Update on Biology and Clinical impact of Occult Hepatitis B Virus Infection

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Statements from the Taormina expert meeting on occult hepatitis B virus infection

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Sessions

1. Virology/Immunology
2. Diagnosis
3. Epidemiology
4. Transmission
5. Liver Disease/Therapeutic Implications
6. General Discussion and Statements
Update of the Statements on Biology and Clinical Impact of Occult Hepatitis B Virus Infection

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*J Hepatol 2019, in press*
Occult HBV Infection (OBI)

DEFINITION

The presence of replication competent HBV DNA (i.e. episomal HBV covalently closed circular DNA [cccDNA]) in the liver and/or HBV DNA in blood of persons testing negative for hepatitis B surface antigen (HBsAg) by currently available assays.

Raimondo et al,
Update of the statements on occult HBV infection
J Hepatol 2019, in press
Schematic representation of HBV serum markers profile in naturally occurring OBI

Update of the statements on occult HBV infection
J Hepatol 2019, in press
OBI-associated HBV variants

- **S-escape variants**
  - HBV DNA levels comparable to overt infection

- **S promoter variants**
  - HBV DNA levels comparable to overt infection

- **Splice variants**
  - HBV DNA levels low or undetectable

*Update of the statements on occult HBV infection*
*J Hepatol 2019, in press*
The molecular basis of OBI is related to the stability and long-term persistence of the HBV cccDNA intermediate, located in the nucleus of infected hepatocytes.

The episomal HBV cccDNA exists as a chromatinized viral minichromosome, which is very stable and long-lasting. Together with the long half-life of hepatocytes, this implies that HBV infection, once initiated, may continue for life even if an efficient immune control is achieved.

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J Hepatol 2019, in press
The vast majority of OBI cases have low levels of HBV cccDNA in the liver and suppression of overall replication activity and viral protein expression is exerted by the host’s immunologic and epigenetic mechanisms.

The low level of transcriptionally active cccDNA in OBI cases results in low or undetectable HBV RNA transcription and subsequent protein translation and expression. However, cccDNA in OBI cases is fully replication competent.
Mechanisms potentially involved in HBV inhibition and OBI induction

Update of the statements on occult HBV infection, J Hepatol 2019, in press
Virology and Immunology (3)

Integrated HBV DNA into the host’s genome may be present and remain in the hepatocytes of HBV-infected individuals after spontaneous or treatment-induced HBsAg clearance. However, integrated HBV DNA is not replication competent and its detection is not required to make a diagnosis of OBI, since OBI is defined as the persistence of replication-competent HBV DNA.
• Immune response to HBV in OBI has not been sufficiently investigated

• OBI is associated with anti-viral immune responses that are continuously stimulated by persistent/intermittent low concentrations of HBV antigens. This mechanism is believed to be important in maintaining HBV control.

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J Hepatol 2019, in press
Diagnosis of OBI is based on the detection of HBV DNA in blood or liver of HBsAg negative individuals.
The diagnosis of OBI is based on the sensitivity of assays used in the detection of HBV DNA and HBsAg.

HBsAg assays with inadequate sensitivity or inability to detect HBV S variants may lead to false negative HBsAg result and misdiagnosis of OBI in persons who have overt HBV infection.

HBV DNA assays with inadequate sensitivity resulting in false negative HBV DNA result may lead to missed diagnosis of OBI.

Update of the statements on occult HBV infection

J Hepatol 2019, in press
The lower limit of detection of most currently available commercial HBV DNA assays is 10-20 IU/ml. It is important that HBV DNA assays have similar performance across HBV genotypes and subtypes.

Because HBV DNA is usually present in low concentrations and may only be intermittently detected in persons with OBI, testing of blood samples collected at more than one time-point as well as testing DNA extracts from not less than 1 ml of serum or plasma is recommended for diagnosis of OBI.

In the setting of blood transfusion, assays used for NAT have high specificity (99.9%) and limit of detection of 2–4 IU/mL HBV DNA when applied to individual units. When NAT screening is conducted in minipools of multiple donations (typically, 6-20 donations per pool), the sensitivity decreases according to the dilution factor introduced by the pooling process.

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J Hepatol 2019, in press
The ideal method of diagnosis of OBI is the detection of replication competent HBV DNA in the liver.

- Standardized and valid assays for HBV DNA detection in the liver are not yet available.
- Studies using in house assays have variable sensitivities and specificities.
- The recommended methods include nested-PCR techniques to amplify at least 3 different viral genomic regions, real-time PCR assays, or droplet digital PCR assays. In each case the assay must include primer sets that allow to detect replication competent HBV DNA.
- Given that HBV DNA is present in low concentrations in persons with OBI, adequate size samples, and fresh frozen – but not formalin fixed – liver tissue should be used.
Detection of anti-HBc in the blood may be used as a surrogate marker for identifying OBI in blood or organ donors, in persons who are about to receive immunosuppressive therapies, and for epidemiological studies.

