Occult Hepatitis B Infection

15

Man-Fung Yuen

Abstract

Occult hepatitis B infection is a unique disease entity defined by detectable hepatitis B virus DNA in the sera and/or livers in subjects who are negative for HBsAg. OBI can be classified by serology. It can also be classified according to the medical history of the subjects. Majority of subjects with OBI have had chronic hepatitis B infection with subsequent loss of HBsAg during the natural history of disease or as a result of antiviral treatment. There are still challenges in diagnosing this condition in many service-based laboratories. The prevalence of OBI was estimated to be ranging from <1 to 18% in general population. The HBV usually replicates at an extremely low level due to various postulated mechanisms. The clinical significance of OBI is particularly prominent in three main areas: (1) transmission of HBV through blood and organ donations, (2) development of chronic liver diseases and liver-related mortality and (3) HBV reactivation during immunosuppressive therapy. With the use of more potent immunosuppressive therapies including B-cell depleting agents, the disease manifestations become more apparent which require more research to establish clinical management guidelines.

Keywords

Hepatitis B virus • Occult hepatitis B infection • Clinical implication • Hepatitis B virus replication • Hepatocellular carcinoma • Hepatitis B reativation

M.-F. Yuen, M.D., Ph.D
Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong
e-mail: mfyuen@hku.hk

J.-H. Kao, D.-S. Chen (eds.), *Hepatitis B Virus and Liver Disease*,
https://doi.org/10.1007/978-981-10-4843-2_15

1 Introduction

Hepatitis B virus (HBV) infection is typically classified into acute infection with full recovery and chronic infection with life-long carriage state. It is generally believed that the former has no long-term clinical sequelae, whereas the latter is associated with a considerable risk of development of long-term liver-related complications. These include disease reactivation with hepatitis flares, cirrhosis leading to liver decompensation and/or hepatocellular carcinoma (HCC). To distinguish these two prognostically distinct conditions, the presence or absence of HBsAg of more than 6 months after the initial infection is the criterion. In other words, disappearance of HBsAg measured by conventional assays within 6 months signifies acute HBV infection, whereas persistence of HBsAg beyond this duration signifies chronic HBV infection. However, challenges were brought out by studies showing that HBV DNA were still present in serum and/or liver of subjects after decades of documented acute HBV infection (Raimondo et al. 2008a, 2013; Michalak et al. 1994; Yotsuyanagi et al. 1998; Yuki et al. 2003; Rehermann et al. 1996; Bläckberg and Kidd-Ljunggren 2000). One of these studies also documented the presence of HBV DNA in the peripheral blood mononuclear cells of subjects up to 70 months after recovery of acute hepatitis B infection (Michalak et al. 1994). This special condition is under the category of "occult hepatitis B infection" (OBI) which was consensually defined in a meeting held in Taormina 2008 as HBsAg-negative subjects with detectable HBV DNA either in serum or the liver (Raimondo et al. 2008b). In fact, this entity of OBI has been firstly described back in 1979 (Tabor and Gerety 1979).

It is noteworthy that majority of patients with OBI are not subjects with acute HBV with persistent viraemia. Patients with chronic hepatitis B (CHB) who lost HBsAg (HBsAg seroclearance) either naturally or through drug treatment indeed attribute for the majority of this population. There is also a small proportion of patients with CHB in which the HBV has hepatitis B surface gene mutations altering the epitopes of HBsAg leading to non-reactivity to the conventional HBsAg assays (Yamamoto et al. 1994; Hou et al. 1995; Carman et al. 1997). This is considered to be the minor group because studies have shown that the HBV in most of the OBI subjects do not have corresponding mutations in the HBsAg genome (Pollicino et al. 2004, 2007; Huang et al. 2014;).

2 Classifications of OBI Patients

OBI patients can be classified by two different ways. First, patients can be classified according to the serology of HBV. Second, patients can be classified according to their medical histories of HBV infection.

2.1 HBV Serology

Sero-positive OBI refers to the presence of anti-HBs and/or anti-HBc in the serum. On the contrary, absence of detectable serum anti-HBs and anti-HBc indicates sero-negative OBI. The hypothesis to explain the total absence of antibodies in

sero-negative OBI is the insufficient specific immune response in the presence of a very minute level of hepatitis B viraemia. According to several studies, up to 20% of OBI subjects are sero-negative (Morales-Romero et al. 2014; Raimondo et al. 2007). At present, there are no detailed studies to compare sero-positive with sero-negative OBI in terms of the disease underlying mechanisms and the clinical disease profiles.

2.2 Medical History of HBV Infection

2.2.1 Subjects with Documented History of Acute HBV Infection

More than 95% adult subjects contracting HBV is associated with "clinical" recovery without HBV chronicity. They would have anti-HBs and anti-HBc positivity with or without clinical symptoms of malaise, loss of appetite and jaundice. Although it is believed that these subjects are free of developing long-term clinical sequelae, it has been shown that HBV DNA was detectable in some subjects even after 70 months of acute HBV infection (Michalak et al. 1994). The findings were subsequently confirmed by two other studies in which HBV DNA were still detectable 6 months to 13 years after the acute HBV infection (Yotsuyanagi et al. 1998; Penna et al. 1996). In spite of the presence of low-grade viraemia in these subjects, their livers are usually with minimal necroinflammation (Yuki et al. 2003; Bläckberg and Kidd-Ljunggren 2000). As such, nearly all these subjects would have normal liver biochemistry. These are compatible with the findings in studies of woodchucks recovering from acute HBV infection with detectable viraemia in that the degree of liver inflammation was low (Mulrooney-Cousins and Michalak 2007).

The clinical significance of OBI subjects after acute HBV infection remains obscure. It is however expected that the chance of developing long-term liver-related complications is low basing on the fact that these subjects have very low viraemic levels, normal histology and alanine aminotransferase levels. However, the clinical implications become important in the aspect of HBV transmission to uninfected subjects through blood product donations. In addition, with the more profound immunosuppressive effects exerted by B-cell depleting monoclonal antibodies for various malignant and immunological diseases, the problem of HBV reactivation as a result of immune reconstitution in these subjects has been increasingly recognized (Yuen 2016). These topics will be discussed below.

