HBsAg quantification:
is useful for staging liver disease?
HBsAg serum levels reflect:
• in HBeAg positive patients, the overall amount of cccDNA
• in HBeAg negative carriers, the transcriptionally active cccDNA

Adapted from Brunetto et al, J Hepatol 2010
The higher is the control of HBV infection, the lower are the serum levels of HBsAg

Nguyen T et al; Jaroszewicz J et al, J Hepatol 2010
<table>
<thead>
<tr>
<th>Latitude</th>
<th>Longitude</th>
<th>Time</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>32°09'18&quot;N, O 40°07'13&quot;</td>
<td>8.15 a.m., 19.01.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29°00'S, E 24°00'</td>
<td>8.15 a.m., 29.01.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3°15'N, E 73°00'</td>
<td>8.15 a.m., 3.2.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALT HBV-DNA IgM anti-HBc HBsAg

Adapted from Brunetto et al, J Hepatol 2010
Use of HBsAg serum levels help to distinguish active from inactive HBV genotype D carriers

209 HBeAg negative carriers

HBV DNA

≤2,000 IU/mL = 79 (73 with normal ALT)

>2,000 IU/mL = 130 (35<20,000 95≥20,000) (52 with normal ALT 78 with abnormal ALT)

Persistently ≤2,000 IU/mL = 56 (all with normal ALT)

Fluctuating >2,000 IU/mL = 23

≥20,000 IU/mL = 9 (all with abnormal ALT*)

<20,000 IU/mL = 17 (all with normal ALT)

≥20,000 IU/mL = 113 (all with abnormal ALT*)

Active Carriers (HBV DNA <20,000 IU/mL)
AC1 31

Active Carriers (HBV DNA ≥20,000 IU/mL)
AC2 122

Inactive Carriers 56

*Occasionally normal ALT in 3;
** Occasionally normal ALT in 35

Brunetto MR et al. Gastroenterology 2010
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
Use of HBsAg serum levels help to distinguish active from inactive HBV genotype D carriers

<table>
<thead>
<tr>
<th>Prediction of:</th>
<th>Inactive infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBsAg levels</strong></td>
<td>&lt;1000 UI/mL plus</td>
</tr>
<tr>
<td><strong>HBV-DNA levels</strong></td>
<td>&lt;2000 IU/mL</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>209</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>91.1%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>95.4%</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>87.9%</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>96.7%</td>
</tr>
<tr>
<td><strong>Diagnostic Accuracy</strong></td>
<td>94.5%</td>
</tr>
</tbody>
</table>

Brunetto MR, Gastroenterology 2010
Serum HBsAg levels helps predict disease progression in patients with low HBV loads

- In HBeAg negative pts with low viral load and gen B or C, a higher HBsAg level can predict disease progression.
- HBsAg < 1000 IU/ml in combination with low levels of HBV-DNA and ALT help define minimal risk HBV carriers

<table>
<thead>
<tr>
<th>HBV-DNA</th>
<th>ALT</th>
<th>HBsAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2000 IU/ml</td>
<td>&lt; 40 U/l</td>
<td>&lt; 1000 IU/ml</td>
</tr>
</tbody>
</table>

Incidence rate of HBeAg neg Hep x 100 person year
- 2.0 (1.8-2.3)
- 1.4 (1.2-1.7)
- 1.1 (0.9-1.4)

Incidence rate of HCC x 1000 person year
- 1.8 (1.3-2.6)
- 1.0 (0.6-1.7)
- 0.3 (0.1-1.1)
Prediction of disease reactivation in asymptomatic HBeAg negative CHB patients using baseline measurements of HBsAg and HBV-DNA

• 129 HBeAg negative carriers (gen. A-E) with normal ALT at BL, followed-up for 1 year with every 3 months ALT and HBV-DNA measurements: 82 resulted to be Inactive Carriers, 23 Active Carriers, 24 with Reactivation

• The optimal cut offs to identify HBV carriers at risk of reactivation were: HBsAg >1000 IU/ml and HBV-DNA >200 IU/ml

• The combined used of the 2 differentiated CHB carriers with reactivation from those without (IC and AC) with 92% sensitivity, 51% specificity, 96% NPV and 30% PPV
### 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>
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</tr>
<tr>
<td><strong>Carriers</strong></td>
<td>Inactive</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
</tr>
</tbody>
</table>

Brunetto MR et al. Gastroenterology 2010
Liver biopsy in 10 patients: **Grading** 3/18 (6 pts); 4/18 (4 pts) // **Staging** 0/6 (8 pts); 1/6 (2 pts)

<table>
<thead>
<tr>
<th></th>
<th>Inactive infection</th>
<th>Active infection</th>
<th>Active Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBV DNA ≤2000 IU/mL</td>
<td>HBV DNA &gt;2000 &amp;</td>
<td>HBV DNA ≥20000 IU/mL</td>
</tr>
<tr>
<td></td>
<td>(IC)</td>
<td>&lt;20000 IU/mL</td>
<td>(AC2)</td>
</tr>
<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>

Brunetto MR et al. Gastroenterology 2010
Log 10 HBV-DNA/HBsAg ratio by phase of infection and disease stage

N. of subjects:                      56                         31                         84                         38
HBV DNA (IU/mL):            ≤2000            >2000 & <20000
Liver disease:                   Absent           Absent           Chr. Hepatitis          Cirrhosis
Carriers:                      IC1                     AC2                     AC2

IC vs AC
P<.001

IC vs AC1
P=NS

AC1 vs AC2
P=.001

CH vs CI
P=.023
A minimum follow-up of 1 year with ALT levels at least every 3–4 months and serum HBV DNA levels is required before classifying a patient as inactive HBV carrier.

