TRANSFUSION COMPLICATIONS

A probable case of hepatitis B virus transfusion transmission revealed after a 13-month-long window period

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BACKGROUND: Transfusion-transmitted hepatitis B virus (HBV) infection in recipients with drug-related immunodeficiency is rarely described in endemic areas. Hepatitis B surface antigen (HBsAg)–negative infectious donor blood can be identified by sensitive nucleic acid testing (NAT). Two immunodeficient patients who received blood components from a single seronegative blood donor subsequently found to contain HBV DNA are described.

MATERIALS AND METHODS: Multiple samples from the implicated donor and the two recipients were tested for HBV serologic and molecular markers. HBV genome fragments were amplified, sequenced, and phylogenetically analyzed.

RESULTS: The implicated donation had low-level HBV DNA due to the donor being in the window period before the donor's seroconversion. Recipient 1 had been vaccinated to HBV and carried anti-HBs but remained negative for all other HBV markers until she developed acute hepatitis B (viral load 2.7×10^8 IU/mL and alanine aminotransferase [ALT] level 1744 IU/L) 13 months after transfusion of red cells. Identical HBV sequences from both donor and recipient provided evidence of transfusion-related infection. Recipient 2, who received platelets from the same donation while receiving major chemotherapy, remained uninfected.

CONCLUSIONS: In unusual circumstances, HBV incubation time can be considerably prolonged. Both active and passive neutralizing antibodies to HBV likely delayed, but did not prevent, acute infection when the immune system was impaired. HBV NAT may have interdicted the infectious unit, although the donation viral load could not be quantified and odds of detection calculated.

Among blood-borne viruses of major concern in transfusion, hepatitis B virus (HBV) presents the highest residual risk,[1] despite several serologic markers available for screening. HBV DNA testing is routinely performed in Germany[2] and Japan[3] and, more recently, in several additional European countries.[4] HBV DNA testing is an expensive alternative to anti-HBc in place for years in several low-prevalence countries but remains cost-prohibitive in areas of higher prevalence to avoid blood shortage. Genomic screening can be performed on individual donations or in plasma pools ranging between 6 and 96, although it was shown that pooling reduces significantly the yield of DNA-containing donations.[4,5] In Brazil, despite relatively high prevalence of the marker, anti-HBc screening is mandatory and a few blood banks also routinely test blood donations for both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) RNA but not for HBV DNA.[6] A fundamental limitation of anti-HBc screening is the inability to detect window-period, highly infectious, donations. The pre-seroconversion window period has been extensively studied in serial plasma donor samples and typically ranges between 37 and 87 days (median, 59 days).[7] Post-transfusion infection was not systematically investigated but the early stages were assumed to be of similar or shorter duration due to the large volume of the inoculum. The protective effect of anti-HBs has been well established as well as the increased susceptibility to HBV infection of

ABBREVIATIONS: BCP = basic core promoter; PC = precore.

