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EDITORIAL

An overview of occult hepatitis B virus infection

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Abstract

Occult hepatitis B virus (HBV) infection (OBI), alternatively defined as occult hepatitis B (OHB), is a challenging clinical entity. It is recognized by two main characteristics: absence of HBsAg, and low viral replication. The previous two decades have witnessed a remarkable progress in our understanding of OBI and its clinical implications. Appropriate diagnostic techniques must be adopted. Sensitive HBV DNA amplification assay is the gold standard assay for detection of OBI. Viral as well as host factors are implicated in the pathogenesis of OBI. However, published data reporting the infectivity of OBI by transfusion are limited. Several aspects including OBI transmission, infectivity and its relation to the development of chronic liver diseases and hepatocellular carcinoma have to be resolved. The aim of the present review is to highlight recent data on OBI with a focus on its virological diagnosis and clinical outcome.

Key words: Hepatitis B virus; Occult infection; Occult hepatitis B virus infection; Occult hepatitis B; Chronic liver disease; Hepatocellular carcinoma; Hepatitis B surface antigen

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INTRODUCTION

Hepatitis B virus (HBV) remains a major public health problem worldwide[1]. Among many transmission routes, transfusion is the one that should be prevented. Implementation of hepatitis B surface antigen (HBsAg) in routine screening of blood donors in the early 1970s has greatly enhanced transfusion safety. The incidence of transfusion-transmitted hepatitis B has been steadily reduced over the last four decades[2]. However, it was demonstrated that HBV transmission by blood components negative for HBsAg can still occur[3] and HBV transmission remains the most frequent transfusion-transmitted viral infection[4-6]; thus, the term occult hepatitis B virus infection (OBI) was introduced. OBI is simply defined as serologically undetectable hepatitis B surface antigen (HBsAg-ve), despite the presence of circulating HBV DNA[7,8]. OBI was reported for the first time almost 30 years ago in a case report of HBV infection through blood transfusion by an antibody to hepatitis B core antigen (anti-HBc) only positive donor[9]. The residual risk of HBV transfusion transmission is mainly related to blood donations negative for HBsAg that have been collected either during the pre-seroconversion "window period" (WP), defined as the time between infection and detection of a viral antigen or antibody marker, or during the late stages of infection[1]. Additionally, OBI has high significance in management of bone marrow and organ transplantations[10-13]. Implementation of HBV DNA screening has the potential to significantly reduce the WP and to reveal OBI or HBV carriage[14].

Allain[15] reported OBI in several clinical contexts including: (1) recovery from past infection indicated by the presence of hepatitis B surface antibody (anti-HBs); (2) chronic hepatitis with surface gene escape mutants that are not recognized by current assays; (3) chronic carriage without any marker of HBV infection other than HBV DNA (referred to as "seronegative"); and (4) most commonly in endemic areas, chronic carriage stage with HBsAg too low to be detected and recognized by the presence of anti-HBc as the only serological marker (referred to as "anti-HBc alone" or "isolated anti-HBc")[15].

DEFINITION OF OCCULT HEPATITIS B INFECTION

Several definitions for OBI have been proposed by many authors. Bremer *et al*[16] emphasized that the term "occult hepatitis B virus infection" has been introduced to describe a pattern with the presence of replication-competent HBV DNA in the liver but without detectable HBsAg in the serum. This often occurs after progressive disappearance of HBsAg in the years after infection[17] and persists in low-level carriers[14]. Early phase of HBV infection before appearance of HBsAg is not considered OBI, as the infection becomes eventually non-occult[18].

A more specific definition was provided by Allain[15] in 2004, who defined OBI as the presence of HBV DNA without HBsAg, with or without the presence of HBV antibodies outside the acute phase window period. This is in accordance with findings by Gerlich *et al*[19], who identified two blood donors whose donations tested HBsAg- and HBV DNA-negative, but transmitted HBV. Both subsequently developed HBsAg and acute hepatitis. It was confirmed that such cases are transient OBI and should not be considered as true OBI. A true OBI remains HBsAg-negative during the entire course[19]. Nevertheless, a 2008 international workshop on occult hepatitis B virus (HBV) infection (OBI), endorsed by the European Association for the Study of the Liver (EASL)[20], as well as The Taormina Consensus Conference in 2008, defined "OBI" as the "presence of HBV DNA in the liver of individuals testing HBsAg-negative with currently available assays"[10] and introduced a cutoff value for serum HBV DNA (< 200 IU/mL). Therefore, cases whose serum HBV DNA levels are comparable to those with different serologically evident (overt) HBV infection are generally due to infection with HBV escape mutants and should be labeled as "false" OBI[10]. As confirmed by Hollinger *et al*[21], this definition implies that infectious viral clones may be present. However, the detection of HBV DNA does not always correspond to infectivity or to the number of HBV progeny viruses released from hepatocytes; therefore, the authors suggested a more comprehensive term "occult hepatitis B (OHB)"[21] rather than OBI. Moreover, nosocomial sources should be carefully excluded before speculating that blood donors with OBI were involved in HBV viral disease transmission[22].

