

What do we know about occult hepatitis B virus infection?

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Abstract

Occult hepatitis B virus (HBV) infection (OBI) is recognized as one of the phases in the natural history of chronic HBV infection and defines the persistence of HBV genomes in the hepatocytes of individuals testing negative for HBV surface antigen (HBsAg) and detectable or undetectable HBV DNA in the serum. Occasionally, OBI is related to the infection with mutant viruses producing a modified HBsAg undetectable by diagnostic kits or with replication-defective variants. However, in most cases OBI is due to replication-competent viruses that are strongly suppressed in their activities by the host's defence mechanisms. Growing evidence indicates that genetic, epigenetic and post-transcriptional mechanisms may be involved in the control of HBV activities and in the OBI occurrence.

OBI may be involved in several different clinical contexts, which can be grouped in four main categories: 1) transmission of the "occult" virus (mainly, through blood transfusion and orthotopic liver transplantation from OBI donors) causing classic forms of hepatitis B in the HBV naïve recipients; 2) reactivation of the HBV infection following the development of an immunosuppressive status, resulting in acute and occasionally fulminant hepatitis; 3) contribution to the progression of patients with various causes of liver disease toward cirrhosis; 4) involvement in hepatocarcinogenesis, likely through the maintenance of the direct and indirect pro-oncogenic properties typical of the overt HBV infection (such as the capacity to integrate in the host's genome and to synthesize pro-oncogenic proteins) and by provoking a mild but persisting necroinflammation which favours cirrhosis development.

KEYWORDS: Hepatitis B virus, Occult hepatitis B infection, Hepatitis B virus reactivation, Liver cirrhosis, Hepatocellular carcinoma.

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Introduction

Recognized as one of the phases in the natural history of chronic HBV infection [1], OBI is defined as the presence of HBV DNA in the liver (with detectable (usually <200 IU/ml) or undetectable HBV DNA in the serum) of individuals testing HBsAg negative by currently available assays. On the basis of the HBV antibody profile, in fact, OBI can be distinguished as:

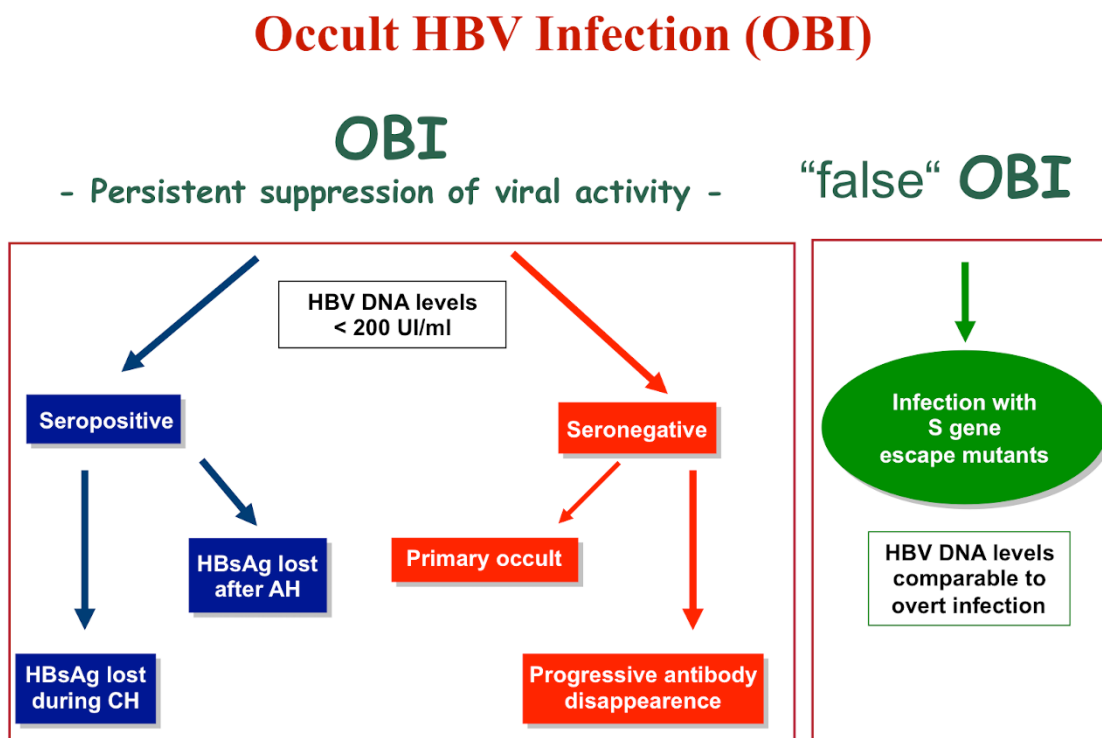
Seropositive-OBI, characterized by the presence of antibodies against the core (anti-HBc) and/or the s antigen (anti-HBs) in the serum.