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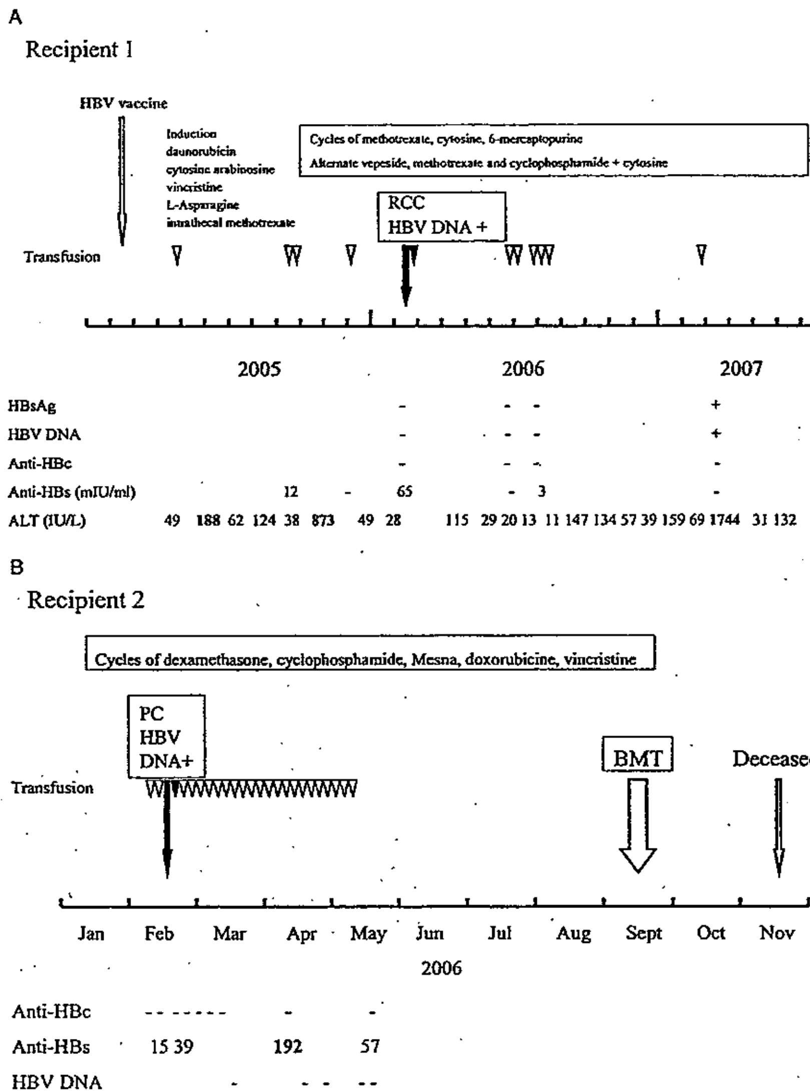

Fig. 1. Case description. (A) Summary of Recipient 1 clinical history. The implicated transfusion of RBCs is indicated by a full arrow. Other transfusions received are indicated as open triangles. The filled triangle indicates the blood product containing high titer of anti-HBs. Bolded ALT levels indicate values above 5 times upper normal level. The HBV infectious component and the PLTs containing high anti-HBs level were transfused on the same day. (B) History of Recipient 2. Symbols are as in Recipient 1 (A). This patient received a PLT concentrate (PC). The interval between receiving the infectious PC and the PC containing high anti-HBs was 3 days. BMT = bone marrow transplantation.

immunodeficient recipients of organs from anti-HBs–carrying donors.

Here are presented two cases of immunodeficient recipients of blood components from a single unit containing very low levels of HBV DNA. One of these recipients developed acute HBV infection 13 months after transfusion despite carrying vaccine-induced anti-HBs while the other was not infected.

CASE REPORT

On March 6, 2007, the hospital notified the blood center that a 9-year-old female child suffering from a high-grade acute lymphoblastic leukemia (Recipient 1), diagnosed in April 2005, was experiencing a clinical episode of acute hepatitis B. Serologic tests confirmed this diagnosis: the presence of hepatitis B surface antigen (HBsAg) and anti-HBc immunoglobulin M (IgM) and an alanine aminotransferase (ALT) level of 1744 IU per L later supported by an HBV DNA load of 2.7×10^8 IU per mL. The patient history revealed 24 transfusions including 13 units of red cell (RBC) and 11 apheresis platelet (PLT) concentrates between April 26, 2005, and August 13, 2006 (Fig. 1A). During this period, she received chemotherapy according to the PROPII-97 protocol consisting of induction by daunorubicin, cytosine arabinosine, vincristine, dexamethasone, and L-asparaginase as well as intrathecal methotrexate/dexamethasone/cytosine-arabinoside. Maintenance treatment consisted of alternate cycles of high-dose methotrexate and cytosine with 6-mercaptopurine, followed by alternate cycles of vepesid plus methotrexate and cyclophosphamide plus cytosine.

Records from the implicated donors were examined and most were excluded as the source of HBV infection because at least one subsequent donation was negative for the presence of HBsAg and anti-HBc. One donor, however, whose RBCs were transfused to the child on February 23, 2006, also donated PLTs by apheresis on March 30, 2006, and subsequent testing results indicated a seroconversion to anti-HBc, without detectable HBsAg, anti-HBs, or HBV-DNA.