In these settings, liver tissue is usually not available, access to tests for HBV DNA in blood may be limited or delayed, and undetectable HBV DNA in blood tested at one timepoint does not rule out OBI.

HBV reactivation has been reported in HBsAg-negative, anti-HBc-positive persons who have undetectable HBV DNA in blood. Similarly, anti-HBc testing may identify some blood donors with OBI who have undetectable HBV DNA in mini-pool nucleic acid testing.

Absence of anti-HBc does not rule out OBI, although the prevalence and clinical significance of seronegative OBI in humans are unknown.
Defining the epidemiology of OBI can be challenging as

- it relies on the performance and sensitivity of HBsAg and HBV DNA detection assays used in the different studies
- it varies with:
  - the presence of risk factors for HBV exposure
  - the presence and severity of liver disease
  - the prevalence of HBV in the general population of a given country
  - with the definition used for OBI.

It is difficult to compare data between studies or to perform meta-analyses across studies.

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J Hepatol 2019, in press
The majority of prevalence studies have been conducted on blood donors and patients with liver disease. The OBI prevalence in these groups is related to the prevalence of overt HBV infection in that geographical area and the population studied.

Due to methodological limitations, OBI prevalence in the general population is still largely undefined.

The prevalence of OBI is higher in chronic liver disease and may be as high as 40% to 75% in HBsAg-negative hepatocellular carcinoma (HCC).

OBI is rarely detected amongst blood donors, with HBV DNA detection rate in HBsAg negative samples typically being less than 0.5%.
The prevalence of OBI varies greatly across the world and across patient populations, with higher rates being reported from Asia.

Higher rates have also been found in persons with risk factors for HBV infection, e.g.

- co-infection with HCV or HIV,
- use of injecting drugs
- hemodialysis
- presence of HCC
- cryptogenic cirrhosis
- OLT for end stage liver disease
• There is a single study that tested HBV DNA in liver to determine the prevalence of OBI in patients with no liver disease. In this study, HBV DNA was detected in 16% of Italians with normal liver histology who underwent abdominal surgery from 2002 to 2006.
Blood Transfusion

- HBsAg negative, HBV DNA positive blood components have to be considered infectious.

- HBV transmission from OBI blood donors is still a major health issue in low- and middle-income countries, where anti-HBc and/or NAT are not implemented.

- A residual risk of transfusion-transmitted OBI exists even in developed countries, because the minimal HBV DNA infectious dose is below the limit of detection of the current NAT assays.
Blood Transfusion (2)

• The incidence of transfusion-related transmission of HBV from OBI donors might be underestimated

This because of several and often concomitant reasons including:

– undetectable or intermittently detectable HBV DNA in donors
– difficulty and reluctance to trace recipients
– lack or limited volume of donor archive samples
– lack of HBV infection diagnosis in recipients without clinically evident acute hepatitis

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J Hepatol 2019, in press
TRANSMISSION

Blood Transfusion (3)

- Presence of anti-HBs in recipients prior to transfusion significantly reduce the risk of infection

- NAT sensitivity required to prevent HBV transmission by transfusion would need to be lowered from the current 3.4 IU/ml to a new lower limit of detection (LLOD) of 0.15 IU/ml.

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Liver Transplantation

- HBV transmission can occur from an OBI seropositive liver donor to a recipient who is HBV susceptible. These recipients should receive lifelong prophylaxis with nucleos(t)ide analogues (NUCs) to prevent hepatitis B.

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Liver Transplantation (2)

- OBI liver donor can transmit HBV infection to an HBsAg negative, anti-HBc negative/anti-HBs negative recipient with possible development of hepatitis B.
- While HBsAg positive infection is prevented, antiviral prophylaxis may not prevent the development of an OBI in the recipient.
- HBV DNA can be detected in the liver of patients who received liver transplantation for hepatitis B and who received anti-HBV prophylaxis.
- OBI of the liver grafts is frequent.
HBV vaccination is one of the most important and most successful medical science accomplishments. WHO has proposed goals to eliminate HBV by 2030. Elimination of mother-to-child transmission of HBV is one of the most important tactics to achieve this goal. OBI in newborns, detection of anti-HBc but not HBsAg after the age of one when passive transfer of maternal antibodies should have disappeared, occurs when immunoprophylaxis failed to completely prevent HBV infection but succeeded in modulating the infection to prevent progression to chronic HBV infection.