POSSIBLE MECHANISMS OF OBI

Several possible mechanisms have been hypothesized for the pathogenesis of OBI and the condition is probably multifactorial. Both host and viral factors are important in suppressing viral replication and keeping the infection under control[21,23,24]. The majority of OBI cases are secondary to overt HBV infection and represent a residual low viremia level suppressed by strong immune response together with histological derangements occurring during acute or chronic HBV infection[25]. It was previously suggested that long-term maintenance of an active anti-viral T cell response several years after clinical recovery from acute hepatitis B could be important, not only for protection against reinfection, but also for keeping the persisting virus under tight control where detection of minute amounts of virus in some recovered subjects was confirmed[26]. Also, in a study to characterize the features of the HBV-specific T-cell response in patients with OBI, 2 different profiles were defined. Anti-HBc-positive patients showed a T-cell response typical of protective memory, suggesting that this condition represents a resolved infection with immune-mediated virus control. In contrast, HBV-specific T cells in anti-HBc-negative patients did not readily expand, suggesting the possibility of a low-dose infection insufficient to allow maturation of protective memory[27]. Additional mechanisms not related to the host response were also extensively studied by many authors, where it was shown that the low level of viral replication was a result of the presence of defective interfering particles or of mutations in transcription control regions or the polymerase domain leading to decrease in HBV DNA replication and HBsAg expression[21,24,28-30].

Humoral and cellular immune pressure on the HBV envelope proteins are major mechanisms generating OBI. Amino acid substitutions are significantly concentrated in the immunologically active parts of the Pre-S/S proteins affecting both cellular CD8 T-cell epitopes and B-cell neutralizing major hydrophilic region epitopes[31]. Escape mutation is one mechanism which also leads to decreased reactivity in HBsAg detection assays[32]. This is confirmed by Gerlich *et al*[19]. van Hemert *et al*[33] in 2008 proposed an evolutionary scenario for occult HBV infection. They identified a novel RNA splicing event (deleting nucleotides 2986-202) that abolishes surface protein gene expression without affecting polymerase, core or X-protein related functions. This 2986-202 splicing generates intracellular virus particles devoid of surface protein, which subsequently accumulate mutations due to relaxation of coding constraints. Such viruses are deficient in autonomous propagation and cannot leave the host cell until it is lysed[33].

Masking of HbsAg by HbsAg-anti-HBs immune complexes is another postulated mechanism for the development of OBI[34,35]. Also, coinfection with hepatitis delta virus or hepatitis C virus (HCV) which results in down-regulation of HBV replication and a reduction in HBsAg synthesis has been reported[21]. Sagnelli *et al*[36] showed an inhibitory effect of HCV on HBV replication. This inhibitory activity of HCV on HBV replication has also

been reported by other investigators in a follow-up study of 6 years duration, where it was shown that the rate of HBsAg clearance is 2.5 times higher in HBsAg/anti-HCV-positive cases than in those with HBV infection alone; it was suggested that HCV is the most important hepatotropic virus that enhances HBsAg clearance in chronic hepatitis B[37]. The underlining molecular mechanism responsible for this suppressive effect has been extensively studied both *in vitro*[38] and *in vivo* studies[39]. Indirect mechanisms mediated by innate and/or adaptive host immune responses have also been postulated as being involved[40]. In this regard, we have recently studied the prevalence of occult HBV among children and adolescents with hematological diseases with or without HCV in an area of high endemicity of HCV infection. It was shown that HCV RNA was a significant predictor for OBI ($P < 0.05$), with an increased frequency of HBV DNA in those who were HBsAg-negative and HCV RNA positive (63.2%) compared with patients negative for HCV RNA (25%) ($P = 0.009$)[41].

Additional mechanisms for OBI have been thoroughly investigated, emphasizing that integration of viral sequence may alter HBsAg expression and decrease HBV replication[42]. Meanwhile, reduced HBV viremia may result from extra-hepatic HBV replication such as that takes place in peripheral blood mononuclear cells (PBMCs)[42]. Patients with long-standing abnormal results of liver function tests with unknown etiology may have HCV RNA or HBV DNA in their PBMCs in the absence of anti-HCV antibodies, HBV markers, serum HBV DNA and serum HCV RNA[43].

