TABLE 3. Demographics of four blood donors with VVR

	Donor			
Characteristic	1	2	3	4
Sex	Male	Male	Female	Female
Age (years)	19	51	18	23
FTD/RD	FTD	RD	RD	RD
VVR history	No	Yes	No	Yes
Height (cm)	160	168	166	166
Body weight (kg)	62	73	57	58
Blood volume (mL)	4232	4890	4072	4135
Water intake (mL)	400	400	200	100
Shifted volume (mL)	197	158	99	163
Symptoms	Nausea, paleness	Nausea, paleness	Nausea, paleness, yawning	Nausea
VVR onset				
Blood pressure	91/58	98/62	94/62	97/58
Pulse rate	61	55	68	77
Treatment	Recumbent rest	Recumbent rest	LRI (500 mL)	Recumbent rest
Recovery time (min)	15	>30	30	20

FTD = first-time donor; LRI = Lactate Ringer infusion; RD = repeat donor.

and 400 mL in Japan, and the frequency of VVR among female donors is higher after collecting 400 mL (1.2% vs. 0.74%; 2010 data from Japanese Red Cross Blood Centers). Thus, the decrease in the circulating blood volume generated by removing a larger volume of blood might contribute to the occurrence of VVR during donation. We therefore investigated the effect of moderate hypovolemia (400 mL of collected blood) on circulating blood volume during blood collection to help identify factors involved in VVR to develop strategies to decrease the occurrence and severity of VVR.

The physiologic mechanisms that control circulating blood volume during hypovolemia caused by acute blood loss are complex, but a rapid, compensatory shift of interstitial fluid from the skeletal muscle and skin into the circulation would increase plasma volume.[16,17] The exchange of fluid at the capillary–interstitium interface is largely determined by alterations in transcapillary Starling forces. The roles played by sympathetic activity and fluid shifts in the maintenance of arterial pressure are of equal importance during hemorrhage of 10% to 40% of total blood volume.[16]

Fluid shifts during blood donation have been described,[19,20] but only in relatively small cohorts. Considering the high variability of the shifted volume, we derived data from 736 donations. Moreover, the previous studies used the Hb concentration upon admission as the Hb value of the collected blood,[19,20] which contradicts the hypothesis that peripheral blood is diluted during donation, because the peripheral blood Hb concentration near the end of collection must be decreased according to the hypothesis. However, as we had no access to the actual Hb concentration in the collected blood, we used the median of concentrations at the beginning and end of blood collection, which should have been closer to reality. To avoid variability, we focused this study within a relatively short period of 11 months, when procedures among phlebotomists were uniform, and Hb was measured after blood sampling using the same instrument at the donation site. The reliability of the Hb measurement is suggested by the minimal difference in the Hb concentration (0.1 g/dL) between that at admission and that at the start of blood withdrawal compared with the large difference (0.6-0.7 g/dL) between that at the start and that at the end of blood withdrawal, although more time was required before starting to collect blood than to actually complete the process of collection.

The mean volume of fluid that shifted from the interstitium to the intravascular compartment during blood donation was 202 mL (adjusted for sex). Only about 200 mL of circulating blood volume was lost at the completion of blood collection, which is similar to described findings.[19,20] Given this finding, blood loss might not be a significant factor in the development of VVR in the setting of a whole blood donation of 400 mL. Moreover, the fluid shift would likely continue after completing the donation. If it continues at the same speed as that during blood collection, circulating blood volume would be restored in most donors within 10 minutes of catheter removal.

We propose that 400-mL donations may not be a direct causative factor, but rather only one of several factors that collectively contribute to low venous return. Donations of 400 mL might comprise a risk factor only when the release of capacitance vessel tone is higher, a change in posture causes a greater venous blood shift to lower extremities, the donor is extremely dehydrated, or the donor has a lower circulating blood volume. The difference in VVR frequency between 400 and 200 mL donations is higher indeed among donor populations with a circulating blood volume of less than 4000 mL. Neurally mediated hypotension might also be triggered more by a donation of 400 mL than of 200 mL. The 11.0% of males and 5.5% of females with a shifted volume of less than

100 mL might represent a subpopulation that is more vulnerable to VVR. This consideration would be applicable to donations of 400 mL, but not 200 mL, because the shifted and collected volumes are too small to elicit VVR.

We found that more interstitial fluid shifted to the intravascular compartment in females than in males. This was evident in differences between shifted volumes in male and female donors within the same body weight ranges (Fig. 1). The reason for this difference is not clear but it might relate to the fact that females have a larger interstitial space. More time was needed to collect blood from females than males (8.8 min vs. 8.0 min), which might have produced a larger shifted volume for females during blood collection. However, the rate of fluid shift is still significantly greater for females than for males when body weight is considered (0.45 mL/min/kg vs. 0.38 mL/min/kg), indicating a greater capacity of blood volume compensation in females than males. The shifted fluid volume in females tended to decrease with increasing age and reach the same level as that in males aged 50 to 69 years. Age-related changes such as increased blood vessel rigidity, decreased interstitial fluid volume, changes in endothelial permeability, or macromolecular composition of interstitial fluid compartments[21-23] might relate to the decreased fluid shift. Regardless of the mechanism, older female blood donors might be more prone to VVR than younger females.

This study underestimated the direct role of blood volume loss in the development of VVR,[20,24] suggesting that strategies for increasing circulating blood volume around the time of donation are ineffective. Several articles, however, have described that consuming water preferentially affects the occurrence of VVR.[25,26] The donors enrolled in this study consumed a mean of 215 mL of water during a mean 19-minute wait before venipuncture. The Hb concentration decreased from 14.4 to 14.3 g/dL during that period, implying that a mean of 29 mL of interstitial fluid shifted to the intravascular space. Consuming about 200 mL of water might be insufficient to elicit an effect, or it might not rapidly influence plasma volume. If an increase in plasma volume is predicted at the time of blood donation, then before donation, individuals should consume a large volume of a beverage with a high sodium concentration and a small amount of sugar, such as the oral rehydration solutions recommended for children with enteritis.[27,28] However, the increase in blood pressure that occurred soon after consuming water might not be a direct effect of an increase in plasma volume, but rather an outcome of stomach wall distension that stimulates the sympathetic tone of blood vessels.[29]

In summary, our findings revealed a rapid shift of interstitial fluid to the intravascular space during blood collection, thus restoring about half of the withdrawn volume by the end of blood collection. The shifted volume is lower in males than in females and tends to decrease with increasing age in females. The direct contribution of blood loss during a blood donation to the development of VVR might be lower in the setting of whole blood donations of 400 mL.

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CONFLICT OF INTEREST

This study received no support in the form of grants, equipment, or drugs. The authors have no conflict of interest regarding this article.

