Review

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Update of the statements on biology and clinical impact of occult hepatitis B virus infection

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Summary

In October 2018 a large number of international experts with complementary expertise came together in Taormina to participate in a workshop on occult hepatitis B virus infection (OBI). The objectives of the workshop were to review the existing knowledge on OBI, to identify issues that require further investigation, to highlight both existing controversies and newly emerging perspectives, and ultimately to update the statements previously agreed in 2008. This paper represents the output from the workshop.

Keywords: Occult HBV infection; OBI; HBV cccDNA; HBV S variants; HBV transmission; HBV reactivation; Hepatocellular carcinoma.

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Introduction

The first international workshop on occult hepatitis B virus (HBV) infection (OBI) was held in Taormina (Italy) in 2008 to review the biology and clinical implications of OBI. A panel of international experts produced a document, the Taormina statements and recommendations on OBI that was published in the *Journal of Hepatology* in 2008.[1]

Subsequently, many studies on OBI have been conducted but only a few of the uncertain issues have been resolved. In fact, many aspects of OBI are still controversial, including prevalence, pathobiology and clinical implications. In addition, new challenges have emerged, such as methods and sensitivities of assays for detection and risks of transmission. Thus, it was considered timely to re-visit and discuss the current understanding of OBI, and so 10 years after the first meeting, a new workshop dedicated to OBI was again held in Taormina, on October 1–2, 2018. This workshop included 5 sessions (Virology and Immunology, Diagnosis, Epidemiology, Transmission, and Liver diseases/Therapeutic implications) with presentations by invited experts followed by panel discussions. A sixth session engaged all the participants, with the goal of reaching a consensus and producing an update to the 2008 statements, culminating in this report.

Definition

- **Occult HBV infection (OBI)** is defined as the presence of replication-competent HBV DNA (*i.e.* episomal HBV covalently closed circular DNA [cccDNA]) in the liver and/or HBV DNA in the blood of people who test negative for hepatitis B surface antigen (HBsAg) by currently available assays.
- Based on the HBV-specific antibody profiles, OBI may be categorised as (Fig. 1):
  Seropositive OBI – hepatitis B core antibody (anti-HBc) and/or hepatitis B surface antibody (anti-HBs) positive.
  Seronegative OBI – anti-HBc and anti-HBs negative.

Among individuals with OBI, the prevalence of detectable HBV DNA in serum/plasma varies depending on the population studied, the sensitivity of the assay used, and whether blood samples at 1 or more time-points are tested. Many studies have found that HBV DNA is only intermittently detected in serum/plasma,[2–6] and when detectable, the concentration is low, usually less than 200 IU/ml (about 1,000 copies/ml).[6–12]

In people with seropositive-OBI, HBsAg may have become negative either following the resolution of acute hepatitis B (thus, after a few months of HBsAg carriage) or after decades of HBsAg positive (namely, "overt") chronic HBV infection with or without disease. It is unknown whether patients with chronic HBV infection/disease who become HBsAg negative following antiviral therapy are comparable to patients who spontaneously clear HBsAg from the virological and immunological points of view, *e.g.* duration of exposure to high viral load and restoration of immune response to HBV. The possible clinical implications of this distinction are also unknown.

People with seronegative OBI (estimated to comprise between 1% and 20% of all individuals with OBI)[7,13–15] might have either progressively lost the hepatitis B antibodies (anti-HBc and anti-HBs) or have been hepatitis B antibody negative since the beginning of the infection. The latter condition has been described in the woodchuck model of occult woodchuck hepatitis virus (WHV) infection.[16]

A subset of people with OBI are infected with HBV S variants carrying mutations in the S gene ('S-escape' mutations), resulting in the production of modified HBsAg that is not recognised by some commercially available HBsAg assays. Circulating HBV DNA levels in these people may be compara-

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Key point

In the vast majority of cases, OBI is characterized by the long-lasting persistence of low levels of HBV cccDNA chromatinised episomes in hepatocytes, with a strong suppression of overall replication activity and viral protein expression exerted by the host's defence mechanisms.