EVALUATION OF DIFFERENT OBI DIAGNOSTIC TECHNIQUES

Most OBIs are asymptomatic and would only be detected by systematic screening of large populations[7]. No published guidelines are provided up till now, categorizing those who should be screened for OBI. However, such investigations should be considered in the following situations: (1) HCV-infected patients with flares in viral replication and liver damage[44]; (2) infected patients becoming immune deficient mainly by receiving immunosuppressive regimens for various clinical conditions[7]; (3) screening of blood donations for immunocompromised recipients[41]; and (4) subjects with unexplained liver diseases. Candotti *et al*[1] further clarified that OBIs are mainly found in older donors, nearly 100% carry anti-HBc, and approximately 50% also carry anti-HBs, suggesting that OBIs occur largely in individuals having recovered from the infection but unable to develop a totally effective immune control[31].

Liver biopsy

Detection of HBV DNA in liver biopsy is the best way for diagnosis of OBI. However, liver biopsy tissue is not always available, and standardized and valid assays for detection of HBV DNA in liver tissue are not FDA approved[21]. A recent Italian study investigated the prevalence of occult HBV in the general population by examining 98 liver specimens from liver disease-free individuals who were HBsAg-negative, and detected HBV DNA in sixteen of them (16.3%); 10/16 (62.5%) were anti-HBc positive[20].

HBsAg testing

The main target for antibodies used in diagnostic tests is the major hydrophilic loop (MHL, amino acids 100-160) that contains the "a" determinant (amino acids 124-147) and is coded by the envelope (S) gene. The existence of mutations in this region could cause diagnostic failure[45]. Current HBsAg screening assays are enzyme immunoassays (EIAs), including enzyme-linked immunosorbent assays (ELISAs), and chemiluminescence immunoassays (CLIAs)[1]. These different assays have sensitivity ranging between < 0.1 and 0.62 ng of HBsAg per mL (1 ng/mL corresponds to approximately 2 IU/mL)[1,46,47]. Performance of commercial assays would be improved by the incorporation of OBI mutants in reagent development[32].

The course of HBV markers during the early phase of true OBI is not well known, where, in spite of transient strong HBV replication, much less HBsAg in the serum than the normal courses is shown[16]. This has been previously confirmed in a Japanese study by Yoshikawa *et al*[48], where 17 million donations were tested for occult infection, and 328 HBV DNA-positive donations were found. From 26 of these donors, sequential samples were examined for the dynamics of viral markers in acute HBV infection. Six of the 26 donors were infected with mutant viruses, and 3 of these 6 donors did not develop detectable HBsAg during the entire observation period, despite a moderately high viral load of 10^4 to 10^5 HBV DNA copies per mL. The authors concluded that HBV nucleic acid amplification test (NAT), even in minipool (MP) configuration, is more effective than HBsAg testing and capable of excluding infected donors in the pre- and post-HBsAg window periods[48].

A novel immunoassay that detects simultaneously HBV PreS1 and/or core-related antigens was developed and evaluated for its potential value for detecting HBsAg variants. The detection limits of the assay were 10 (2.9 ± 0.5) copies/mL (mean ± SD) for HBsAg-positive sera with different genotypes, and 10 (3.5 ± 1.2) copies/mL for HBsAg variants containing sera. The specificity of the assay was 99.9% (95% CI: 99.7-99.9, 4551 healthy individuals). The sensitivities were 93.9% (95% CI: 92.8-94.9), 59.3% (95% CI: 38.7-77.6) and 80% (95% CI: 44.4-97.5) in three independent groups which included: 2065 hepatitis patients, 27 patients with OBI and 10 HBsAg variants, respectively. In addition, a novel premature stop code mutation at position 112 of HBsAg was observed in two patients with chronic hepatitis B with different genotypes[49].

Anti-HBc testing

Serological profiling of HBV infection showed that OBI may be antibody (anti-HBc alone or together with anti-HBs) positive (seropositive OBI) or antibody negative

(seronegative OBI)[13]. The HBV DNA detection rate is highest in subjects who are anti-HBc-positive but anti-HBs-negative, and these individuals are more likely to be infectious[21].

Recently, Urbani *et al*[50] illustrated that the serological assay for the long-lasting antibody response to the highly immunogenic HBV core antigen (anti-HBc) represents a qualified candidate as a surrogate for DNA amplification, or for increasing overall sensitivity when assessing the risk of occult hepatitis in peripheral blood. The risk of occult hepatitis associated with anti-HBc seropositivity has been demonstrated extensively, and the presence of antibody response to HBc can be considered a sentinel marker of occult HBV infection[50].

