New indicators for the evaluation of hepatitis B therapy

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Virus suppression and sustained disease control

**Definition of Cure**

Durantel & Zoulim, J Hepatol 2016;
Biomarkers in evaluation to assist drug development

Testoni et al, Semin Liver Dis, 2017
Classic biomarkers

➢ HBsAg

➢ HBeAg

➢ HBV DNA
HBsAg levels can be used to guide therapy decisions in HBeAg-positive patients treated with PEG-IFN

Application of the prediction rules based on HBsAg levels at week 12 (A) and 24 (B) and HBsAg declines at weeks 12 (C) and 24 (D).

Response rates were low:

- in patients with genotypes A or D if there was no decline of HBsAg by week 12 (NPV: 97%-100%)

- in patients with genotypes B or C if HBsAg at week 12 was >20,000 IU/mL (NPV: 92%-98%).

At week 24, nearly all patients with HBsAg >20,000 IU/mL failed to achieve a response (NPV: response and HBsAg loss: 99% and 100%).
HBsAg decline at week 12 is an effective predictor of response rate in CHB patients treated with Peg-IFN

Flowchart showing chances of sustained response (SR)

Combination of HBsAg and HBV DNA levels at week 12 identifies HBeAg-negative patients with a very low chance of SR.
The baseline level of qHBsAg and the on-treatment decline of qHBeAg in HBeAg positive patients were highly useful in predicting VR and SR.

- **Baseline HBsAg**: cutoff level of 3.98 IU/mL, yielded the highest predictive value for VR;
- **HBeAg decline**: 1.00 PE IU/mL, yielded the highest predictive value for SR.
HBsAg decline in HBeAg positive CHB patients treated with TDF suggests the possibility of HBsAg loss

Exploratory measures of HBsAg decline at weeks 12 and 24 for HBeAg-positive patients on TDF and corresponding positive and negative predictive values for ultimate HBsAg loss.

<table>
<thead>
<tr>
<th>Measure of decline in HBsAg level</th>
<th>Study time point</th>
<th>Percentage of patients (n/N)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.5 log_{10} IU/ml</td>
<td>Week 12</td>
<td>55 (94/171)</td>
<td>6.4</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>Week 24</td>
<td>39 (66/170)</td>
<td>0</td>
<td>83.7</td>
</tr>
<tr>
<td>≥0.5 log_{10} IU/ml</td>
<td>Week 12</td>
<td>26 (44/171)</td>
<td>25.0</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>Week 24</td>
<td>34 (57/170)</td>
<td>29.8</td>
<td>100</td>
</tr>
<tr>
<td>≥1.0 log_{10} IU/ml</td>
<td>Week 12</td>
<td>13 (23/171)</td>
<td><strong>34.8</strong></td>
<td><strong>93.9</strong></td>
</tr>
<tr>
<td></td>
<td>Week 24</td>
<td>17 (29/170)</td>
<td><strong>44.8</strong></td>
<td><strong>97.2</strong></td>
</tr>
</tbody>
</table>

- A reduction in HBsAg levels of ≥ log_{10} IU/ml at week 12 and 24 has a very high NPV (97%) and modest PPV (45) for ultimate HBsAg loss.

Baseline HBV DNA and ALT level are predictors of virologic response in HBeAg negative CHB patients treated with Peg-IFN

Predictors of virologic response (HBV DNA 10,000 copies/mL) at 6 months and 3 years after treatment.

- **Baseline ALT level** is independent predictor of virological response after 3 years of treatment;
- **Baseline HBV DNA level** is independent predictor of virological response after 6 months of treatment.

MARCELLIN et al. Gastroenterology 2009
New biomarkers

- HBV-RNA
- HBcrAg
- Anti-HBc
- cccDNA

EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection
HBV RNA
Circulating viral particles

Classic
- HBsAg
- filament
- Dane particle
- sphere
- HBeAg

Newly described
- DNA free particles
- RNA containing particles

Van Bommel et al, Hepatology 2015; Hu et al, J Viral Hepatitis 2015
Predictive value of serum HBV RNA during antiviral therapy

- Independently predict virologic response;
- Predict HBV reactivation after discontinuation of NAs;
- Reflect antiviral potency of NAs;
- Predictor of early emergence of viral mutation during LAM therapy.

HBV RNA is associated with a response to Peg-IFN and NAs

During long-term NA treatment of patients with CHB, HBV RNA levels remained higher than HBV DNA levels;

Peg-IFN–based treatment induced a stronger decrease in HBV RNA load than NA monotherapy;

Responders showed a stronger and earlier decline in HBV RNA during Peg-IFN treatment.

Jansen et al. JID 2016
Serum HBV RNA is an early predictor of HBeAg seroconversion in patients with CHB treated with NAs

- Those patients with subsequent HBeAg seroconversion showed a significantly stronger decline in HBV RNA levels during treatment;
- Patients with HBeAg negative chronic HBV infection have a marked decline in HBV RNA levels.
Serum HBV RNA is an early predictor of HBeAg seroconversion in patients with CHB treated with PegIFN α-2a

- Baseline HBV RNA levels are lower in responders
- During treatment, responders show markedly lower or undetectable (○) HBV RNA levels

van Bömmel F, et al. AASLD 2015, San Francisco
The association of HBV pgRNA virion levels and viral rebound after the discontinuation of NAs-therapy

Wang et al, J Hepatol 2016

Viral rebound 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.
Hepatitis B core-related antigen (HBcrAg)
HBcAg

- HBcAg quantification may provide additional information concerning the translational activity of the HBV infection beyond HBsAg quantification.