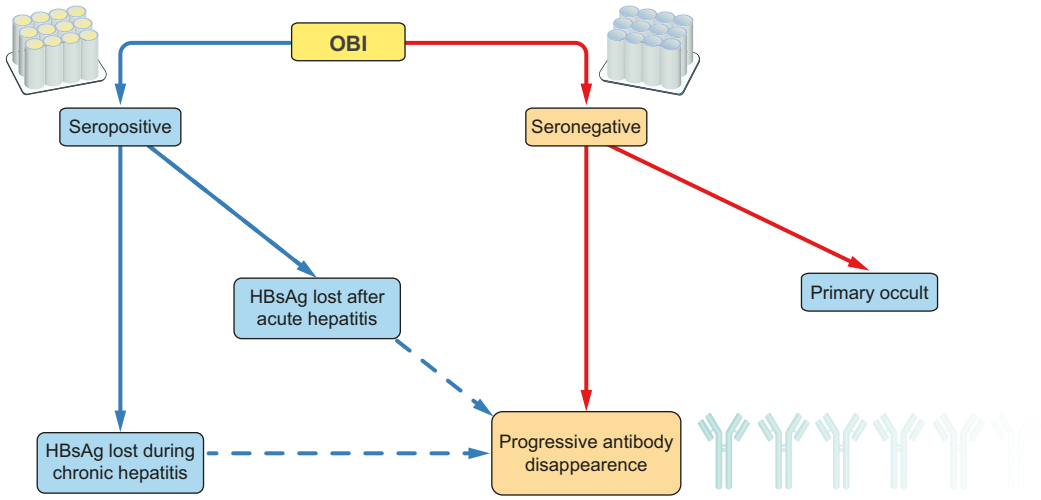

Fig. 1. Schematic representation of HBV serum marker profile in naturally occurring OBI. AH, acute hepatitis; CH, chronic hepatitis; HBV, hepatitis B virus; OBI, occult HBV infection.

ble to those detected in HBsAg positive individuals. Additional HBV variants with mutations in the S-gene promoter and splice variants have also been reported to affect HBsAg production/secretion and to be responsible for some cases of OBI[17–27] (Fig. 2).

Virology and immunology

- The molecular basis of OBI is related to the stability and long-term persistence of cccDNA in the nucleus of infected hepatocytes (Fig. 3).

The episomal HBV cccDNA exists as a chromatinised 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 efficient immune control is achieved.[15,28–36]

- 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.[15,37] However, cccDNA in OBI cases is fully replication competent.[38] HBV DNA may be integrated into the host's genome 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 antiviral immune responses that are believed to be important in maintaining HBV control.

The high prevalence of OBI worldwide suggests that the immune system is effective in controlling HBV (even if it is not definitively cleared) in the majority of people with OBI.[39] Antiviral immune responses in OBI are continuously stimulated by persistent/intermittent low concentrations of HBV antigens.[40,41] Studies performed after spontaneous resolution of acute or chronic HBV infections indicate that different profiles of antiviral T cell response are associated with the acquisition of HBV control. This difference in efficiency of antiviral protective mechanisms is probably related to the short or long-term duration of exposure of the immune system to high antigen and viral loads.[42] In these studies, however, the presence of OBI was only inferred from anti-HBV antibody positivity but not directly diagnosed by the detection of HBV DNA. In individuals with OBI, the immune response was confirmed by detection of HBV DNA in a single study which showed different profiles of T cell immune response against HBV epitopes in OBI-seropositive and OBI-seronegative individuals. While *ex vivo* responses were similarly weak, *in vitro* T cell expansion following specific peptide stimulation was generally more efficient among individuals with seropositive OBI than seronegative OBI.[43] Not only T cells, but also humoral antibody responses appear to be important in host control of OBI,[44] as indicated by the frequent HBV reactivations observed in patients treated with B-cell selective monoclonal based therapies (anti-CD19/20; *e.g.* rituximab and ofatumumab). Finally, innate immunity is also likely to contribute to HBV control in occult infection, but no data are available on this issue at present.