2.2.2 Subjects with Chronic Hepatitis B Infection

As mentioned above, majority of subjects with OBI are CHB patients who undergo an uncommon event of HBsAg seroclearance. This event happens either spontaneously or with the assistance of antiviral therapy. In the former situation, patients undergo the different phase of CHB infection from immunotolerance through immune clearance before entering into the residual phase where HBsAg seroclearance may occur (Fig. 15.1). It has been shown that HBsAg seroclearance occurs in these subjects at an annual rate of 0.1–2% (Lai and Yuen 2009; Liu et al. 2010). In patients receiving antiviral therapy, the rate of HBsAg seroclearance is around 10% in long-term follow-up studies (Yuen et al. 2016).

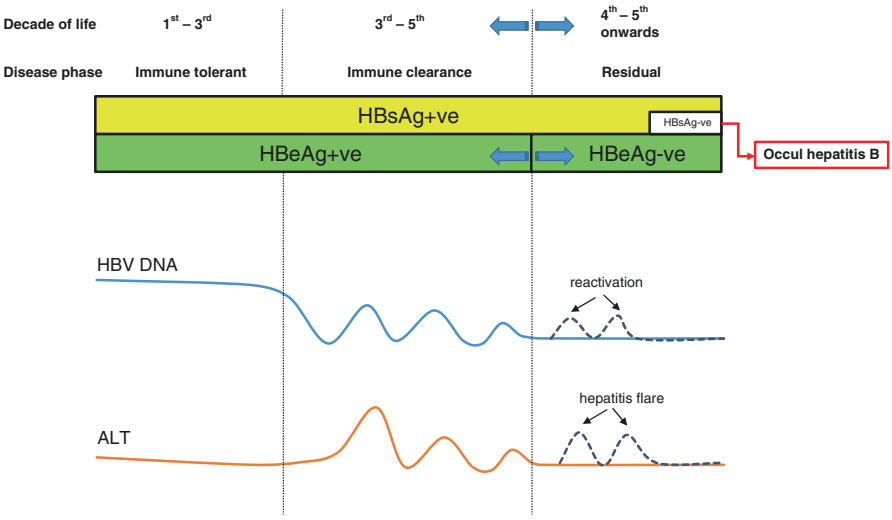

Fig. 15.1 Different phases of chronic hepatitis B infection

Around 60% of CHB patients with HBsAg seroclearance would develop anti-HBs at the time of HBsAg seroclearance or at the subsequent follow-up after HBsAg seroclearance (Yuen et al. 2008). CHB patients with HBsAg seroclearance usually have undetectable HBV DNA in the serum. The serum HBV DNA detection rate inversely correlates with the time of measurement after HBsAg seroclearance. For instance, serum HBV DNA was detectable in 13%, 6% and 3.7% when the measurements were carried out at 1, 5–10 and >10 years after HBsAg seroclearance (Yuen et al. 2008). It is worth to note that the intrahepatic HBV DNA was detectable (although at low levels) practically in all subjects with HBsAg seroclearance at all times (Yuen et al. 2008; Ahn et al. 2005). The HBV transcriptional activities however are minimal as indicated by negligible mRNA expression of the core, surface and X genes (Yuen et al. 2008; Kuhns et al. 1992). It is therefore an expected observation among 29 patients with liver biopsies that two-third of them had no necroinflammation and fibrosis and the remaining one-third only had mild necroinflammation or fibrosis (Yuen et al. 2008). It has further been shown that for those with early HBsAg seroclearance, e.g. <40 years old, none of these subjects had fibrosis in the liver (Yuen et al. 2004).

With regard to the chance of the development of long-term liver-related complications in CHB patients with HBsAg seroclearance, it largely depends on the clinical status at the time of HBsAg seroclearance. According to an early study (Huo et al. 1998), HBsAg seroclearance did not reduce the risk of development of complications as the cumulative probability of developing them was as high as 30% after 4 years of HBsAg seroclearance. Nevertheless, a subsequent study showed that this risk would be lower if there were no other viral co-infection (e.g. HCV infection) and no pre-existing cirrhosis (Chen et al. 2002). A study conducted in Hong

Kong showed that those who achieved HBsAg seroclearance before the age of 50 years had a significantly lower risk of development of complications compared with patients who had the HBsAg seroclearance after the age of 50 years (Yuen et al. 2008). None of the former group developed HCC compared to 10% ($p = 0.004$) of patients developing HCC in the latter group at the median follow-up of 3 years after HBsAg seroclearance. It is likely related to the lower proportion of patients having severe fibrosis or cirrhosis when the HBsAg seroclearance occurring at a younger age.

2.2.3 Subjects with Unknown HBV History

There are subjects in whom OBI is diagnosed by routine screening especially during the blood donation surveillance. There are many blood donation centres worldwide routinely performing nuclear acid testing either in pooled or individual samples which are firstly tested negative for HBsAg. The absence of the medical history of HBV infection in these subjects makes it impossible to differentiate unnoticed clinically silent acute HBV subjects from CHB patients with HBsAg seroclearance who are never known to themselves or their physicians.

In the context of OBI subjects diagnosed incidentally in the blood donation services, a study with 40 OBI blood donors showed that the liver biochemistry was normal in all subjects. They also had negligible liver necroinflammation (median necroinflammatory score: 1 out of 18) and fibrosis (median Ishak's fibrosis score: 1 out of 6) (Wong et al. 2016). The viral replicative status was extremely low. Only 13% of OBI subjects had detectable pre-genomic RNA, and the median intrahepatic HBV DNA was 0.22 copies/cell compared to 260 copies/cell ($p < 0.0001$) in age- and sex-matched HBsAg-positive CHB patients. Only 45% of OBI subjects had serum HBV DNA > 1.1 IU/mL.

