HBV integrated sequences may produce subgenomic RNAs and proteins (HBsAg)

Elimination of cccDNA reservoir would lead to a sterilizing cure
Reduction and inactivation of cccDNA may lead to a functional cure (HBsAg loss)
Determination of HBV intrahepatic cccDNA amount and activity remains challenging

A liver biopsy is required to quantify cccDNA in the liver (invasive technique)

Coexistence of nearly identical HBV DNA molecules (replicative forms) in infected cells (technical limitations)

Concerted harmonization efforts of HBV cccDNA quantification (ICE-HBV / ANRS / DZIF collaborative project)
HBV serum markers of cccDNA amount and activity

As new therapies are developed, there is an urgent need for non-invasive biomarkers reflecting cccDNA amounts and expression in the liver

- To monitor therapy efficacy
- To identify patients that may achieve sustained virological control off-treatment
- To predict HCC risk

*Coffin, Gastroenterology 2019*
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To understand the mechanisms regulating cccDNA activity....
Without biopsies – the liver remains a black box yet!
Serum biomarkers: HBsAg

HBsAg is a classical marker mostly derived from the high production of SVPs.

HBsAg clearance is considered the most important clinical endpoint.

HBsAg loss is associated with histological improvement and reduced HCC risk.

Impact of NAs on cccDNA and HBsAg

HBV polymerase inhibitors (NAs) efficiently lower HBV DNA in serum.

NAs do not affect directly cccDNA activity.

RNA production and HBsAg levels are not expected to change.
Impact of NAs on cccDNA and HBsAg

- BL: median 7.28 cccDNA copies/cell
- Year 1: 1 log decline
- Last biopsy (6-12y; n= 43): 2.94 log reduction (49% below LLoD)


Patients studies show that NAs have no (very limited) impact on HBsAg levels

cccDNA amounts decrease slowly in NA-treated patients

HBV DNA suppression is not complete under NAs (Boyd, J.Hepatol. 2016)

Correlation between qHBsAg and cccDNA levels varies between studies

HBV integrations may contribute to HBsAg production (i.e. HBeAg-negative)

Serum biomarkers: HBsAg

Low HBsAg levels (BL) ↔ Low cccDNA levels (+/- integrated sequences)

Low HBsAg levels → Best predictor in prevention of relapse after stopping NAs

HBsAg decrease → Good predictor of IFN response / efficacy of siRNA therapy

Less HBV RNAs (degradation)
Decrease of activity / silencing
„cccDNA levels might not change“

(Volz et al. Gastroenterol. 2007; Pfefferkorn Gut 2017; Pfefferkorn J. Hepatol. 2020; Gane et al. EASL 2020)
**Impact of peg-IFNα on cccDNA and viral markers**

**IFN-α decreases the acetylation status of cccDNA-bound histones**

**Peg-IFNα can induce substantial epigenetic suppression of cccDNA transcription (and partial degradation) even in the absence of immune responses**

**Reduction of HBV transcripts and reappearance of SMC5/6 complex play a key role in cccDNA silencing**

(Belloni, JCI 2012; Allweiss, J.Hepatology 2014; Tropberger PNAS 2015; Lucifora et al. Science 2014; Allweiss et al. EASL 2018; Allweiss L., Giersch K., in revision)
The HBcrAg assay measures the combined antigenic reactivity resulting from:
1) HBeAg (predominant component)
2) HBV core antigen (HBcAg)
3) 22KDa precore/core protein (p22cr);

Heterogeneous protein mixture

Translated from pgRNA / precore RNA

cccDNA derived

Mak LY et al. Gut and Liver 2019

Hadziyannis & Laras Genes 2018
Serum biomarkers: HBcrAg

Studies show that HBcrAg correlates well with cccDNA loads ... 

... and transcriptional activity (pgRNA/ccDNA)

HBeAg-neg. patients: HBcrAg+ show higher cccDNA transcriptional activity than HBcrAg−
Serum biomarkers: HBcrAg

- 124 NUC treated patients with paired liver biopsies at baseline and year 1,
- 43 with third biopsy year 6-12
- Serum HBcrAg (Lumipulse)

HBcrAg correlates with intrahepatic cccDNA levels in NUC treated patients

HBcrAg was shown to predict clinical relapse after stopping NA treatment

The sensitivity of current HBcrAg needs to be improved
Serum biomarkers: HBV RNA

- pgRNA, spliced RNAs and X RNAs are found in serum
- HBV RNAs can be found in naked capsids and enveloped

Adapted from Testoni et al. Seminars in Liver Disease 2017

Can be released into serum as enveloped 3.5kb pregenomic RNA containing virions

(Wang et al. J Hepatol. 2016)

HBV RNA in serum is present in viral particles with a similar density as HBV DNA particles

HBV RNA particles are produced at almost as high rates as viral particles containing DNA.

Prakash et al. Virology Journal 2018

Serum biomarkers: HBV RNA
HBV RNA levels vary according to HBeAg status, disease status…

VAN CAMPENHOUT, VAN BöMMEL, ET AL. Hepatol. 2018


Prakash et al. Virology Journal 2018

HBV genotype differences:
> reverse transcription efficiency in GT-D

HBeAg-neg. patients show lower cccDNA activity and loads …… lower HBV RNA in serum
Serum pgRNA can serve as a biomarker of cccDNA activity

Giersch et al J.Hepatol. 2017
Serum pgRNA can serve as marker of cccDNA activity

**Serum pgRNA levels strongly correlates with the amount of pgRNA in the liver and with cccDNA amounts in untreated mice (or NA-treated mice) – where cccDNA activity is not affected**

IFN lowers HBV RNA levels in liver and serum:

- good correlations between serum pgRNA and intrahepatic pgRNA
- but no with cccDNA amounts (reduced cccDNA activity)

Serum pgRNA measurement may serve as a surrogate marker to assess the impact of drugs affecting RNA levels: transcription / stability (i.e. IFNs; siRNAs; …. CpAMs)

![Image](image_url)
NVR 3-778 & PEG-IFNα, but not Entecavir, lower the levels of HBV RNA in serum without causing substantial changes in cccDNA loads

Zhang M, et al. EASL dILC2020
Serum biomarkers: HBV RNA

Low serum pgRNA levels ↔ Low pgRNA in liver ↔ low cccDNA levels

Low HBV RNA (BL) → Good predictor of sustained virological control off-treatment

HBV RNA decrease → Good predictor of IFN response
Good marker to monitor therapy efficacy (CpAMs, siRNA)

Less HBV RNAs (degradation)
Decrease of activity / silencing
“cccDNA levels might not change!”

Conclusion: HBV serum markers of cccDNA expression

Different non-invasive HBV serum biomarkers reflecting cccDNA amounts and activity in the liver have been identified.

HBcrAg and pgRNA are very promising since these are more likely cccDNA-derived.

- To monitor therapy efficacy
- To identify patients that may achieve sustained virological control off-treatment
- Composition of HBV RNA in serum may serve as a marker of cccDNA mutations and turnover

More translational studies (biopsies / in vivo models) are needed to fully judge the potential of distinct HBV serum biomarkers and to understand the impact of novel therapies on cccDNA loads and activity.

Coffin, Gastroenterology 2019