In a recent review conducted by Candotti *et al*[1] in 2009[1], it was emphasized that approximately 90% of blood donors carrying anti-HBc also carry anti-HBs, indicating recovered HBV infection[51]. The remaining 10% are either false-positive anti-HBc due to poor assay specificity and the lack of confirmatory assays, or true anti-HBc (anti-core antigen alone)[1,52,53]. Anti-HBc only samples may originate either from recovered infections having lost detectable anti-HBs or from late stage chronic infections having lost detectable HBsAg[1]. Recent studies have confirmed the existence of occult HBV infection in samples with anti-HBc alone[54,55]. Nevertheless, low levels of HBV DNA were reported not only in anti-HBc alone positive blood donations but also in some blood units carrying low-level anti-HBs[1]. A serologic testing algorithm with anti-HBc followed by anti-HBs (anti-HBs $\geqslant$ 100 IU/L probably non-infectious) or implementation of highly sensitive HBV DNA screening are adopted in different countries; however, this is still an area of debate by many authors. In our recent study, OBI was detected in blood units from healthy volunteer blood donors showing adequate level of anti-HBs (under publication).

OBI is observed in anti-HBc-positive patients with chronic HBV infection following the decline of HBsAg to an undetectable level that is sometimes associated with the appearance of anti-HBs. This serological pattern occurs at a rate of 0.7%-1.3% per year and is associated with older age and hepatitis B e antibody (anti-HBe) reactivity[21,56-58]. In an experimental study to determine the relationship between anticore detection and the molecular status of virus replication in a primary woodchuck hepatitis virus (WHV) surface antigen (WHsAg)-negative infection or long after resolution of WHV hepatitis, it was shown that the long-term presence of anticore antibodies alone is a consequence of sustained restimulation of the immune system by virus nucleocapsid produced during low-level hepadnaviral assembly[59]. On the other hand, it was shown that about 20% of OHB sera are negative for all serological markers of HBV infection except HBV DNA[21].

HBV nucleic acid (DNA) testing

The gold standard test for detection of OBI is the amplification of HBV DNA[50]. At present, the optimal standard for diagnosis is the analysis of HBV DNA extracts from plasma performed by real-time, nested polymerase chain reaction (PCR) techniques[21]. False results of these assays could be avoided by choosing PCR primers that span at least three genomic regions of the HBV genome such as the S, X and core genes, and validation should require detection from at least two regions of the genome[20]. Unfortunately, this suggestion is not usually fulfilled, and only one segment of a region is amplified. The preferred lower limit of detection (LLOD) for HBV DNA is 5 IU/mL[21]. Some investigators prefer to repeat extraction and testing under the assumption that according to Poisson distribution, repeated testing increases the chances of detecting a low number of template sequences[7]. Nucleic acid testing (NAT) for HBV DNA detection that combines simultaneous detection of human immunodeficiency virus (HIV) RNA, HCV RNA, and HBV DNA ("multiplex" NAT assays) and use of an automated testing platforms have made HBV NAT blood screening feasible[1]. In order to standardize these newly developed assays, the World Health Organization International Standard for hepatitis B virus DNA (NAT)-based assays was created (code 97/750) with a potency of 10^6 IU/mL (500 000 IU/vial)[60].

Biswas *et al*[46] showed that pooled-sample NAT would reduce the WP by 9 to 11 days; and single-sample NAT would reduce the WP by 25 to 36 d, compared to currently licensed HBsAg tests[46]. This leaves WPs of 40-50 d and 15-34 d with minipool (MP) and individual donor (ID) HBV NAT, respectively[1]. As emphasized by Candotti *et al*[1], the ability of NAT to reduce the WP depends not only on the sensitivity of both the molecular and serological tests, but also on the sample volume (200 or 500 μL) as well as the dilution factor introduced by pooling samples, the prevalent HBV genotype at the location and the level of HBV endemicity[7,46,61-63]. Beyond shortening the WP, NAT screening, particularly in individual units, has uncovered a relatively large number of HBsAg-negative "occult" HBV infection or carriage[11,14].

OBIs are usually characterized by very low HBV DNA load in plasma (< 200 IU/mL)[1]. Detection of OBI requires assays of the highest sensitivity and specificity with a lower limit of HBV DNA detection of less than 10 IU/mL and < 0.1 ng/mL for hepatitis B surface antigen (HBsAg)[21].

Regarding estimation of HBV residual transfusion transmission risk, Candotti *et al*[1] in their recent review clarified that HBV DNA yield appears directly related not only to the analytical sensitivity and serum pool size used for the HBV NAT assay, but also to the analytical sensitivity of the HBsAg test used for screening and to the general HBV prevalence in the donor population. They further added that HBV NAT yields reported from countries with low, moderate, and high HBsAg prevalence range between 1:4 000 and 1:730 000[52,64-69], 1:4000 and 1:20 300[70-74], and 1:192 and 1:5200[51,75-80], respectively[1].

Role of Anti S

It is believed that occult HBV carriers without detectable

antibodies to the surface antigen could be infectious[45]. Indeed, Candotti *et al*[1] emphasized that the presence of anti-HBs following natural infection, vaccination, or passive immunoprophylaxis prevents *de novo* HBV infection in transplanted patients receiving anti-HBc positive livers[81-84]. Experiments in chimpanzees showed no HBV infection in animals transfused with blood from three anti-HBs positive human plasma samples, despite exposure to an HBV DNA dose known to be infectious in the absence of anti-HBs[85]. However, it has been reported by many authors that among individuals positive for anti-HBs, 0.5%-15% still tested positive for serum HBV DNA, though at a very low titer[3,86]. Countries such as Germany, Austria and Japan allow transfusion of units with anti-HBs titers higher than 100 IU/L[87].