3 Prevalence of OBI

The true prevalence of OBI worldwide has not been well studied because of several unresolved issues. First, the HBV DNA level in serum is notoriously low for it to be measurable by standard commercial assays. Although the HBV DNA level was described to be usually less than 200 IU/mL (Morales-Romero et al. 2014), studies have shown that the HBV DNA levels were typically less than 20 IU/mL (Yuen et al. 2010, 2011). Second, the fluctuations of low HBV DNA levels in OBI subjects often cast doubts on the reliability of the sensitive HBV DNA assays. These two difficulties may be partly overcome by using large-volume samples for testing. Finally, although the yield of detectable HBV DNA can be greatly enhanced by measuring it in the liver tissues, invasive nature of liver biopsy makes it an unpopular methodology in many studies. With all these issues to be considered, prevalence of OBI was estimated to be <1 to 18% (Yuen et al. 2008, 2010; Song et al. 2009; Bhatti et al. 2007; Werle-Lapostolle et al. 2004; Reesink et al. 2008; Georgiadou et al. 2004; Fang et al. 2009; Minuk et al. 2005; Kim et al. 2007; Svicher et al. 2012). According to a large-scale study in

Hong Kong with 9990 blood donors, the prevalence of OBI was found to be 0.11% (Yuen et al. 2010). It suggests that the prevalence of OBI may not be as high as one would expect even in region with high CHB endemicity.

4 Pathogenesis of OBI

There are several mechanistic postulations for the existence of OBI. These include the viral and host factors. It has long been proposed that mutations over the major surface gene, e.g. G145R, may lead to detection escape from the standard HBsAg assays (Yamamoto et al. 1994; Hou et al. 1995; Carman et al. 1997). It is however not supported by three studies in which corresponding mutations as suggested were not found in OBI subjects (Pollicino et al. 2004, 2007; Huang et al. 2014). In addition, a study also showed that most of the standard assays were able to detect various mutated HBsAg epitopes (Yuen and Lai 2006). Another hypothesis is the persistently low viral replication in OBI subjects because of two possible but interlinking factors. The HBV may be intrinsically replicate at an extremely low level because of multiple genomic mutations or rearrangements in splice donor sites of pre-S1, pre-S2, S genes as well as their regulatory genomic regions (Candotti et al. 2012; El Chaar et al. 2010). This is supported by studies showing greater sequence diversities in both nucleoside and amino acid levels in subjects with OBI compared with HBsAg-positive CHB subjects (Huang et al. 2014; Pollicino et al. 2004; Lai and Yuen 2009). The viral replication capacity may be much restrained with all these changes which may be additive or synergistic. At the post-transcriptional level, it was found that Pre-S2/S RNA splicing may also reduce the pre-S2/S and HBsAg transcripts (Candotti et al. 2012; Hass et al. 2005). Epigenetic factor exerting on the virus has also been postulated. It has been shown that the cccDNA-bound histones in OBI subjects were highly hypoacetylated which may result in low HBV replication (Pollicino et al. 2006).

The other factor interlinking with viral replication is the more intense immune control of the HBV in OBI subjects. There is an indirect but very strong evidence from the phenomenon of HBV reactivation in HBsAg-negative, anti-HBc-positive subjects (potential OBI subjects) under immunosuppressive therapy especially with potent anti-B-cell depletive effect, e.g. anti-CD 20 (Larrubia 2011; Bes et al. 2012). It suggests that OBI status is successfully kept by adequate immune control and the virus reactivates upon withdrawal of this controlling factor under immunosuppression. To explore this, studies to compare the HBV-specific T-cell responses between OBI and CHB subjects are deemed to be important. In fact, intense immune response has been shown to be detectable many years after the recovery of acute HBV infection (Rehermann et al. 1996; Penna et al. 1996).

Finally, there may be external factors inhibiting HBV replication, e.g. co-infection with other viruses, leading to the OBI state. In HCV-HBV co-infection, HBV replication is often diminished because HCV core protein has been shown to exert inhibitory effects on HBV replication (Chen et al. 2003; Raimondo et al. 2005; Schüttler et al. 2002; Shih et al. 1995). It seems to be in accordance to the observation

that OBI is apparently more commonly found in HCV patients than other populations. Therefore, HCV may play a role in inducing OBI status (Pollicino and Raimondo 2014).

5 Clinical Significance of OBI

There are three major clinical areas where OBI plays an important role (Fig. 15.2). They include (1) the possibility of transmission of HBV disease through blood and organ donations, (2) the pathologic role on chronic liver disease and liver-related complications and (3) the HBV reactivation during immunosuppressive therapy.

5.1 Transmission of HBV

OBI donors, although usually having very low HBV viraemia, may transmit the HBV to other individuals in the setting of blood donation and organ transplantation. It is because any products containing full HBV viral particles are considered to be potentially infectious. According to a chimpanzee study, HBV DNA of only ten copies can already achieve the minimum 50% infectious dose of HBV (Komiya et al. 2008). In another study using severe immunodeficient chimeric mice with human hepatocytes, serum HBV DNA, intrahepatic HBV DNA as well as covalently closed circular (ccc) DNA (the viral replication template) were detectable in one of the four mice inoculated with the sera from OBI subjects (Yuen et al. 2011). The mouse hepatocytes were also stained positive for HBcAg. Whether these findings are applicable to human would require actual human studies to verify as the immune components in animals are different and the mice are basically immunodeficient. In addition, there are other confounding factors in human situations influencing the chance of HBV transmission from OBI subjects. These

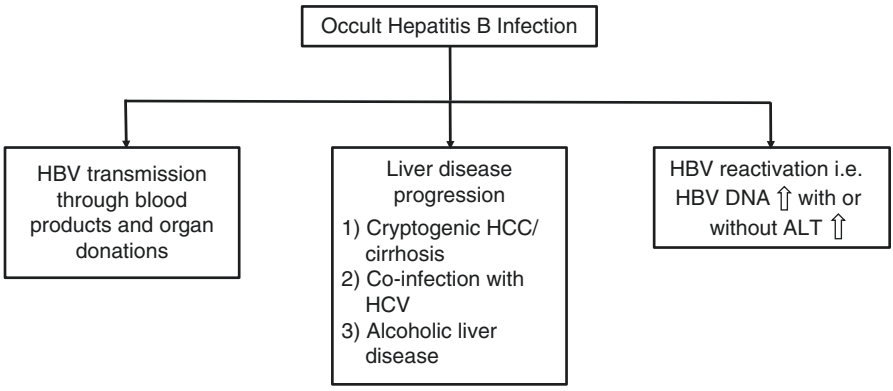

Fig. 15.2 Clinical scenarios of occult hepatitis B infection

include anti-HBs status of both the donors and the recipients, the kind and the volume of the blood products being transfused to the recipients and the immunological status of the recipients (Raimondo et al. 2013; Mosley et al. 1995; Satake et al. 2007). Finally, the actual circulating viral load (which may fluctuate with times) of the OBI donors at the time of donation may also affect the outcome (Chemin et al. 2009).

