The role of anti-core antibody response in the detection of occult hepatitis B virus infection

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Abstract

Occult hepatitis B virus (HBV) infection is characterized by the presence of HBV DNA in serum and/or in the liver of patients negative for hepatitis B surface antigen (HBsAg). Occult infection may impact in several different clinical contexts including the risk of HBV transmission with transfusion or transplantation, and endogenous viral reactivation. The gold standard test for detection of occult infection is the amplification of HBV DNA. However, 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.


Keywords: anti-core antibody response; HBV core (HBc); hepatitis B virus (HBV); occult hepatitis B infection; reactivation; transfusion; transplantation.

Introduction

Hepatitis B virus (HBV) infection is a major health problem worldwide, with an estimated 400 million people infected chronically and a leading cause of mortality (1–3). Chronic infection is a primary risk for development of cirrhosis and hepatocellular carcinoma (HCC) (1–3) by way of direct and indirect pro-oncogenic properties (4–7). However, only in a minority of cases, HBV primary infection evolves into chronic infection (1–3). Instead, primary infection with its wide spectrum of severity from asymptomatic to extremely severe fulminant hepatitis, in most cases evolves in apparently benign long-term persistence of HBV (8–11). Modern molecular analysis has demonstrated that even when HBV surface antigen (HBsAg) is no longer detectable, the viral genome of HBV persists indefinitely within previously infected host cells. Latency of the HBV genome at the molecular level in the absence of detectable HBsAg has been characterized recently as occult hepatitis B infection (OBI) (8–11). The definition of HBV latency in the context of OBI poses new challenges at the clinical level due to the inherent risks of endogenous reactivation following immune suppression (12, 13), of HBV transmission due to blood transfusion (14) or transplantation (13, 15, 16) of cells, tissues and solid organs, and in terms of the potential risk for development of chronic liver diseases (17–19). Accordingly, HBV latency also poses new challenges at the laboratory level for specific and definitive diagnosis of OBI itself, which imposes controlled nucleic acid amplification from liver tissue extracts (8–11). Unfortunately, in most instances, liver biopsy may not be readily available for diagnosis, while conventional nucleic acid amplification from peripheral blood may not be sufficiently sensitive because HBV latency within liver cells produces only sporadic HBV DNA in blood. Thus, a complementary and potentially sensitive assay for preliminary cost-effective screening can be provided by detection of antibodies reacting to HBV core antigen (anti-HBc). In this review, we will summarize the currently available evidence supporting the indirect but important role of the traditional anti-HBc assay for the identification of subjects previously exposed to HBV in the context of the modern definition of OBI.

The persistence of HBV genome in occult hepatitis B infection

The general definition of OBI is referred to as persistence of latent HBV infection with undetectable HBsAg. Therefore, molecular analysis is usually required for uncovering HBV viral genomes in liver tissue and/or in blood in individuals negative for HBsAg (8–11). However, the demonstration of the existence of OBI is based not only on detection of HBV
DNA at the molecular level inside hepatocyte nuclei following primary infection, but also on indirect evidence observed at the clinical level. For example, subjects without HBsAg may still undergo reactivation of HBV infection after immunosuppression. In addition, HBV infection occurring in HBV-naive recipients following liver transplantation from HBcAb-positive donors provides convincing evidence of OBI.

The molecular basis of OBI is related to the peculiar life cycle of this hepatotropic virus. The fundamental step of its replication is represented by the conversion of a 3.2-kb related circular DNA in a covalently closed circular (ccc) DNA in the nucleus of the infected hepatocyte, where it is then conjugated with nuclear proteins forming a minichromosome (20). cccDNA is the template for transcription leading to the production of new infectious virions in infected cells. cccDNA is probably the basis for persistence of HBV infection, even after complete clinical recovery from acute hepatitis. This is because cccDNA is highly stable and resistant to digestion by cellular enzymes (21). Different mechanisms have been proposed to explain why HBV remains confined primarily to the liver, with only transient low levels of viremia. These mechanisms include interference with HBV replication of concomitant viral infection (22, 23), regulation of HBV gene transcription by cellular mechanisms, impaired replicative competence of HBV as a result of specific virus mutation (24), and control of HBV replication by the adaptive immune response (25, 26). Consistent with the possibility that HBV replication in occult infection is controlled by the cell-mediated immune response, a recent study showed the existence of an HBV-specific T-cell response in patients with occult infection, even when serum HBV markers are completely negative (27). The essential role of immune surveillance for maintenance of the residual HBV genome in a state of latency is underscored at the clinical level by the high frequency of endogenous reactivation occurring in OBI with intense immunosuppression (12, 13). However, the potential for reactivation of dormant HBV DNA from subjects with OBI is expressed not only endogenously, but may be transmitted and generate the classical form of hepatitis B in newly infected individuals following transplantation of organs, tissues, cells, and transfusion of blood (8–19).