Diagnosis

- Diagnosis of OBI is based on the detection of HBV DNA in the blood or the liver of HBsAg-negative individuals.
  - o Detection of HBV DNA in the liver is the gold standard.
  - o Detection of HBV DNA in the blood is commonly used.
  - o Detection of anti-HBc in the blood is often used as a surrogate.

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 a false negative HBsAg result and misdiagnosis of OBI in people with overt HBV infection. On the other hand, HBV DNA assays with inadequate sensitivity can result in false negative HBV DNA results and may lead to a missed diagnosis of OBI.

The lower limit of detection of most currently available commercial HBsAg assays is 0.05 IU/ml. Recent studies found that between 1% and 48% of samples that tested negative using these assays test positive using more sensitive HBsAg assays with a lower limit of detection of 0.005 IU/ml.[45–47] Besides sensitivity, commercial HBsAg assays differ in their ability to detect S-escape variants.[45,48–50] The use of anti-HBs probes targeting multiple epitopes of HBsAg should be mandatory for all HBsAg assays to ensure the detection of HBV S variants.

Moreover, all serological assays detect excess HBsAg in the presence of immune complexes. Co-occurrence of anti-HBs in excess in those with HBV infection may thus interfere with the detection of HBsAg. In that situation, some people with OBI may become HBsAg positive if tested using assays that can dissociate HBsAg from immune complexes that bind HBsAg to anti-HBs.[51]

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 people with OBI, testing blood samples collected at more than one time-point, as well as testing DNA extracts from no less than 1 ml of serum or plasma is recommended for the diagnosis of OBI.

In the setting of blood transfusion, assays used for nucleic acid testing (NAT) of blood products have high specificity (99.9%) and a 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.[52] One study using a highly sensitive HBV DNA assay with a limit of detection of 3.4 IU/ml identified 3 blood donors who previously tested negative for HBsAg and HBV DNA and who were shown to have transmitted HBV.[6] As much as 2–24 ml of donor plasma was used for testing and not all archived samples from these donors tested positive for HBV DNA.

The ideal method of diagnosis of OBI is the detection of replication-competent HBV DNA in the liver. However, standardised 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.[1,7,15,53,54] In each case the assay must include primer sets that enable detection of replication-competent HBV DNA. It is important that the liver samples are properly processed to avoid cross-contamination and that appropriate negative controls are included to confirm the specificity of the assays. It is also important that a panel of HBV standards is included to validate the sensitivity of the assay. Given that HBV DNA is present in low concentrations in people with OBI, adequately sized 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 to identify OBI in blood/organ donors, in people who are about to receive immunosuppressive therapies, and for epidemiological studies. In these settings, liver tissue is often not available, access to tests for HBV DNA in the blood may be limited or delayed, and undetectable HBV DNA in blood tested at one time-point does not rule out OBI. Indeed, HBV reactivation has been reported in HBsAg-negative, anti-HBc-positive individuals who have undetectable HBV DNA in the blood.[55] Similarly, anti-HBc testing may identify some blood donors with OBI who have undetectable HBV DNA in mini-pool NAT.[11] Although earlier anti-HBc assays had high rates of false positive results, the specificity of most currently available commercial assays is very high (≥99%).[56]

It should be noted that the absence of anti-HBc does not rule out OBI, although the prevalence and

Key point

The lack of sensitive, standardised, and validated assays for the diagnosis of OBI is a major limitation, and available data across studies cannot be properly compared and combined.