A plateletpheresis concentrate prepared from the index automatic blood donation of February 23 (Trima, Gambro BCT, Lakewood, CO) was transfused to a second patient (Recipient 2), a 65-year-old female diagnosed with high-risk myelodysplastic syndrome evolving to biphenotypic leukemia. At the time of the suspect transfusion, she was receiving Hyper-CVAD (ondosetin, dexamethasone, cyclophosphamide, Mesna, doxorubicine, and vincristine) plus intrathecal QT (meth-

TABLE 1. HBV markers in samples from the implicated donor

Date of sample collection	HBsAg	Anti-HBc* sample OD/cut-off	Anti-HBs	HBV DNA
February 17, 2006	Negative	Negative	ND	ND
Repository samples February 17, 2006	Negative	Negative (0.866/0.407)	ND	Positive
March 31, 2006	Negative	Reactive (0.142/0.382)	Negative	Negative

* Hepanostika anti-HBc Uniform, BioMerieux, Boxtel, the Netherlands. Specificity in package insert is 99.85 percent.
OD = optical density; ND = not done.

otrexate and aracytin). She was negative for the presence of HBsAg and anti-HBc but had a low level of anti-HBs (13 mUI/mL). In September 2006, she received marrow transplantation in another hospital where no clinical or laboratory evidence of HBV infection was observed. She died of sepsis in November 2006.

Unfortunately, when retrospective investigation was initiated, the archive sample of the implicated donation had already been discarded from the repository according to the national policy mandating the storage of a sample from nonreactive donations for 1 year. Two separate aliquots of 230 µL of plasma, however, had been archived for potential investigation, allowing us to perform polymerase chain reaction (PCR) amplification and DNA sequencing for comparison with recipient data.

MATERIALS AND METHODS

Serologic testing

Anti-HBc (Abbott/Murex, Delkenheim, Germany), HBsAg (Axsym MEIA, Abbott Laboratories, Abbott Park, IL), and anti-HBs (Axsym MEIA, Abbott) testing was performed according to the manufacturer's instructions. Anti-HBs levels are expressed in mIU per mL.

Molecular testing

DNA was extracted from 200 µL of serum and/or plasma with a DNA blood mini kit (QIAamp, Qiagen, Hilden, Germany) in Brazil and either tested locally or shipped to the UK in dry ice. HBV DNA was detected initially by one-step PCR using 7 µL of extract DNA submitted to a fast PCR protocol (Applied Biosystems, Foster City, CA) in the presence of 1 µmol per L of each primer OY1 sense (5′-CAAGGTATGTTGCCCGTTTG-3′) and OY2 antisense (5′-AAAGCCCTGACCACTGA-3′),[8] in a final volume of 25 µL. Nested PCR was performed on 12.5 µL of DNA in a 25-µL reaction (final volume) as previously described.[9] All PCR procedures were performed in a thermocycler (Model 9700, Applied Biosystems). Two nested PCR procedures were used to amplify a 276-bp fragment located in the basic core promoter (BCP) and precore (PC) regions and a 1434-bp fragment spanning the whole pre-S/S gene, as previously described.[10] Sequences of BCP/PC and pre-S/S regions were obtained by direct sequencing of amplicons. Sequences were aligned with reference HBV genotype A to H sequences using computer software (Clustal W software implemented in Mac Vector Version 7.2, Accelrys, San Diego, CA), and the alignments were confirmed by visual inspection. Phylogenetic analysis was performed using computer software (PAUP 4.0b10, Sinauer Associates, Inc., Sunderland, MA) after exclusion of positions containing an alignment gap from pairwise sequence comparisons. Nucleotide distances were analyzed by neighbor-joining algorithm based on Kimura two-parameter distance estimation. To confirm the reliability of the phylogenetic trees, bootstrap resampling was performed for each analysis (1000 replicates).