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Residual risk of transfusion-transmitted hepatitis B virus (HBV) infection caused by blood components derived from donors with occult HBV infection in Japan

Rikizo Taira, Masahiro Satake, Shun'ya Momose, Satoru Hino, Yoshiharu Suzuki, Hiroyuki Murokawa, Shigeharu Uchida, and Kenji Tadokoro

BACKGROUND: Nucleic acid amplification testing (NAT) for hepatitis B virus (HBV) during blood screening has helped to prevent transfusion-transmitted HBV infection (TT-HBV) in Japan. Nevertheless, 4 to 13 TT-HBV infections arise annually.

STUDY DESIGN AND METHODS: The Japanese Red Cross (JRC) analyzed repository samples of donated blood for TT-HBV that was suspected through hemovigilance. Blood donations implicated in TT-HBV infections were categorized as either window period (WP) or occult HBV infection (OBI) related. In addition, we analyzed blood from 4742 donors with low antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs) titers using individual-donation NAT (ID-NAT) to investigate the relationship between anti-HBc titer and proportion of viremic donors.

RESULTS: Introduction of a more sensitive NAT method for screening minipools of 20 donations increased the OBI detection rate from 3.9 to 15.2 per million, while also the confirmed OBI transmission rate increased from 0.67 to 1.49 per million. By contrast the WP transmission rate decreased from 0.92 to 0.46 per million. Testing repository samples of donations missed by minipools of 20 donations NAT showed that 75 and 85% of TT-HBV that arose from WP and OBI donations, respectively, would have been interdicted by ID-NAT. The ID-NAT trial revealed that 1.94% of donations with low anti-HBc and anti-HBs titers were viremic and that anti-HBc titers and the frequency of viremia did not correlate.

CONCLUSIONS: The JRC has elected to achieve maximal safety by discarding all units with low anti-HBc and anti-HBs titers that account for 1.3% of the total donations.

The prevalence of hepatitis B virus (HBV) surface antigen (HBsAg) in Japan is slightly higher than the average for developed countries. A recent screening of blood donors, local residents, and school pupils found an estimated national prevalence of HBsAg of 0.71%.[1] However, the prevalence was higher during the 1990s, being 1.5% among first-time blood donors aged in their 40s.[2] Taking into account horizontal transmission and a birth cohort effect, a relatively large cohort with historical HBV infection might persist among older individuals in Japan.

To prevent transfusion-transmitted HBV (TT-HBV) infection, Japanese Red Cross (JRC) blood centers introduced HBsAg screening for all blood donations in 1972. In 1989, antibody to hepatitis B core antigen (anti-HBc) testing was introduced to exclude donations by people with prior HBV infection. Because total elimination of anti-HBc–reactive donations might have seriously reduced the blood supply, donations with high antibody to hepatitis B surface antigen (anti-HBs) titers and those

ABBREVIATIONS: CLEIA(s) = chemiluminescence enzyme immunoassay(s); ID = individual donation; JRC = Japanese Red Cross; LOD = limit of detection; OBI = occult hepatitis B virus infection; PC(s) = platelet concentrate(s); S/CO = signal-to-cutoff ratio; TT-HBV = transfusion-transmitted hepatitis B virus infection; TTI = transfusion-transmitted infection; WP = window period.

From the Blood Service Headquarters and Central Blood Institute, Japanese Red Cross; and the Japanese Red Cross Kanto-Koshin'etsu Block Blood Center, Tokyo, Japan.

Address correspondence to: Masahiro Satake, MD, Japanese Red Cross Central Blood Institute, Tatsumi 2-1-67, Koto-ku, Tokyo 135-8521, Japan; e-mail: m-satake@jrc.or.jp.

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with low anti-HBc titers have been accepted, and only donations with low anti-HBs and high anti-HBc titers were excluded.

In addition to this serologic screening algorithm, the JRC implemented multiplex nucleic acid amplification testing (NAT) for HBV, hepatitis C virus (HCV), and human immunodeficiency virus Type 1 (HIV-1) in 1999.[3] Although NAT has greatly reinforced blood safety regarding TT-HBV infection, 4 to 13 TT-HBV infections continue to arise annually. While some occur as a result of transfusion with blood components obtained during the window period (WP), others arise due to components being derived from donors with occult HBV infection (OBI) defined as detectable HBV DNA in peripheral blood but no detectable HBsAg.[4,5] Although donations from donors with OBI have helped to maintain an adequate blood supply, such donations have also raised a concern about the risk of TT-HBV. Here, we describe the current status of TT-HBV under the NAT screening system as well as problems inherent in the current HBV screening algorithm, especially with regard to OBI-derived blood donations. We also discuss the feasibility of strategies that could increase HBV safety in countries such as Japan with a slightly elevated prevalence of HBV.

MATERIALS AND METHODS

Screening donated blood at JRC blood centers

The JRC blood centers are the only facilities authorized to handle blood collection, processing, testing, and delivery in Japan. Donated blood is screened at these centers for HBsAg, anti-HCV, anti-HIV-1 and -2, anti-human T-lymphotropic virus type 1, anti-*Treponema pallidum*, and human parvovirus B19 antigen. Whereas HBsAg-positive blood is rejected, HBsAg-negative samples are further tested for anti-HBc and anti-HBs (Table 1). Blood with a high anti-HBs titer (≥200 IU/L) is accepted irrespective of the anti-HBc titer and that with a low anti-HBc titer is also accepted irrespective of the anti-HBs titer. Blood with high anti-HBc and low anti-HBs titers (<200 IU/L) is disqualified. All blood had been serologically tested before 2008 using the agglutination method with the initial cutoff for a high anti-HBc titer being a dilution factor of 2^6, which was later revised to 2^5. All agglutination tests were replaced with chemiluminescence enzyme immunoassays (CLEIAs, CL4800 testing system, Fujirebio, Tokyo, Japan) in 2008 and the threshold for anti-HBc positivity is currently a signal-to-cutoff ratio (S/CO) of 12.0. This value was validated as being essentially equivalent to an agglutination titer of 2^5. Blood donations with elevated serum alanine aminotransferase (>60 IU/mL) are also rejected.

TABLE 1. HBV screening algorithm applied at JRC blood centers*

	Anti-HBc titer	
Anti-HBc reactive 4.9% 261,000, *49,000*	Low <2^6(2^5) or S/CO≥1.0 but <12.0	High ≥2^6(2^5) or S/CO ≥12.0
Anti-HBs ≥ 200 IU/L	Accepted 2.04% 108,000, *20,000*	Accepted 1.38% 73,000, *14,000*
Anti-HBs < 200 IU/L	Accepted 1.31% 69,000, *13,000*	Rejected 0.19% 10,000, *2,000*

* HBsAg-negative donations are tested for both anti-HBc and anti-HBs. Dilution factors for anti-HBc titers were applied for agglutination testing. Dilution factors in parentheses were applied between 1997 and 2007. The S/CO ranges are currently used for CLEIA. Ratios (%) of donations for each category are shown (2010 data). Observed number and number per million (italics) of donations are also included.