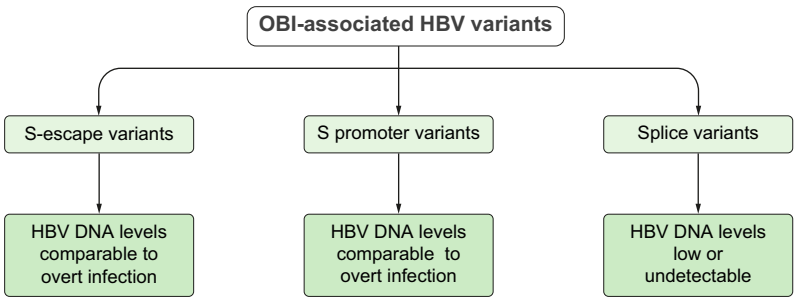

Fig. 2. HBV genetic variants leading to the synthesis of HBsAg unrecognised by available assays or affecting its production/secretion. HBV, hepatitis B virus; HBsAg, HBV surface antigen.

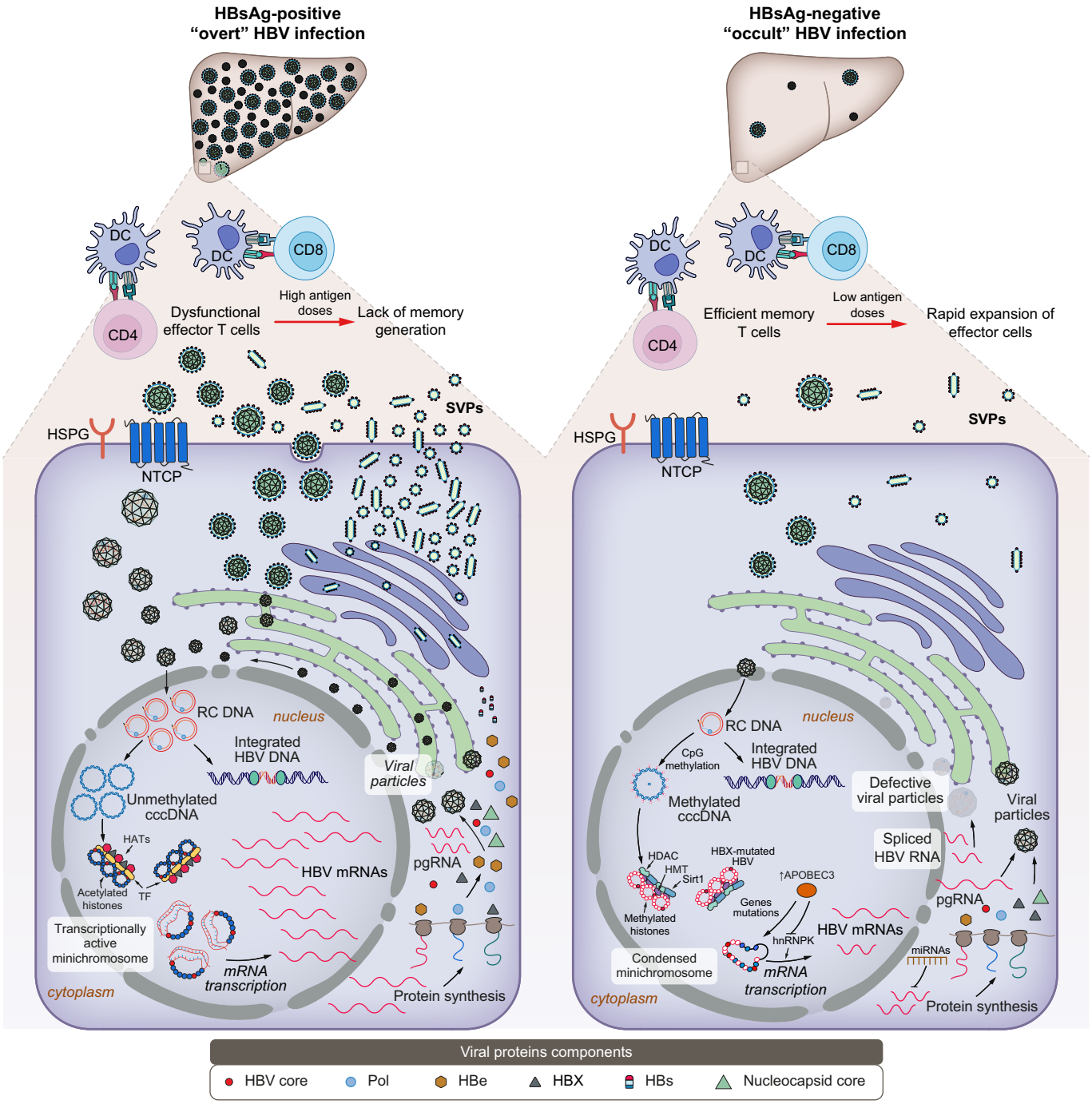

Fig. 3. Schematic comparison of overt and occult HBV infection. Differences in amounts of total viral DNA, transcripts, proteins, formation and secretion of virions are exemplified. Diversity in packaging and transcriptional activities of HBV minichromosomes is also represented. Modified from Pollicino T. and Raimondo G., *Journal of Hepatology* 2015.[131] cccDNA, covalently closed circular DNA; DC, dendritic cell; HATs, histone acetyltransferases; HDAC, histone deacetylase; HMT, histone methyltransferase; HBV, hepatitis B virus; HBsAg, HBV surface antigen; HSPG, heparan sulfate proteoglycan; NTCP, Na+-taurocholate cotransporting polypeptide; pgRNA, pregenomic RNA; rcDNA, relaxed circular DNA; SVPs, sub-viral particles; TF, transcription factor.

clinical significance of seronegative OBI in humans are unknown.

Epidemiology

- Defining the epidemiology of OBI can be challenging as it relies on the performance and sensitivity of HBsAg and HBV DNA detection assays; it also 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, and the definition used for OBI.
- 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 patients with chronic liver disease and may be as high as 40% to 75% in those with HBsAg-negative hepatocellular carcinoma (HCC).
