Occult Hepatitis Infection in Transfusion Medicine: Screening Policy and Assessment of Current Use of Anti-HBc Testing

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Summary
HBV still represents a global risk factor in transfusion medicine. The residual risk of HBV is not limited to pre-seroconversion window period but it extends to donors with occult HBV infection (OBI) characterized by the presence of HBV DNA in liver and by the absence of the virus surface antigen. Each country developed an appropriate blood screening policy according to local HBV prevalence, yields of infectious units per different screening methods and cost-effectiveness. We underline the need of maintaining a high level of attention for OBI carrier identification in all blood banks worldwide where the screening procedures are generally based on a combination of both serological markers and nucleic acid amplification test. In this context, markers such as hepatitis B surface antibodies and hepatitis B core antibodies (anti-HBc) might be useful, although the use of this latter is highly debated and still controversial. Our aim is to give an overview on the relevant diagnostic approaches for the routine screening for HBV focusing on the feasibility of anti-HBc testing as precautionary measure in preventing OBI transmission worldwide. In our tailored algorithm, the loss of about 1% of ‘anti-HBc only’ donors, does not significantly affect the blood supply while improving recipient safety.

Introduction

Blood transfusion is considered a valuable support for cardiovascular and transplant surgery as well as for treating massive trauma and related injuries or solid and hematological malignancies, becoming a life-saving procedure.

According to the latest 2016 data by the Italian National Blood Center, which refer to the period 2013–2015, about 2,500,000 red blood cell units/year, 351,533 total plasma units/year and 134,488 platelet concentrates/year have been transfused, and more than 750,000 plasma units have been sent to the industrial processing for plasma products [2, 3]. Over the last two decades, great steps forward have been made on the ‘zero-risk road’ in transfusion medicine. Nevertheless, the likelihood of contracting viral infections such as that of HBV is still present [4, 5]. This DNA virus replicates through a reverse transcriptase (RT) with an intermediate viral RNA. After host cell infection, the circular, partially dou-
ble-stranded DNA is repaired and completed by both viral and cellular enzymes for the transcription. The small genome of about 3.3 kb contains four open reading frames consisting of a pre-core/core (pre-C/C), env or surface (pre-S/S), polymerase (DNA polymerase), and X genes; Hepatitis B surface antigen (HBs-Ag) is the major antigenic determinant composed of the three integral transmembrane glycoproteins of the envelope L, M, and S.

According to the prevalence of the first serologic marker HBs-Ag, it has been possible to identify several geographic areas of major diffusion of HBV classified in highly endemic areas such as Sub-Saharan Africa, South East Asia, China, and Amazon Basin, with a prevalence of ≥8%, countries including Mediterranean areas, Eastern Europe and Middle East with intermediate endemism (2–8%), and areas of low endemism (<2%) such as Western and Northern Europe, North America, South America, and Australia [7–9].

Several studies reported HBV transmission through blood components from asymptomatic apparently healthy individuals such as blood donors that were later revealed as affected by occult HBV infection (OBI) [10–14].

Over the last decades, the continuous improvement of health and hygiene conditions, the increasingly enhanced techniques for screening pregnant women and blood donors as well as the compulsory use of vaccines since the 1990s have considerably decreased the risk of HBV infection. Nevertheless, HBV continues to be the most common posttransfusion infection because the residual risk is not limited to pre-seroconversion window period, but it extends to donors with OBI [6, 10–14]. These donors do not exhibit significant levels of HBs-Ag in the serum, with fluctuating low levels of viremia. HBV DNA could play a central role and reveal OBI or chronic carriers thus shortening the window period [16, 17].

In this review we summarize and critically assess the role of the current serological and molecular methods used for detecting HBV in blood donors by reporting the more recent and different laboratory approaches in the world. In this context, we focus on the opportunity of implementation of HBV core antibody (anti-HBc) testing in order to reduce the residual risk of disease transmission following transfusion from OBI carriers. Besides, we report our in-house screening strategy including anti-HBc to strengthen the routine molecular assays for HBV detection.

**Occult Hepatitis B Infections**

More than 30 years ago, it was shown that negative HBs-Ag and anti-HBc positive blood donors were able to transmit HBV [18, 19]. For the first time in 1994, Michalak et al. [20] observed the persistence of HBV DNA in the serum and in peripheral blood mononuclear cells despite of a clinical biochemical and serological recovery from an acute viral hepatitis, with important epidemiological and pathogenic implications in the development of chronic diseases. The detectability of all serological and molecular markers during HBV infection is reported in figure 1a.