NAT

Samples that were qualified by the testing algorithm for anti-HBc and anti-HBs described above as well as by HBsAg testing are then screened using NAT. The JRC started NAT in 1999 using a real-time multiplex polymerase chain reaction system with a minipool format that originally comprised 500 samples (Ampli-NAT MPX system, Roche, Indianapolis, IN).[6] The pool size was decreased to 50 in 2000 and to 20 in 2004. The JRC implemented the Roche TaqScreen MPX system for NAT in 2008 with a pool size of 20, but with an approximately threefold increase in sensitivity because of the increased sample volume required for nucleic acid extraction and improvements in reagents. The screening sensitivity of HBV is 650, 260, and 76 copies/mL (50% limit of detection [LOD]; JRC data) for 50- (50p) and 20- (20p) sample pools using AmpliNAT and 20p using TaqScreen, respectively.

A trial screening using individual-donation NAT (ID-NAT) proceeded at the Tokyo Blood Center between December 2010 and May 2011 to verify the distribution of the rate of donations containing HBV DNA relative to anti-HBc titers and the residual TT-HBV risk that could arise from transfusion with blood donations that have low anti-HBc and anti-HBs titers. All available donations with both an anti-HBc titer between 1.0 and 12.0 S/CO and an anti-HBs titer of less than 200 IU/L were screened by ID-NAT using the Roche TaqScreen MPX system with a 50% LOD of 3.8 copies/mL (JRC data). The sensitivity of ID-NAT used in lookback studies (described below) was 13 copies/mL (50% LOD) using AmpliNAT until July 2008 and 3.8 copies/mL (50% LOD) using TaqScreen from August 2008.

Hemovigilance system

The JRC established a hemovigilance system in 1993 and has since collected reports on adverse effects caused by blood transfusion. Through blood screening the JRC obtains information about repeat donors who have recently acquired infection.[7] Their previous donations are evaluated for transfusion-transmitted infection (TTI) risk by considering donation timing and performing ID-NAT on repository samples (lookback studies). If they are judged as harboring a TTI risk, the JRC notifies the relevant facilities that used the component at risk and requests that physicians investigate whether any patient who received a transfusion of the component has acquired the corresponding infection.

The JRC also obtains information about TTI in transfused patients through voluntary reports by physicians who are involved in blood transfusion at medical facilities.[7] Upon receiving such information, the JRC analyzes repository blood samples obtained from implicated donations using ID-NAT. The TTI risk of cocomponents derived from the implicated and previous donations provided by implicated donors is assessed. The JRC notifies the relevant medical facilities of the findings. Implicated blood components are interdicted if they have not yet been used for transfusion.

The JRC headquarters and central laboratory determine the causal relationship between the implicated donation and posttransfusion infection considering patient clinical course, results of virologic analysis including ID-NAT and sequence analysis, serologic viral markers, and donation timing. Even if all repository samples implicated for TTI are verified as being ID-NAT-negative, implicated donors are followed up for repeat donation thereafter for sero- or NAT conversion, because the possibility that the index donation was provided during the ID-NAT WP persists. All processes for lookback studies are defined in national guidelines[8] that describe in detail the test items and timing of testing for donated blood and at-risk patients in addition to the roles of the relevant physicians, blood centers, and blood authority.

Sequence analysis

The HBV genome sequence identity is assessed between implicated repository blood samples and patient samples by sequencing 1550 bp of the alpha region within the HBV pre-S and S regions using a genetic analyzer (ABI 3130XL, Life Technologies Japan, Tokyo, Japan). When the viral load is too low to sequence, viral nucleic acid is further extracted from larger plasma volumes if the accompanying plasma bag is available. When findings are ambiguous, HBV obtained from donor or patient samples is cloned, amplified, and sequenced.

Estimation of current risk of TT-HBV

Although universal pre- and posttransfusion testing of patient samples for TTI has been recommended, the likelihood that all transfused patients undergo this evaluation is low. Moreover, the JRC hemovigilance system described above is voluntary. Therefore, TTI might be underreported to JRC blood centers. The exact amount of TT-HBV infections that could occur under the current screening system must be defined to assess novel TT-HBV–mitigating strategies. This study therefore reevaluated the current risk of TT-HBV infection based on data obtained under current system.

The projected number of ID-NAT–positive donations derived from OBI donors was calculated using the ID-NAT positivity rate obtained in the ID-NAT trial screening described above and the number of donations with low anti-HBc and anti-HBs titers. The additional WP yield in donations determined by ID-NAT was calculated based on rates of detection of recently infected donors.[9,10] Assuming that the frequency of donation is constant at any time during the presymptomatic phase of acute infection, the yields by tests for an infection marker are in direct proportion to the length of time during which each test gives a yield. The potential ID-NAT yield (screening NAT negative) was calculated herein by multiplying the screening NAT yield by the ratio of the interval between ID-NAT detection and 20p-NAT detection (11.2 days) to that between 20p-NAT detection and HBsAg detection (9.7 days). The interval covered by each NAT strategy (11.2 and 9.7 days) was calculated using the value for the detection limit of each test (3.8, 76, and 1000 copies/mL for ID-NAT, 20p-NAT, and CLEIA detection, respectively) and the doubling time of HBV in human peripheral blood (2.6 days).[9,11] The number of donations that could appear in the ID-NAT–negative WP was similarly calculated separately for each component type taking into account both the interval between 1 copy/bag and ID-NAT detection deduced from the mean plasma volume of each component type and the number of each component issued to hospitals.

We estimated the number of TT-HBV infections with reference to our previous systematic lookback study.[7] The infectivity of ID-NAT–positive and screening NAT-negative components was calculated in that study as being 3% (95% confidence interval [CI], 0%-17.2%, n = 33) and 50% (95% CI, 28.2%-71.8%, n = 22) for OBI- and WP-derived components, respectively. The incidence rate for TT-HBV infections was thus obtained by multiplying the number of estimated at-risk donations deduced using the above method by the infection rates (0.03 or 0.5).

Statistical analysis

Data were statistically analyzed using computer software (SSRI for Windows, Excel Statistics Version 8, Social Survey Research Information Co. Ltd, Tokyo, Japan). Significance was determined using the chi-square test except for associations between total viral load in the components and alanine aminotransferase (ALT) levels in patients that were evaluated using the Mann-Whitney U test.