- OBI is rarely detected amongst blood donors, with HBV DNA detection rates 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 reported in Asia.[13] Yet, despite high endemicity, a low prevalence of OBI has been found by various groups in Asia and in Africa.[57–60] Prevalence rates have varied from as low as <1% to as high as 87% but these results need to be interpreted with caution[2,59] because a number of factors can influence rates of OBI including the particular risk group studied, sampling issues, assay sensitivity, and the prevalence of HBsAg in the geographical region in which the study was conducted. Higher rates have also been found in individuals with risk factors for HBV infection, *e.g.* those coinfected with hepatitis C virus (HCV) (15%–33%)[7,61] or human immunodeficiency virus (HIV) (10%–45%),[62–65] people who inject drugs (45%),[66] and people on dialysis (27%).[67] Prevalence rates are also higher in patients with HCC (62%),[68] cryptogenic cirrhosis (32%),[69] or those who have undergone liver transplantation (64%).[70] In carefully conducted studies of blood donors,[71] HBV DNA was detected in 0% to 4.6% of those who were HBsAg-negative and anti-HBc positive, with or without anti-HBs, with a median prevalence of 1%.[11,22,55,56,67,68,72,73] There is a single study that tested HBV DNA in the 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.[74]

As discussed earlier, the performance and sensitivity of the HBsAg and HBV DNA assays, the characteristics of the study population, the prevalence of HBsAg positive infection in the general population, and the criteria used to define OBI can all influence OBI prevalence rates. Thus, it is difficult to compare data between studies or to perform meta-analyses across studies.