CLINICAL SIGNIFICANCE

Continuous progress in molecular biology techniques has led to greater recognition and diagnosis of OBI. It has been reported in healthy blood donors, patients with chronic liver disease and patients with hepatocellular carcinoma (HCC)[21], in viral reactivation following immunosuppression, accidental transmission through transplantation, transfusion or experimental transmission to chimpanzees[42]. Therapy should be considered during reactivation and in cirrhotic settings[25].

As illustrated by Shi *et al*[88], a dynamic balance between viral replication and host immune response is pivotal to the pathogenesis of liver disease. Most HBV infections are spontaneously resolved in immunocompetent adults, whereas they become chronic in most neonates and infants who are at great risk of developing complications such as cirrhosis, chronic liver disease (CLD) and HCC. Those with chronic HBV infection may present in one of the four phases of infection: immune tolerance, immune clearance (HBeAg-positive chronic hepatitis B), inactive carrier state, and reactivation (HBeAg-negative chronic hepatitis B)[88].

OBI is a complex biological entity with possible relevant clinical implications, mainly related to the intrahepatic persistence of viral covalently closed circular DNA (cccDNA) and to a strong suppression of viral replication and gene expression[13]. Detection of virus-specific nucleic acid does not always translate into infectivity, and the occurrence of primer-generated HBV DNA that is of partial genomic length in immunocompetent individuals who have significant levels of anti-HBs may not be biologically relevant[21]. Several authors concluded that as a general rule, immune individuals who have recovered from acute hepatitis B have no clinical evidence of liver disease despite the detection of traces of HBV DNA in their blood, PBMC and/or liver decades later[20,21,23,26].

Cross-sectional studies across the spectrum of HBV infection have revealed a marked increase in OBI prevalence towards patients with cirrhosis or HCC[25,42,89]. However, data collected in Poland indicated that approximately 50% of OBIs occur in asymptomatic, apparently healthy blood donors carrying anti-HBs[70]. Levels of DNA and anti-HBs are variable[90].

OBI infectivity by transfusion

It is well known, and recently confirmed by Candotti *et al*[1], that the estimated residual risk of HBV transfusion transmission remains significantly higher than the risk of either HIV-1 or HCV. Whether residual risk estimates translate into true rate of infection is largely unknown since estimates are generally based on the simplification that all HBV DNA-containing donations are infectious[1].

All forms have been shown to be infectious in immunocompromised individuals, such as organ- or bone marrow-transplant recipients. In immunocompetent recipients, there is no evidence that anti-HBs-containing components (even at low titer) are infectious. Anti-HBc only, with HBV DNA, can be associated with infectivity, as can rare cases of HBV DNA without any serological HBV marker[14].

HBV transmission was previously reported from OBI donors who had circulating HBV DNA at a low level[74,91,92]. However, as reported by Candotti *et al*[1], in some cases units from WP and OBI donors were not infectious even though viral load ranging between < 20 and > 500 IU/mL (< 100 and > 2500 geq/mL) was transfused[86,91,93]. These authors emphasized that the lack of a clear relationship between infectivity and viral load in blood components may be related to immune factors affecting the susceptibility to infection in recipients. In addition, HBV infectivity is related to the amount of plasma transfused and the viral load in the product[1].

Few data regarding the infectivity of blood components or donated organs containing both anti-HBc and anti-HBs are available. Theoretically, if HBV particles are present in the peripheral blood of subjects with high-titer anti-HBs, the anti-HBs may neutralize the infectivity of the viral particles[3]. Nevertheless, an OBI carrier with anti-HBs was found to have transmitted HBV to two immunocompetent transfusion recipients[90]. Gerlich *et al*[94] reported five donors (4 genotype D, one genotype A2) with OBI, also carrying only anti-HBc, transmitting HBV to recipients. Candotti *et al*[1] examined the infectivity of HBV-containing blood products according to the immune status of recipients and concluded that: (1) WP and anti-HBs-positive and negative OBI units can transmit HBV; (2) the confirmed HBV transmission rate of WP-derived donations is higher than by occult carriers (81% *versus* 19%) but may be biased by the large number of Japanese cases identified, with a peculiar set of anti-HBc and DNA screening protocols[91]; (3) viral transmission can be associated with extremely low levels of HBV DNA in anti-HBc-positive only units (< 20 IU/mL) or blood collected during the very early phase of acute infection (eclipse phase) in which neither HBsAg nor HBV DNA is detectable[86,95]; (4) HBV DNA load is similar in infectious and non-infectious anti-HBc-positive donations, suggesting that viral load is not the only factor for infectivity; and (5) the presence of anti-HBs seems to largely protect from transmission[91,94], except in rare cases[1,90].