The current general view is that subjects with primary HBV infection that has apparently resolved without persistence of HBsAg, generally harbor OBI. This is characterized by long-term (possibly permanent) persistence of HBV DNA in a latent form, whose destiny depends on endogenous mechanisms of surveillance, and transfer to other subjects through transplantation or transfusion. Under these circumstances, OBI is a very frequent condition that should concern any individual with a history of previous exposure to HBV, and which has impact in selected clinical settings.

The clinical impact of the occult hepatitis B infection

The natural impact of OBI has been significantly modified with the advent of new therapeutic strategies in modern medicine including transplantation, intense immunosuppression, and blood transfusion. With these new strategies, the impact of OBI has become particularly evident for its inherent risk of endogenous reactivation and its potential risk for transmission. Another possible impact of OBI has been suggested for development and progression of chronic liver diseases, and is a matter of intense current research.

The clinical setting most vulnerable to HBV transmission from subjects with OBI is liver transplantation (12, 15, 16, 28–32). In this context, before the advent of prophylactic strategies, "de novo" post-transplantation hepatitis B infection had been reported to occur in the vast majority of recipients transplanted with livers from donors with OBI (28–30), especially if the recipient was negative for all HBV serum markers. Hopefully, prophylaxis with antiviral agents, such as lamivudine prevents de novo hepatitis B in most, but not all cases (31, 32). However, although the risk for "de novo" hepatitis B infection following transplantation of other solid organs appears to be theoretically possible, there is no firm evidence supporting such a hypothesis at present (33). This discrepancy between the high risk occurring with orthotopic liver transplantation and the extremely lower risk with transplantation of other solid organs constitutes proof that viral latency inside liver cells is the basic origin of this straightforward mechanism for transmission.

Another clinical setting exposed to OBI consists of endogenous reactivation following immune suppression (12, 13). Indeed, the advent of diseases and various therapeutic strategies inducing immune deficiency or suppression have modified the natural history of OBI, with possible reemergence of HBsAg and acute hepatitis of unpredictable intensity. In particular, since the degree of suppression and the specificity of immune mechanisms deficiency allowing HBV reemergence in OBI are not known, any patient with OBI undergoing treatments that induce immunosuppression should be considered at potential risk for HBV reactivation. In addition, the risk for reactivation of occult HBV infection could be even higher in patients undergoing immunosuppressive therapies for treating diseases involving the immune system itself (13, 34–39). A recent report by Persico et al. (38) showed that prevalence of anti-HBc antibodies among patients with non-Hodgkin lymphoma could reach 35%, and that such patients could have significant risk of severe HBV reactivation when treated with cytotoxic chemotherapy. Similarly, reactivation of HBV with reemergence of HBsAg and acute hepatitis is not uncommon in human immunodeficiency virus (HIV)-infected subjects, especially following withdrawal of antiretrovirals whose activity is also directed against HBV (40). Thus, endogenous reactivation of HBV represents a substantial risk for the expanding number of patients debilitated by immune suppression.

Another emerging clinical implication of OBI takes place in post-transfusion infection. In particular, blood donations lacking HBsAg but containing HBV DNA need to be considered potentially infectious. Consistently, OBI has emerged as the major cause of transfusion transmitted HBV in several countries where routine screening for HBV in blood donation...
relies solely on serological testing for HBsAg (14, 41–43). Also, regardless of the different strategies for blood donation screening, the residual risk of transmission of HBV by transfusion is significantly higher than hepatitis C virus (HCV) or HIV. Most likely, these residual cases are dependent primarily on transmission from donors with unrecognized OBI (14).

Finally, apart from endogenous reactivation and allogeic transmission, OBI is also suspected for contributing to progression to chronic liver disease and hepatocarcinogenesis in immunocompetent individuals. At present, there is no clear evidence that patients who have persistently low levels of HBV DNA after primary infection develop progressive liver disease. However, many studies suggest that OBI may be a risk factor for HCC (7–11, 18, 44). Also, the highest prevalence of OBI has been reported in patients with chronic hepatitis C, suggesting that cryptic HBV may favor progression towards cirrhosis in patients with HCV (45). Although available evidence is still incomplete, the common perception is that OBI alone may not sustain clinically relevant liver injury. However, when other causes of liver damage coexist, there might be synergy that negatively influences the chronic progression of liver disease.

