Il Virus: categorie virologiche e stadiazione

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UO Epatologia – Azienda Ospedaliero Universitaria Pisana - Centro Riferimento Regionale “Diagnosi e trattamento delle epatopatie croniche e del tumore di fegato”
To optimize the management of HBV carriers
HBV replication and life Cycle

Adapted from Wands JR, NEJM 2004

HBV receptor

modulation of cccDNA activity
Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus

- By using near zero distance photo-cross-linking and tandem affinity purification, the receptor-binding region of pre-S1 was shown to specifically interact with sodium taurocholate cotransporting polypeptide (NTCP), a multiple transmembrane transporter predominantly expressed in the liver.

- Silencing NTCP inhibits HBV and HDV infections, while exogenous NTCP expression rendered nonsusceptible hepatocarcinoma cells susceptible to these viral infections.

- Myrcludex B – HBV entry inhibitor (lipopeptide representing part of the pre-S1 domain of L-HBsAg) blocks NTCP-mediated bile salt transport.

- Cyclosporin A and Ezetimibe interfere with HBV infection by inhibiting NTCP.

   - New tools to study in vitro HBV infection
   - New target for antiviral therapy

HBV replicative cycle

The longer the replication phase the higher the integration rate

cccDNA dilution by hepatocyte turnover once new infection of liver cells is stopped

“Cell cure” by IFN gamma and TNF alpha

However, the long half life of both cccDNA and hepatocytes explains the persistence of “occult HBV infection” even after a resolved acute hepatitis B.
From RC-HBV-DNA to cccDNA formation and modulation

- Host DNA repair mechanisms (tyrosil-DNA-phosphodiesterase 2 and additional components of the host DNA repair machinery) are though to be exploited by HBV to convert RC-DNA into cccDNA.
- **HBV cccDNA** is organized as a minichromosome in the nucleus of infected cells by histone and non-histone proteins.
- long half-life (not established)
- HBV replication is regulated by the acetylation status of cccDNA-bound H3/H4 histones
- Viral (HBx, HBc) and cellular regulatory proteins bind to and modulate cccDNA functions

cccDNA epigenetic control in HBV carriers

- the epigenetic control of cccDNA activity foster the transition from low-replicative to occult HBV infection

- IFN-α inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome

- Active IFN-α repression of HBV transcription associated with 60-70% reduction of 3.5 kb pgRNA, 2.4-2.1 mRNA (pres/s RNA), without affecting levels of cccDNA copies per cell

Pollicino T, 2006; Belloni et al., J Clin Invest 2012 ; Palumbo AASLD 2014
A multispecific T cell response directed to different HBV proteins is detectable ex-vivo in subjects with occult HBV Infection independently from the serological status.
Outcome of HBV infection and liver damage

Drugs

direct or indirect
cccDNA epigenetic control in HBV carriers

- The epigenetic control of cccDNA activity fosters the transition from low-replicative to occult HBV infection.

- Treatment with drugs able to modify histones’ acetylation may modulate cccDNA activity, inducing HBV reactivation (i.e., Romidepsin, histone deacetylase inhibitor, HDAC) or silencing cccDNA (Histone Acetyl Transferase Inhibitor, Sirt1/2 or Ezh2 histone methyltransferase activity activators).
Immune response to hepatitis B

HBV Immune response

Acute/Resolved infection
Presence of an integrated activation of both cellular and humoral arms of adaptive immunity.

Chronic infection
Impaired T and B cell response
Functional defects

Ability to mount an efficient T and B cell response is the main mechanism responsible for HBV control.
Natural history of Chronic Hepatitis B Virus Infection

**Immune tolerance**
- HBeAg positive
- HBsAg positive

**Immune clearance**
- HBeAg negative
- HBsAg negative

**Immune control**
- HBeAg or anti-HBe
- Inactive carriers
- OBI carriers

**Markers**
- HBV DNA (log_{10} IU/ml)
- IgM anti-HBc (PEI Units)
- ALT (U/L)
The Virus: HBV-DNA  
HBsAg qt 
HBeAg 
HBcrAg 
HBV-RNA

The host’s immune response: T cell response  
anti-HBc qt 
NK phenotype

The virus/host interplay:  
mRNA profile 
MiR-B-Index

R. Magritte, The masterpiece or the mysteries of the horizon 1955
Serum HBV-DNA decline: reduction of replication

Serum HBsAg decline: reduction of the cccDNA amount or of the cccDNA transcription /mRNAs translation

Virions + defective particles (exceeding virions by a factor of $10^2$-$10^5$)

Brunetto et al, J Hepatol 2010
HBV-DNA and HBsAg quantification in clinical practice

- HBsAg production follows cellular pathways distinct from viral replication

- HBsAg production depends, at least in part, on constitutive viral features, such as genotype and quasispecies.