No transmission of HBV has ever been demonstrated in blood donors who developed anti-HBc and anti-HBs following acute hepatitis B[24]. Satake *et al*[91] in Japan found that no HBV infections occurred in 22 recipients of HBsAg-negative, HBV DNA-positive blood that contained anti-HBs compared to 10 HBV infections that occurred among 37 recipients (27%) of OHB units that were devoid of anti-HBs[21].

OBI in blood donors

It is generally admitted that pre-seroconversion WP infections are most likely to transmit HBV but transmission from occult HBV infection remains a debated subject[11]. Occult HBV is transmissible through blood transfusion in HBV-naive recipients[96]. Post-transfusion hepatitis B virus (HBV) infection still occurs, although its incidence has been found to be substantially reduced since the introduction of screening for HBsAg in blood donors[97]. A similar study was recently conducted in India and showed that a considerable number of HBV-infected donors remain undetected, if only HBsAg is used for screening[98].

Occult HBV in blood donors has a wide range of potential origin within the natural history of the infection. It may originate from previous infections with development of anti-HBs, but be accompanied by persistent, low-level, viral replication and/or escape mutants undetected by the HBsAg assays or healthy chronic carriage. The latter situation is mostly found with anti-HBc only. Over time, antibody markers may become undetectable leaving HBV DNA as the only marker of the infection[15].

A European study conducted by Candotti *et al*[31] confirmed that 91% of 77 donor samples of European origin were HBV DNA-positive/HBsAg-negative. Viral load ranged between unquantifiable and 5640 IU/mL (median 25 IU/mL).

A recent study conducted in Taiwan showed that in HBV hyperendemic areas, occult hepatitis B transfusion might not lead to HBsAg carriage or post-transfusion hepatitis. The risk of transfusion-transmitted HBV infection was probably lower than that in non-endemic areas because most recipients had already experienced HBV infection[96]. Infection of vaccinated individuals favors development of OBI, as was observed in 6 blood donors. HB vaccination may solve the problem of overt HBV infection but may favor OBI[19].

Addition of anti-HBc testing for donor screening, although leading to rejection of a large number of donor units, will definitely eliminate HBV-infected donations and help in reducing HBV transmission with its potential consequences, especially among the immunocompromised population[98].

OBI blood donors have very low HBV replication, and normal liver biochemistry and histology, conferring a favorable prognosis[99].

Donations carrying anti-HBc only and HBV DNA can be infectious and this is a threat where anti-HBc is not screened. Anti-HBc screening identifies most OBI but not all. HBV NAT needs either extreme sensitivity or to be performed on individual donations to eliminate HBV DNA-containing units[15]. Reduction of HBV residual risk depends upon developing more sensitive HBsAg tests, adopting anti-HBc screening when appropriate, and implementing HBV NAT, either in minipools or more efficiently in individual samples[1].

Liu *et al*[3] emphasized that anti-HBc screening has the potential to exclude the vast majority of OHBs, leaving only the probably rare cases with HBV DNA alone undetected. This approach, however, has two main drawbacks: it does not detect the seronegative WP infections; and most importantly, it would not be practical in most parts of the world where the prevalence of anti-HBc is > 10%, as too many otherwise healthy donors will be ineligible[3].

The transmission risk of OBIs is not well defined, although some cases of OBIs with anti-HBc only which were infectious by transfusion have been described[91,94]. HBV transmission by blood components from a single anti-HBs-positive OBI donation to two recipients was recognized and it was clearly illustrated that the neutralizing capacity of low-level anti-HBs is limited, reinforcing the validity of considering anti-HBs below 100 IU/L to be poorly protective from infectivity when HBV DNA is present[90]. Authors further emphasized that even in the presence of higher levels of anti-HBs in a severely immunodeficient recipient, HBV DNA-containing blood might be infectious and the clinical expression severe.

However, as emphasized by Candotti *et al*[1], iatrogenic sources of infection should be systematically investigated before concluding that HBV-infected blood donors are involved in viral transmission[22,100,101]. They further added that adequate donor follow-up and laboratory testing have to be performed, and more importantly, pre- and post-transfusion testing of recipients has to be completed[1]. Definitive evidence of transfusion transmission can be obtained by genomic analysis of the viral strains present in both donor and recipient[1]. In addition, sequencing, which might be informative, becomes very difficult to perform at levels of viremia below 200 IU/mL[7]. Limited but convincing evidence that OBIs can be infectious and can be detected by HBV DNA screening should be carefully considered by the health authorities of countries where neither anti-HBc nor HBV NAT are implemented[90].