Transmission

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

Several and often concomitant reasons may be underestimated due to: a) undetectable or intermittently detectable HBV DNA in donors; b) difficulty and reluctance to trace recipients; c) lack or limited volume of donor archive samples; d) HBV infection in recipients without clinically evident acute hepatitis, which generally goes unnoticed, and may represent the majority of cases of transfusion-related transmission from OBI donors.[75]

The presence of anti-HBs in recipients prior to transfusion significantly reduces the risk of infection.[76] A recent study from Candotti and colleagues[6] investigated 3 repeat HBsAg-negative donors from Slovenia who had undetectable HBV DNA by highly sensitive NAT and who transmitted HBV to 9 recipients following transfusion of blood components. This study has enabled a revised estimation of the minimal HBV infectious dose from the previous 20 IU/ml to approximately 3.0 IU/ml of HBV DNA. The 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 of 0.15 IU/ml.

Transfusion transmission of HBV could be reduced by implementing anti-HBc screening (if donor loss rate is not too high) or HBV NAT with a lower limit of detection of 0.15 IU/ml (technologically demanding), NAT screening of individual donation (rather than mini-pool) with larger volumes, or pathogen reduction strategies.

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.

HBV persists in the liver of people who had been infected even after HBsAg clearance. Thus, an OBI liver donor can transmit HBV infection to an HBsAg-negative, anti-HBc negative and anti-HBs negative recipient with possible development of hepatitis B.[77,78] Long-term prophylactic antiviral therapy with a NUC, such as entecavir or tenofovir, is recommended. However, while HBsAg positive infection is prevented, antiviral prophylaxis may not prevent the development of an OBI in the recipient.[79,80] HBV DNA can be detected in the liver of patients who received liver transplantation for hepatitis B and who received anti-HBV prophylaxis. Despite the absence of detectable

Key point

The prevalence of OBI varies greatly across the world, with a higher prevalence in areas of elevated HBV endemicity and among those subjects at higher risk of parenteral transmission of the viruses.

HBsAg and HBV DNA in serum, OBI of the liver grafts is frequent. Thus, lifelong prophylaxis with NUCs is recommended for all patients who had liver transplantation for hepatitis B.[80–82]

Mother-to-child transmission

- OBI may occur in newborns from HBsAg positive mothers despite proper active/passive immunoprophylaxis at birth.[83–91]

HBV vaccination is one of the most important and most successful accomplishments in medical science. The World Health Organization has proposed goals to eliminate HBV by 2030.[92] 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 fails to completely prevent HBV infection but succeeds in modulating the infection to prevent progression to chronic HBV infection.

Clinical implications

- 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.

Key point

Although OBI potentially maintains the pro-oncogenic properties usually attributed to the HBsAg-positive HBV infection, its role as a risk factor for hepatocellular carcinoma development still needs to be confirmed.

A still widely debated topic is whether OBI may accelerate the progression toward cirrhosis and the development of HCC in patients with chronic liver disease caused by other factors (*e.g.* HCV, alcohol, non-alcoholic steatohepatitis).[13,93,94] While many studies, including some studies from Europe and the United States where the prevalence of HBV infection in the general population is low, have shown a significant association between OBI (as determined by detection of HBV DNA in the liver) and HCV-related cirrhosis as well as HCV-related HCC,[95–97] other studies in these locations found no association.[98] Most studies have found a high prevalence of anti-HBc among patients with HCV-related HCC, but not all studies have shown a significantly higher prevalence compared to patients with chronic HCV and no HCC.[97,99–102] Similarly, among the few studies where detection of HBV DNA in liver was performed, some but not all studies showed a difference in detection rates between patients with HCV, with and without HCC.[15,96,98]

In the woodchuck model, the clearance of the WHV surface antigen is invariably associated with persistent detection of WHV DNA in the liver as well as mild persistent necroinflammation and a high rate of HCC development.[103–105] Both virus- and host-related differences may explain the higher association rates compared to HBV infection in humans.