Seronegative-OBI, characterized by the total absence of antibodies anti-HBV [2], condition

accounting for more than 20% of cases [3] (**Figure 1**).

OBI prevalence is strongly dependent on HBV endemicity, and generally higher in populations at high risk of parenterally transmitted infections [4, 5]. A high prevalence of occult infected individuals has been found among patients affected by chronic liver disease, in particular HCV-related or cryptogenic (up to 50% of cases)[3, 6-8].

Figure 1. Schematic representation of HBV profile in OBI and “false” OBI (adapted from Taormina’s statements on occult HBV infection. Raimondo et al. J Hepatol 2008).



Virology and detection

HBV is a Hepadnavirus consisting of a partially double-stranded relaxed circular DNA of approximately 3,200 nucleotides. In the nucleus of the hepatocytes, the viral genome it is converted into a covalently closed circular DNA (cccDNA) [9]. Similarly to retroviruses, HBV DNA can integrate in the genome of the host’s hepatic cells. The stability and long-term persistence of cccDNA molecules and the long half-life of hepatocytes imply that HBV infection, once occurred, may continue forever [10, 11].

Standardized, valid assays for OBI detection are not yet available. The analysis of DNA extracts from liver tissues by highly sensitive techniques (real time or nested-polymerase chain reaction) and oligonucleotide primers specific for HBV genomic regions are currently the gold standard

[2]. Due to the limited availability of liver biopsy, the most common approach to identify cases of OBI is the serial extraction of HBV DNA from serum/plasma samples [2]. When HBV DNA testing is not accessible, serum anti-HBc antibody can be used as a surrogate marker, but may provide false positive results [2, 12].

Recently, IFN signalling-related microRNAs have been proposed as a potential marker for OBI diagnosis [13], but further research is needed to confirm this finding.

Mechanisms potentially involved in the induction of obi

Both viral and host factors can be responsible of OBI status.

Viral factors

In some cases, OBI is associated with viruses with defective replication activity or S proteins synthesis (“S-escape mutants”), undetectable by HBsAg commercial assays [2, 6, 14, 15]. This condition, usually characterized by serum HBV DNA levels comparable to those detectable in the overt infection, has been described as “false” OBI [2]. In the majority of cases, however, the main responsible of OBI status is the strong suppression of the HBV replication and gene expression by host’s factors [2, 16, 17].

Host-related factors

Immunological factors

There is strong direct and indirect evidence of the involvement that the host’s immune surveillance (both adaptive and innate) plays an important role in the OBI development. Long-lasting memory CD4 and CD8 lymphocytes against HBV antigens have been detected several years after clinical recovery from acute hepatitis B. It has been hypothesised that, in the occult phase of the infection, the persistence of traces of virus and the subsequent synthesis of very small quantities of antigens allow the maintenance of an HBV-specific T-cell response [18, 19]. Other proofs of the fundamental role played by the host’s immune system in the control of the viral replication are the potent HBV-specific T-cell response found in OBI positive blood donors [20], the lower CD4 count (reflecting cellular immune deficiency) demonstrated in HIV patients in cases of occult infection [21] and the fact that all the conditions inducing immunosuppression can lead to OBI reactivation, with the subsequent reappearance of the serological profile of overt active infection [3, 6, 22]. Finally, it has been recently demonstrated that liver cells can produce inflammatory cytokines in response to HBV infection [23], implying a possible role of the innate immune response in the control of HBV activities.