Occult infection may have impact in several different clinical situations. Extensive studies have evaluated the risk of acquiring OBI in several clinical entities including the following.

OBI and chronic liver diseases

The long-lasting persistence of the virus in the liver may provoke a very mild but continuing necro-inflammation that (if other causes of liver damage coexist) may contribute over time to the progression of the chronic liver damage towards cirrhosis[13].

In studying the situation of OBI and HCV coinfection, Hollinger *et al*[21] reviewed several cross-sectional studies where it was suggested that HBV replication accounts for many of the ALT flares that occur in patients with HCV[40]. OHB is also known to decrease the

response to interferon therapy when employed in patients with chronic hepatitis C[102] and to accelerate the progression of cirrhosis, hepatic decompensation and HCC[40,103]. A strong association was noted between the presence of OHB in 204 patients with chronic hepatitis C and the development of HCC when compared to HCV monoinfected patients[21,104].

Chemin *et al*[42] previously put forward the theory that estimating the percentage of OBI among non-A-E hepatitis cases depends on several parameters including: (1) the method of detection, including PCR primer selection; (2) patient recruitment; (3) patients from countries highly endemic for HBV are more likely to develop occult HBV infections; and (4) prevalence may also vary depending on the nature of biological material tested, with a higher proportion for liver compared to serum specimen[42].

Occult hepatitis B and fulminant hepatic failure

The state of suppression of viral replication and gene expression may be discontinued when an immunosuppressive status occurs, leading to typical hepatitis B with severe - and sometimes fulminant-course[13,105]. Gerlich *et al*[19] studied 5 blood donors with OBI and 55 of their recipients. In 22 recipients, transmission was probable, but they remained healthy. However, in 3 recipients, who were immunosuppressed at the time of transfusion, fatal fulminant hepatitis B developed. The majority of anti-HBc-positive healthy individuals have HBV DNA in the liver which may start replication under severe immunosuppression.

Occult hepatitis B and hepatocellular carcinoma

OBI is supposed to be an important risk factor for HCC development since it maintains the pro-oncogenic properties typical of the overt infection[13]. It has been suggested that the occult viral strains, maintaining the transcriptional activity and the pro-oncogenetic assets of the clear HBV infection (HBsAg+), may harbor a potential risk for liver cancer development[8]. A recent study conducted in Japan confirmed the existence of serum HBV DNA in OBI as a predictor of a high hepatocellular carcinogenesis rate in a cohort of patients with non-B, non-C cirrhosis. Eighty-two consecutive Japanese patients with cirrhosis, who showed negative HBsAg and negative anti-hepatitis C virus, were observed for a median of 5.8 years. The carcinogenesis rates in the patients of the positive HBV DNA group and negative DNA group were 27.0% and 11.8% at the end of the 5th year, and 100% and 17.6% at the 10th year, respectively ($P = 0.0078$)[106]. The mechanisms leading to HCC in OBI seem similar to those in overt HBV-infected patients with low-grade but diagnosable HBV replication that retains its pro-oncogenic properties[13,42].

Occult hepatitis B infection and immune suppression

Patients with an OBI undergoing immunosuppression are at risk of HBV reactivation. As emphasized by Allain[7], the severity of the immunosuppression and its duration play a considerable role in triggering reactivation of HBV infection. Reactivation during relatively mild and short immunosuppression for homologous bone marrow transplantation or solid tumor chemotherapy elicits lower frequency of reactivation than more severe regimens such as employed in allogeneic bone marrow or organ transplantation[7]. The reactivation of OBI in hematological malignancies (< 5%), although at a lower rate than that of HBsAg-positive cases, carries a significant risk of mortality and morbidity[107], which is much higher in the setting of stem cell transplantation[108].

Occult HBV infection harbors potential risk of HBV transmission through hemodialysis. A recent study conducted in Italy showed that occult HBV infection is frequent among hemodialysis patients, particularly correlated to the presence of isolated anti-HBcAg and anti-HCV antibodies. The authors recommended that the presence of isolated anti-HBcAg should prompt the clinician to evaluate a possible occult HBV infection, especially if anti-HCV antibodies are also detectable[109].

Furthermore, another recent Iranian study assessed OBI in 289 hemodialysis patients with isolated hepatitis B core antibody (18 subjects). HBV DNA was detected quantitatively in 9 of 18 patients (50%) where plasma HBV DNA load was less than 50 IU/mL[110]. Meanwhile, a recent study conducted in Brazil found that OBI was not observed in hemodialysis patients and immunosuppression in HIV-positive patients was not a determining factor for occult HBV infection[111].

On the other hand, Demir *et al*[112] showed that the prevalence of occult HBV infection is higher in diabetics compared with healthy controls, which may contribute to the increased prevalence of primary HCC in diabetics[112].