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In the vast majority of cases, OBI does not appear to lead to any clinical sequelae. However, OBI may result in transmission of HBV infection to blood or organ transplant recipients, and reactivation of HBV replication in patients receiving cancer chemotherapy or other immunosuppressive therapies.
HBV Reactivation in OBI patients

Definition

• HBsAg seroreversion and/or increase of serum HBV DNA by at least one log above the low level of detection of the assay in a person who had previously undetectable HBsAg and HBV DNA in serum.

• A more than 1 log increase in serum HBV DNA in persons who had detectable HBV DNA at baseline.

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CLINICAL IMPLICATIONS

HBV Reactivation in OBI patients (2)

• Can occur in up to 40% of persons with OBI when potent immunosuppressive therapies are use.

- The risk is high (>10%) in persons who receive anti-CD20 containing regimens and myeloablative regimens for hematopoietic stem cell transplantation.

- The risk is low (<1%) to moderate (1-10%) in persons who receive other cancer chemotherapies, high dose corticosteroids, or anti-rejection therapies for solid organ transplantation.

• Can occur in persons coinfected with HIV when antiretroviral regimens are modified and drugs active against HBV are withdrawn.

• Prophylactic antiviral therapy should be used in all OBI persons at high risk of HBV reactivation.

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CLINICAL IMPLICATIONS

• A still widely debated topic is whether OBI may accelerate the progression toward cirrhosis and the development of HCC in persons with chronic liver disease due to other causes (e.g. HCV, alcohol, NASH).

• OBI retains several of the oncogenic mechanisms of overt HBV, including production of pro-oncogenic proteins and propensity of the viral DNA to integrate into the host’s genome.

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Clinical Implications

Occult HBV infection may...

... be transmitted *(blood transfusion; organ transplantation)*
   Consequence: typical hepatitis B in the recipient

... reactivate *(under immunosuppression)*
   Consequence: typical hepatitis B in the occult carrier

... contribute to liver fibrosis progression toward cirrhosis

... favour HCC development
ANTIVIRAL THERAPY

• Currently, antiviral therapy is not recommended for persons with OBI.

Proposed definition of HBV functional cure, clearance of HBsAg, may suggest a conversion from overt HBV infection to OBI, but a key differentiation is that definition of HBV functional cure requires that HBV DNA is not detected in blood. While low amounts of HBV DNA can persist in the liver, replication is suppressed possibly by host immune response. The risk of HCC and liver mortality is lower in persons with chronic HBV infection, who cleared HBsAg.

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To eliminate HBV from persons with OBI, elimination of HBV-infected hepatocytes or curing these cells of the infection would be necessary. Several paths could theoretically be investigated:

• Elimination of cccDNA within infected hepatocytes (i.e. curing infected cells) through cccDNA targeting strategies such as the gene editing approaches including the CRISPR/Cas9 technologies, or cytokine mediated degradation of cccDNA;

• Specific killing of infected hepatocytes using strategies aiming at restoring HBV specific T cell responses (check point inhibitors, restoration of HBV specific T cell metabolism), therapeutic vaccination strategies, engineered T cell therapies such as chimeric antigen receptor (CAR-T) cells technologies or HBV-T cell receptor (TCR) engineered T cells to kill the residual infected cells in the liver.
Main Objectives of Future Research Studies

- Definitively clarify whether, how, and in which circumstances OBI might be involved in liver injury and/or hepatocarcinogenesis
- Understand the immunological mechanisms driving the development of OBI
Future Research Studies

Epidemiology and Clinical research:

• Adopt standardized methods of reporting for studies on OBI such that results across studies can be compared and combined.
• Development of more sensitive, standardized, and validated assays for detection of HBV DNA in blood and in liver.
• Development of more sensitive, standardized, and validated assays for detection of HBsAg in blood, including detection of HBV S variants, and HBsAg present in immune complexes with anti-HBs. Determine the clinical implications of the detection of low concentrations of circulating HBsAg, in particular risk of transmission, liver disease progression and risk of HCC.
• Determine the prevalence of OBI in different parts of the world, specifically among blood donors – using standardized definitions and appropriate assays.
• Define the risk of transmission of HBV from OBI blood, organ, tissue and cell donors and determine the best strategies to prevent such transmissions, tailoring to local prevalence and resources available.

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FUTURE RESEARCH STUDIES

Basic/translational research:

• Virology
  - Determine which are the molecular virology determinants driving OBI
  - Determine how common is OBI due to HBV variants

• Immunology
  - Determine what is the difference between immune control in OBI versus overt HBV infection
  - Determine how to harness the immune control mechanisms in OBI to achieve functional HBV cure
  - Establish what disturbs the immune control in OBI leading to HBV reactivation.

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Conclusions

OBI is a frequent condition that deserves attention from the scientific, medical, and public health communities. We hope these communities will collaborate to address the key questions we identified and answers to many of these questions will be satisfactorily addressed at the next workshop on Occult HBV Infection a decade from now.