RESULTS

Reports of possible TT-HBV infections

The JRC blood centers received 789 reports of possible TT-HBV infections between 2001 and 2010 (Fig. 1). The number of such reports obviously increased in 2004 and 2005 because a nationwide systematic retrospective study started in 2004 that also identified patients with TT-HBV infection that would have previously been unrecognized. Causality was investigated in all but two of these possible TT-HBV infections. The possibility of TT-HBV infection was precluded in 97 (12.3%) of the 789 reported patients without testing repository samples based on evaluation of the patient's clinical course and the transfusion setting for each. For all of the remaining patients, repository samples were tested serologically and by ID-NAT to detect the HBV genome. Of the 789 initial reports, 98 (12.4%) were determined to be TT-HBV infections after the introduction of 50p-NAT. The HBV sequence identity was established between donor and recipient in 88 of these cases, and TT-HBV was determined considering other HBV markers and the clinical setting in the remaining 10. An HBV genome was not detected in repository samples for 587 (74.4%) potential TT-HBV infections. Although HBV was detected in four repository samples, HBV sequence identity was not confirmed between donors and recipients. Forty-two (43%) of the established TT-HBV infections were discovered through lookback studies that were started based on risk information provided by JRC blood centers. The remaining 56 (57%) were initially recognized by physicians at medical facilities. The number of established TT-HBV infections ranged from 4 to 13 per year between 2006 and 2010.

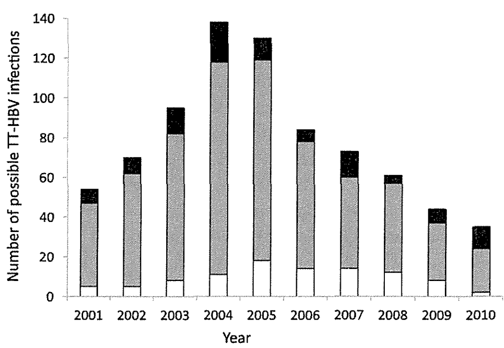

Fig. 1. Annual number of potential TT-HBV infections. (□) Patients in which possibility of TT-HBV was excluded (n = 97, 12.3%) without testing repository samples. (■, ▨) Patients in whom HBV DNA was identified or not, respectively, in the repository samples corresponding to a donation from the donor of the implicated blood components. Four patients are not included as HBV DNA sequence identity was not established.

Infection status of donors implicated in TT-HBV infection

The sensitivity of NAT screening improved through the three phases described above (50p-AmpliNAT, 20p-AmpliNAT, and 20p-TaqScreen). With the increased sensitivity of 20p-TaqScreen, the NAT yield of OBI donations increased from 3.9/million to 15.2/million, whereas the yield of WP donation decreased from 13.2/million to 5.7/million (Table 2). This was caused by the simultaneous introduction of CLEIA in 2008 for serologic screening including HBsAg detection, which effectively shortened the period that could be covered by 20p-NAT.

The established TT-HBV infections that occurred during each period were categorized based on the presence or absence of the HBV genome in the implicated component (that is, ID-NAT positive or negative) and the infection status of the donation (WP related [anti-HBc nonreactive] or OBI related [anti-HBc reactive]). Table 2 also shows the numbers of established TT-HBV infections associated with each group during each period. Figure 2 shows the incidence (per million donations) of estab-

TABLE 2. NAT yield and number of TT-HBV infections relative to three phases of screening NAT*

Screening system	50p-AmpliNAT	20p-AmpliNAT	20p-TaqScreen
Duration of screening period	Feb. 2000– Jul. 2004 (4.5 year)	Aug. 2004– Jul. 2008 (4.0 year)	Aug. 2008– Mar. 2010 (1.67 year)
Sensitivity of screening NAT (copies/mL)†	650	260	76
Sensitivity of ID-NAT used for lookback study (copies/mL)†	13	13	3.8
Number of donations tested	24,702,784	19,513,054	8,746,037
Confirmed WP donations (/million)	473 (19.1)	258 (13.2)	50 (5.7)
Confirmed OBI donations (/million)		76 (3.9)	133 (15.2)
Number of donations causing established HBV transmission			
ID-NAT–negative WP	5	6	1
ID-NAT–positive WP	28	12	3
ID-NAT–negative OBI	4 (1)‡	1 (0)‡	2 (1)‡
ID-NAT–positive OBI	13 (1)‡	12 (1)‡	11 (5)‡

* Yields by ID-NAT trial conducted from December 2010 are not included in the table.
† 50% LOD.
‡ Numbers of donations with anti-HBs of greater than 10 mIU/mL.

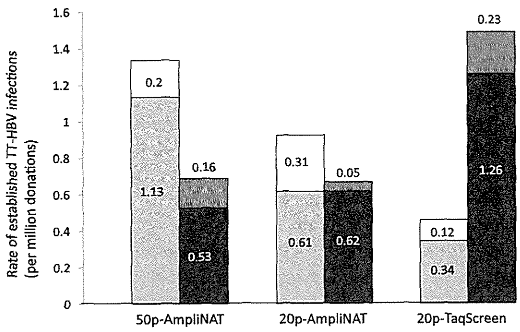

Fig. 2. Number of established TT-HBV infections grouped according to pool-based NAT screening systems. See Table 2 for intervals when indicated NAT systems were applied, sensitivities of NAT systems used, and actual yields for each category at each interval. (□, □) Infections caused by transfusion with ID-NAT–negative and -positive WP-derived components, respectively. (■, ■) Infections caused by transfusion with ID-NAT–negative and -positive OBI-derived components, respectively.

lished TT-HBV infections relative to the three periods. The rate of infections caused by transfusion with a WP-derived component notably decreased with increasing NAT sensitivity, but that caused by transfusion with OBI-derived components rather increased despite the increased NAT sensitivity. Current NAT screening protocols indicated that TT-HBV infections occur more frequently due to transfusion with OBI- than with WP-related components (1.49/million vs. 0.46/million donations; Fig. 2). Nine TT-HBV infections occurred as a result of transfusion with blood components containing more than 10 mIU/mL anti-HBs during the past decade (Table 2). Two of them were caused by donations with negative ID-NAT.

The number of TT-HBV infections caused by transfusion with ID-NAT–negative components accounts for 15% (2/13) and 25% (1/4) of OBI- and WP-related TT-HBV infections, respectively, according to the current NAT system (Table 2). These infections involving ID-NAT–negative donations were determined as TTI by analyzing repository blood samples obtained before the index donation and/or by following up with the implicated donors after the index donation. Details of the clinical course of a typical TT-HBV infection caused by ID-NAT–negative OBI-related blood components are shown in Tables 3 and 4.