In each of the settings mentioned above, OBI may have substantial but distinct clinical relevance and possibly distinct diagnostic requirements.

The diagnosis of occult hepatitis B infection

The apparently simple definition of OBI based on detection of HBV DNA in subjects without HBsAg does not translate easily into standardized criteria for diagnosis (2, 8, 10, 11). The key to diagnosis of occult HBV infection is molecular detection of HBV DNA by PCR in the absence of HBsAg. Therefore, HBV DNA is the only reliable and unanimously accepted marker of OBI (2, 8, 10, 11). However, most PCR assays, including those commercially available, have variable sensitivity. In addition, other factors related to the starting biological material can affect the detection rates of HBV DNA (10). For example, the volume of sample could be a relevant factor. Also, OBI tests must be performed only using samples collected and stored under the most appropriate conditions, paying particular attention to avoid cross-contamination. Higher HBV DNA detection rates have been reported using cellular samples from liver or peripheral blood mononuclear cells when compared to serum or plasma. Also, snap-frozen liver samples have higher rates of HBV DNA detection compared with samples of liver embedded in paraffin.

Considering that OBI is dependent on the long-lasting persistence inside hepatocytes, the most sensitive and appropriate strategy is provided by analysis of DNA from snap-frozen liver extracts (11). Unfortunately, this sensitive and specific approach may not be readily available for most patients outside selected units dedicated to study of liver diseases. Rather, peripheral blood is usually the only sample available to rule out or confirm OBI in the vast majority of donors and patients.

In fact, although the analysis of liver DNA extracts represents the gold standard method for OBI since liver biopsy samples are not available for healthy individuals, most of the available data come from studies performed on blood. Again, HBV DNA amplification from peripheral blood, or more commonly from serum, still remains the standard for diagnosis of OBI, and the search for OBI from peripheral blood on a routine basis should be conducted with this technique (11). Nucleic acid testing (NAT) is indeed a standard method, widely used for preventing not only HBV, but also HIV and HCV transmission through blood components (14). However, highly sensitive NAT has not yet reached extensive application outside blood transfusion. Also, even when used extensively, its sensitivity is not absolute since many subjects may not release viral DNA into blood on a regular basis, in spite of intrahepatic latency. Therefore, a readily available serological assay for identification of subjects with OBI is a necessity in the absence of highly sensitive HBV DNA amplification. The detection of the long-lasting antibody response to the highly immunogenic anti-HBc represents a qualified candidate, either as a surrogate for DNA amplification, or to increase the overall sensitivity when looking for risk of OBI in peripheral blood.

The characteristics of persistent antibody response to hepatitis B core antigen

Antibodies to HBc antigen (HBcAg), the nucleocapsid that encloses HBV DNA, are detected in virtually all patients who have ever been exposed to HBV (46). This may depend on the robust immunogenicity of HBcAg, which appears remarkable not only for sustaining humoral response, but also for cellular response (27, 46–49). Indeed, when HBcAg-derived peptides are expressed on the surface of hepatocytes, they induce a cellular immune response that is crucial for killing infected cells (50). The anti-HBc antibody response is usually the first to be detected in the pre-acute phase of hepatitis, before the appearance of HBsAg. Subsequently, anti-HBc coexists with HBsAg in symptomatic infection. Later, it can persist for life following recovery from hepatitis, either with or without antibodies to HBsAg (51, 52). Unlike anti-HBsAg antibodies, anti-HBc are not protective, and their presence does not allow differentiation of acute from chronic infection. Acute infection is associated with the anti-HBc immunoglobulin M (IgM) subtype, which disappears in a few months. However, since some patients with chronic hepatitis may also become positive for IgM anti-HBc during flares in their disease, their presence is not an absolute reliable marker for acute primary hepatitis (53). Eventually, patients who have persistent HBV infection are positive for antibodies to HBcAg, as are those who have apparently recovered but still have OBI. The absence of anti-HBc antibodies in any phase of HBV infection is rare and possibly related to aberrant immunological response to HBV, or to infection with viral variants. Thus, the presence of antibody response to HBc can be considered a sentinel marker of exposure to HBV infection.
Unlike anti-HBcAg antibodies, anti-HBs are protective, although their presence may not be persistent in spite of permanent OBI along with anti-HBc. In some cases, it has been suggested that nucleotide substitutions in the gene coding for HBsAg could alter its own antigenicity, and consequently, its detection in serum. The serological pattern consisting of "anti-HBc alone", namely anti-HBc without both HBsAg and anti-HBs, has gained increased attention as a possible marker of occult HBV infection (54–56). The majority of individuals characterized by this antibody profile are cases that develop following resolved HBV infection, with loss of anti-HBs. Some may identify chronic HBV of "non- or low-productive infection", where HBsAg is probably masked because it is complexed with anti-HBs antibodies. Considering that the presence of HBsAg and/or anti-HBsAg can be influenced by different molecular or immunological mechanisms, the only reliable marker, at least at the immunological humoral level, for detecting previous contact with HBV is the presence of anti-HBc. However, the serological pattern of "anti-HBc alone" is currently receiving much attention because of the inherent risk in the absence of the protective effect provided by anti-HBs. Accumulated data suggest that a proportion of individuals with this serological pattern are carriers of HBV and may transmit HBV by either blood or organ donation to both immunocompetent and immunosuppressed recipients (56–59). The majority of these individuals with "anti-HBc alone" may be considered as cases of OBI developed from unresolved HBV infection, or chronic HBV carriage with non- or low-productive infection (59).