RESULTS

Analysis of the implicated donation sample and donor

Upon retesting, the repository sample gave the same serologic results as in the screening (anti-HBc and HBsAg nonreactive) but HBV DNA was detected by two distinct PCR methods, both single-step and nested PCR. The first assay has a limit of detection of 500 IU per mL and the second of 100 IU per mL, and both showed clear amplicons, suggesting that, although not properly quantified, the viral load was above 500 IU per mL. Viral load, however, could not be quantified due to the limited sample availability. Of note, the patient and the donor samples were processed 3 weeks apart, the donor sample first, and were kept in different freezers, limiting considerably the possibility of cross-contamination. On the basis of phylogenetic analysis of the pre-S/S gene, the sample was classified as genotype A1. Translation of the "a" region of the S gene indicated a wild-type amino acid sequence when compared to the genotype consensus sequence. The BCP/PC region was also wild type without mutation in either the 1762 to 1764 doublet or the 1896 nucleotide of PC codon 18 or in any of the start codons for PC or core sequences.

When retested from a sample collected 6 weeks after the index donation, the donor plasma showed clear anti-HBc seroconversion but no HBsAg or anti-HBs detectable (Table 1). Other HBV serologic markers such as IgM anti-HBc could not be tested for lack of available sample volume.

The donor was a 39-year-old male who denied risk factors. He was of mixed race, partly of African origin. His donation did not react for anti-HIV and anti-HCV.

Recipient 1

A summary of the Recipient 1 data is presented in Fig. 1A. Before transfusion of the implicated component, anti-HBs was present at low levels on two occasions as expected in a child previously vaccinated to HBV. ALT levels were fluctuating around upper normal levels except on two occasions in May and October 2005 and 2006 when levels reached 188 and 873 IU per L. In the subsequent absence of markers of HBV infection, these high ALT levels could be attributed to the underlying disorder and the chemotherapy. In the period after the transfusion of the implicated component, HBV DNA or serologic markers were never detected until the acute HBV infection 13 months later. During this period, as in the preceding year, ALT levels fluctuated but did not exceed four times upper normal levels. Between transfusion in February 2006 and the acute episode in March 2007, the patient received seven blood components. A single dose of PLT concentrate obtained from a double unit of PLTs prepared by apheresis containing an anti-HBs titer of greater than 1000 mIU per mL was transfused on February 23, 2006, the same day as the implicated HBV DNA containing RBCs. The amount of plasma transfused with the PLTs was approximately 125 mL.

Seven samples collected from Recipient 1 between February 2006 and August 2006 did not contain detectable HBV DNA. After a period of 7 months without transfusion, a sample collected on March, 30, 2007 contained a viral load of 2.7×10^8 IU per mL. This strain was sequenced in the BCP/PC and pre-S/S regions. The latter sequence was phylogenetically analyzed and revealed a genotype A1. When these sequences were aligned with the corresponding sequences obtained from the suspected donation, the 276- and 1202-nucleotide-long sequences, respectively, were identical except for one ambiguity. Within the pre-S/S region, Sample SL167648 (donor) showed a sequence ambiguity (adenosine/guanine) at nucleotide 231 starting from the ATG of the S protein. This suggested the presence of quasispecies in the donor while at position 231 only guanine was detected in the recipient sequence. Phylogenetic analysis of the pre-S/S region showed that recipient and donor sequences clustered with HBV genotype A1 reference sequences of African origin, supported by bootstrap values of 100 percent over 1000 replicates. On that basis, the relationship between donor and recipient HBV infection was clearly established. Since HBV genotype A1 in Brazil is essentially found in Brazilians with African ancestry, racial origins of donor and recipient were examined. The donor was of mixed African origin and the recipient was Caucasian.

Recipient 2

Recipient 2 received the PLT concentrate prepared from the same donor and donation transfused to Recipient 1. Follow-up samples collected up to June 2006 (3 months after transfusion) did not reveal the presence of any serologic or molecular marker of HBV infection (Fig. 1B). Before receiving the PLT concentrate from the suspected blood unit, a low titer of anti-HBs was detected acquired either from active or from passive immunity to HBV. The elevation of anti-HBs titer to 192 mIU per mL observed in April 2006 was probably related to passive immunization since, coincidentally, the second unit of a double-plateletpheresis concentrate collected from the same strongly anti-HBs–reactive donation (>1000 mIU/mL) whose PLTs were transfused to Recipient 1 was transfused to Recipient 2. This concentrate contained approximately 125 mL of plasma and was transfused 3 days after the implicated PLT concentrate. Overall, despite receiving PC from an infectious blood donation, no evidence of HBV infection was found in this immunosuppressed adult patient to date.

