Immunosuppression-driven HBV Reactivation in Patients with Resolved HBV Infection Correlates with a Relevant Risk of Evolution Towards Active Chronic Infection and Death


Abst#_O_09

The authors declare that there are not conflict of interest
Background

How do we define immunosuppression-driven HBV reactivation?

**HBV reactivation** is defined as:

- a marked rise of serum HBV-DNA (> 2 log IU/ml from baseline level) in patients with **chronic HBV infection** (both in the active and in the inactive form)

  or

- a reappearance of serum HBV-DNA (>100 IU/ml) in patients with **apparently resolved HBV infection** during or after the administration of immunosuppressive therapy

Hwang & Lok, Nat Rev Gastroenterol Hepatol. 2014
Background

Clinical manifestations of HBV reactivation according to pre-reactivation serological status

<table>
<thead>
<tr>
<th>Active carrier</th>
<th>Inactive carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>(defective immunological control)</td>
<td>(partial immunological control)</td>
</tr>
<tr>
<td><strong>Hepatitis exacerbation</strong></td>
<td><strong>Hepatitis B reactivation</strong></td>
</tr>
<tr>
<td>(progression of liver disease)</td>
<td>(from transient liver damage up to fulminant hepatitis)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inactive carrier</strong></td>
<td><strong>HBsAg- and anti-HBc+ subject</strong></td>
</tr>
<tr>
<td>(partial immunological control)</td>
<td>(optimal immunological control)</td>
</tr>
</tbody>
</table>
Aim of the study

To provide a snapshot of virological and clinical features of patients, undergoing HBV-reactivation driven by immunosuppressive-therapy with a focus on reactivated patients with apparently resolved HBV infection
This study includes **80 patients** with immunosuppression driven HBV-reactivation (HBV-R) defined according to Hwang, 2014.

**Statistical analysis**
Mann-Whitney test and Fischer’s Exact test were used to assess statistically significant differences between factors positively or negatively associated with HBV-R.

**Survival analysis**
Kaplan-Meier analysis was used to estimate cumulative probability after HBV-reactivation of:
- transaminases normalization,
- undetectability of serum HBV-DNA,
- loss of HBsAg,
- death (competing risk analysis).

**Genetic analysis**
Mean genetic distance was used to estimate the extent of genetic variability in HBsAg in a subset of 55 HBV-reactivated patients infected with genotype D.

Presence of HBsAg-mutations associated with HBV-R (Salpini, 2015) was investigated.
Results
# Patients’ characteristics at HBV-reactivation

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>N=80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, N (%)</td>
<td>53 (66.2)</td>
</tr>
<tr>
<td>Italian nationality, N (%)</td>
<td>64 (80)</td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>63 (54-71)</td>
</tr>
</tbody>
</table>

**HBV characteristics at reactivation:**

- Median HBV-DNA, log IU/ml (IQR): 6.7 (4.5-7.9)
- Median quantitative HBsAg, IU/mL (IQR): 8679 (1069-25776)
- Median ALT, IU/L (IQR): 117 (40-621)
- Median AST, IU/L (IQR): 91 (32-286)
- Median MELD score (IQR): 9 (7-14)

**Serological profiles at reactivation**

- HBsAg positive/Anti-HBs negative N (%): 54 (69.2)
- **HBsAg positive/Anti-HBs positive, N(%)**: 9 (11.5)
- HBsAg positive with Anti-HBs unknown, N(%): 6 (7.7)
- **HBsAg negative/Anti-HBs positive, N(%)**: 4 (5.1)
- HBsAg negative/Anti-HBs negative, N(%): 5 (6.5)

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*a Datum available for 78 patients*
Serological status of HBV infection at screening before starting immunosuppressive therapy

- Active carriers: 3%
- Inactive carriers: 26%
- Anti-HBs alone: 4%
- Anti-HBc + Anti-HBs: 20%
- Anti-HBc alone: 43%
- Negative for all serological markers: 4%
- Occult HBsAg+: 70%

Percentages were calculated on overall population of 80 patients. a Inactive carrier state was defined as HBV-DNA levels <2000 IU/ml with persistently normal transaminases.
Occult hepatitis B virus in liver tissue of individuals without hepatic disease

Giovanni Raimondo¹,*, Giuseppe Navarra², Stefania Mondello¹, Lucy Costantino¹, Guido Colloredo³,†, Eugenio Cucinotta⁴, Gaetano Di Vita⁵, Claudio Scisca⁴, Giovanni Squadrito¹, Teresa Pollicino¹

16 individuals
HBsAg - and Anti-HBc+

62.3% Anti-HBc positive with occult infection

82 individuals
negative for all HBV markers without a clinical history of liver disease

7.3% HBV-seronegative individuals with occult infection

“A not negligible portion of OBI cases are negative for all HBV serum markers: they might have either progressively lost the anti-HBV antibodies or might be HBV antibody negative since the beginning, as a consequence of a very limited number of virions in the infecting inoculums”
Immunosuppressive conditions associated with HBV-reactivation

Treatments with corticosteroids and chemotherapies include: methotrexate+methylprednisolone, vincristine+dexamethasone, dexamethasone + thalidomide, chlorambucil+prednisone.