Although there are already many human studies examining the transmissibility rate of HBV from OBI blood donors, most of these studies are retrospective in nature. This greatly limits the effectiveness of contact tracing to the recipients. In areas where HBV is of high endemicity, meticulous phylogenetic analysis of the viral sequence between the donors and recipients which are especially essential to establish the HBV transmission is required. This is however difficult to achieve in context of the retrospective nature of the studies. In addition, the difficulty in obtaining good quality of viral sequence (from amplification of the low HBV DNA levels) also impinges the study accuracy.

Nevertheless, even with all these problematic issues, there are several studies performed to determine the HBV transmission rate from OBI donors. It was found that the transmission rate was low at around 1–3% (Yuen et al. 2011; Mosley et al. 1995; Candotti and Allain 2009). One the studies further showed that the risk was greatly reduced if the donor serum was anti-HBs positive (Mosley et al. 1995). From a study conducted in Japan, of the identified 12 subjects with transfusion-related hepatitis B, all received the blood products from donors who were negative for both anti-HBs and anti-HBc (Satake et al. 2007). It is likely that these donors were at the window period of the acute HBV infection when all the antibodies were yet to be detectable. This group of donors may also be sero-negative OBI donors if their sera remain to be negative for both anti-HBs and anti-HBc.

There are studies showing that the HBV transmission is possible from anti-HBc-positive donors (Hoofnagle et al. 1978; Lander et al. 1978; Koziol et al. 1986). The transmissible rate is around 2.4–3.0% according to one study (Lai and Yuen 2009). It is compatible with another study conducted in Hong Kong using detailed phylogenetic relatedness methodology. This study showed that the transmission rate of 2.2% (Yuen et al. 2011).

In the context of organ transplantation in which the recipients receive organs from OBI donors, HBV transmission may also be of concern. Unlike the situation in blood product donations given to usually immunocompetent recipients. Patients receiving organ transplantation have an additional risk factor of acquiring the HBV from OBI donors because of the immunosuppressive status rendered by the immunosuppressive therapy for prevention of organ rejection. In the setting of liver transplantation, it has been estimated that 22–100% of liver-transplanted recipients would have de novo HBV infection of the liver grafts from HBsAg-negative, anti-HBc-positive donors (Dickson et al. 1997; Muñoz 2002). It seems that recipients positive for both anti-HBs and anti-HBc are at a relatively low risk for de novo HBV infection. It should also be noted that HBV reactivation from pre-transplanted, "hidden" HBV of the OBI recipients is also possible especially after the use of intensive immunosuppressive therapy for the liver transplantation.

Finally, unlike the situation in liver transplantation, the risk of HBV transmission from OBI donors to recipients of kidneys, hearts and bone marrows seems to be lower (Wachs et al. 1995; De Feo et al. 2005). It is now a routine procedure in many centres that prophylactic antiviral therapy would be given to patients who are going to receive organs from OBI donors. Some centres are also extending this prophylactic measure to recipients receiving anti-HBc-positive donors with undetectable HBV DNA.

5.2 Role on Chronic Liver Disease and Complications

For chronic liver diseases, the initial activities can be assessed by the liver biochemistry and the liver histology, the more reliable parameter for assessment of liver damage. According to various studies (Yuen et al. 2010; Fung et al. 2010; Wong et al. 2014), OBI subjects usually have normal liver biochemistry as well as very minimal histological inflammation and fibrosis. However, it has been shown in early 1980s that HBV DNA were detectable in HBsAg-negative patients with chronic liver disease implying a possible pathologic role of OBI (Brechot et al. 1981, 1985). It is therefore not surprising to observe that around 5–50% of cirrhotic patients without identifiable cause have documented presence of OBI (Pollicino et al. 2004; Hou et al. 2001; Bréchot et al. 2001; Alavian et al. 2012).

The pathologic roles of OBI are firstly described in HBsAg-negative alcoholic patients with HCC (Bréchot et al. 1982). This suggests a possible synergism between these two factors leading to the development of HCC. According to a recent study, 56% of HBsAg-negative alcoholic patients with HCC had the presence of HBV DNA in the liver (Wong et al. 2011). There are even more studies examining the role of OBI in patients with chronic hepatitis C virus (HCV) infection. They have shown the pathological component of OBI in causing cirrhosis and HCC in patients co-infected with HCV (Bréchot et al. 1985; Cacciola et al. 1999; Fukuda et al. 1999; Tanaka et al. 2004; Squadrito et al. 2006; Shetty et al. 2008; Adachi et al. 2008; Shi et al. 2012). For instance, HCV patients with OBI had more advanced fibrosis compared to HCV patients without OBI (Matsuoka et al. 2008). Liver cirrhosis was found in 33% of HCV patients with OBI compared to 19% of HCV mono-infected HCV patients (Cacciola et al. 1999). In addition, HBV DNA and anti-HBc positivity were found to be two independent risk factors for the development of HCC in HCV patients (Adachi et al. 2008; Matsuoka et al. 2008; Ikeda et al. 2007). All these findings suggest that HCV patients with OBI carry a more progressive course for the development of cirrhosis and HCC. A recent meta-analysis study showed that HCV patients with OBI had a 2.8-folds higher risk of development of HCC compared to mono-infected HCV patients (Shi et al. 2012). However, there are studies which did not show an additively or synergistically increased risk for the development of liver complication by OBI (Sagnelli et al. 2008; Kao et al. 2002; Tsubouchi et al. 2013). As these studies are all cross-sectional and also of small scale, the best way to confirm whether OBI has additive or synergistic effects on development of liver complications in HCV patients is to have large-scale longitudinal studies.

Apart from the possible role of OBI in patients with alcoholic liver disease and patients with HCV infection, OBI may also be the primary aetiological cause for liver disease patients with apparently no identifiable cause, i.e. cryptogenic cirrhosis or HCC. It has been shown by very early studies that HBV DNA was detected in HBsAg-negative patients with HCC (Bréchot et al. 1981; Shafritz et al. 1981). The proportion of patients with cryptogenic HCC having OBI is in a range between 48% and 73% according to two studies (Wong et al. 2011; Yotsuyanagi et al. 2000). In fact, OBI was shown to be an independent risk factor for liver carcinogenesis with the hazard ratio of 8.3 (Ikeda et al. 2007). According to a longitudinal study performed in Japan, there was an association between OBI and HCC. In a meta-analysis study including 16 retrospective and prospective studies, OBI was confirmed to be a risk factor for the development of HCC with the hazard ratio of three when the analyses were limited to the five prospective studies (Shi et al. 2012). Another meta-analysis revealed a ninefold increased risk of development of HCC in OBI subjects (Covolo et al. 2013).

