

The clinical significance of occult HBV infection

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Abstract

The presence of hepatitis B virus (HBV) DNA in HBV surface antigen (HBsAg)-negative individuals is defined as occult HBV infection (OBI). OBI is related in some cases to infection with variant viruses (S-escape mutants) undetectable by HBsAg commercial kits. More frequently, however, it is due to infection with wild-type viruses that are strongly suppressed in their replication activity. OBI may be involved in different clinical contexts, including the transmission of the infection by blood transfusion or liver transplantation and its acute reactivation when an immunosuppressive status occurs. Moreover, much evidence suggests that it may contribute to the development of cirrhosis and may have an important role in hepatocarcinogenesis.

Keywords Hepatitis B virus, occult hepatitis B virus infection, hepatitis B virus transmission, hepatitis B virus reactivation, cirrhosis, hepatocellular carcinoma

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Introduction

According to the guidelines on the management of chronic hepatitis B virus (HBV) infection issued by the European Association for the Study of the Liver (EASL), one may schematically distinguish five phases (not necessarily sequential and stable) in the natural history of chronic hepatitis B (CHB) (Table 1) [1]. The fifth of these phases corresponds to occult HBV infection (OBI). OBI is characterized by the persistence of HBV genomes in the liver tissue (and in some cases also in the serum) of hepatitis B surface antigen (HBsAg)-negative individuals [2]. This particular form of HBV infection appears to have a fairly frequent occurrence, not only in individuals with circulating antibodies to HBsAg (anti-HBs) and/or hepatitis B core antigen (anti-HBc), but also in subjects negative for all HBV serum markers [2-4]. In some cases, OBI is associated with mutant viruses (S-escape mutants) that cannot be detected by HBsAg commercial assays [2-6], but it is much more often caused by the host mechanisms producing a strong suppression of the HBV replication and gene expression [2,6,7] (this could also explain why no or very low levels of viremia are usually detected in most OBI carriers). Why HBV suppression occurs is not yet entirely

clear, although the host immune surveillance and epigenetic mechanisms are probably involved [5]. The molecular bases of OBI appear to be related to the long-lasting persistence in the nuclei of the hepatocytes of the HBV cccDNA, an intermediate form of the virus life cycle that serves as a template for gene transcription [2,8,9].

OBI may be involved in many different clinical conditions that may be schematically summarized in four main contexts (Fig. 1): a) it can be transmitted (through blood transfusion and organ - mainly liver - transplantation), causing typical hepatitis B in newly infected individuals; b) the development of an immunosuppressive status (i.e., by immune-therapy) may induce OBI reactivation and development of acute and sometimes fulminant hepatitis; c) a large body of data suggests that OBI can contribute to the progression of the chronic liver disease toward cirrhosis, in particular in HCV-infected patients; and d) much evidence suggests that OBI can be involved in hepatocellular carcinoma (HCC) development.

We herein provide a comprehensive review of the published data concerning the possible clinical impact of OBI.

Transmission of OBI

Blood transfusion

Owing to the enormous development of specific and sensitive diagnostic assays, the risk of HBV transmission through blood transfusion has become a very rare occurrence, at least in the western world, although some anecdotal cases are occasionally still reported [6,10]. However, one should always take into account that OBI carriers may potentially be a source of HBV transmission in the case of blood donation [6,10-13],

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Table 1 Phases of the natural history of chronic HBV infection according to EASL guidelines on management of patients with chronic HBV infection [1]

Phases	Characteristics
HBeAg positive, immune-tolerant phase	High levels of HBV replication; normal aminotransferases levels; no or mild liver necroinflammation; no progression of fibrosis
HBeAg-positive, immune-reactive phase	High serum HBV DNA levels; increased or fluctuating levels of aminotransferases; from moderate to severe liver necroinflammation; rapid progression of fibrosis
HBeAg-negative, inactive HBV carrier state	Low or very low serum HBV DNA levels; normal aminotransferases levels; very low risk of cirrhosis or HCC; HBsAg loss and anti-HBs seroconversion may occur spontaneously and more frequently compared to the other HBsAg positive phases
HBeAg-negative CHB phase	Persistent or fluctuating high levels of HBV DNA and aminotransferases; active hepatitis at histology
HBsAg-negative phase (occult infection)	Persistence of HBV DNA in the liver in the absence of HBsAg; undetectable or very low levels of HBV DNA in the serum

CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B s antigen; HBV, hepatitis B virus

thus it appears of relevance that the alert in blood banks in identifying OBI-positive donors is maintained at high levels. In fact, the introduction of Nucleic Acid Testing (NAT) for HBV has confirmed that a limited - but not negligible - number of HBsAg-negative blood donors are found to be HBV DNA positive worldwide, although the NAT-based studies indicate that the frequency of serum HBV DNA positivity in HBsAg-negative donors is clearly related with the HBV prevalence in the different countries [13,14].

Schematically, OBI may be involved in the transfusional transmission of HBV in two conditions: a) The donor is infected with wild-type HBV populations suppressed in their replication activity (typical “OBI carrier”). In this context, it has to be considered that chronic occult infection frequently shows phases of low levels of viremia alternating with periods of absence (or undetectability) of HBV DNA in the serum [11,15,16]. Consequently, the potential blood infectivity of

an “OBI carrier” may fluctuate over time. In this context, however, it appears of importance to stress that it is unknown whether the minute amounts of HBV DNA that may be present in the blood of OBI donors are sufficient to induce a typical acute hepatitis B in the recipient. b) The donor is infected with S-escape mutant HBVs able to replicate but synthesize a mutated HBsAg not detected by the routinely available diagnostic assays. This is the most frequent condition associated with the residual cases of hepatitis B related to blood transfusion [10,11].

Liver transplantation

HBV infection may be transmitted also in cases of orthotopic liver transplantation (OLT) if the donor is OBI-positive, as an obvious consequence of the fact that the hepatocytes are

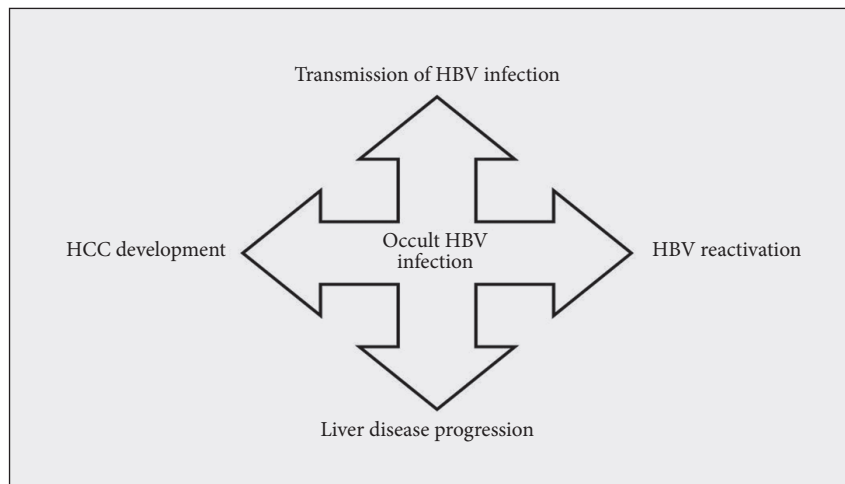


Figure 1 Schematic representation of the possible clinical implications related to occult HBV infection
HCC, hepatocellular carcinoma; HBV, hepatitis B virus

the reservoir of the viral cccDNA. Even in these situations the recipients may develop *de novo* hepatitis B, particularly when they are HBV naïve [4,17]. Indeed, OBI transmission in the event of OLT may frequently occur and it largely justifies the anti-HBV prophylaxis currently applied in HBsAg-negative transplanted patients receiving livers from anti-HBc-positive donors. This prophylaxis is very efficient in preventing hepatitis B in the recipients [18]. It is based on the use of anti-HBs immunoglobulin alone or in combination with lamivudine, although monotherapy with lamivudine appears also to be very efficient and cost-effective [18]. However, the prophylaxis cannot completely prevent HBV re-infection as shown by several reports that have documented the frequent occurrence of chronic occult infection in transplanted patients who received the new organ from an OBI carrier [17]. Indeed, viral genomes (including HBV cccDNA) have been identified in the transplanted liver. These viral genomes may derive from an OBI previously occurring in the recipient, in the donor or even in both [19]. At present, there is a strongly debated argument over whether OBI might have any clinical impact in the long-term outcome of OLT patients. In this context, the recent evidence suggesting a possible role of OBI in the progression toward cirrhosis of the post-OLT liver disease in patients with HCV infection appears of relevance [20,21].