DISCUSSION

Posttransfusion viral infection has been the focus of considerable scrutiny after the occurrence of HIV infections related to transfusion. Although receiving considerably less attention, reporting of HBV posttransfusion infection has been limited by screening for specific HBV markers such as HBsAg and anti-HBc in some low-prevalence countries. More recently, genomic screening for HBV has become available and was implemented in several countries either in pools of plasma from blood donations or in individual donations. Most anti-HBc screening countries, however, do not feel that it is necessary to screen for HBV DNA and hence do not address the risk of window period. Countries where HBV infection is relatively high (European Mediterranean countries or Poland) as well as some relatively affluent countries with high infection prevalence (Southeast Asia) started screened for DNA to avoid deferring a number of donors that would endanger the blood supply to patients.

Few studies describe the duration of the window period in humans. Most investigate blood donors where the origin of the infection was mostly unknown or posttransfusion. The latter situation had the peculiarity of a large volume of inoculum (100-250 mL) compared to no more than 5 mL in the situation of intravenous drug use, nosocomial infection, or vertical or sexual transmission. In a study conducted in the 1950s, inmates were inoculated with Australian antigen-positive serum; the interval between infection and detection of HBV antigen was 45 to 92 days (mean, 77 days) but longer when the inoculum was diluted 1:1000 (92-130 days).[11] The infectious dose seems therefore to influence the duration of the window

period. Other elements possibly interfering in the time interval between viral contact and seroconversion to HBsAg (window period) such as the state of the immune system of the infected individual or the presence of specific neutralizing antibodies to HBsAg have not yet been systematically examined. Only in the situation of transplantation of organs from donors carrying anti-HBs with or without detectable HBV DNA was evidence of infection provided in patients receiving immunosuppressive drugs for liver transplantation.[12] In contrast, experiments conducted in chimpanzees indicated that, in immunocompetent animals, low levels of HBV in the presence of anti-HBs were not infectious.[13] It has also been well known for many years that the risk of developing chronic HBV infection was inversely proportional to the immunocompetence of children.[14] In none of these circumstances, however, was the duration of the window period or the level of preseroconversion viral load addressed.

In the complicated and discrepant cases presented here, several areas of uncertainty require discussion. First is the authentication of the donation as source of Recipient 1 infection and as a window-period donation. This implication is based on two main elements: 1) the presence of HBV DNA in the donation and 2) the identity of pre-S/S and BCP/PC sequences between donor and recipient. The presence of HBV genome in the implicated donation was found in two separate laboratories in Brazil and in England using different amplification methods and targeted regions. These positive results are strongly supported by obtaining sequences from two such regions. The hypothesis of laboratory contamination is unlikely because the prevalence of chronic hepatitis is 0.2 percent in blood donors in the São Paulo blood center (limiting the possibility of sample to sample cross-contamination) and amplification of HBV in the donor and recipient samples was performed 3 weeks apart from samples stored in different freezers. Finally, being of genotype A1 in a donor of partial African origin is the most plausible since in an unpublished study of 33 strains of HBV from the same blood center, 52 percent of strains were of genotype A1 (J.P. Allain and M. Premnath, unpublished). This dominance of genotype A1 was confirmed by several other studies in Brazil.[15,16] The donor seroconversion to anti-HBc 42 days after the implicated donation without anti-HBs or HBsAg is not totally convincing (Table 1). While HBV DNA as sole evidence of HBV recent infection strongly suggests being in the window period, the negativity of HBV DNA, HBsAg, and anti-HBs in the second sample is unexpected, unless the stage of infection in the follow-up sample corresponds to the second window period, after disappearance of HBsAg and possibly DNA before the occurrence of anti-HBs. Unfortunately, no further sample was obtained from this donor.