Treatments with only chemotherapeutics include: fludarabin, carboplatin, radiotherapy, everolimus, mycophenolate.

Treatment with corticosteroids include: prednisone, deltacortene, methylprednisolone. Median (IQR) dosage of corticosteroids, mg: 5 (4-25). The duration of corticosteroids therapy ranges from 3-36 months.

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**Table: HBV-reactivation Causes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV induced immune depletion</td>
<td>3%</td>
</tr>
<tr>
<td>Unknown</td>
<td>4%</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>11%</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>10%</td>
</tr>
<tr>
<td>Corticosteroids &amp; chemotherapy</td>
<td>22%</td>
</tr>
<tr>
<td>LMV yes</td>
<td>50%</td>
</tr>
<tr>
<td>LMV no</td>
<td>40%</td>
</tr>
<tr>
<td>LMV no</td>
<td>11%</td>
</tr>
<tr>
<td>Rituximab</td>
<td>10%</td>
</tr>
</tbody>
</table>

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*a* Treatments with corticosteroids and chemotherapies include: methotrexate+methylprednisolone, vincristine+dexamethasone, dexamethasone + thalidomide, chlorambucil+prednisone.

*b* Treatments with only chemotherapeutics include: fludarabin, carboplatin, radiotherapy, everolimus, mycophenolate.

*c* Treatment with corticosteroids include: prednisone, deltacortene, methylprednisolone. Median (IQR) dosage of corticosteroids, mg: 5 (4-25). The duration of corticosteroids therapy ranges from 3-36 months.
In our population, a large fraction of patients develops HBV reactivation after completing immunosuppressive therapy.

- HBV reactivation during immunosuppression: 29/65 (44.6%)
- HBV reactivation after completing immunosuppressive therapy: 36/65 (55.4%)

Datum available for 65 patients.
Factors **positively or negatively** associated with HBV-reactivation after completing immunosuppressive therapy

<table>
<thead>
<tr>
<th>Patients’ characteristics at reactivation</th>
<th>HBV reactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age, years (IQR)</strong></td>
<td>60 (50-64)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>HBV characteristics at reactivation:</strong></th>
<th>[N=29]</th>
<th>[N=36]</th>
<th>[P value&lt;sup&gt;a&lt;/sup&gt;]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median quantitative HBsAg, mIU/mL (IQR)</td>
<td>1135 (91-12871)</td>
<td>16526 (1553-32972)</td>
<td>0.03</td>
</tr>
<tr>
<td>Median AST, IU/L (IQR)</td>
<td>43 (31-153)</td>
<td>138 (39-420)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Immunosuppressive therapy:</strong></th>
<th>[N=29]</th>
<th>[N=36]</th>
<th>[P value&lt;sup&gt;a&lt;/sup&gt;]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids, N(%)</td>
<td>6 (21)</td>
<td>1 (2)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Pathology requiring immune-suppressive therapy:</strong></th>
<th>[N=29]</th>
<th>[N=36]</th>
<th>[P value&lt;sup&gt;a&lt;/sup&gt;]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onco-hematological disease&lt;sup&gt;b&lt;/sup&gt;, N(%)</td>
<td>20 (69)</td>
<td>33 (92)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Statistically significant differences were assessed by Mann-Whitney Test and by Fisher’s exact test.

<sup>b</sup> Onco-hematological disease: chronic lymphocytic leukaemia (LLC), multiple myeloma (MM), mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), bone marrow aplasia.

- The following variables were considered for the analysis: age; sex; HBsAg levels; HBV-DNA; ALT; AST; MELD; HBV serological profiles before reactivation; lamivudine prophylaxis; immunosuppressive therapies; pathologies requiring immunosuppressive therapy; exitus.
After HBV-reactivation, most patients were treated with high genetic barrier drugs with a median time of follow-up (IQR) of 30 (14-46) months.

a Datum available for N=72 patients.
Despite ALT normalization and HBV-DNA undetectability, HBsAg loss is observed in only 34%.

- Kaplan-Meier analysis was used to estimate cumulative probability after HBV-reactivation of achieving transaminases normalization, undetectability of serum HBV-DNA, loss of HBsAg after starting anti-HBV therapy. Patients were followed from the date of HBV-reactivation.