Concerning the best methodology for detection, different studies use different experimental protocols targeting for different genomic regions of HBV. In general, the detection rate is higher in non-tumorous parts compared to tumorous parts of the liver. In addition, sequencing the X gene compared to other genomic regions also yields a higher positive rate (Wong et al. 2011).

Similar to the oncologic mechanisms of overt CHB disease, mechanisms of OBI causing HCC include the following:

1. Persistently low-grade necroinflammation leading to cirrhosis of the liver (it may be possible that there are unrecognized high-grade necroinflammation during the overt stage of CHB disease before entering into the OBI state) (Yuki et al. 2003; Bläckberg and Kidd-Ljunggren 2000).
2. Integration of viral sequence from OBI into the human genome triggering the oncogenic cascade (Pollicino et al. 2004; Bréchot et al. 1981, 2000; Shafritz et al. 1981; Tamori et al. 1999; Paterlini et al. 1995). Integration of the HBV gene into the human genomes is also commonly found in OBI patients (Brechot et al. 1981). It has also been shown that the process of HBV DNA integration can happen even at the time of acute HBV infection (Murakami et al. 2004; Kimbi et al. 2005).
3. OBI may have HBV transcriptional activities producing oncogenic proteins, namely, protein X and truncated pre-S-S protein (Cougot et al. 2005).

5.3 HBV Reactivation

HBV reactivation can be associated with significant liver-related morbidity and mortality. Under normal circumstance, spontaneous HBV reactivation from OBI is rare. In most of the OBI subjects, the serum HBV DNA level remains undetectable, and if it is detectable at baseline, it usually remains very low throughout subsequent follow-up. However, there are circumstances where HBV may

reactivate from this low viraemic state. It is well known that HBsAg-positive CHB patients undergoing chemotherapeutic treatment and immunosuppressive therapy, in particular steroid containing regimes, have a considerable risk of HBV reactivation (Yuen 2016; Cheung et al. 2016). As such, it is already firmly established that all HBsAg-positive CHB patients before receiving immunosuppressive therapy are recommended to receive antiviral prophylaxis to prevent HBV reactivation. However, this recommendation has not been universally implemented in OBI subjects yet.

As a result of the increasing use of biologics and monoclonal antibodies with potent immunosuppressive effects for autoimmune diseases and haematological malignancies, HBV reactivation from OBI subjects have emerged to be a more serious problem nowadays. It was found that there was no HBV reactivation from HBsAg-negative, anti-HBc-negative subjects with lymphoma receiving chemotherapy (Yeo et al. 2009). However, approximately 24% of anti-HBc-positive subjects receiving cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) plus anti-CD 20 (rituximab) had HBV reactivation resulting in one death. On the other hand, there was no HBV reactivation in anti-HBc-positive subjects if they received rituximab-free treatment regime. It strongly suggests that rituximab significantly increases the risk of HBV reactivation in OBI subjects. This issue was studied in detail by a prospective study recently (Seto et al. 2014). Of the 63 HBsAg-negative, anti-HBc-positive subjects (all with undetectable serum HBV DNA) receiving rituximab, 42% of subjects develop HBV reactivation (Seto et al. 2014). It was also found that the rate of HBV reactivation was lower in anti-HBs-positive subjects compared with anti-HBs-negative subjects (34% vs. 68%, respectively, $p = 0.012$). Prompt treatment using entecavir at the time of HBV reactivation was able to control the HBV DNA level to undetectable level in all patients, and therefore no hepatitis flares were observed.

Concerning the biologics, the rate of HBV reactivation from OBI subjects was lower than that of using rituximab. According to a large-scale study with HBsAg-negative and anti-HBc-positive, the use of anti-tumour necrosis factor was associated with the HBV reactivation rate of 1.7% (Lee et al. 2013). The rate of HBV reactivation is however, considerably higher (15–35%) in HBsAg-positive subjects receiving anti-tumour necrosis factor (Perez-Alvarez et al. 2011).

Patients with haematological malignancy are usually at a higher risk of HBV reactivation because of the high degree of bone marrow suppression by intense immunosuppressive therapy. Among those, patients receiving haematopoietic stem cell transplantation (HSCT) are having the highest rate of HBV reactivation. The rate of HBV reactivation in HSCT patients who were HBsAg-negative and anti-HBc-positive is in a range of 3 and 43% as reported by different studies (Vigano et al. 2011; Hammond et al. 2009; Nakamoto et al. 2014). A recent prospective study with 62 HSCT patients showed that the HBV reactivation was as high as 40% with 2 years of follow-up (Seto et al. 2017). In addition, patients with chronic graft-versus-host disease (requiring more intense and long-term immunosuppressive therapy) and patients older than 50 years old would have a higher rate of HBV reactivation compared to subjects without these factors.

The underlying mechanisms involved in the HBV reactivation in OBI subjects are believed to be the same as for CHB subjects. These include direct increase in HBV replication related to the agents of the therapy. It has been well known that steroid can increase HBV replication as it can act through the glucocorticoid-responsive element in the HBV genome. As the immune control may be further jeopardized by the B-cell depleting agent, e.g. rituximab, HBV may reactivate whenever under a less intensive immune control environment. Finally, at the later stage upon cessation of the immunosuppressive therapy, the immune reconstitution may exert an even more overwhelming immune-mediated attack to the hepatocytes where the HBV load is high.