Many authors proposed different definitions of OBI [21–23]. During the International Workshop on OBI in Taormina (Italy) in 2008, the European Association for the Study of the Liver defined...
the OBI by detectable levels of HBV DNA in liver or plasma with undetectable HBs-Ag with or without anti-HBc or anti-HB surface antibodies (anti-HBs) outside the pre-seroconversion window period [24].

Despite biopsy is considered the gold standard, HBV DNA hepatic detection is often less feasible than that in the serum. Besides, blood samples are more used allowing a sufficient sensitivity for OBI diagnosis in the clinical practice [24–26].

According to the International Workshop meeting on OBI, several serological profiles have been distinguished:

- The seropositive OBI present anti-HBc and/or anti-HBs, but not HBs-Ag. HBs-Ag seroclearance may occur either after the acute phase that takes few months or after years of chronic infection [24, 27, 28].
- The seronegative OBI do not present anti-HBc and anti-HBs antibodies; anti-HBs antibodies progressively could be disappeared with an anti-HBc seroconversion over time without development of detectable HBs-Ag [24, 29].

In the false OBI, HBV DNA levels are similar to those detected in the different phases of an evident HBV infection; the lack of detectable HBs-Ag could be attributed to the viral genome variability (mutations in the 'hot spots' of viral gene region as the immunodominant ‘α’ determinant of S protein) thus altering the antigenicity and the immunogenicity of the HBs-Ag [30]. Mutations that can occur in the major hydrophilic region (MHR) would seem to be correlated with serum HBs-Ag decrease in OBI blood donors [31]. HBV genomic variability was reported in figure 1b. Then, to allow an optimal detection of the virus, it would be necessary to identify all S-gene variants by using multivalent antibodies in HBs-Ag commercially available kits (fig. 1b) [24].

**Mechanism and Infectivity of Occult Hepatitis B Infections in Blood Donors**

In the case of OBI, the DNA is found in the hepatocyte nuclei in a stable form of covalently closed circular DNA (cccDNA). In the long term, the stability of cccDNA together with the long half-life of hepatocytes ensures the persistence of viral infection throughout life [32]. Additionally, the replicative intermediate DNA acts as a template for the genome transcription; hence, most OBI cases are infected by a HBV competent for replication of wild-type virus but are also characterized by a low rate of HBV replication in vivo, although they are competent in vitro [32]. Conversely, a small number of OBI cases are linked to particular circumstances such as some replication deficiencies, genetic S gene variability, integration of viral DNA into host genome, infection of peripheral blood mononuclear cells, and presence of immune complexes leading to decreased reactivity in HBs-Ag detection assays [21, 32].

In recent years, several studies on the infectivity of OBI donors via blood transfusion were reported [10–14]. In Italy, two cases of posttransfusion hepatitis were found in which the sequence identity of donor-recipient by phylogenetic analysis was confirmed [16]. Lookback and backtrack studies performed in some European countries demonstrated 99% sequence homology of HBV DNA in 10 donor-recipient pairs, confirming the infectivity of blood products of OBI carriers [11]. Also outside Europe, through the hemovigilance system of the Japanese Red Cross, the relationship between blood donation and the onset of posttransfusion infections was assessed, thus confirming that the residual risk of OBI did not decrease over the years; in this regard, it was possible to identify HBV infection in 19 patients that had previously been unrecognized by molecular testing [33].

The OBI prevalence in blood donors was well reported [34]. This prevalence is quite variable in different parts of the world and depends on a number of factors such as the considered population, HBV endemism in particular areas, and the screening procedure (serological and/or molecular) used as described in literature [35–38]. Even considering these variables, the worldwide OBI prevalence in blood donors was lower than that observed in the general population [34, 39]. This was likely due to the strict and accurate selection procedures of blood donors excluding high-risk groups such as hemodialysis and transplanted patients, HIV/HCV co-infected subjects, drug users, and blood product recipients [34, 35]. However, the risk linked to HBV transmission by apparently healthy donors with OBI remains high since these individuals have no other concomitant liver diseases and do not show any clinical signs of hepatic damage [40].