### The association between anti-HBc antibody response and occult hepatitis B

The characteristics of OBI and anti-HBc antibody response lead to a similar destiny of long-lasting persistence in subjects who have been exposed to HBV infection. Thus, many studies have considered anti-HBc to be highly predictive for OBI because the risk of OBI is certainly consistent in any subject found seropositive for anti-HBc. Also, several reports of either HBV transmission with transfusion and transplantation, or endogenous viral reactivation, have reiterated the risk of OBI associated with anti-HBc seropositivity. Unfortunately, the estimation of the negative vs. positive predictive value and the sensitivity vs. specificity of anti-HBc as a surrogate marker of OBI is still pending, and currently based on analysis obtained from small numbers of subjects.

Some of the most informative studies for deriving data on the association between OBI and anti-HBc can be represented by liver transplantation (28–32). In most cases of liver transplantation, an entire liver is placed in an immunosuppressed patient without previous exposure to HBV, and, at least in early trials, without antiviral prophylaxis. This condition might be considered an experiment in nature for maximized transmission of HBV from subjects with OBI. One of the most extensive studies allowing for indirect estimation of specificity and sensitivity of anti-HBc seropositivity for retrospective identification of donors’ OBI is the liver transplantation observational trial reported by Dickson et al. (29). Interestingly, in 674 US individuals undergoing liver transplantation without prophylactic antiviral therapy, hepatitis B developed in 90% (18/20) of seronegative liver recipients from anti-HBc positive donors, whereas only 0.5% (3/651) of liver recipients developed hepatitis B when receiving organs from anti-HBc negative donors. Assuming that the efficiency of HBV transmission could have been nearly absolute, these data indicate that a fraction (about 10%) of subjects with anti-HBcAb did not transmit HBV, suggesting they might have been due to false, possibly cross-reacting, seropositive results. Indeed, the risk that anti-HBc tests may provide false positive results has been reported (60–62). Likewise, the fraction of false negative results seemed of similar extent (14.3%). Three cases of hepatitis B developed from anti-HBc seronegative donors out of 21 total cases of hepatitis. Thus, limitedly to data from this report, anti-HBc seropositivity showed a high positive predictive value (90%), and an even higher negative predictive value (99%), with acceptable sensitivity (85%) for screening purposes. Recent similar studies reported data that were often, but not always, consistent with those mentioned above (31, 32, 60, 63). The discrepancies might have been due to many factors, including the characteristic of patients, donors, and the serological assay (32). In spite of their incomplete reproducibility, these data may represent proof of the potential diagnostic value of anti-HBc in the identification of OBI carriers.