Impact of blood product on transmission rate

Table 5 shows the numbers of implicated donations by either ID-NAT negative or positive for groups categorized by the type of component and WP/OBI status. During the past decade, ID-NAT–positive donations have caused 79 TT-HBV infections. Transfusion with red blood cells (RBC), fresh-frozen plasma (FFP), and platelet concentrate (PC) was associated with infections in 42, 22, and 15 of them, respectively. Of 19 TT-HBV infections associated with ID-NAT–negative donations, 2, 4, and 13 were caused by transfusion with RBCs, FFP, and PC, respectively. Transfusion with blood components containing a larger plasma volume (FFP and PC, but not RBCs) caused more frequent TT-HBV infections among patients who received ID-NAT–negative donations (17/19, 89%) than among those who received ID-NAT–positive donations (37/79, 47%; $p < 0.01$), which could be a reflection of the plasma volume effect on infectivity.

Table 5 also shows that if ID-NAT had been implemented during the screening, 81% of established TT-HBV infections would have been avoided. The introduction of ID-NAT would have been the most (95%) and least (54%) effective for preventing TTI caused by RBC- and PC-related transfusions, respectively. Under the current 20p-TaqScreen system, 75 and 85% of TT-HBV infections arising from WP and OBI donations, respectively, are ID-NAT positive and will be interdicted by ID-NAT. In particular, the effect of ID-NAT will be 100% for OBI-related infections caused by RBC transfusion.

Outcomes of patients with TT-HBV infection

ALT levels during TT-HBV infection were determined in 68 transfusion recipients who developed TT-HBV infection. Table 6 shows the maximal ALT values relative to WP or OBI donations and ID-NAT–positive or -negative donations. Almost half (47%, 32/68) of the patients had maximal ALT values of more than 1000 IU/L. The proportion of patients with maximal ALT of more than 1000 IU/L was greater in OBI-related (61%, 19/31) than in WP-related (35%, 13/37; $p < 0.05$) infections. Total viral load in the implicated components did not significantly differ between patients with ALT values above and below 1000, which was true for both WP- and OBI-related infections. Although barely insignificant, total infused viral load tended to be lower in OBI- than in WP-related patients among groups with maximal ALT values of more than 1000.

Three patients with TT-HBV infection died of fulminant hepatitis after the introduction of NAT. One was caused by transfusion with PC derived from an ID-NAT–negative, WP-related donation (Genotype A with wild type precore region). The other two developed hepatitis after transfusion with RBCs derived from ID-NAT–positive, OBI-related donations (Genotype B with a G1898A precore mutation and Genotype C with a G1896A precore mutation).

ID-NAT screening trial in donations with low anti-HBc and anti-HBs titers

During a 6-month ID-NAT trial, 4742 (0.74%) of 640,628 blood donations at the Tokyo Blood Center with low anti-

HBc and anti-HBs titers were analyzed by ID-NAT. The number of donations analyzed by ID-NAT decreased as the anti-HBc titer increased (Fig. 3). HBV DNA was detected in 92 (1.94%) of the 4742 donations. Figure 4 shows the frequency of ID-NAT–positive donations relative to the anti-HBc titer. The frequency of ID-NAT positivity for HBV did not correlate with the anti-HBc titer and did not tend to increase with an increasing anti-HBc titer. The proportions of anti-HBs–positive (>10 mIU/mL) donations among those that were ID-NAT positive and negative were 77 and 75%, respectively, and did not significantly differ. The proportion of anti-HBs–positive donations increased with increasing anti-HBc S/CO values among ID-NAT–negative donations (67.5, 82.0, and 87.5% for anti-HBc S/CO 1.0-3.9, 4.0-7.9, and 8.0-11.9, respectively; $p < 0.01$ between any two groups). The frequency of ID-NAT positivity between males (1.8%) and females (2.4%) did not significantly differ. Eighty-three (90.2%) of the 92 ID-NAT–positive donors were at least 50 years of age. Fifteen had a viral load of less than 100 copies/mL, whereas quantitative NAT could not detect HBV DNA loads in samples from the remaining 77. The distribution of HBV genotypes among the ID-NAT–positive donations did not differ from that among the general Japanese population: Genotypes A, B, C, and D, n = 1, 24, 45, and 1, respectively (21 were undetermined).

TABLE 3. Representative TT-HBV infection caused by OBI-derived, ID-NAT–negative blood component: clinical course of a patient who received an implicated blood component.

Date	Clinical events and test results
Nov. 10, 2008	Surgery to treat head injury*
	HBsAg negative, anti-HBc negative, HBV DNA negative, preoperatively
	Transfused until Nov. 20 with 21 RBC units, 5 PCs, and 11 FFP† including one derived from the donation of Mar. 27, 2008, shown in Table 4
Mar. 05, 2009	AST 15, ALT 32
Mar. 25, 2009	AST 517, ALT 1273
Mar. 30, 2009	AST 1312, ALT 3110, HBsAg positive, IgM-anti-HBc positive
	Reported to JRC blood center
Mar. 31, 2009	AST 695, ALT 2396
Apr. 01, 2009	HBsAg negative, anti-HBs positive, HBV DNA positive

* Recipient was a teenage boy who was injured in a traffic accident.
† HBV DNA was not detected based on ID-NAT for the repository samples from these 37 blood components transfused. These results were obtained in the first lookback study performed in April 2009.

Estimation of current TT-HBV risk in Japan

From the frequency of ID-NAT–positive (1.94%) donations among those with low anti-HBc and anti-HBs titers (69,000/year or 13,000/million; see Table 1 and below), we calculated that 1339/year or 252/million donations should be ID-NAT positive among screening NAT-negative donations with low anti-HBc and anti-HBs titers. Using an infectivity rate of 3%[7] among components derived from OBI donations that were screening NAT negative and ID-NAT positive, we calculated that 40/year or 7.6/million OBI-related TT-HBV infections should arise. If TT-HBV infections related to OBI-derived ID-NAT-negative donations are taken into account, then the total number of TT-HBV infections should be 47/year or 8.9/million. This estimate was based on the observation that TT-HBV infection caused by ID-NAT–negative components during the

TABLE 4. Representative TT-HBV infection caused by OBI-derived, ID-NAT–negative blood component: HBV marker profile of blood donor responsible for the outcome shown in Table 3

Date of donation	Date of testing	Test results
Oct. 17, 2007*	Oct. 17, 2007 (screening)	Pool NAT negative, anti-HBc 2^4 (negative), anti-HBs negative
	Feb. 24, 2010 (repository sample tested in second lookback study)	ID-NAT negative
Mar. 27, 2008† (index donation)	Mar. 27, 2008 (screening)	Pool NAT negative, anti-HBc 2^4 (negative), anti-HBs negative
	Apr. 7, 2009 (repository sample tested in first lookback study)	ID-NAT negative (negative result reported to corresponding facility)
Feb. 05, 2010‡	Feb. 05, 2010 (screening)	Pool NAT negative, anti-HBc 15.4 S/CO§ (positive), anti-HBs negative
	Feb. 10, 2010 (donated blood sample tested in second lookback study)	ID-NAT positive (high probability of TT-HBV infection in Patient A reported to corresponding facility)

* RBCs derived from this donation were transfused to an HBsAg-negative patient. Patient continued to be HBsAg-negative until May 2008 when he died.
† FFP derived from this donation was transfused to patient shown in Table 3. Cocomponent (RBCs) processed from this donation was transfused to a patient who died of the primary disease soon after transfusion. Whether TT-HBV occurred remains unknown.
‡ This donation was rejected due to anti-HBc seroconversion and a second lookback study was conducted on the donation of October 17, 2007.
§ Because of very low HBV load in donated blood sample of February 5, 2010, HBV sequence was assessed in donor blood only at 193 bp (Nucleotides 475-667) of S region. HBV sequence in that region was identical except for nt. 654 between the blood samples from donor and patient on April 01, 2009.