Although OBI is widespread worldwide, its transmission by blood transfusion is still limited. The transmission rate by hemo-components from donors with pre-seroconversion window period infections was 10-fold greater than that by blood products from OBI carriers [13]. The fact that not all recipients developed hepatitis as well as the fact that methods used for OBI diagnosis showed different levels of sensitivity and specificity could be logical explanations for low transmission rate of OBI [12, 13]. Nevertheless, the lack of acute hepatitis development did not exclude the transmission of OBI through blood transfusion with a subsequent HBV infection in the recipient [12]. According to a recent study, blood donors negative for both HBs-Ag and HBV DNA but reactive for anti-HBc might be carriers of viral loads below the detection level [35]. Noteworthy, the donor HBV serological status (including anti-HBc and/or anti-HBs), the viral load, and the type of blood component transfused as well as the recipient immune status could contribute to OBI infectivity. Because immunosuppressed or immunodeficient subjects represent a substantial percentage of recipients, a certain degree of caution is advised in transfusing these subjects with ‘anti-HBc only’ blood components [42, 43]. According to Allain et al. [11], the presence of anti-HBc and anti-HBs antibodies in blood donors would reduce the risk of HBV infection by a factor of about 5; OBI carriers with high anti-HBs levels are unlikely to transmit the infection, whereas those with ‘anti-HBc only’ might transmit the infection [11, 13, 35, 44, 45]. Indeed, in HBV hyperendemic areas, most of the recipients have already experienced HBV infection; so, the posttransfusion risk was low while it remained high in HBV-naive patients [46]. In order to clarify and solve these apparent inconsistencies, it was shown that anti-HBs-neutralizing power was limited by the titer, considering
an anti-HBs less than 100 IU/l as poorly protective if HBV DNA is present [16, 33, 45]. However, the viral load and the actual infectious power of an OBI carrier is still an issue. The viral load given to the recipient is related to the transfused plasma volume; so for equal volume, a unit of fresh frozen plasma from an infected donor would bear a greater risk of HBV transmission when compared with a platelet or a red blood cell concentrate. The transmission rates vary from 24% for red blood cells to 51% for platelets to 85% for fresh frozen plasma [7, 11, 47].

**Detection of Occult Hepatitis B Infections in Blood Donors**

It is necessary to maintain a high level of attention for identifying OBI carriers, especially in blood banks [10–14]. For this reason, it is necessary to use advanced testing to detect both common and uncommon HBV genotypes as well as escape mutants (fig. 1b).

While OBI definition is clear enough, a shared global algorithm for OBI detection does not exist yet. HBV detection through HBs-Ag testing was the first mandatory assay in all blood banks worldwide. In developed countries, the current HBs-Ag screening tests consist mostly of immunoassays such as enzyme-linked immunosorbent assays (ELISAs) and chemiluminescence immunoassays (CLIAs) with sensitivity limits lower than 0.1 ng/ml of HBs-Ag and enhanced power of detection for HBs-Ag mutants [48, 49]. The sensitivity of the employed assay is still a pressing problem involving multiple aspects. OBI prevalence could be estimated incorrectly in some rural areas with limited resources and low-sensitivity serological assays leading to an underestimation of HBs-Ag presence and an overestimation of OBI carriers. Some of these cases, if subsequently confirmed with more sensitive and specific serological testing, proved to be HBs-Ag reactive revealing the inconsistence of previous findings [50].

Since the 1990s, the development of molecular methods such as nucleic acid amplification testing (NAT) led some European countries to adopt a specific directive prescribing NAT screening for HBV, HCV, and HIV of all blood products [51–53]. Compared with classical methods such as immunoassays, NAT allows for higher levels of sensitivity and for standardization of results [54]. In blood banks, NAT is usually based on transcription-mediated amplification (TMA) or a multiplex polymerase chain reaction (PCR) that allows the simultaneous detection of HIV-1/2 RNA, HCV RNA, and HBV DNA on individual samples (ID-NAT) even though it is quite expensive. To limit costs, NAT screening was done with pooled samples of 6–50 blood donations that, if reactive, are further tested to identify the reactive sample [55–59]. Some assays have been specifically designed to provide the amplification of the viral genome and the identification of reactive samples in only one step [60, 61]. Furthermore, it is worth considering that using NAT a significantly higher number of samples can be processed per day and that results are readily available allowing for the release of all blood components, even those with a short half-life such as platelets [62]. Although this procedure allowed a reduction of costs, it was not always rewarded by efficacy; OBI carriers identified by NAT range from 1:1,000 to 1:50,000, depending on the epidemiology and whether single samples or pooled samples are tested [63]. At least two factors should be considered with regard to the molecular detection of HBV: i) HBV replication has a slow doubling time of viral load of approximately 2.56 days even during the initial phase of infection; so HBV NAT is less effective than HIV or HCV NAT [64]. ii) in OBI subjects, the viral load is very low even if HBV DNA testing is still considered as gold standard.