While the identical sequence of more than 1500 cumulated bases between donor and recipient HBV strains leaves little doubt about the donor being responsible for the infection, once contamination of the donor sample has been excluded, the discrepancy of the outcome of HBV contact between the two recipients raises multiple questions. Although both patients received chemotherapy accompanied with assumed substantial immunosuppressive effects and similar volumes of HBV DNA–containing plasma (110 and 180 mL for Recipients 1 and 2, respectively), only Recipient 1 developed infection. Neither age nor volume of the inoculum could significantly affect the ability to develop an immune response since, at age 9, the maturity of the immune system is comparable to that of an adult. The presence of low levels of anti-HBs before the implicated transfusion in both recipients might have played a protective role, particularly as the blood component viral load was low, below 1000 to 3000 copies per mL, which is considered the limit of detection for HBsAg.[17,18] Coincidentally, both recipients received passive antibodies to HBV in the form of 125 mL of plasma containing high-titer anti-HBs from the same double-plateletpheresis donation. One difference between the two patients was that Recipient 1 received 125 mL of this plasma the day of transfusion with the implicated product while Recipient 2 received the same volume of plasma 3 days after being in contact with the implicated PLT concentrate. Since the suspected viral strain was wild type in the S region, there is a high likelihood that anti-HBs either raised by vaccine or passively transmitted was neutralizing the circulating virus.

Recipient 1 did not receive any transfusion during the 7 months preceding the episode of acute hepatitis B and, therefore, no reinforcement of her low level of anti-HBs. During the same period of time, the immunosuppressive effect of the chemotherapy accumulated and one can speculate that at one point, the precarious protection offered by low-level neutralizing antibodies became insufficient to contain the virus that started actively replicating.

Posttransfusion HBV infection window period typically ranges between 37 and 87 days in HBV-only infection and between 80 and 110 days when HCV coinfection was present.[7] The prolongation of the interval between infectious contact and evidence of active viral replication in Recipient 1 was unexpected and remains difficult to explain. Conflicting factors are at play. First the chemotherapy received by the patient to treat leukemia had likely some immunosuppressive effect, which was expected to shorten the window period and facilitate viral replication. In contrast, prior HBV vaccination and passive immunization was expected to prevent or at least delay the clinical expression of the infection. One hypothesis to explain the evidence is that most of the virus received by transfusion was complexed by neutralizing antibodies either actively acquired by vaccination or passively transmitted. Some free virus, however, may have persisted in the liver, escaping the immune system until the level of immunodefi-

ciency was such that viral replication could take place. This hypothesis is compatible with the surprising absence of detectable HBV DNA in the middle of this long window period in two samples collected in July and August 2006, 5 and 6 months after the infectious contact. Typically, after the eclipse period of approximately 2 weeks during which no evidence of viral DNA is found, low levels of HBV DNA without detectable HBsAg are detectable during the window period.[17,19,20] Recently a very similar case to ours was published, reporting a 19-week window period in a leukemia patient receiving unspecified chemotherapy regimen and carrying anti-HBs passively transmitted by PLT transfusion (58 mIU/mL) at the time of receiving the low-viral-load window-period donation.[21]

In view of these inconsistencies, the hypothesis of an HBV reactivation from a previously recovered HBV infection can be formulated. Strains mutated in the antigenic "a" region of the S gene, however, are usually found together with anti-HBc.[22] In this case, the absence of detectable anti-HBc and the wild-type genotype A1 (Recipient 1 was Caucasian) of the sequenced strain are strong argument against such hypothesis.

These two recipients in contact with a relatively low amount of HBV illustrated that human intervention, whether preventive such as HBV vaccination or passive immunization or to the contrary facilitating infection such as chemotherapy or immunosuppression can considerably modify the variables classically defining the early stages of a viral infection. As a result, in complicated situations such as described here, advanced molecular methods can be most helpful to resolve cases where transfusion, reactivation, and nosocomial elements may need to be separated.