CI: confidence interval. NA: not available.
Among patients with a past occult infection (HBsAg neg, N=35) only 31.4% of patients returns to the pre-reactivation status, after a median time of 3.5 years

<table>
<thead>
<tr>
<th>N of patients</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of treatment median (IQR), months</td>
<td>41 (IQR: 25-84) (min-max:9-95)</td>
</tr>
<tr>
<td>Transaminases normalization N(%)</td>
<td>26 (74.3%)</td>
</tr>
<tr>
<td>Undetectable serum HBV-DNA N(%)</td>
<td>15 (42.9%)</td>
</tr>
<tr>
<td>Still HBsAg positive</td>
<td>24 (68.6%)</td>
</tr>
<tr>
<td>HBsAg-loss</td>
<td>11 (31.4%)</td>
</tr>
</tbody>
</table>

All patients had a pre-serological status compatible with occult HBV infection, and were treated with TDF and/or ETV at the time of HBV reactivation.

Chronicization of HBV infection after reactivation, requiring long-term (life-time) antiviral treatment
In our cohort of 67 patients developing HBV reactivation, \textbf{8.9\% (6/67)} die for hepatic failure related to HBV reactivation:

- 5 patients with occult HBV infection
- 1 inactive carrier patient

\textbf{Competing risk analysis} was used to estimate the cumulative probability of exitus. Cumulative probability was evaluated in 64 patients with follow up and (when occurred) date of exitus available.
This study includes **80 patients** with immunosuppression driven HBV-reactivation (HBV-R) defined according to Hwang, 2014.

**Statistical analysis**

Mann-Whitney test and Fischer’s Exact test were used to assess statistically significant differences between factors positively or negatively associated with HBV-reactivation.

**Survival analysis**

Kaplan-Meier analysis was used to estimate cumulative probability after HBV-reactivation of:
- transaminases normalization,
- undetectability of serum HBV-DNA,
- loss of HBsAg,
- death (competing risk analysis).

**Genetic analysis**

Mean genetic distance was used to estimate the extent of genetic variability in HBsAg in a subset of 55 HBV-reactivated patients infected with genotype D.

Presence of HBsAg-mutations associated with HBV-R (Salpini, 2015) was investigated.
Of 51 patients negative to HBsAg at screening before immunosuppression, 17.7% (9/51) patients remains HBsAg-negative despite HBV-reactivation.

At reactivation:
- HBsAg neg
  - N(%)=9 (17.7)
  - (HBV-DNA: 3.0-7.5 log IU/ml)

In 9/9, >1 new N-linked glycosylation HBsAg site is detected at position 113, 115, 123, 131 (all residing in Major Hydrophilic Region).
Additional N-linked glycosylation sites are anchors for glycan attachment.

The hyperglycosylation of HBsAg might mask HBsAg epitopes interfering with its recognition by immunity and diagnostic antibodies (anti-HBs).
N-Glycosylation mutations strongly affect HBsAg recognition and quantification by diagnostic tests.

The histogram reports the quantification of strep-tagged HBsAg released in supernatants of HepG2 cell cultures by different ELISAs are shown. For each mutant, the amount of strep-tagged HBsAg released in supernatants was expressed as a percentage, considering the amount of the WT strep-tagged HBsAg as 100%. Dotted line indicates a 90% inhibition in HBsAg recognition and quantification.

Salpini et al., Hepatology 2015; Colagrossi et al., AISF 2015
An higher degree of genetic variability in HBsAg correlates with rituximab-related immunosuppression and higher ALT levels.

- Genetic distance was estimated as the extent of nucleotide substitutions per site determined by the Tajima-Nei model of MEGAv5.
- Box plots were used to report the distribution of GD values. Box plots report median, 25\textsuperscript{th} percentile, 75\textsuperscript{th} percentile, lower and upper whiskers, minimum and maximum values.
- Statistically significant differences were assessed by Mann-Whitney Test.
Q129R correlates with rituximab-related immunosuppression, suggesting a role in promoting HBV-reactivation in the setting of B-cell depleting drugs.


Statistically significant differences were assessed by Fisher Exact Test.
Conclusions

- Immunosuppression-driven HBV reactivation, can occur in a large variety of anti-HBV serological profiles and immunosuppressive settings.
- A relevant proportion of patients remains HBsAg-negative despite HBV-reactivation, highlighting the importance of HBV-DNA (more than HBsAg) in HBV-reactivation diagnosis.
- A higher degree of genetic variability and specific mutations in HBsAg, such as Q129R, are correlated with Rituximab use and may favor HBV reactivation in the setting of drug-induced B-cell depletion.
- Overall, these data support the need of an optimized management of HBV-reactivation in terms of adequate monitoring before and during immunosuppression, and improved prophylaxis.
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