OBI reactivation

It is well documented that chronic HBV infection may be reactivated in patients with disease-related or therapeutically induced conditions of strong immune suppression. Indeed, the general interest in this field is growing also as a consequence of the increasing use of potent immunological therapies in various clinical contexts [22-24]. HBV reactivation is a very frequent occurrence when HBsAg-positive subjects undergo immune-suppression, but it may also occur in OBI patients who may develop fulminant hepatitis [25,26]. This event may be explained by the fact that the strong inhibition of HBV replication and protein synthesis commonly observed in the course of OBI may be interrupted in immune-suppressed patients who may show a reactivation of the viral activities because of the fall of immunological control. This important, indirect confirmation of the role played by the host immune system in leading to the OBI development, however, does not exclude the possibility that other mechanisms may be involved in OBI reactivation. In fact, recent evidence suggests that even therapies with histone deacetylase inhibitors may provoke OBI reactivation [27,28], thereby representing indirect proof of the involvement of epigenetic mechanisms in the control of HBV cccDNA minichromosome.

As mentioned above, HBV reactivation occurs more rarely in OBI than HBsAg-positive patients. Hematological malignancies, hematopoietic stem cell transplantation and therapeutic schedules comprising Rituximab appear to be the clinical conditions with the highest risk of OBI reactivation [4,29,30], usually diagnosed when it is followed by the occurrence of a typical acute hepatitis B, whereas when

the clinical sequels are less severe it is likely to be underdiagnosed [31,32].

Anti-HBV prophylaxis with nucleos(t)ide analogues (NA) is a commonly accepted approach to prevent viral reactivation in HBsAg-positive individuals undergoing immunosuppressive therapies. In HBsAg-negative/anti-HBc-positive patients - who represent the individuals at the highest risk of OBI - the opportunity to perform that prophylaxis is still debated. According to EASL guidelines, these patients should be carefully evaluated by means of HBV DNA and alanine aminotransferase (ALT) testing before and during immunosuppressive treatments and also for several months after stopping it: NA therapy must be immediately started in patients who become HBV DNA positive even before any ALT elevation, since the objective of this strict surveillance is to identify the HBV reactivation in a phase prior to the start of liver injury and thus to start NA treatment on time to prevent acute hepatitis [1]. Of note, the EASL guidelines also suggest to consider the possibility of a preemptive use of Lamivudine in the above-mentioned cases with the highest risk of OBI reactivation (hematological malignancies treated with regimens including Rituximab; bone marrow or stem cell transplantation) or if HBV DNA monitoring is not feasible for practical reasons [1].

OBI and chronic liver disease

There is clear evidence that HBV genomes may persist over time in the liver of subjects who have recovered from self-limited acute hepatitis without inducing any clinical and biochemical abnormality [4,33]. However, all the studies evaluating the histology in such apparently healthy individuals have revealed that a mild necroinflammation persists for many years after the resolution of the acute hepatitis [34,35]. Likewise, studies performed on the woodchuck model, showed that animals that have recovered from acute woodchuck hepatitis virus (WHV, the HBV-like hepadnavirus infecting that rodent) are lifelong infected with viruses replicating at low levels and provoking a mild but persistent liver necroinflammation [36]. This premise appears to be useful in explaining the observations - also confirmed by a recent meta-analysis - that OBI is associated with the progression toward cirrhosis of the chronic liver disease (CLD) occurring in HCV-infected patients as well as in individuals with liver disease of unknown origin [3,4,37,38]. Of note, our very recent long-term observational cohort study not only confirmed the role of OBI in HCC development occurring in HCV patients (see below) but also showed that OBI is associated with the most severe complications of CLD even independently of the HCC occurrence and that chronic HCV patients with OBI have a significantly increased risk of liver-related death compared to OBI-negative patients [39]. Finally, it is worth mentioning that in patients with combined chronic hepatitis C and OBI, the phases of ALT occurrence correspond to the reappearance of circulating HBV DNA, thereby suggesting