Because of the potential mortality from HBV reactivation, prevention using prophylactic antiviral agents is generally recommended. It is now a universally accepted protocol to prescribe antiviral agents for HBsAg-positive patients undergoing chemotherapy or immunotherapy. For OBI subjects, this practice is also widely implemented for those with detectable HBV DNA. A controversy, however, exists in HBsAg-negative, anti-HBc-positive patients with undetectable HBV DNA. Some clinicians advocate the prophylactic approach, whereas others adopt a close surveillance strategy. The latter approach recommends HBV DNA monitoring at a 4–12 weekly interval. According to a study of lymphoma patients receiving rituximab, prompt administration of entecavir at the time of detectable HBV DNA checked at 4 weekly intervals is able to render HBV DNA undetectable without hepatitis flare in all patients (Seto et al. 2014). This strategy is also recommended by treatment guidelines (European Association for the Study of the Liver 2012). At present, there are no studies to determine the best monitoring intervals for HBV DNA. Practical logistics, manpower and costs are the major factors for the consideration. Another issue is whether the "interval" of monitoring would be the same for all kinds of chemotherapy and malignancy. It is because the risk of HBV reactivation would largely be determined by the type of chemotherapy and to a lesser extent the specific malignancy.

In summary, OBI is now a well-recognized disease entity with increasing attention in various aspects of liver diseases. The prevalence is yet to be studied in greater detail. The diagnosis of OBI is greatly dependent on the clinicians' awareness and the availability of standard and accurate HBV DNA testing. The presence of anti-HBc would trigger off the investigative steps. Subjects with OBI are potentially infectious with a low transmissibility rate in which the level of viraemia and the immune status of the recipients are the two major determinants. OBI can lead to end-stage liver disease and HCC, and this potential aetiology should be actively sought for in patients with cryptogenic cirrhosis and/or HCC. HBV reactivation from OBI is now being increasingly recognized in the present era of using potent B-cell depleting therapy for various malignant and immunological conditions. While effective antiviral therapy reducing the morbidity and mortality from the HBV reactivation is already in place, the best strategy of prevention of HBV reactivation in these subjects remains to be defined.

References

Adachi S, Shibuya A, Miura Y, et al. Impact of occult hepatitis B virus infection and prior hepatitis B virus infection on development of hepatocellular carcinoma in patients with liver cirrhosis due to hepatitis C virus. Scand J Gastroenterol. 2008;43:849–56.

Ahn SH, Park YN, Park JY, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol. 2005;42:188–94.

Alavian SM, Miri SM, Hollinger FB, et al. Occult hepatitis B (OBH) in clinical settings. Hepat Mon. 2012;12:e6126.

Bes M, Vargas V, Piron M, et al. T cell responses and viral variability in blood donation candidates with occult hepatitis B infection. J Hepatol. 2012;56:765–74.

Bhatti FA, Ullah Z, Salamat N, et al. Anti-hepatitis B core antigen testing, viral markers, and occult hepatitis B virus infection in Pakistani blood donors: implications for transfusion practice. Transfusion. 2007;47:74–9.

Bläckberg J, Kidd-Ljunggren K. Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation. J Hepatol. 2000;33:992–7.

Brechot C, Hadchouel M, Scotto J, et al. Detection of hepatitis B virus DNA in liver and serum: a direct appraisal of the chronic carrier state. Lancet. 1981;2(8250):765–8.

Bréchot C, Hadchouel M, Scotto J, et al. State of hepatitis B virus DNA in hepatocytes of patients with hepatitis B surface antigen-positive and -negative liver diseases. Proc Natl Acad Sci U S A. 1981;78:3906–10.

Brechot C, Pourcel C, Louise A, et al. Detection of hepatitis B virus DNA sequences in human hepatocellular carcinoma in an integrated form. Prog Med Virol. 1981;27:99–102.

Bréchot C, Nalpas B, Couroucé AM, et al. Evidence that hepatitis B virus has a role in liver-cell carcinoma in alcoholic liver disease. N Engl J Med. 1982;306:1384–7.

Bréchot C, Degos F, Lugassy C, et al. Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. N Engl J Med. 1985;312:270–6.

Bréchot C, Gozuacik D, Murakami Y, et al. Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol. 2000;10:211–31.

Bréchot C, Thiers V, Kremsdorf D, et al. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? Hepatology. 2001;34:194–203.

Cacciola I, Pollicino T, Squadrito G, et al. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N Engl J Med. 1999;341:22–6.

Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. J Hepatol. 2009;51:798–809.

Candotti D, Lin CK, Belkhiri D, et al. Occult hepatitis B infection in blood donors from South East Asia: molecular characterisation and potential mechanisms of occurrence. Gut. 2012;61:1744–53.

Carman WF, Van Deursen FJ, Mimms LT, et al. The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa, and Sardinia. Hepatology. 1997;26:1658–66.

Chemin I, Guillaud O, Queyron PC, et al. Close monitoring of serum HBV DNA levels and liver enzymes levels is most useful in the management of patients with occult HBV infection. J Hepatol. 2009;51:824–5.

Chen YC, Sheen IS, Chu CM, et al. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology. 2002;123:1084–9.

Chen SY, Kao CF, Chen CM, et al. Mechanisms for inhibition of hepatitis B virus gene expression and replication by hepatitis C virus core protein. J Biol Chem. 2003;278:591–607.

Cheung KS, Seto WK, Lai CL, Yuen MF. Prevention and management of hepatitis B virus reactivation in cancer patients. Hepatol Int. 2016;10:407–14.