The possibility of detecting HBV DNA in liver tissue from apparently healthy subjects, with or without anti-HBc, has been also explored. In particular, Marusawa et al. (64) found HBV DNA in 14/17 (82%) of liver sample from anti-HBc seropositive subjects. By contrast, they found no positive viral DNA from 20 seronegative control subjects. The combined results in this cohort would imply that anti-HBc had 86.9% specificity with a positive predictive value of 82.3%. Also, sensitivity and negative predictive value would both reach 100%. However, these latter figures seem to be overestimated because only 20 seronegative control subjects were evaluated. More recently, Raimondo et al. (65) investigated the persistence of HBV DNA in liver biopsies from apparently healthy anti-HBc seropositive subjects without evidence of hepatic disease. They reported that only 62.5% (10/16) of anti-HBc seropositive cases were associated with positive HBV DNA amplification from liver biopsy. Conversely, only 7.3% (6/82) of anti-HBc seronegative individuals were unsuspected carriers of OBI. Again, the combined results from this last cohort would imply that anti-HBc had a specificity of 92.7% and a positive predictive value of 62.5%. Also, sensitivity was 62.5%, whereas the negative predictive value was 92.7%. Interestingly, this study was conducted on a cohort considered representative of the national population. Therefore, this indicated the possibility that ~1/6 (namely 10 million people) of the general Italian population might be represented by OBI carriers, with significant correlation with anti-HBc seropositivity.

The emerging generally accepted concept is that there is a significant, but not absolute, correlation between persist-
ence of OBI and anti-HBc, due to the possibility of finding either false positive or false negative serological data. These false serological data may be due either to technical problems, related for example to antigen specificity and/or the sensitivity of commercially available assays, or to aspects pertinent to the natural history of OBI. With respect to the latter, it should be noted that as indicated by Bréchot et al. (18), the HBV DNA detection rate is highest in subjects who are positive for anti-HBc alone, intermediate in subjects who are positive for both anti-HBc and anti-HBs and lowest in seronegative subjects. This observation is particularly important when considering the risk of blood transmissions. Although a systematic analysis is beyond the scope of this review, a huge amount of data has been reported on the correspondence between anti-HBc sero-positivity and OBI as determined by HBV DNA amplification from peripheral blood (14). The implications of donors’ anti-HBc seropositivity in terms of risk for HBV transmission can be highly relevant. Indeed, the importance of anti-HBcAg has been suggested since the late 1970s, when a blood donor negative for HBsAg, but positive for anti-HBc, was reported to transmit HBV resulting in acute hepatitis (66). Currently, the introduction of NAT has drastically reduced the risk of transmission during the window phase, and from subjects without detectable HBsAg (67). Nevertheless, even after NAT, the residual risk of transfusion-transmitted HBV remained higher than HCV or HIV (14), and donors’ OBI is considered the main factor responsible for these residual cases. In particular, the ability of blood tested by NAT to identify OBI may not be sufficient for the scope, because long-term intrahepatic latency is often accompanied by intermittent viremia in “isolated anti-HBc” positive subjects (18, 55), and/or because the sensitivity of the method is not always adequate to detect small amounts of DNA as those present in OBI (68–70). Therefore, the question is whether such low viral load transmission from donors with OBI could be prevented by current NAT testing, by routine anti-HBc tests, or both (71, 72).

Several large-scale studies using contemporary NAT assays demonstrated the residual rate of HBV-DNA in anti-HBc-positive/HBsAg-negative units, as well as the need for anti-HBc donor screening, especially if no HBV-NAT is performed (73). Thus, it is noteworthy that in areas with low HBV prevalence, not more than 5% of HBsAg (–)/anti-HBcAb (+) blood units contains HBV-DNA (74). In contrast, in high prevalence areas (such as India and Taiwan), serum HBV-DNA is found in 4%–25% of the HBsAg (–) and anti-HBc (+) population (42, 43, 75). Therefore, the role of anti-HBc in detecting blood donors with OBI at risk for HBV transmission needs to be considered separately in various countries according to their routine use of NAT (76). In fact, when NAT is used, anti-HBc may have a complementary role in reducing the residual risk from donors with intrahepatic OBI without transiently detectable HBV DNA in blood (77). However, when NAT is not available, anti-HBc may have a much more relevant impact, which however should be considered against the possibility of deferring an excessive number of subjects from donation.

Conclusions

In conclusion, if a screening test needs to identify subjects at risk for OBI, given the peculiar natural history of this latency, the ideal test should identify subjects previously exposed to HBV and potentially bearing significant risk for HBV reactivation. In an era characterized by increasing availability of new immunosuppressive therapies for neoplastic and hematological disorders, and by increasing emigration of people from highly endemic HBV areas to Western countries, occult HBV infection may become an important problem for public health. While waiting for more sensitive methods for blood HBV DNA detection, the use of the anti-HBc assay as a surrogate marker for diagnosis of occult hepatitis B appears to be a cost-effective approach for identifying occult carriers, not only among organ and blood donors, but also in subjects at risk of immune suppression and possibly, the entire general population.

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