OBI retains several of the oncogenic mechanisms of overt HBV, including production of pro-oncogenic proteins and the propensity of the viral DNA to integrate into the host's genome.[15,106,107] Further studies on molecular epidemiology and onco-pathogenesis are required to confirm the role of OBI in HCC development, and to determine the mechanisms by which it might exert a pro-oncogenic activity.

HBV reactivation

The definition of HBV reactivation in patients with OBI generally includes: i) HBsAg seroreversion and/or an increase of serum HBV DNA by at least 1 log above the lower limit of detection of the assay in a person who had previously undetectable HBsAg and HBV DNA in serum, and ii) a more than 1 log increase in serum HBV DNA in people who had detectable HBV DNA at baseline.

People with OBI can experience reactivation of HBV replication when they receive cancer chemotherapy or other immunosuppressive therapies. Although the incidence is lower than in those with chronic HBV infection, HBV reactivation can occur in up to 40% of people with OBI when potent immunosuppressive therapies are used. The risk is high (>10%) in patients receiving anti-CD20 containing regimens and myeloablative regimens for hematopoietic stem cell transplantation.[108–111] The risk is low (<1%) to moderate (1–10%) in people who receive other cancer chemotherapies, high dose corticosteroids, or anti-rejection therapies for solid organ transplantation.[112] Earlier studies suggested that the risk is modest in individuals with OBI receiving tumor necrosis factor inhibitors, but recent studies found that the risk of HBV reactivation is very low in patients receiving these therapies.[103] Similarly, the risk of HBV reactivation is very low in individuals with OBI receiving direct-acting antiviral therapy for hepatitis C.[113–115]

Given the shared transmission routes for HIV and HBV, and the immune impairment produced by HIV, OBI and HBV reactivations were more frequently reported in patients with acquired immunodeficiency syndrome. Following the widespread use of potent antiretroviral therapies, including antiretroviral agents with anti-HBV activity, reactivation of OBI has become negligible in the HIV population receiving appropriate therapy. However, HBV reactivation can occur in patients coinfected with HIV when antiretroviral regimens are modified and drugs active against HBV are withdrawn.

Most studies on HBV reactivation in people with OBI relied on detection of anti-HBc. In studies where HBV DNA in the blood is tested, the risk of HBV reactivation is higher in those with detectable HBV DNA but the risk is also present in those with undetectable HBV DNA in serum.[55] Anti-HBs antibody – when present – may progressively decrease during immunosuppressive therapy,

and HBV reactivation can also occur in people who are anti-HBs and anti-HBc positive.[55,116,117] Prophylactic antiviral therapy with NUCs with a high barrier to resistance, *i.e.*, entecavir or tenofovir, should be used in all patients with OBI at high risk of HBV reactivation. Those at moderate risk may receive prophylactic antiviral therapy and if not, they should be closely monitored and antiviral therapy initiated at the earliest sign of HBV reactivation. Those at low risk do not require prophylactic antiviral therapy but they need to be monitored. Risk stratification, indications for prophylactic antiviral therapy and frequency of monitoring are described in professional society guidelines.[118–120]

Antiviral therapy

- Currently, antiviral therapy is not recommended for individuals with OBI.

The 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 the 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 the host immune response. The risk of HCC and liver mortality is lower in patients with chronic HBV infection who have cleared HBsAg.[121]

The current drug discovery efforts and clinical trials aim at developing novel antiviral strategies to achieve a cure for HBV. Sterilising cure with eradication of cccDNA and integrated HBV DNA is likely not possible. Thus, current efforts focus on achieving functional cure defined as HBsAg clearance in a high proportion of patients after a finite course of therapy.[122] Patients with overt HBV infection who achieve functional cure would be HBsAg negative, anti-HBc positive with or without anti-HBs. While HBV DNA is not detected in the blood, the majority if not all of these patients would still have detectable HBV DNA in the liver. Multiple studies have shown that spontaneous or treatment-induced HBsAg clearance in patients with chronic HBV infection result in decreased necroinflammation of the liver and, in turn, in a reduced risk of cirrhosis, HCC, and HBV-related mortality.[118,119]

The development of quantitative and more sensitive assays based on digital droplet PCR for cccDNA detection has shown the possibility of detecting as little as 1 cccDNA copy/10^5 liver cells[54,123] in patients with chronic hepatitis B and viral suppression during NUC therapy, as well as in liver donors with OBI. If these results are confirmed, these assays will help to evaluate antivirals aimed at HBV cure, while highlighting the very high bar required to demonstrate eradication of HBV.