Cougot D, Neuveut C, Buendia MA. HBV induced carcinogenesis. J Clin Virol. 2005;34(Suppl 1):S75–8.
Covolo L, Pollicino T, Raimondo G, et al. Occult hepatitis B virus and the risk for chronic liver disease: a meta-analysis. Dig Liver Dis. 2013;45:238–44.
De Feo TM, Poli F, Mozzi F, et al. Risk of transmission of hepatitis B virus from anti-HBC positive cadaveric organ donors: a collaborative study. Transplant Proc. 2005;37:1238–9.
Dickson RC, Everhart JE, Lake JR, et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases liver transplantation database. Gastroenterology. 1997;113:1668–74.
El Chaar M, Candotti D, Crowther RA, et al. Impact of hepatitis B virus surface protein mutations on the diagnosis of occult hepatitis B virus infection. Hepatology. 2010;52:1600–10.
European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167–85.
Fang Y, Shang QL, Liu JY, et al. Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. J Infect. 2009;58:383–8.
Fukuda R, Ishimura N, Niigaki M, et al. Serologically silent hepatitis B virus coinfection in patients with hepatitis C virus-associated chronic liver disease: clinical and virological significance. J Med Virol. 1999;58:201–7.
Fung J, Lai CL, Chan SC, et al. Correlation of liver stiffness and histological features in healthy persons and in patients with occult hepatitis B, chronic active hepatitis B, or hepatitis B cirrhosis. Am J Gastroenterol. 2010;105:1116–22.
Georgiadou SP, Zachou K, Rigopoulou E, et al. Occult hepatitis B virus infection in Greek patients with chronic hepatitis C and in patients with diverse nonviral hepatic diseases. J Viral Hepat. 2004;11:358–65.
Hammond SP, Borchelt AM, Ukomadu C, et al. Hepatitis B virus reactivation following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15:1049–59.
Hass M, Hannoun C, Kalinina T, et al. Functional analysis of hepatitis B virus reactivating in hepatitis B surface antigen-negative individuals. Hepatology. 2005;42:93–103.
Hoofnagle JH, Seeff LB, Bales ZB, et al. Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. N Engl J Med. 1978;298:1379–83.
Hou J, Karayiannis P, Waters J, et al. A unique insertion in the S gene of surface antigen--negative hepatitis B virus Chinese carriers. Hepatology. 1995;21:273–8.
Hou J, Wang Z, Cheng J, et al. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. Hepatology. 2001;34:1027–34.
Huang FY, Wong DK, Seto WK, et al. Sequence variations of full-length hepatitis B virus genomes in Chinese patients with HBsAg-negative hepatitis B infection. PLoS One. 2014;9:e99028.
Huo TI, Wu JC, Lee PC, et al. Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology. 1998;28:231–6.
Ikeda K, Marusawa H, Osaki Y, et al. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. Ann Intern Med. 2007;146:649–56.
Kao JH, Chen PJ, Lai MY, et al. Occult hepatitis B virus infection and clinical outcomes of patients with chronic hepatitis C. J Clin Microbiol. 2002;40:4068–71.
Kim SM, Lee KS, Park CJ, et al. Prevalence of occult HBV infection among subjects with normal serum ALT levels in Korea. J Infect. 2007;54:185–91.
Kimbi GC, Kramvis A, Kew MC. Integration of hepatitis B virus DNA into chromosomal DNA during acute hepatitis B. World J Gastroenterol. 2005;11:6416–21.
Komiya Y, Katayama K, Yugi H, et al. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype a and genotype C. Transfusion. 2008;48:286–94.
Koziol DE, Holland PV, Alling DW. Antibody to hepatitis B core antigen as a paradoxical marker for non-a, non-B hepatitis agents in donated blood. Ann Intern Med. 1986;104:488–95.

Kuhns M, McNamara A, Mason A, et al. Serum and liver hepatitis B virus DNA in chronic hepatitis B after sustained loss of surface antigen. Gastroenterology. 1992;103:1649–56.
Lai CL, Yuen MF. Occult hepatitis B infection: incidence, detection and clinical implications. ISBT Sci Series. 2009;4:347–51.
Lander JJ, Gitnick GL, Gelb LH, et al. Anticore antibody screening of transfused blood. Vox Sang. 1978;34:77–80.
Larrubia JR. Occult hepatitis B virus infection: a complex entity with relevant clinical implications. World J Gastroenterol. 2011;17:1529–30.
Lee YH, Bae SC, Song GG. Hepatitis B virus (HBV) reactivation in rheumatic patients with hepatitis core antigen (HBV occult carriers) undergoing anti-tumor necrosis factor therapy. Clin Exp Rheumatol. 2013;31:118–21.
Liu J, Yang HI, Lee MH, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010;139:474–82.
Matsuoka S, Nirei K, Tamura A, et al. Influence of occult hepatitis B virus coinfection on the incidence of fibrosis and hepatocellular carcinoma in chronic hepatitis C. Intervirology. 2008;51:352–61.
Michalak TI, Pasquinelli C, Guilhot S, et al. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest. 1994;93:230–9.
Minuk GY, Sun DF, Uhanova J, et al. Occult hepatitis B virus infection in a north American community-based population. J Hepatol. 2005;42:480–5.
Morales-Romero J, Vargas G, García-Román R. Occult HBV infection: a faceless enemy in liver cancer development. Virus. 2014;6:1590–611.
Mosley JW, Stevens CE, Aach RD, et al. Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. Transfusion. 1995;35:5–12.
Mulrooney-Cousins PM, Michalak TI. Persistent occult hepatitis B virus infection: experimental findings and clinical implications. World J Gastroenterol. 2007;13:5682–6.
Muñoz SJ. Use of hepatitis B core antibody-positive donors for liver transplantation. Liver Transpl. 2002;8(10 Suppl 1):S82–7.
Murakami Y, Minami M, Daimon Y, et al. Hepatitis B virus DNA in liver, serum, and peripheral blood mononuclear cells after the clearance of serum hepatitis B virus surface antigen. J Med Virol. 2004;72:203–14.
Nakamoto S, Kanda T, Nakaseko C, et al. Reactivation of hepatitis B virus in hematopoietic stem cell transplant recipients in Japan: efficacy of nucleos(t)ide analogues for prevention and treatment. Int J Mol Sci. 2014;15:21455–67.
Paterlini P, Poussin K, Kew M, et al. Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. Hepatology. 1995;21:313–21.
Penna A, Artini M, Cavalli A, et al. Long-lasting memory T cell responses following self-limited acute hepatitis B. J Clin Invest. 1996;98:1185–94.
Perez-Alvarez R, Dı'az-Lagares C, Garcı'a-Herna'ndez F, et al. Hepatitis B virus (HBV) reactivation in patients receiving tumor necrosis factor (TNF)-targeted therapy: analysis of 257 cases. Medicine (Baltimore). 2011;90:359–71.
Pollicino T, Raimondo G. Occult hepatitis B infection. J Hepatol. 2014;61:688–9.
Pollicino T, Squadrito G, Cerenzia G, et al. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. Gastroenterology. 2004;126:102–10.
Pollicino T, Belloni L, Raffa G, et al. Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. Gastroenterology. 2006;130:823–37.
Pollicino T, Raffa G, Costantino L, et al. Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. Hepatology. 2007;45:277–85.
Raimondo G, Cacciamo G, Saitta C. Hepatitis B virus and hepatitis C virus co-infection: additive players in chronic liver disease? Ann Hepatol. 2005;4:100–6.
Raimondo G, Pollicino T, Cacciola I, et al. Occult hepatitis B virus infection. J Hepatol. 2007;46:160–70.