ALT levels should remain persistently within the normal range according to traditional cut-off values (approximately 40 IU/ml) and HBV DNA should be below 2000 IU/ml.

Some inactive carriers, however, may have HBV DNA levels greater than 2000 IU/ml (usually below 20000 IU/ml) accompanied by persistently normal ALT levels.

Patients with HBV-DNA <2000 IU/ml and elevated ALT should be advised to undergo liver biopsy for the evaluation of the cause of liver injury.
Natural history of Chronic HBV Infection

Immune tolerance

Immune clearance

Immune control

HBeAg or anti-HBe

Anti-HBe

HBV DNA (log 10 gen. Eq./ml)

IgM anti-HBc (PEI Units)

ALT (U/L)
A Longitudinal Study on the Natural History of serum HBsAg changes in CHB

117 chronic hepatitis B patients
FU for 99±16 months; number of visits 17 (8-49)

- Immune tolerance (7)
- e+ active CHB (25)
- e seroconversion (17)
- e- active CHB (46)
- e- inactive CHB (22)

High HBsAg levels predict insignificant fibrosis in HBeAg positive CHB

Seto W-K ET al, PLOS ONE 2012

140 HBeAg positive patients, 56 (40%) of whom had ALT levels \(\leq 2 \times \text{ULN}\), 72 (51.4%) had fibrosis score \(\leq 1\) and necro-inflammation grading \(\leq 4\)

In pts with ALT \(\leq 2 \times \text{ULN}\) HBsAg levels achieved a ROC curve of 0.869 in predicting fibrosis score \(\leq 1\)

### Diagnostic performance of different HBsAg levels for predicting insignificant fibrosis among patients with ALT \(\leq 2 \times \text{ULN}\) (n.56)

<table>
<thead>
<tr>
<th>HBsAg IU/ml</th>
<th>N° pts</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\geq 10.000)</td>
<td>45</td>
<td>90.9</td>
<td>58.3</td>
<td>88.9</td>
<td>63.6</td>
</tr>
<tr>
<td>(\geq 25.000)*</td>
<td>41</td>
<td>86.4</td>
<td>75</td>
<td>92.7</td>
<td>60.0</td>
</tr>
<tr>
<td>(\geq 50.000)</td>
<td>32</td>
<td>68.2</td>
<td>83.3</td>
<td>93.8</td>
<td>41.7</td>
</tr>
<tr>
<td>(\geq 75.000)</td>
<td>24</td>
<td>52.3</td>
<td>91.7</td>
<td>95.8</td>
<td>34.4</td>
</tr>
<tr>
<td>(\geq 100.000)</td>
<td>16</td>
<td>36.4</td>
<td>100</td>
<td>100</td>
<td>30.0</td>
</tr>
</tbody>
</table>

* The 7.3% (3 of 41) of patients with HBsAg serum levels \(\geq 25000\) IU/m, but fibrosis stage \(>1\), had only stage 2
HBsAg marker of the overall cccDNA transcriptional activity

Marker contributing to define the phase of the infection

Marker useful to define the severity of liver disease?
Clinical significance of serum HBsAg levels and association with liver histology in HBeAg positive chronic hepatitis B

198 HBeAg positive naive CHB patients (125 gen. B/ 73 gen. C)

• lower HBsAg serum levels were associated with gen. C, pre-core mutations, Knodell necroinflammation grading ≥ 7 and advanced fibrosis (Ishak score 4-6)

• HBsAg serum levels were the only independent factor correlating with advanced fibrosis (coefficient: - 0.975, p=0.039, OR 0.377, CI 0.149-0.953)

• At ROC curve analysis 3.580 log IU/ml (3810 IU/ml) HBsAg serum levels identified advanced fibrosis with 82% sensitivity, 56% specificity (AUROC: 0.716, p 0.001)
HBsAg serum level is associated with fibrosis severity in treatment-naive, e antigen-positive patients

406 CHB patients, 101 HBeAg pos (38% gen. B-C)

- In HBeAg positive patients HBsAg serum levels strongly correlate with fibrosis ($r=0.43$, $p<0.0001$) with higher correlation in patients with normal ALT.

Martinot-Peignoux M et al J Hep 2013 (58):1089-1095
HBsAg serum level is associated with fibrosis severity in treatment-naive, e antigen-positive patients

406 CHB patients, 101 HBeAg pos (38% gen. B-C)

- HBeAg positive patients with moderate to severe fibrosis showed lower HBsAg serum levels (** \( p<0.0001 \), F0-1 vs F2-F4, by METAVIR)

- In gen B-C patients at ROC curve 3.85 log IU/ml (7000 IU/ml) HBsAg serum levels identified advanced fibrosis with 100% sensitivity, 86% specificity (AUROC: 0.89)

- No correlation between HBsAg serum levels and severity of fibrosis was observed in HBeAg negative patients (** HBV-DNA= \( p<0.001 \), F0-1 vs F2-F4)

Martinot-Peignoux M et al J Hep 2013 (58):1089-1095
A statistical association can be found between HBsAg serum levels and the stage of liver disease in specific clinical setting.

However, we must be aware that HBsAg serum levels reflect the virological status of the HBV carrier.

Whereas, the extent of liver damage results from multiple factors and not only from the virologic status.
Natural history of Chronic HBV Infection

- Immune tolerance
- Immune clearance
- Immune control

**HBsAg** (log 10 IU/ml)

**HBV DNA** (log 10 gen. Eq./ml)

**IgM anti-HBc** (PEI Units)

**ALT** (U/L)