TABLE 5. Blood components implicated in established TT-HBV infection*

	ID-NAT+/ID-NAT–						
	WP transmissions established			OBI transmissions established			
Screening period	RBCs	FFP	PC	RBCs	FFP	PC	
50p-AmpliNAT	15/0	6/1	7/4	5/0	5/1	3/3	
20p-AmpliNAT	8/2	0	4/4	7/0	5/1	0	
20p-TaqScreen	2/0	0	1/1	5/0	6/1	0/1	
Total	25/2	6/1	12/9	17/0	16/3	3/4	
	WP plus OBI				All components		
	RBCs	FFP	PC	FFP + PC	WP	OBI	Total
50p-AmpliNAT	20/0	11/2	10/7		28/5	13/4	41/9
20p-AmpliNAT	15/2	5/1	4/4		12/6	12/1	24/7
20p-TaqScreen	7/0	6/1	1/2		3/1	11/2	14/3
	100%	86%	33%		75%	85%	82%
Total	42/2	22/4	15/13	37/17	43/12	36/7	79/19
	95%	85%	54%		78%	84%	81%

* Ratios (%) in the two bottom rows represent rates of ID-NAT–positive events or effectiveness of ID-NAT implementation.

TABLE 6. Maximal values for ALT in patients with TT-HBV infection and total viral load contained in implicated components

	ALT	
	<1000	>1000
WP* (n‡)	24 (7)§	13
OBI† (n‡)	12 (2)§	19
Total viral load (copies/bag)ǁ		
WP		
n‡	21	10
Min	40	100
Max	260,000	560,000
Median	1,400	9,100
Mean	20,460	74,790
OBI		
n‡	8	16
Min	60	40
Max	6,240	19,200
Median	630	1,470
Mean	1,440	3,750
ID-NAT status		
Positive‡	30	28
Negative‡	6	4
Component types		
RBCs‡	19	16
FFP‡	7	13
PC‡	10	3

* Patients transfused with WP-related components include 11, 8, and 18 patients with malignant hematologic disorder, solid tumor, and others, respectively.
† Patients transfused with OBI-related components include 7, 11, and 13 patients with malignant hematologic disorder, solid tumor, and others, respectively.
‡ Numbers of patients.
§ Numbers in parentheses, patients with maximal ALT values of less than 100 IU/L.
ǁ Total viral load was calculated using viral concentrations in implicated donations and average plasma volume of each component type. When viral load was less than 100 copies/mL, total viral load in the component was calculated assuming that viral concentration is logarithmically distributed between 1 and 100 copies/mL.

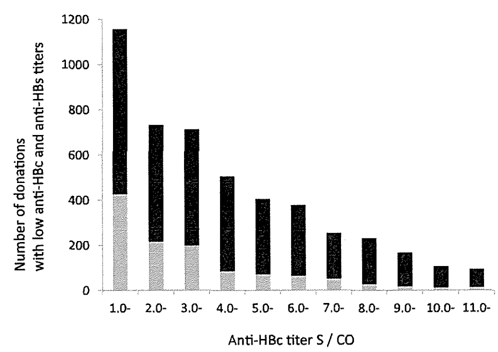

Fig. 3. Number of donations screened by ID-NAT trial categorized by anti-HBc titer. All donations tested had low anti-HBc (S/CO 1.0-11.9) and anti-HBs (<200 IU/L) titers and were qualified serologically based on algorithm applied at JRC blood centers. Donations verified to be ID-NAT-positive were disqualified. (■, □) donations with anti-HBs titers of more than and not more than 10 mIU/mL, respectively.

20p-TaqScreen period accounted for 15% (2/13) of all OBI-related infections (Table 2).

We estimated how many more WP-related TT-HBV infections would be prevented by introducing ID-NAT. The current screening NAT yield (30 donations/year or 5.7/million, Table 2) was multiplied by the ratio of the interval between ID-NAT and 20p NAT detection (11.2 days) to that between 20p NAT and HBsAg detection (9.7 days). We then deduced that 34.6/years or 6.6/million more viremic donations would be captured by ID-NAT. The number of ID-NAT–negative WP donations was calculated separately for each component type. Based on the plasma volume of each component (20, 200, 240, 450, and 120 mL for RBCs, PC, FFP-3, FFP-5, and FFP1.5,

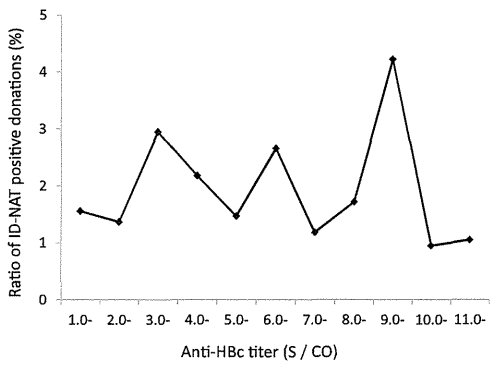

Fig. 4. Ratios (%) of ID-NAT–positive donations with low anti-HBc and anti-HBs titers relative to anti-HBc titer.

respectively), the deduced intervals between 1 copy/bag and ID-NAT detection were 16.3, 24.9, 25.5, 28, and 23 days, respectively. The ratio of the number of those components issued to hospitals is 6.3:2.2:2.1:0.7:0.1. The incidence of ID-NAT–negative WP donations calculated from these data was 59.5/year or 11.4/million. Adding ID-NAT–positive WP donations (34.6/year or 6.6/million), current risk related to WP donations amounts to 94.1/year or 18.0/million. The effect of ID-NAT on the reduction of all WP donation would be 37% (6.6/18.0). If the infectious risk (50%) of ID-NAT–positive, screening NAT-negative WP-related components is also applied to ID-NAT–negative WP-related components, the total number of WP-related TT-HBV infections would be 47.1/year or 9.0/million. Together, these estimates for WP- and OBI-related TT-HBV infections indicate that 94.1/year or 17.9/million TT-HBV infections are likely to occur in Japan.