OBI and organ transplantation

OBI often leads to HBV transmission and subsequent infection during organ transplantation[21]. In studying occult HBV infection in HBsAg-negative patients undergoing liver transplantation, Ghisetti *et al*[113] found that OBIis not associated with increased episodes of acute rejection, coinfection with hepatotropic viruses, different responses to HBV vaccination, or the development of *de novo* hepatitis B. In OBI, a particular virus-host interaction can explain the low intrahepatic HBV content and the lack of extrahepatic HBV replication, thus justifying the low risk of hepatitis B reactivation, in absence of specific prophylaxis, once the recipient liver is removed[113]. On the other hand, Hollinger *et al*[21] emphasized that liver transplant recipients with serological evidence of past infection with hepatitis B (anti-HBc-positive) may have reactivation of OHB under immunosuppression in the post-transplant period[21]. A recent systematic review by Cholongitas *et al*[114] covering the last 15 years, identified 39 studies including 903 recipients of anti-HBc-positive liver grafts. They found that liver grafts from anti-HBc-positive donors can be safely used, preferentially in HBsAg-positive or anti-HBc/anti-HBs-positive recipients. HBsAg-negative recipients should receive prophylaxis with lamivudine, while both anti-HBc- and anti-HBs-positive recipients may need no prophylaxis at all[114].

Transmission of HBV after kidney and heart transpla-

ntation from an anti-HBc-reactive donor occurs at a much lower rate[21]. A study in the USA which included 1067 cadaveric kidneys, 38 of them from HBsAg(-)/HBcAb(+) donors, showed that recipients of kidneys from HBsAg(-)/HBcAb(+) donors are at a small risk of hepatitis B seroconversion and are at no excess risk of graft failure or short-term morbidity or mortality[115]. This low viral transmission risk was also confirmed in transplantation of hearts from donors with hepatitis-B core antibodies[116].

Currently, the critical issue in transfusion safety is to identify blood or tissue donors with OHB, and then to block this transmission route. Liu *et al*[3] concluded that the strategy to prevent transmission of HBV by OHB carriers will be different in endemic and nonendemic areas; in low-endemic areas it is still a subject of debate whether anti-HBc screening should be implemented[117]. Whether ID-NAT would eventually be able to replace HBsAg or anti-HBc testing also remains to be studied[3]. On the other hand, in HBV endemic areas, the priority is to examine the prevalence of OHB in blood donors on a large scale, and so establish the cost-effectiveness of implementing sensitive ID-HBV NAT blood screening technology in order to reduce the risk of HBV transmission[3].

PREVALENCE OF OCCULT HEPATITIS B

The prevalence of occult HBV is unclear and depends in part on the sensitivity of the HBsAg and DNA assays used as well as the prevalence of HBV infection in the study population[117]. OHB varies significantly between different geographical regions[11]. Studies have shown that the prevalence of occult HBV infection is closely related to the endemicity of HBV infection[118,119]. Patients from countries highly endemic for HBV are more likely to develop occult HBV infections[42]. As in highly endemic countries, the majority of infections are contracted perinatally or in early childhood; a higher proportion of the infected adults have late chronic HBV with undetectable HBsAg. This may account for the higher rate of OHB in anti-HBc-positive populations in these areas[3]. Prevalence may also vary depending on the nature of biological material tested, with a higher proportion for liver compared to serum specimens[42].

Occult HBV infection has been reported in 0.1%-2.4% of HBsAg-negative, anti-HBc-positive (± anti-HBs) blood donors in Western countries such as the United States, where only 5% of the population has prior exposure to HBV, and in up to 6% of a similar cohort of donors who reside in endemic areas where 70%-90% of the population has been exposed to HBV[11,21]. When anti-HBc only data is evaluated, the rates range from 0% to 15% (median of 1.1%)[11]. In this regard, our recent unpublished data show that OBI is present among 15% of HBsAg-negative, anti-HBc-positive (± anti-HBs) healthy blood donors in an area of intermediate prevalence for HBV.

CONCLUSION

OBI is defined as the presence of HBV DNA in liver/serum with undetectable HBsAg. Advanced progress in molecular biology techniques helps in early detection of OBI and paves the way for implementing a detecting strategy to eliminate post-transfusion occult HBV infection, with consideration for the immune status of blood recipients. Evidence is accumulating supporting the prevalence of OBI among blood donors and in CLD patients. However, current data emphasize the low prevalence of OBI, implying a low impact on transfusion services. Detection of HBV DNA does not always indicate infectivity. Available data encourage testing for OBI in HCV-infected patients, in patients under immunosuppression, in people with unexplained liver diseases and in blood units for immunocompromised recipients where proper recruitment and selection of donors are highly recommended. Further work is needed to clarify the clinical significance of OBI, infectivity, possible transmission and its pathogenic consequences, reactivation and progression to chronic liver disease or hepatocellular carcinoma.

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