Currently, the efficiency of each NAT system is closely related to the size of the pool [65]. Many countries have chosen to reduce the number of samples/pool by using mini pool (MP) testing, thus increasing the analytical sensitivity [66]. The detection of OBI donations by NAT is improved when the MP is reduced from 50 to 20 samples [33]. However, the increased sensitivity of MPs may cause false-positive results, particularly in those pools containing samples with a high HBV viral load. Then, the prior removal of reactive samples from the pool can lead to lowering the false-positive rate compared with those pools with HBs-Ag positive samples [34]. Yet, ID-NAT algorithm could be safer than MP-NAT showing high sensitivity since the percentage of HBs-Ag positive/NAT negative samples dropped off [63, 67]. From these data, it becomes clear that the pooling strategy influences the analytical sensitivity of NAT because it implies a dilution factor approximately equal to the sample number in the pool [49]. Nonetheless, the use of small MPs may not result in substantially increased detection probabilities [68]. Another concern refers to the detection limit that should be considered in donor screening programs. In a recent study [69], it was shown that a hypothetical detection limit below 5 IU/ml could be advisable since in a cohort of Swiss donors 1.2% of the reactive samples had a viral load below 10 IU/ml.

In summary, in countries where the viral prevalence is low MP-NAT is considered a better choice, while in those countries where the exposure to the virus is also up to 90% ID-NAT should be preferred to guarantee the safety of the blood supply [70]. It is conceivable that previously HBs-Ag positive donors have an extremely low level of HBV DNA (below the sensitivity limit of the currently available tests) that comes along with a hidden infection risk for blood product recipients.

**Anti-HBc: to Test or Not to Test, That Is the Question**

Many authors supported the use of serological markers such as anti-HBc to compensate less sensitive NAT assays [71, 72]. These markers are assumed to play a crucial role, especially when taking into account that HBV DNA in OBI donors was often detectable only intermittently [72].

Introduced in the 1980s, the marker anti-HBc was initially considered for identification of ‘non-A non-B’ hepatitis [73]. Subsequently, and after the introduction of a specific test for anti-HCV, anti-HBc was used to check for both a previous exposure to HBV and those OBI cases in which HBV DNA was only intermittently
It is well known that anti-HBc is detectable during asymptomatic infections as well as throughout life after recovery from hepatitis with or without anti-HBs production [44]. It thus is considered a key marker for OBI.

In the literature, the use of anti-HBc testing is highly debated and still controversial both for its validity and the cost-benefit ratio [39, 72, 74]. It is well-known that anti-HBc testing is not particular useful in areas with high prevalence [78]. The prevalence for anti-HBc in blood donors considerably varies in different countries: USA 0.23%, Italy 8.3%, India 7.5%, South Korea 13.5%, Iran 16.4%, Japan 38%, and East Asia and sub-Saharan Africa over 50% [44, 79–84].

One drawback of the serological assays compared with NAT is their inability to detect HBV pre-seroconversion infections as reported in International Survey on NAT testing of blood donations about the existence of NAT+/HBs-Ag–/anti-HBc– donors outside the window period (2–11% of donations) [52]. Moreover, a clear consensus about their use could not be reached due to the lack of confirmation testing [72]. The Italian Society for Transfusion Medicine and Immunohematology (SIMTI) working-group argued that there was little evidence of infectivity of donations with ‘anti-HBc only’ [8]. If anti-HBc testing is not suspected to result in increased safety, its adoption should be cautiously proven as it might result in the deferral of valid donors [8]. Liver biopsies performed in the Italian general population showed that the majority of individuals with anti-HBc had OBI, with a percentage close to 10% of unresolved MPs containing a low viral load [68, 85].

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<th>Supplemental screening markers</th>
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HBs-Ag = Hepatitis B surface antigen; anti-HBc = hepatitis B core antibody; Anti-HBs = hepatitis B surface antibody; HBe-Ag = hepatitis B e antigen; Anti-HBe = hepatitis B e antibody; T = tested; // = not tested; MP-NAT= minipool-nucleic acid amplification test; ID-NAT = individual -nucleic acid amplification test; PCR = polymerase chain reaction.