DISCUSSION

Infection with HBV results in a wide spectrum of clinical manifestations ranging from asymptomatic liver dysfunction with only slightly elevated transaminase levels or acute self-limiting hepatitis to chronic hepatitis that in some patients progresses to cirrhosis, liver failure, or hepatic cell carcinoma. In rare cases, HBV infection can cause fulminant hepatitis that is associated with high mortality. Fulminant hepatitis in Japan is frequently associated with primary infection by HBV carrying precore or core-promoter mutations.[12,13] These HBV mutants are frequently found among chronic HBV carriers[14,15] who typically have an anti-HBc–positive serostatus. To prevent fulminant hepatitis arising as a result of blood transfusion,[16] the JRC incorporated anti-HBc testing into blood screening in 1989.

The agglutination method had been used for all serologic testing at JRC blood centers before 2008. Although this method was somewhat insensitive to HBsAg, it could semiquantify anti-HBc. Thus, the cutoff point for the anti-HBc titer had been set at 2^6, and donations with an anti-HBc titer of at least 2^6 and an anti-HBs titer of less than 200 mIU/mL were disqualified.[17] Although this anti-HBc testing had essentially prevented transfusion-transmitted fulminant hepatitis since 1989,[18] reports of fulminant or acute severe hepatitis continued for an additional 7 years. These conditions were attributed to transfusion with components with a 2^5 anti-HBc titer.[19] Consequently, the JRC lowered the anti-HBc cutoff from 2^6 to 2^5 in 1997. The agglutination method for serologic screening was replaced in 2008 with CLEIAs, which can also semiquantify anti-HBc. The policy described above is maintained in the algorithm for HBV screening with CLEIA; the range defined as a low anti-HBc titer includes S/CO values between 1.0 and 11.9, and donations with anti-HBc S/CO values within this range are currently accepted.

The highly sophisticated strategy of multiplex NAT was designed to decrease the incidence of TTI. Implementing HBV NAT into blood screening was important in Japan mainly because of the unsatisfactory sensitivity of the standard agglutination method to HBsAg. The JRC implemented multiplex NAT targeting HBV DNA, HIV RNA, and HCV RNA during 1999[6] and improved the sensitivity of the test at three points. The 98 infections described herein had been confirmed as TT-HBV since the introduction of 50p-AmpliNAT in 2000. The HBV genome was not detected in donor repository samples of 587 (74.4%) suspected TT-HBV infections. The JRC has informed the appropriate physicians of the ID-NAT results of viral detection that imply a low or high probability of TT-HBV infection.

In parallel with the increase in the screening NAT sensitivity, the incidence of WP-related TT-HBV infection has decreased as predicted, whereas that of OBI-related TT-HBV infection has not decreased (Fig. 2). To explain the increasing number of OBI-related TT-HBV infections, the increase in the sensitivity of NAT used in JRC laboratories for retrospective studies might have helped to identify TT-HBV infections, thus sustaining the number of OBI-related TT-HBV infections despite improvements in screening NAT sensitivity.[4]

This consideration could encourage the speculation that most of the 587 infection reports that had been excluded from established TTI (Fig. 1) based on negative results from repository samples might have been confirmed as TT-HBV had more sensitive NAT and a larger sample volume been analyzed. With regard to this notion, the outcomes of recent hemovigilance for TT-HBV are described below. During the 20p-Taqscreen period, the JRC received 61 clinical reports of possible HBV-TTI. Seventeen were determined as TTI, among which, three repository samples were ID-NAT negative. Historical HBV infection was confirmed in 10 patients by retesting pretransfusion samples. Results from HBV tests of posttransfusion samples from five patients were false positive. The possibility of TT-HBV was ruled out in two patients related to ID-NAT–negative donations because repeated blood donations from two of two and three of three implicated donors were not sero- or NAT-converted. The remaining 27 patients related to ID-NAT–negative donations are inconclusive for TTI as follow-up studies have not yet been completed. Some of the 587 reported infections had been confirmed to be associated with passive anti-HBc transfer from infused components. Thus, it is unlikely that a considerable proportion of the infections excluded from the TTI category were real TT-HBV infections.

Among 19 patients with TT-HBV infections associated with ID-NAT–negative donations, 17 (89%) of them were caused by transfusion with FFP or PC that contained a larger plasma volume (120 to 450 mL) than RBCs (20 mL). In contrast, 37 (47%) were caused by FFP or PC among 79 infections associated with ID-NAT–positive donations (Table 5). This finding suggests that because HBV infectivity is extremely high, the relationship between infectivity and plasma volumes contaminated with HBV could only be established in the era of ID-NAT screening when the viral load in the donation is low enough to escape ID-NAT screening. This might explain why we could not previously establish such a relationship using viral loads around the sensitivity of the pool-based NAT system or serology.[7] If ID-NAT is introduced as routine screening, it will prevent 75 and 85% of WP- and OBI-related infections. In particular, all RBC-related TT-HBV could be prevented because of the small plasma volume involved. The finding also suggests that novel viral reduction technologies[20,21] could be an attractive strategy to decrease the incidence of TT-HBV because these technologies are presently more applicable to FFP or PC than to RBC.

The maximal ALT levels of patients with TT-HBV infection showed that transfusion with components harboring an extremely low HBV load that escaped NAT screening is not necessarily associated with mild clinical illness. This seems particularly true for OBI-related infection (Table 6). The frequency with which transfusion causes severe hepatitis (i.e., ALT > 1000) is significantly higher for OBI- than for WP-derived components. Moreover, OBI-derived components tend to cause severe hepatitis despite lower total viral loads compared with those in the WP-derived components. These findings should be further substantiated by analyzing samples from patients that are regularly obtained after transfusion because most of the maximal ALT values described in this article were found after occasional sampling.

Three patients died of TT-HBV fulminant hepatitis caused by transfusion with blood that had escaped NAT screening. Two of them were notably caused by transfusion of OBI-derived RBCs, and the other was caused by an ID-NAT–negative WP donation. Although a larger plasma volume might generally be required to establish TT-HBV infection under the NAT screening system, plasma volume or the total infused viral load might not be determining factors in fulminant hepatitis. Although viral genome mutations such as those in precore or core-promoter regions are frequently associated with the development of fulminant hepatitis in Japan,[12,13] other crucial factors have not clearly been demonstrated despite considerable investigation.