<table>
<thead>
<tr>
<th>Secreted particles and proteins</th>
<th>Dane particles</th>
<th>Midair particles, Filamentous and spherical particles</th>
<th>HBeAg</th>
<th>HBsAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBcAg (HbcAg, HBeAg, p22cr proteins)</td>
<td>detectable</td>
<td>detectable</td>
<td>detectable</td>
<td>detectable</td>
</tr>
<tr>
<td>HBsAg</td>
<td>detectable</td>
<td>detectable</td>
<td>detectable</td>
<td>detectable</td>
</tr>
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</table>

EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection
HBcrAg

- HBcrAg is a potential surrogate marker of cccDNA.
- It may soon become a useful marker for disease monitoring, predicting treatment response and disease outcome of chronic hepatitis B.

CLEIA® HBcrAg assay kit with a fully automated analyzer system (Lumipulse System, Fujirebio, Inc.)

L-Y Mak et al, Aliment Pharmacol Ther. 2017
Monitoring HBcrAg levels during PEG-IFN therapy help identify patients with a very low probability of response

<table>
<thead>
<tr>
<th>HBcrAg and HBsAg levels to predict virological response at week 72</th>
</tr>
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<tbody>
<tr>
<td><strong>Cut-off values</strong></td>
</tr>
<tr>
<td>Log$_{10}$ HBcrAg (U/ml)</td>
</tr>
<tr>
<td>Week 12</td>
</tr>
<tr>
<td>7.0</td>
</tr>
<tr>
<td>7.5</td>
</tr>
<tr>
<td>8.0</td>
</tr>
<tr>
<td>8.5</td>
</tr>
<tr>
<td>Log$_{10}$ HBsAg (IU/ml)</td>
</tr>
<tr>
<td>4.3</td>
</tr>
<tr>
<td>Combination of both markers*</td>
</tr>
<tr>
<td>Week 24</td>
</tr>
<tr>
<td>Log$_{10}$ HBcrAg (U/ml)</td>
</tr>
<tr>
<td>7.0</td>
</tr>
<tr>
<td>7.5</td>
</tr>
<tr>
<td>8.0</td>
</tr>
<tr>
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<tr>
<td>Log$_{10}$ HBsAg (IU/ml)</td>
</tr>
<tr>
<td>4.3</td>
</tr>
<tr>
<td>Combination of both markers*</td>
</tr>
</tbody>
</table>

*Combined HBcrAg (log$_{10}$ 8.0 U/ml) and HBsAg (log$_{10}$ 4.3 IU/ml or 20,000 IU/ml).

NPV, negative predictive value; PPV, positive predictive value.

Correlations of HBcrAg and cccDNA at baseline

The quantitative HBcrAg represented a reliable marker of intrahepatic cccDNA.
Baseline HBsAg and HBcrAg predict response/HBsAg loss in HBeAg negative CHB patients treated with Peg-IFN

<table>
<thead>
<tr>
<th></th>
<th>HBsAg</th>
<th>HBcrAg</th>
<th>HBsAg+HBcrAg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> Prediction of response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>0.716 (0.578–0.855)</td>
<td>0.668 (0.524–0.811)</td>
<td>0.745 (0.612–0.878)</td>
</tr>
<tr>
<td>Cut-off</td>
<td>3.141 (2.941–3.592)</td>
<td>3.450 (2.150–5.050)</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>0.857 (0.571–0.971)</td>
<td>0.829 (0.400–1.000)</td>
<td>0.886 (0.600–1.000)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.667 (0.407–0.889)</td>
<td>0.593 (0.296–0.889)</td>
<td>0.667 (0.444–0.889)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.742 (0.645–0.855)</td>
<td>0.710 (0.613–0.806)</td>
<td>0.774 (0.677–0.855)</td>
</tr>
<tr>
<td>PPV°</td>
<td>0.762 (0.590–0.947)</td>
<td>0.714 (0.533–1.000)</td>
<td><strong>0.800 (0.611–1.000)</strong></td>
</tr>
<tr>
<td>NPV°</td>
<td>0.756 (0.660–0.889)</td>
<td>0.718 (0.630–0.857)</td>
<td><strong>0.765 (0.675–0.889)</strong></td>
</tr>
</tbody>
</table>

|      |                |                |                |
| **B** Prediction of HBsAg loss |                |                |                |
| AUC  | 0.771 (0.576–0.965) | 0.552 (0.324–0.779) | 0.763 (0.599–0.927) |
| Cut-off | 3.141 (2.630–3.317) | 2.550 (2.150–3.550) |                |
| Specificity | 0.796 (0.6531–0.980) | 0.878 (0.653–0.980) | 0.837 (0.490–1.000) |
| Sensitivity | 0.846 (0.538–1.000) | 0.462 (0.231–0.769) | 0.692 (0.385–1.000) |
| Accuracy | 0.806 (0.694–0.919) | 0.790 (0.645–0.887) | 0.806 (0.580–0.919) |
| PPV° | 0.526 (0.385–0.917) | 0.500 (0.286–0.800) | 0.533 (0.305–1.000) |
| NPV° | 0.949 (0.875–1.000) | 0.863 (0.811–0.932) | 0.917 (0.852–1.000) |

°Positive Predictive Value.  
°Negative Predictive Value.