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<th>Table 1. An overview of different approaches for HBV detection in blood donors in the last decade</th>
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*Only for cited study purpose.
§Tested if negative HBs-Ag.
HBc [16]. However, anti-HBc testing has a low specificity with a high rate of false-reactive results and a number of donors indefinitely deferred [75]. Candotti and Allain [7] reported that 90% of anti-HBc reactivity in blood donors indicated resolved HBV infection since these same donors were also positive for anti-HBs; the remaining 10% could be attributed to false-positive or true ‘anti-HBc only’ donors. Moreover, since high titer of anti HBs (≥100 IU/l) in association with anti-HBc suggested a resolved infection, new re-entry strategies should be considered in transfusion medicine, also taking into account the possibility of a further determination to confirm the results [53, 71, 86].

Already in 1991, the Food and Drug Administration recommended the repetition of the anti-HBc testing before the definitive donor deferral. In Germany, the second anti-HBc confirmation did not determine the permanent deferral, allowing to the donor to return in donor programs at any later date if not reactive. The result of anti-HBc testing is not the single criterion for donor re-entry in anti-HBc reactive donors. Also negative ID HBV DNA testing and HBs-Ag testing are considered [87]. The German Paul Ehrlich Institute suggested to perform two other anti-HBc assays when HBV DNA and HBs-Ag are not detectable; if at least one supplemental is reactive, the anti-HBs titer must be ≥100 IU/l for releasing blood products [75, 88]. Other researchers have proposed the use of the anti-HBc IgM test by skipping alternative screening assays. In addition to anti-HBc and anti-HBs titer, they proposed to identify the donors in the post-HBs-Ag early recovery phase of infection. This reactivity was supported by HBe-Ag or by the detection antibody (anti-HBe), but only an anti-HBe positive result confirmed the anti-HBc reactivity while a negative result did not exclude the real exposure to HBV [84].

Supplemental serological HBV markers can be of considerable help in blood donors as well. In table 1 we report several approaches for HBV detection that were applied worldwide during the last decade. Many laboratories adopted anti-HBc as a marker mostly where NAT has not been implemented for its high cost and/or as additional criterion for the evaluation of the overall risk of OBI in the peripheral blood [44]. Further data indicated that HBV DNA (by MP or ID NAT) together with anti-HBc testing could be considered complementary [38, 89]. For these reasons, in some countries such as the USA, Germany, France, Korea, Japan, Thailand, and Australia anti-HBc has been routinely screened together with HBV DNA detection according to each National Blood Bank policy, while in others countries the diagnostic approach may be different (table 1) [13, 29, 62, 71, 75, 77, 79–82, 86, 90, 94]. Moreover, anti-HBc testing was used only for research purposes (table 1) [91–93].

In Italy, there is still great discretion regarding the use of anti-HBc on the basis of its past and present utilization and of economic resources of each laboratory. In blood donors, anti-HBc testing, in the absence of other serological markers, is currently not recommended for its relatively high prevalence that would lead to the rejection of an unacceptable number of donors (table 1) [8, 29]. According to data of 2013, the prevalence of HBV infection among Italian first-time blood donors is clearly lower than in the past, and it is expected to gradually decrease in the future. The donor loss can be conquered by both vaccination programs and the reduced incidence of new infections [39]. The Italian health authorities advised to adopt a screening algorithm based on HBs-Ag with last-generation immunoassay kits and HBV DNA detection in blood donors [95, 96].