To eliminate HBV from people with OBI, HBV-infected hepatocytes would either need to be eliminated or cured. 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,[124,125] or cytokine-mediated degradation of cccDNA;[126–128]
- Specific killing of infected hepatocytes using strategies aimed 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[129] or HBV-T cell receptor (TCR) engineered T cells[130] to kill the residual infected cells in the liver.

This would require not only the adaptation of these cutting-edge technologies to this clinical application, but also more understanding of the biology of cccDNA and immune control, as well as of the number of infected hepatocytes in the setting of OBI.

More research will be necessary to achieve these goals, but such research is clearly warranted in light of the expected clinical benefit in terms of prevention of viral reactivation, transmission, and complications of the underlying liver disease.

Key point

Clinical implications of OBI concern: (i) HBV transmission, inducing a typical hepatitis B in the recipients, by transfusion of blood products or by liver transplantation; (ii) HBV reactivation with consequent development of hepatitis B, as in cases undergoing potent immune-suppressive therapies.

Further research studies

Several clinical and biological aspects of OBI need to be further explored. In this context, a fundamental objective is to definitively clarify whether, how, and in which circumstances OBI might be involved in liver injury and/or hepatocarcinogenesis. Studies aimed at understanding the immunological mechanisms that drive the development of OBI would be of seminal importance, as they might help in understanding the deficiencies in host immune control which lead to the development of productive HBV infection, and provide insights into new directions to cure HBV infection.

The 2018 OBI workshop speakers identified the following questions that should be addressed to resolve the controversies and uncertainties surrounding prevalence, clinical implications, and virologic/immunologic mechanisms of OBI.

Epidemiology and clinical research:

- Adopt standardised methods of reporting for studies on OBI such that results across studies can be compared and combined.
- Development of more sensitive, standardised, and validated assays for detection of HBV DNA in the blood and liver.
- Development of more sensitive, standardised, and validated assays for detection of HBsAg in the blood, including detection of HBV S variants, and HBsAg present in immune complexes with anti-HBs. Determine the clini-

cal implications of low concentrations of circulating HBsAg, in particular the risk of transmission, liver disease progression, and HCC.
- Determine the prevalence of OBI in different parts of the world, specifically among blood donors – using standardised 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 them to local prevalence and available resources.
- Define the clinical significance of OBI, in particular its role in HCC and in accelerating (or not) progression to cirrhosis in patients with other identifiable causes of liver disease as well as those with no identifiable cause (cryptogenic liver disease).
- Harmonise international guidelines on recommendations for preventing HBV reactivation in people with OBI.

Basic/translational research:
- Virology – Identify the molecular mechanisms implicated in the occurrence of OBI, and define how frequently OBI is due to infection with HBV variants.
- Immunology – Identify what is different between immune control in OBI versus overt HBV infection, and determine how to harness the immune control mechanisms in OBI to achieve functional HBV cure. Establish what disturbs immune control in OBI, leading to HBV reactivation.

Conclusions

The primary objectives of the 2018 Taormina workshop were to review the existing knowledge about OBI and – through vigorous discussion among a large number of international experts with complementary expertise – to both update the previous statements and to highlight emerging perspectives that need to be more extensively investigated.

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 that the answers to many of these questions will be satisfactorily addressed at the next workshop on Occult HBV Infection a decade from now.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

The Taormina Workshop on Occult HBV Infection Faculty Members

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Supplementary data

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