Epigenetic factors

Similarly to host cell chromatin, HBV cccDNA molecules accumulate in the nucleus of infected hepatocytes as stable minichromosomes packed into nucleosomal arrays by histone and non-histone proteins [10] and are targeted by epigenetic regulatory mechanisms. In particular, the acetylation status of H3/H4 histones bound to the viral cccDNA in the nuclei of the infected hepatocytes [24], the recruitment of chromatin modifying enzymes onto the viral minichromosome and the methylation of CpG-rich regions within the HBV genome seem to be involved in the control of HBV replication and gene expression, and thus in the OBI occurrence [10, 25, 26].

Co-infection

There are conflicting data as to whether HBV replication may be inhibited in case of co-infection with other agents, in particular HCV and HIV [27-30]. Also, *Schistosoma mansoni* seems to be potentially able to suppress HBV replication [31], and to increase the incidence of OBI in patients affected by chronic hepatitis C in endemic areas [32].

Clinical implications

Transmission of OBI

Blood transfusions

OBI carriers may potentially be a source of HBV transmission in the case of blood donation [15, 33, 34]. Thanks to the development of highly sensitive diagnostic assays, (i.e. Nucleic Acid Testing [35]), in the developed countries this risk has become a very rare occurrence.

Transfusional transmission of HBV may occur in essentially three conditions:

- 1) The HBV-infected donor is in the *window period* of HBV infection [33];
- 2) The donor is a typical “OBI carrier” with a wild-type virus whose replication activity is suppressed [15, 36, 37];
- 3) The donor is infected with S-escape mutants - condition accounting for the major cause of HBV transmission by blood transfusion [12, 33].

Organ transplantation

The fact that the hepatocytes are the reservoir of the viral cccDNA implies, in cases of orthotopic liver transplantation (OLT) from OBI positive donor, a possible transmission of the HBV infection with the subsequent development of *de novo* hepatitis B in HBV naïve recipients [4, 38]. Anti HBV prophylaxis with hepatitis B immunoglobulin and lamivudine or with lamivudine alone is very effective and therefore needs to be performed in all HBsAg-negative patients receiving livers from anti-HBc positive donors [4, 39]. The clinical impact of OBI on long-term

post-transplant outcome is still unclear, but recent evidence suggest it may play a role in the progression toward cirrhosis of post-OLT liver disease in HCV positive patients [40, 41].

Occurrence of HBV transmission in cases of kidney, heart and bone marrow transplantation has also been described, however this appears to be a much less frequent event [42].

Hepatitis B reactivation

Similarly to what frequently happens in immunocompromised HBsAg-positive subjects, but less commonly, HBV reactivation may occur in patients with OBI and significant impairment of their immune function, who may subsequently develop fulminant hepatitis [43-47]. Recent data suggest that OBI reactivation may be associated with the use of histone deacetylase inhibitors [48, 49], confirming the involvement of epigenetic mechanisms in the control of HBV cccDNA minichromosome.

Conditions such as hematological malignancies, hematopoietic stem cell transplantation and immunosuppressive treatments comprising anti-CD20 monoclonal antibody (Rituximab), CHOP regimen [46] or fludarabine [50] are at high risk for OBI reactivation [51-53]. Luckily, the changes in the HBV serological profile commonly occurring in immunocompromised patients are followed by serious clinical sequelae only in a minority of cases [54, 55].