that a transient HBV reactivation might be involved in the hepatocyte damage in these patients [15,16]. Summarizing, all these data appear to be in accordance with the hypothesis that OBI *per se* is inoffensive in immune-competent individuals, but if other causes of liver disease co-exist (in particular HCV infection) then the minimal lesions produced by the immune response to the occult virus might negatively influence the outcome of the disease [40]. In this context, it should also be considered that a portion of OBI patients with CLD previously had the classical forms of overt HBV infection and chronic hepatitis B. Subsequently, HBV replication and gene expression may progressively decrease and the HBsAg may even disappear over time. However, if advanced CLD has already been established, OBI such as overt B hepatitis is able to maintain liver damage.

OBI and HCC

HBV is a well-known oncogenic virus and the main risk factor for HCC development. Actually, a large number of epidemiological and molecular studies show that chronic HBV infection may also be involved in HCC development in cases with OBI [41,42]. In fact, there is evidence that OBI may favor or accelerate the HCC development in patients with chronic hepatitis of different etiologies including the HCV infection that appears to be a condition particularly prone to HCC development in case of concomitant OBI [4,43-45]. Among HCV-negative patients, OBI seems to exert its tumorigenic effect in subjects with cryptogenic CLD as well as in alcoholics and patients with genetic diseases [4,46-49]. Of note, the pro-oncogenic role played by OBI has recently been confirmed by a meta-analysis showing that the occult infection is an important risk factor for HCC development both in HCV-infected and HCV-negative patients with CLD [50]. Finally, studies performed in rodents susceptible to infections with hepadnaviruses (essentially, woodchucks and ground squirrels) showed that these animals maintain a high risk of developing HCC even after recovery from the acute infection with the clearance of the viral surface antigens and the appearance of the corresponding antibodies [42].

HBV may be potentially involved in most of the complex pathogenetic mechanisms underlying the development of HCC. In particular, chronic HBV infection may exert its carcinogenic effects through both indirect mechanisms (i.e., its propensity to chronically induce necroinflammation of the liver and to favor the progression of the CLD toward cirrhosis which is the main risk factor for HCC development) and direct tumorigenic mechanisms (i.e., the capacity of HBV to integrate into the host genome and to produce proteins – such as the X protein and the mutant pre-S proteins – potentially able to induce hepatocyte transformation. The belief that HBV may be involved in hepatocellular transformation also when the OBI occurs is based on the consideration that the direct and indirect mechanisms underlying HCC development in HBsAg-positive chronic infection are maintained

in cases with occult infection. In particular, in the OBI: a) there is a long-lasting persistence of viral genomes into the hepatocytes both as integrated DNA and as free episome; b) very low levels of HBV replication and transcriptional activity with protein synthesis are maintained; c) a mild necroinflammation chronically persists and possibly contributes to the occurrence of cirrhosis.

Concluding remarks

OBI is a phenomenon essentially attributed to the long-lasting presence of HBV cccDNA into the hepatocytes and to a strong inhibition of HBV replication and protein synthesis. Considering the very low levels of serum HBV DNA, its detection requires the use of highly sensitive and specific molecular biology techniques. The inhibition of HBV replication may be reversible and the occult infection may be reactivated, leading to acute and severe forms of classical hepatitis B, which may also occur after transmission of OBI by blood transfusion or organ transplantation. The long-term persistence of the virus in the liver may induce a very mild but continuing necroinflammation that – if other causes of liver injury co-exist – may favor the progression of the chronic liver disease toward cirrhosis. Moreover, OBI seems to maintain the tumorigenic properties typical of the overt infection, and it is in fact an important risk factor for HCC development. Considering all the above-mentioned data, we believe that clinicians should take into consideration the presence of OBI in several categories of patients, such as the immunosuppressed and cirrhotic individuals with OBI, who have a very high risk of viral reactivation and liver cancer development, respectively.

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