  - *In-vitro* HBsAg production differs among HBV genotypes and *in vivo* HBsAg serum levels are higher in genotype A than genotype B, C and D infected individuals.

  - Pre-S/S mutants may impact on HBsAg production as they are negatively correlated with HBsAg serum levels (r=-0.431; P <0.005).

- Both HBV-DNA and HBsAg production are modulated by the interplay between virus and host's immune response

Only the combined quantification of HBsAg and HBV-DNA can contribute to pinpoint accurately the single HBV carrier within the highly dynamic phases of chronic HBV infection, provided that all variables interfering with HBsAg production are carefully considered.
Natural history of Chronic Hepatitis B Virus Infection

- **Immune tolerance**
  - HBsAg positive
  - Patients with CHB
  - HBeAg positive or negative
  - Immune tolerant carriers

- **Immune clearance**
  - HBsAg negative
  - Inactive carriers
  - OBI carriers

- **Immune control**
  - HBsAg negative
  - Patients with CHB
  - HBeAg positive or negative
  - Immune tolerant carriers

- **HBV DNA** (log$_{10}$ IU/ml)
  - High and stable HBsAg (4.5-5 log$_{10}$ IU/ml) and HBV-DNA (8 log$_{10}$ IU/ml) serum levels are the virologic hallmark of Immune tolerant phase

- **IgM anti-HBc** (PEI Units)
  - Low HBsAg serum levels (3.85 log$_{10}$ IU/ml) in genotype B and C infected HBeAg positive patients are associated with significant fibrosis (≥F2)

- **ALT** (U/L)

Natural history of Chronic Hepatitis B Virus Infection

- **HBsAg serum levels** <1000 IU/ml in low viremic HBeAg negative carriers are associated with better outcomes

- **Combined** HBsAg (<1000 IU/ml) and HBV-DNA (<2000 IU/ml) serum levels in genotype D show high diagnostic accuracy (94.3%) in the identification of Inactive Carriers

Long term outcome of Inactive and Active, Low Viremic HBeAg negative carriers: benign one direction course towards spontaneous HBsAg clearance

In 89 Inactive Carriers (84% genotype D) 31% had HBsAg levels >1,000 IU/mL as compared to 9% of a previous study (56 IC Inactive Carrier, all genotype D)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Category/Unit</th>
<th>HBsAg BL (IU/mL)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤1000</td>
<td>&gt;1000</td>
<td>P</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>36 (73.5%)</td>
<td>13 (26.5%)</td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24 (63.2%)</td>
<td>14 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>≤40 years</td>
<td>11 (45.8%)</td>
<td>13 (54.2%)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>&gt;40 years</td>
<td>49 (77.8%)</td>
<td>14 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>Country of Origin</td>
<td>Italy</td>
<td>55 (76.4%)</td>
<td>17 (23.6%)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>5 (33.3%)</td>
<td>10 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>Genotype D</td>
<td>54 (73.0%)</td>
<td>20 (27.0%)</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>Genotype non-D</td>
<td>3 (33.3%)</td>
<td>6 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Surace L & Oliveri F.et al, submitted
Use of Hepatitis B Surface Antigen Serum Levels Help to Distinguish Active From Inactive Hepatitis B Virus Genotype D Carriers

<table>
<thead>
<tr>
<th>Subjects (N)</th>
<th>56</th>
<th>31</th>
<th>84</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA (IU/mL)</td>
<td>≤2,000</td>
<td>2,001 - 19,999</td>
<td>≥20,000</td>
<td>≥20,000</td>
</tr>
<tr>
<td>Liver disease</td>
<td>Absent</td>
<td>Absent</td>
<td>Chr Hep</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>Carriers</td>
<td>Inactive</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
</tr>
</tbody>
</table>