The JRC accepted 5.3 million donations in 2010, of which 4.9% (261,000) was anti-HBc reactive (Table 1), 0.19% (10,000) was rejected because of high anti-HBc and low anti-HBs titers. Another 3.4% (182,000) was accepted because of high anti-HBs titers (≥200 IU/L). The notion that blood components with an anti-HBs titer of more than 100 IU/L are not infectious is generally accepted.[22-25] The relationship between anti-HBs titer and TT-HBV infection will be discussed elsewhere (manuscript in preparation). Importantly, 1.3% of donations (69,000) with low anti-HBc and anti-HBs titers were accepted, and this category included all donations to which OBI-related TT-HBV infections were attributed. Our ID-NAT trial verified that 1.94% of the donations with low anti-HBc and anti-HBs titers were HBV DNA positive.[26] Accordingly, an estimated 1339/year or 252/million viremic OBI donors and 47/year or 8.9/million TT-HBV infections caused by OBI-derived components would be missed by the current screening algorithm. When estimates for WP-related TT-HBV infections are included, the calculated number of TT-HBV infections was 94.1/year or 17.9/million. Whole blood withdrawn from donors in Japan is split into RBCs and FFP, and the total number of components processed averages 23% more than the number of donations. However, because of outdating and rejection by testing or processing problems, the number of components finally issued by JRC becomes almost the same as the number of donations. Therefore, the calculated number of TT-HBV infections was not significantly influenced by the issue of splitting.

The considerable discrepancy between the estimated and established TT-HBV incidence per million (8.9 to 1.49 and 9.0 to 0.46 for OBI-related and WP-related infections, respectively) might be due to the following factors. A clinical manifestation of HBV infection is often unclear in patients transfused with blood components harboring a low viral load and low proliferative ability. Physicians might thus be likely to overlook infection under such circumstances. Medical practitioners are not compliant with national guidelines for lookback investigations. Indeed, only 30% to 40% of transfused patients were reportedly traced for TTI even after the guidelines were established.[27] A considerable proportion of patients who receive blood transfusions die before TTI evaluation.[28] In fact, when we inquired about the outcomes of transfusions with components containing verified HBV at medical facilities, 99 (42%) of 238 patients who had been transfused with such components had already died (JRC data from 2009 to 2010). The transmissibility of ID-NAT–positive donations might require reevaluation because of the low numbers of patients analyzed in the previous study[7] (30 and 22 for OBI- and WP-related cases, respectively). The fact that a large proportion of elderly patients are immune to HBV due to prior infection might also contribute to the low figure for established TT-HBV and, finally, anti-HBs in cotransfused components neutralizes HBV. Classified WP donation that is anti-HBs positive and could be attributed to possible vaccine breakthrough infection or anti-HBc–negative chronic OBI could also be a factor influencing infectivity. However, we have not encountered any implicated WP donations with anti-HBs among established TT-HBV infections.

Because of the high probability of a residual risk of TT-HBV, novel strategies that reinforce the safety of blood components but do not damage the blood supply should be implemented. Transfusion with ID-NAT–negative infectious components currently cause 15 and 25% of OBI- and WP-related TT-HBV infections, respectively (Table 2), and screening with ID-NAT would interdict 85 and 75% of these infections, respectively (Table 5). With respect to this, the ID-NAT screening of only donations with low anti-HBc and anti-HBs titers that are currently qualified has been suggested.[29] However, screening with ID-NAT might not be as effective as expected. For example, the variability in viral load in individuals with OBI might allow persistent OBI-related TT-HBV infection; some individuals might have an intermittently elevated viral load.[30-33] Such donations could be identified as HBV positive only when the viral load exceeds the detection threshold of ID-NAT screening. Alternatively, the detection of intermittent viremia might reflect the stochastic phenomenon inherent in NAT technology, particularly at very low viral concentrations. Moreover, one report describes a donor in whom viral load increased in blood samples over a period of several years.[34] Nine among 48 blood donations from this donor were ID-NAT positive, and two of four ID-NAT–positive and three ID-NAT–negative blood transfusions had caused TT-HBV infections. The diverse fluctuation of viremia described above has supposedly hindered the efficient detection of viremic donations by pool-based NAT screening,[35] which is predictable even in the event of ID-NAT screening. Table 5 shows that ID-NAT is not sensitive enough in 16% of established OBI-related transmission events although most of those events are caused by FFP or PC transfusions and ID-NAT screened RBC transfusions are relatively safe. Moreover, although viremia is considered undetectable in most individuals with OBI, this assumption might be dependent on the sensitivity of the NAT used; a considerable number of donations might have viremia with a viral load below the ID-NAT detection limit.

Another strategy that might increase the safety of OBI-derived donations could be to accept only those OBI-derived donations with a profile that is safer than the current standards, if such a profile can be found and systematically applied. We initially expected to find that OBI donations with a very low anti-HBc titer would be safer based on ID-NAT. However, the finding from the ID-NAT trial was that the frequency of viremia does not correlate with anti-HBc titers in the range of S/CO 1.0 to 11.9. Therefore, we concluded that the risk of TT-HBV infection will not be mitigated by implementing a strategy that qualifies only donations with very low anti-HBc titers such as S/CO between 1.0 and 3.0.

We speculated during 2003 that more than 4% of donations would be disqualified if the anti-HBc cutoff were set at 2^1, that is, if all donations with low anti-HBc and anti-HBs titers are rejected. We thought that the loss of so many donations would cause catastrophic damage to the blood inventory and thus that cutoff was not implemented. However, based on current data, the number of donations received in 2010 with low anti-HBc and anti-HBs titers was 69,000, which accounts for 1.31% of all donations in Japan. Given this ratio, we consider that to eliminate all donations with low anti-HBc and anti-HBs titers is feasible. We verified that severe hepatitis is caused more often by OBI- than WP-derived blood. The fact that two patients died of fulminant hepatitis related to OBI-related donations is also serious. Rejecting this category of donations would eliminate nearly all those harboring a risk of OBI-related infection.[26] However, a slight, but distinct risk of TT-HBV infection might persist because a small fraction of OBI donors have an anti-HBc titer of less than 1.0 S/CO, and these donors as well as NAT WP donors present a TT-HBV risk.[36] A committee of the Ministry of Health, Labour and Welfare of the Japanese government has just discussed and authorized the implementation of a new policy in which all donations with low anti-HBc and anti-HBs titers would be rejected.

In conclusion, ID-NAT screening of donations with low anti-HBc and anti-HBs titers revealed that nearly 2% of these donations were associated with low-level viremia and that viremia was identified over the entire range of anti-HBc titers. Importantly, anti-HBc titer did not correlate with the frequency of viremia. The elimination of all donations with low anti-HBc and anti-HBs titers would be important to any strategy aimed at preventing OBI-related TT-HBV infections in countries such as Japan that have a slightly elevated HBV prevalence in blood donations. If this strategy is implemented, the only acceptable donors with OBI in Japan will be those with high anti-HBs titers (≥200 IU/L).

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CONFLICT OF INTEREST

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