In the attempt to reduce the risk of HBV transmission and to increase the safety in the blood bank of the Università degli Studi della Campania, Italy, all donors are routinely evaluated for HBs-Ag, HBV DNA and also for anti-HBc. Those presenting anti-HBc reactivity are further tested for anti-HBs. All these serological markers are performed by chemiluminescent microparticle immunoassay (CMIA) with an automated analyzer Architect i2000SR (Abbott Diagnostics, Wiesbaden, Germany) as depicted in figure 2a. We reported our experience related to 1 year of donation from 4,300 voluntary (first-time and repeated) blood donors enrolled in our center; of these blood donors, 10.54% were HBc-Ab positive / NAT negative, and only 1.26% showed ‘anti-HBc only’ with no anti-HBs, while 9.28% were positive for anti-HBc and also for anti-HBs; moreover, 3.00% had an antibody titer between 10 and 100 IU/l showing a poor protection while 6.28% had a titer > 100 IU/l (fig. 2a,b). All anti-HBc positive donors were HBV DNA negative (Tri-NAT screening for HBV, HCV, and HIV-1/2 in mp-6 samples (Roche Diagnostics, Branchburg, NJ, USA)) (fig. 2a). On the basis of our results, we have considered discontinuing all serological profiles with ‘anti-HBc only’ with consequent donor discard. In our opinion, the discard of 1.26% of ‘anti-HBc only’ donors does not mean a significant loss of donors but enhances the safety of recipient. Moreover, this percentage is expected to drop off after adopting a secondary confirmation system for in-house strategy, such as Cobas e411, an automated electro-chemiluminescence immunoassay-ECLIA (Roche Diagnostics, Mannheim, Germany) [97, 98]. Indeed, even if improved assays are now available, the repetition of an initially reactive anti-HBc testing with a different method is considered a good laboratory practice [71, 75, 87, 99]. The introduction of this supplemental system allowed us to confirm ‘anti-HBc only’ positive samples as well as to verify those that, despite presenting an anti-HBs positive titer (>10 IU/l), are not finally protected (<100 IU/l), as previously reported in the literature [45, 75]. Using our algorithm, donors with an anti-HBs>100 IU/l are considered accepted donors, those being ‘anti-HBc only’ are permanently deferred, while donors with anti-HBs between 10 and 100 IU/l are temporarily deferred waiting for the repetition test after some weeks from the first determination to assess a reliable anti-HBs seroconversion to regain the status of accepted donor [100]. Moreover, we noted that in our total blood donor population, 0.30% (n = 13) were anti-HBc reactive and HBs-Ag positive donors confirmed by neutralization testing (CMIA, Abbott Diagnostics, Wiesbaden, Germany) (fig. 2a,b). Out of these donors, only 11 were also NAT positive while the remaining 2 NAT negative donors presumably had a viral loads below the limit of sensitivity of the NAT test used, which might be due to further dilution in the MP setup used.

HBV prevalence is quite different from region to region in Italy, but, since Campania is one of the regions with the highest HBV...
prevalence, the discard of blood donors with ‘anti-HBc only’ (1.26%) observed in our region, potentially takes in account the general Italian trend [101].

**Conclusions**

In recent years, various diagnostic strategies were designed and implemented to optimize the management of blood supply.

The combination of serological and molecular methods for the prevention of transfusion-related transmission of HBV was considered by many operators both as an ideal solution to resolve the crucial problem of the window period and as a valuable support in identifying even low concentrations of virus in asymptomatic individuals, e.g. blood donors. Several cases of undiagnosed ‘occult’ infection can still arise. The analysis of HBV DNA in liver biopsy samples is generally never feasible in healthy blood donors. For this reason, the OBI identification is mainly based on HBV DNA detection in serum samples by NAT; indeed, HBV DNA is detectable long before the presence of HBs-Ag or anti-HBc remaining even after the disappearance of other serological markers (fig 1a).

OBI is considered a rare event in developed countries while it still represents a serious problem in developing areas. Indeed, in high HBV endemic countries, it is advisable to implement ID-NAT rather than MP-NAT while in those with a low endemism MP-NAT can be sufficient. In some countries with an intermediate endemism such as Italy the debate remains open. However, molecular assays still exhibit some pitfalls that inhibit their extensive use in all blood bank settings worldwide. When a highly sensitive NAT is lacking or when in many individuals the viremia is often intermittent, a readily available serological method becomes necessary for identification of OBI carriers.

To date, the strategies for HBV detection in blood donors are multiple but not fully fitting. In the light of the recent findings, through an in-house adopted strategy that preserves the total safety of recipients with a small deferral of about 1% of blood donors, the anti-HBc supplemental marker could be considered an ideal candidate both for OBI identification in countries where NAT cannot be implemented and for an overall sensitivity increase of OBI risk evaluation in countries where it is carried out (fig. 2a,b). Then, anti-HBc testing and HBV NAT should be considered as complementary assays.

In conclusion, even though a more sensitive ID-NAT is strongly recommended, it will be still a long way to full blood safety. Until then, markers such as anti-HBc testing could be a simple precautionary measure for preventing transfusion-transmitted OBI, especially in the immunocompromised recipients.

**Disclosure Statement**

All authors have no conflict of interest to declare.
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