Brunetto MR et al. Gastroenterology 2010
<table>
<thead>
<tr>
<th></th>
<th>Inactive infection (HBV DNA ≤2000 IU/mL (IC))</th>
<th>Active infection (HBV DNA &gt;2000 &amp; &lt;20000 IU/mL (AC1))</th>
<th>Active Infection (HBV DNA ≥20000 IU/mL (AC2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers Number</td>
<td>56</td>
<td>31</td>
<td>122</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 (20 – 75)</td>
<td>43 (21 – 64)</td>
<td>47 (18 – 77)</td>
</tr>
<tr>
<td>Male/female</td>
<td>29 / 27</td>
<td>10 / 20</td>
<td>65 / 58</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>38 (24 – 104)</td>
<td>33 (24 – 106)</td>
<td>33 (6 – 110)</td>
</tr>
<tr>
<td>Baseline HBsAg (IU/mL)</td>
<td>62.12 (0.1 – 4068)</td>
<td>883 (0.5 – 7838)</td>
<td>4233 (164 – 82480)</td>
</tr>
<tr>
<td>End of f.u HBsAg (IU/mL)</td>
<td>40.92 (n.d. – 4143)</td>
<td>613 (0.41 – 7754)</td>
<td>3887 (172 – 65160)</td>
</tr>
<tr>
<td>Baseline HBV DNA (IU/mL)</td>
<td>49 (n.d. – 1990)</td>
<td>2758 (n.d. – 19524)</td>
<td>389500 (98 – 166000000)</td>
</tr>
<tr>
<td>End of f.u HBV DNA (IU/mL)</td>
<td>30 (n.d. – 1114)</td>
<td>1483 (n.d. – 14532)</td>
<td>396450 (15 – 151000000)</td>
</tr>
<tr>
<td>Baseline ALT (U/L)</td>
<td>21 (10 – 35)</td>
<td>22 (11 – 39)</td>
<td>68 (11 – 722)</td>
</tr>
<tr>
<td>End of f.u ALT (U/L)</td>
<td>20 (13 – 38)</td>
<td>23 (12 – 40)</td>
<td>98 (15 – 2056)</td>
</tr>
<tr>
<td>Liver elastometry by Fibroscan (kPa)</td>
<td>4.3 (3.1 – 5.6)</td>
<td>4.7 (3.2 – 5.8)</td>
<td>11.2 (3.2 – 59.8)</td>
</tr>
</tbody>
</table>

Liver biopsy in 10 patients: **Grading 3/18 (6 pts); 4/18 (4 pts) // Staging 0/6 (8 pts); 1/6 (2 pts)**
Long term outcome of Inactive and Active, Low Viremic HBeAg negative carriers: benign one direction course towards spontaneous HBsAg clearance.

Initial follow-up (12 months)

- 153 HBsAg carriers HBeAg-/anti-HBe+ with HBV-DNA ≤20,000 IU/mL and normal ALT
  - 133 (86.9%) carriers with HBV-DNA ≤20,000 IU/ml
  - 20 (13.1%) carriers with HBV-DNA >20,000 IU/mL and elevated ALT

Additional follow-up [57.2 (8.5-158.3) months]

- 87 IC (HBV-DNA ≤2000 IU/mL and normal ALT)
  - 84 IC (96.6%)
  - 3 IC (3.4%) transition to LV-AC
  - 19 HBsAg loss (21.8%)

- 46 LV-AC (HBV-DNA 2000-20,000 IU/mL) and normal ALT
  - 20 IC (43.5%)
  - 25 LV-AC (54.3%)
  - 1 Hepatitis Reactivation (2.2%)

Surace L & Oliveri F.et al, submitted
Natural history of Chronic Hepatitis B Virus Infection

Immune tolerance  Immune clearance  Immune control

HBsAg positive

HBeAg
HBeAg or anti-HBe
Anti-HBe

HBsAg neg

HBV DNA (log_{10} IU/ml)

IgM anti-HBc (PEI Units)

ALT (U/L)