End-of-treatment response (EOT) was defined as undetectable serum HBV DNA or serum HBV DNA ≤3 log IU/mL

Baseline levels of HBsAg and HBcrAg and the combination of the two markers identify patients with high probability of response (PPV: 80.0%) and low probability of response (NPV: 76.5%)

Martinot-Peignoux et al. Journal of Viral Hepatitis 2016
HBcrAg+ patients show higher cccDNA amount and activity

cccDNA

cccDNA transcriptional activity

pgRNA

Testoni et al, AASLD 2016
Quantitative hepatitis B core antibody
Baseline quantitative hepatitis B core antibody

Baseline anti-HBc titer may serve as a useful predictor of Peg-IFN and NA therapy efficacy in HBeAg-positive CHB patients, which could be used for optimizing the antiviral therapy of CHB.
Patients who achieved response had a higher baseline qAnti-HBc level (>30,000IU/ml)

The response rate (SR, VR, CR) gradually rose with increasing baseline qAnti-HBc levels

ALT normalization rates in patients with different baseline qAnti-HBc levels

The HBc-high (>30,000IU/ml) patients had a higher ALT normalization rate at the end of the follow-up

Baseline anti-HBc titre is a useful predictor of Peg-IFN and NUC therapy efficacy in HBeAg positive CHB patients

Patients with baseline anti-HBc $\geq 4.4\log_{10}$ IU/mL (a) and baseline HBV DNA <9 log10 copies/mL (b) had 65.8% and 37.1% rates of HBeAg seroconversion in the Peg-IFN and NUC cohorts, respectively.

Baseline anti-HBc titre is a useful predictor of long-term entecavir therapy efficacy in HBeAg positive CHB patients

(A) AUROCs of baseline parameters in predicting HBeAg seroconversion at w144

(B) AUROCs of baseline parameters in predicting HBeAg seroconversion at w240

Patients with baseline anti-HBc $\geq 4.65$ log10 IU/mL had 28.0% and 35.5% chance of seroconversion at weeks 144 and 240, respectively.

cccDNA
Monitoring of the cccDNA pool and its transcriptional activity

Testoni et al, Sem Liver Dis, 2017
cccDNA

• The main limitation is the requirement of liver biopsy;

• Persist in the liver of infected patients even after long-term NA therapy and even after HBsAg loss and seroconversion;

• Quantification of cccDNA levels and its transcriptional activity will be important in clinical trials evaluating novel treatment concepts to cure HBV infection.
Correlation between cccDNA and HBV RNA

A) Correlation of serum pgRNA and intrahepatic cccDNA/cell in HBV-infected mice

B) Serum HBV RNA correlations with intrahepatic cccDNA in HBeAg positive patients

C) Serum HBV RNA correlations with intrahepatic cccDNA in HBeAg negative patients

- Serum HBV RNA correlated well with intraphepatic cccDNA in HBeAg-positive patients (B), but not in HBeAg-negative patients (C).

Other potential serum biomarkers

- Hepatitis B virus large surface protein (LHBs)
- QS by Next Generation Sequencing (NGS)
On-treatment quantification of serum LHBs may be a more useful parameter for predicting VR in patients on Peg-IFN than those on ETV.

Combining LHBs, HBsAg and HBV DNA can predict VR and SR more effectively and earlier.
HBV exists as quasispecies with different fitness
Characterization of viral QS by Complexity and Diversity

Quasispecies Complexity
(Shannon Entropy, $S_n$)
$$S_n = -\sum_i (p_i \ln p_i) / \ln N$$

Quasispecies Diversity
$d$: mean genetic distance, Hamming distance

$d_S$: the number of synonymous substitutions per synonymous site
$d_N$: the number of non-synonymous substitutions per non-synonymous site
Positions of the 3 sequential and overlapping contigs on RT and S gene
Prediction of VR by pretreatment HBV RT QS heterogeneity: the advantage of using NGS
• NGS predict better than CBS
• AUC rt2 Max

Han Y, Gong L, ... Zhang XX. Clin Microbiol Infect. 2015 Aug;21(8):797.e1-8.
Conclusion

• The effectiveness of the classic biomarkers HBsAg, HBeAg, HBV DNA, mentioned in the guidelines in predicting response during antiviral therapy have been confirmed by many studies.

• The value of the new biomarkers HBV RNA, HBcrAg, qAnti-HBc and cccDNA, in predicting response still need more experiments to provide evidence.

• More other potential biomarkers are still needed to guide therapy, optimize CHB treatment strategies, and improve clinical outcomes in CHB patients.
Thank you

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