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研究報告の概要

○B型肝炎ウイルス（HBV）DNAおよびHBV表面抗原の新規濃縮方法：オカルトHBV感染検出方法への応用

背景：輸血後B型肝炎ウイルス（HBV）感染のリスクは、HBV核酸増幅技術（NAT）の導入後減少したが、HBV DNA陽性かつ表面抗原（HBsAg）陰性オカルトHBV感染の問題は未解決である。その理由の一つは、オカルトHBV感染はミニプールNATにより検出するにはHBV DNA量が少なすぎることである。HBVコア抗体（HBcAb）の検査は、オカルトHBV感染を完全には排除していない。そのため、検出感度を上げるために、HBV DNAとHBsAgを同時に濃縮する新規方法を開発した。

方法：二価金属存在下でpoly-L-lysineを使用し、ウイルス凝集反応を増強させ、ウイルスを濃縮する。濃縮処理時間を短縮するためにpoly-L-lysineでコートした磁気ビーズ法を用いる。HBcAb陽性およびHBsAg陰性供血血液77本について、酵素免疫法（EIA；AxSYM、Abbott社）および赤血球凝集阻害検査（日本赤十字社）により、HBsAgおよびHBcAbをそれぞれ調べた。

結果：HBV DNAとHBsAg量は、最高4～7倍に濃縮された。この方法により、HBcAb陽性およびHBsAg陰性供血者77名のうち35名は個別NATにてHBV DNA陽性となり、更に供血者5名はHBVの濃縮によりHBV DNA陽性となった。オカルトHBV感染者40名のうち27名は、HBsAgの濃縮によりHBsAg陽性となった。

結論：HBV DNAおよびHBsAgを濃縮する我々の新しい方法により、EIAとHBV NATの感度が上昇し、HBsAg EIAを用いてオカルトHBV感染者40名のうち27名を検出することができた。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
HBV DNAとHBsAg量を、同時に最高4～7倍に濃縮することで、EIAとHBV NATの感度が上昇し、HBV DNA量が少ないオカルトHBV感染者40名のうち27名を検出することができたとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法（CLEIA）及び精度を向上させた新NATシステムを導入した。HBV検査に関する新たな知見等について今後も情報の収集に努める。

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A new method of concentrating hepatitis B virus (HBV) DNA and HBV surface antigen: an application of the method to the detection of occult HBV infection

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Vox Sanguinis

Background The risk of post-transfusion hepatitis B virus (HBV) infection has been reduced after the implementation of HBV nucleic acid amplification technology (NAT). However, the problem of HBV DNA-positive and HBV surface antigen (HBsAg)-negative occult HBV infections remains to be solved. This is in part due to the HBV DNA load being too low to detect these occult HBV infections using mini-pool NAT. In Japan, the assay for the antibody against the HBV core antigen (anti-HBc) has not completely excluded occult HBV infection. To solve this problem, we have developed a new method of concentrating HBV DNA and HBsAg simultaneously to increase the sensitivity of detection tests.

Methods Virus concentration is achieved by the enhancement of the agglutination of viruses using poly-L-lysine in the presence of a bivalent metal. Poly-L-lysine-coated magnetic beads are used to shorten the time of each step of the concentration procedure. Seventy-seven anti-HBc-positive and HBsAg-negative donations were examined. HBsAg and anti-HBc were tested by enzyme immunoassay (EIA) (AxSYM; Abbott) and haemagglutination inhibition test (Japanese Red Cross), respectively.

Results HBV surface antigen and HBV DNA levels were concentrated up to four- to sevenfold. Using this method, 35 of the 77 anti-HBc-positive and HBsAg-negative donors were HBV DNA-positive by individual NAT and a further five donors became HBV DNA-positive by HBV concentration. Twenty-seven of 40 occult HBV infections became HBsAg-positive by HBsAg concentration.

Conclusion Our new method of concentrating HBV and HBsAg increased the sensitivities of EIA and HBV NAT, and enabled us to detect 27 of 40 occult HBV infections by HBsAg EIA.

Key words: anti-HBc, concentration of HBV DNA, concentration of HBsAg, occult HBV infection, poly-L-lysine-coated magnetic beads.

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Introduction

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) [1]. HBV is one of the most important viral infections transmitted by transfusion. Nucleic acid amplification technology (NAT) screening has widely been introduced for hepatitis C virus (HCV) and human immunodeficiency virus, and has greatly reduced the risk of transfusion-transmitted infection by these viruses. In contrast, HBV NAT has not been widely implemented, in part due to assay sensitivity issues. HBV therefore remains a source of post-transfusion infection. The risk of post-transfusion HBV infection has been reduced after the implementation of

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