Raimondo G, Navarra G, Mondello S, et al. Occult hepatitis B virus in liver tissue of individuals without hepatic disease. J Hepatol. 2008a;48:743–6.
Raimondo G, Allain JP, Brunetto MR, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008b;49:652–7.
Raimondo G, Caccamo G, Filomia R, et al. Occult HBV infection. Semin Immunopathol. 2013;35:39–52.
Reesink HW, Engelfriet CP, Henn G, et al. Occult hepatitis B infection in blood donors. Vox Sang. 2008;94:153–66.
Rehermann B, Ferrari C, Pasquinelli C, et al. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med. 1996;2:1104–8.
Sagnelli E, Imparato M, Coppola N, et al. Diagnosis and clinical impact of occult hepatitis B infection in patients with biopsy proven chronic hepatitis C: a multicenter study. J Med Virol. 2008;80:1547–53.
Satake M, Taira R, Yugi H, et al. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. Transfusion. 2007;47:1197–205.
Schüttler CG, Fiedler N, Schmidt K, et al. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein. J Hepatol. 2002;37:855–62.
Seto WK, Chan TSY, Hwang YY, et al. Hepatitis B reactivation in patients with prior HBV exposure undergoing rituximab-containing chemotherapy for lymphoma: a prospective study. J Clin Oncol. 2014;32:3736–43.
Seto WK, Chan TS, Hwang YY, et al. Hepatitis B reactivation in occult viral carriers undergoing hematopoietic stem cell transplantation: a prospective study. Hepatology. 2017.; (in press)
Shafritz DA, Shouval D, Sherman HI, et al. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. N Engl J Med. 1981;305:1067–73.
Shetty K, Hussain M, Nei L, et al. Prevalence and significance of occult hepatitis B in a liver transplant population with chronic hepatitis C. Liver Transpl. 2008;14:534–40.
Shi Y, Wu YH, Wu W, et al. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int. 2012;32:231–40.
Shih CM, Chen CM, Chen SY, et al. Modulation of the trans-suppression activity of hepatitis C virus core protein by phosphorylation. J Virol. 1995;69:1160–71.
Song EY, Yun YM, Park MH, et al. Prevalence of occult hepatitis B virus infection in a general adult population in Korea. Intervirology. 2009;52:57–62.
Squadrito G, Pollicino T, Cacciola I, et al. Occult hepatitis B virus infection is associated with the development of hepatocellular carcinoma in chronic hepatitis C patients. Cancer. 2006;106:1326–30.
Svicher V, Cento V, Bernassola M, et al. Novel HBsAg markers tightly correlate with occult HBV infection and strongly affect HBsAg detection. Antivir Res. 2012;93:86–93.
Tabor E, Gerety RJ. Non-a, non-B hepatitis: new findings and prospects for prevention. Transfusion. 1979;19:669–74.
Tamori A, Nishiguchi S, Kubo S, et al. Possible contribution to hepatocarcinogenesis of X transcript of hepatitis B virus in Japanese patients with hepatitis C virus. Hepatology. 1999;29:1429–34.
Tanaka T, Inoue K, Hayashi Y, et al. Virological significance of low-level hepatitis B virus infection in patients with hepatitis C virus associated liver disease. J Med Virol. 2004;72:223–9.
Tsubouchi N, Uto H, Kumagai K, et al. Impact of antibody to hepatitis B core antigen on the clinical course of hepatitis C virus carriers in a hyperendemic area in Japan: a community-based cohort study. Hepatol Res. 2013;43:1130–8.
Vigano M, Vener C, Lampertico P, et al. Risk of hepatitis B surface antigen seroreversion after allogeneic hematopoietic SCT. Bone Marrow Transplant. 2011;46:125–31.
Wachs ME, Amend WJ, Ascher NL, et al. The risk of transmission of hepatitis B from HBsAg(−), HBcAb(+), HBIgM(−) organ donors. Transplantation. 1995;59:230–4.
Werle-Lapostolle B, Bowden S, Locarnini S, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126:1750–8.

Wong DK, Huang FY, Lai CL, et al. Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. Hepatology. 2011;54:829–36.
Wong DK, Fung J, Lee CK, et al. Hepatitis B virus serological and virological activities in blood donors with occult hepatitis B. Hepatol Int. 2014;8(Suppl 1):S149.
Wong DK, Fung J, Lee CK, et al. Intrahepatic hepatitis B virus replication and liver histology in subjects with occult hepatitis B infection. Clin Microbiol Infect. 2016;22:290.e1-3.
Yamamoto K, Horikita M, Tsuda F, et al. Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. J Virol. 1994;68:2671–6.
Yeo W, Chan TC, Leung NW, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol. 2009;27:605–11.
Yotsuyanagi H, Yasuda K, Iino S, et al. Persistent viremia after recovery from self-limited acute hepatitis B. Hepatology. 1998;27:1377–82.
Yotsuyanagi H, Shintani Y, Moriya K, et al. Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. J Infect Dis. 2000;181:1920–8.
Yuen MF. Need to improve awareness and management of hepatitis B reactivation in patients receiving immunosuppressive therapy. Hepatol Int. 2016;10:102–5.
Yuen MF, Lai CL. HBsAg seroclearance in the natural history of chronic hepatitis B infection. Curr Hepatitis Rep. 2006;5:23–6.
Yuen MF, Wong DK, Sablon E, et al. HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology. 2004;39:1694–701.
Yuen MF, Wong DK, Fung J, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
Yuen MF, Lee CK, Wong DK, et al. Prevalence of occult hepatitis B infection in a highly endemic area for chronic hepatitis B: a study of a large blood donor population. Gut. 2010;59:1389–93.
Yuen MF, Wong DK, Lee CK, et al. Transmissibility of hepatitis B virus (HBV) infection through blood transfusion from blood donors with occult HBV infection. Clin Infect Dis. 2011;52:624–32.
Yuen MF, Ahn SH, Chen DS, et al. Chronic hepatitis B virus infection: disease revisit and management recommendations. J Clin Gastroenterol. 2016;50:286–94.
Yuki N, Nagaoka T, Yamashiro M, et al. Long-term histologic and virologic outcomes of acute self-limited hepatitis B. Hepatology. 2003;37:1172–9.