HBeAg Immune tolerant carriers

CHB

Low viremic Active Carriers

Inactive Carriers

OBI

Surface L & Oliveri F et al, submitted
Use of Hepatitis B Surface Antigen Serum Levels Help to Distinguish Active From Inactive Hepatitis B Virus Genotype D Carriers

Subjects (N) | 56  | 31  | 84  | 38  
---|---|---|---|---
HBV DNA (IU/mL) | ≤2,000 | 2,001 - 19,999 | ≥20,000 | ≥20,000 
Liver disease | Absent | Absent | Chr Hep | Cirrhosis 
Carriers | Inactive | Active | Active | Active 

Brunetto MR et al. Gastroenterology 2010
HBsAg serum levels according to the prevalence of pre-s mutants during the natural history of chronic HBV infection

Stabile A et al, EASL 2011
<table>
<thead>
<tr>
<th>HBV</th>
<th>Diagnostic categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-HBs</td>
<td>immunity</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>exposure</td>
</tr>
<tr>
<td>HBsAg, HBV-DNA</td>
<td>infection</td>
</tr>
<tr>
<td>HBeAg, HBV-DNA</td>
<td>replication</td>
</tr>
<tr>
<td>IgM anti-HBc, HBV-DNA</td>
<td>disease</td>
</tr>
</tbody>
</table>
Identification of virus induced liver disease

- Viral infection
- Liver disease

HEPATITIS

Other factors causing liver damage:
- dismetabolisms
- drugs
- other hepatotropic viruses
Management of chronic HBV carriers

Laboratory
- **ethiology** *(viral markers, autoantibodies, Fe and Cu metabolism, glucose and lipids metabolism)*
- **liver injury** *(AST/ALT; Aph GGT)*
- **liver function** *(albumin, bilirubin, PT, PCHE)*
- serum globulins
- complete blood count

Morphology
- Liver biopsy

Instrumental evaluation
- liver elastometry
- ultrasound
Liver stiffness in the hepatitis B virus carrier: A non-invasive marker of liver disease influenced by the pattern of transaminases

Study Population: 268 CHB carriers

Table 2 Correlation between phase of infection, stage of liver disease and liver stiffness values

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Fibroscan values (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>50</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td>Acute Hepatitis^1</td>
<td>9</td>
<td>12.3 ± 3.3</td>
</tr>
<tr>
<td>Untreated HBsAg carriers overall^1</td>
<td>188</td>
<td>8.9 ± 8.0</td>
</tr>
<tr>
<td>Inactive carriers without LD^2</td>
<td>51</td>
<td>4.3 ± 1.0</td>
</tr>
<tr>
<td>Inactive carriers with LD^2</td>
<td>17</td>
<td>6.9 ± 2.3</td>
</tr>
<tr>
<td>CHB S0-S2</td>
<td>71</td>
<td>6.4 ± 2.4</td>
</tr>
<tr>
<td>CHB S3-S4</td>
<td>12</td>
<td>10.1 ± 3.8</td>
</tr>
<tr>
<td>CHB S5-S6</td>
<td>14</td>
<td>15.7 ± 9.0</td>
</tr>
<tr>
<td>US Cirrhosis^3</td>
<td>23</td>
<td>23.6 ± 11.8</td>
</tr>
<tr>
<td>Treated CHB overall^1</td>
<td>80</td>
<td>13.4 ± 9.7</td>
</tr>
<tr>
<td>CHB S0-S2</td>
<td>14</td>
<td>6.1 ± 1.7</td>
</tr>
<tr>
<td>CHB S3-S4</td>
<td>7</td>
<td>8.5 ± 2.8</td>
</tr>
<tr>
<td>CHB S5-S6</td>
<td>16</td>
<td>11.7 ± 5.2</td>
</tr>
<tr>
<td>US Cirrhosis^3</td>
<td>43</td>
<td>17.2 ± 11.4</td>
</tr>
</tbody>
</table>

Oliveri F et al, World J Gastroenterol 2008
Liver stiffness in the hepatitis B virus carrier: A non-invasive marker of liver disease influenced by the pattern of transaminases

Study Population: 268 CHB carriers

Cut-off levels for identification of:

≥S3 Fibrosis  7.5 KPa
Cirrhosis  11.8 KPa

Oliveri F et al, World J Gastroenterol 2008
HBV infection:

- virologic characterization
- staging of liver disease
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