OBI reactivation appears to be an infrequent event in patients with rheumatologic diseases undergoing treatments including biologics or corticosteroids administered at high doses and for long periods [52, 56]. Only very few cases of OBI reactivation have been reported in patients with liver cancer undergoing trans-arterial-chemo-embolization and in patients with inflammatory bowel diseases under treatment with biological agents [2], while it is increasing the number of cases of HBV reactivation in patients treated with chemotherapy for solid tumours [57, 58], however studies on the topic are not conclusive and the complete dimension of risk remains unknown [59].

Finally, recent reports have raised the possibility of HBV reactivation in OBI patients undergoing treatment for HCV with direct acting antivirals. However, the risk in this setting appears to be negligible, and without clinical or virological sequelae [60-62].

Prevention of HBV reactivation

Patients at high risk of reactivation, including anti-HBc-positive/HBsAg-negative individuals who need to be treated with rituximab in the onco-haematological setting or those undergoing stem cell transplantation and those who need extended duration treatment with highly immunosuppressive regimens should receive antiviral prophylaxis [1] with Nucleos(t)ide

analogues (NA), such as lamivudine, entecavir or tenofovir (TDF or TAF). Prophylaxis with NA can be also considered in anti-HBc positive patients receiving highly immunosuppressive regimens of extended duration [1, 63]. Since HBV reactivation may occur long time (up to 27 months) after stopping chemotherapy [43, 56, 64, 65], antiviral prophylaxis should be continued for at least 18 months after stopping immunosuppression, with a post-treatment monitoring of at least 12 more months [1].

Table 1. Risk stratification and treatment/surveillance recommendations for HBV reactivation in occult hepatitis B infection.

	HBsAg-/HBcAb+ subjects		
	Antiviral therapy	Treatment duration	Monitoring
High risk (>10%) <ul style="list-style-type: none"> • Onco-haematological malignancies under treatment • R-CHOP treatments • Liver transplantation (from HBcAb positive donors) • Haematopoietic stem cell transplantation 	Lamivudine or ETV/tenofovir prophylaxis.	Continue for at least 18 months after stopping immunosuppression.	Serum ALT +/- HBV DNA for at least 12 months after prophylaxis withdrawal.
Moderate risk (1-10%) <ul style="list-style-type: none"> • Rheumatological diseases treated with biological agents or high dosage of steroids for prolonged time • HIV infection • Kidney transplantation (from HBcAb positive donors) • Bone marrow transplantation • Compliance to monitoring • Unknown risk of viral reactivation for new biologicals 	Lamivudine prophylaxis or narrow serum ALT +/- HBV DNA monitoring.	Continue for at least 6 months after completion of treatment.	Serum ALT +/- HBV DNA every 1-3 months during and after immunosuppression.
Low risk (<1%) <ul style="list-style-type: none"> • Dermatological and inflammatory bowel diseases treated with biologics • Solid tumors treated with chemotherapy • Organ transplantation other than liver and kidney • Transarterial chemoembolization for treatment of hepatocellular carcinoma 	No NA prophylaxis. Serum ALT +/- HBV DNA every 3 months.	-	-

HBsAg, Hepatitis B s antigen; Hepatitis B anti-core antibodies; R-CHOP, Rituximab with cyclophosphamide, hydroxydaunorubicin, oncovin and predniso(lo)ne; ETV, entecavir; ALT alanine aminotransferase; HIV, human immunodeficiency virus; NA, Nucleos(t)ide analogues.

Pre-emptive use of lamivudine in these cases should be considered also when HBV DNA monitoring is not feasible for practical reasons [66].

For HBsAg-negative/anti-HBc-positive subjects at moderate or low risk of HBV reactivation, regular monitoring HBsAg and/or HBV DNA every 1–3 months during and after immunosuppression is instead recommended [1, 67] (**Table 1**). NA therapy must immediately be started in patients who become HBsAg and/or HBV DNA positive even before any ALT elevation [1].

OBI and chronic liver disease

OBI is characterized by phases of absent viraemia alternating with phases of very low but detectable viral load, possibly associated with a slight increase of transaminase levels [36, 37]. These episodes of transient, partial viral reactivation may account for mild but persistent liver necroinflammation, detected on histology up to several decades after the resolution of the acute hepatitis [68-70]. The minimal lesions chronically produced by the immune response to the occult virus, inoffensive in itself, might contribute, when other causes of liver damage co-exist (HCV infection, alcohol abuse, etc.), to make the course of the liver disease worse over time [71]. Accordingly, the presence of OBI has been associated with the most severe forms of chronic hepatitis, suggesting that it might negatively influence the outcome, accelerating the progression toward cirrhosis of patients with various causes of liver disease, in particular those affected by chronic hepatitis C [4, 72, 73] and cryptogenic liver disease [3, 4, 74].

OBI and cancer

A number of studies indicate that OBI is an important risk factor for hepatocellular carcinoma (HCC) [75-77]. The oncogenic properties and the role of HBV in the development of HCC are well known [78, 79], and it seems, that most of the pro-oncogenic properties of the overt HBV infection are maintained in its occult form, in particular:

1. Long-lasting persistence of viral genomes into the hepatocytes both as integrated DNA and as free episome;
2. Maintenance of very low levels of HBV replication and transcriptional activity with subsequent synthesis of viral proteins;
3. Chronic persistence of a mild necroinflammation into the liver, possibly contributing to the development of cirrhosis [68].

Some recent evidence suggests a possible involvement of HBV and OBI in malignancies other

than HCC, such as intrahepatic cholangiocarcinoma and non-Hodgkin lymphoma [80-84], although the underlining tumorigenic mechanism hasn't been completely elucidated.

Conclusions

Occult HBV infection is a phenomenon characterized by the long-lasting presence of HBV free viral genomes (cccDNA) in the nuclei of the infected hepatocytes and by the strong inhibition of viral activities such as replication and protein synthesis.

OBI can present with different virological and immunological profiles (sero-positive or sero-negative OBI) and, given the very low levels of serum HBV DNA, its detection requires the use of highly sensitive and specific molecular biology techniques.

The molecular mechanisms underlining OBI need still to be fully elucidated. Both viral and host-related factors appear to be involved, however it appears clear that factors associated with the host immunological status play a key role in the suppression of the viral activities in the majority of cases. This implies that an alteration of the host's defence mechanisms by environmental factors, such as exposure to immune-suppressive therapies or drugs able to interfere with the epigenetic control of the HBV replication, may lead to breakdown of the host-virus balance and determine viral reactivation, with subsequent possible development of acute severe forms of classical hepatitis B, potentially fatal.

In analogy, because of their replication-competence, when occult viruses are transmitted to other individuals they may induce hepatitis B. These cases include blood transfusions and liver (or, less frequently, other organs) transplantation from patients with OBI to HBV naïve recipients. Prevention of *de novo* hepatitis B in the recipient by careful screening of the blood units by highly sensitive diagnostic assays and the use of anti-HBV prophylaxis, respectively in the first and in the second circumstance, is recommended. The long-term persistence of the virus in the liver may provoke a very mild but continuing necroinflammation potentially favouring the progression of the chronic liver disease toward cirrhosis when other causes of liver injury co-exist. Finally, OBI has been recognised as an important risk factor for the development of HCC, however the underlining pathophysiological mechanisms need further elucidation.

In consideration of its potentially severe clinical impact, it appears to be of paramount importance to increase clinicians' awareness about the possible presence of OBI, in particular in categories of patients such as immunosuppressed individuals or subject affected by other chronic liver disease. International, prospective trials to improve knowledge and definition of risk factors, monitoring and surveillance procedures, as well as type and duration of antiviral approaches, should be conducted. Finally, it needs to be remarked that, given the potential lifelong persistence the

cccDNA molecules in the hepatocytes, OBI patients remain a reservoir of HBV infection. In this light, the extension of HBV neonatal vaccination to all the countries, particularly where the virus is endemic, appears to be the best effective intervention to contrast the HBV spread.

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