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TRANSFUSION

CASE REPORT

Transfusion-transmitted HBV infection with isolated anti-HBs-positive blood

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Abstract

Background: Hepatitis B virus (HBV)-positive individuals with isolated anti-HBs are found among HBV vaccine recipients and healthy blood donors with no vaccination history. HBV infectivity from blood transfusions derived from such individuals remains unclear.

Case Presentation: A male patient who received transfusion with blood negative for individual donation-NAT, HBsAg and anti-HBc but weakly positive for anti-HBs developed typical transfusion-transmitted (TT)-HBV with anti-HBc response. The responsible blood donor was a frequent repeat donor showing a marked increase in anti-HBs titer without anti-HBc response 84 days after index donation. Test results for his past donations showed transient viremia with very low viral load and fluctuating low-level anti-HBs. The HBV vaccination history of this donor was unknown.

Discussion: Anti-HBs and anti-HBc kinetics of the donor suggest a second antibody response to new HBV challenge, representing a vaccine breakthrough case. On the other hand, transient low-level viremia and fluctuating anti-HBs in the test results of past donations suggested chronic occult HBV infection with isolated anti-HBs.

Conclusion: Whatever the basic infection state, blood donors with isolated weak anti-HBs may include a small population with a risk of causing TT-HBV. Identifying individuals harboring such TT-HBV risk among individuals positive only for anti-HBs is difficult under current screening strategies. Active surveillance for the occurrence of TT-HBV with blood positive only for anti-HBs is necessary.

KEYWORDS

anti-HBc, anti-HBs, NAT, OBI, vaccination

1 | INTRODUCTION

Blood donors harboring a risk of transfusion-transmitted HBV infection (TT-HBV) can be broadly categorized into two groups: donors within the window period of acute infection and donors in a state of occult HBV infection (OBI).[1,2] Blood included in the former category is detectable by the sensitive nucleic acid amplification (NAT) technique, although very low viral loads can still be undetectable by the NAT.[3] Blood in OBI sometimes

exhibits an extremely low viral load that is undetectable even with highly sensitive individual donation-NAT (ID-NAT), but most such cases could be detected by positivity for antibody to HBV-core antigen (anti-HBc).[4]

Japanese Red Cross (JRC) Blood Centers deal with a nationwide blood program from blood procurement to blood product delivery. They have been conducting HBV serological tests for anti-HBc and antibody to HBV-surface antigen (anti-HBs), as well as for HBV-surface antigen (HBsAg). Anti-HBc-positive blood were acceptable for anti-HBs titers ≥200 mIU/mL. These tests were conducted using CL4800 (Fujirebio) up to 2018 and have been performed by Architect (Abbott) since 2019. They also introduced ID-NAT (Procleix Ultrio Elite Assay with Panther system; Grifols) in 2014. Such screening strategies have greatly contributed to decreasing TT-HBV in Japan to an annual frequency of less than one case. We report herein a case of TT-HBV caused by the transfusion of blood that appeared negative for all of ID-NAT, HBsAg, and anti-HBc, but was positive for anti-HBs.

2 | CASE PRESENTATION

A man in his 60s received multiple blood transfusions during a great vessel operation in December 2019. He had not been in an immunocompromised condition before transfusion and his pre-transfusion blood sample had been negative for HBV NAT, HBsAg, anti-HBc and anti-HBs. Regular testing of blood from the patient on day 72 post-transfusion revealed positivity for HBV-DNA with a viral load >1.7E+08 IU/mL, and liver injury was noted on day 121 in the form of elevated

TABLE 1 Test results for the blood donor related to the HBV infection.

Sample no.	Donation year	NAT[a]	ID-NAT in repository samples	HBsAg[b]	Anti-HBc[c]	Anti-HBs[d] (mIU/mL)	ALT (U/L)	Highly sensitive HBsAg[e]
1	2020	+		0.1	0	95.8	34	
2	Dec 2019 index donation	–		0.1	0.1	10.7	20	–
3	2019–2	–		0.3	0	0.5	16	
4	2019–1	–		0.2	0.1	1.3	23	
5	2018	–		0.1	0.1	1.6	19	
6	2017	–		0.1	0.1	1.4	160	
7	2016–2	–		0.2	0.1	5.8	75	
8	2016–1	–		0.2	0.1	11.4	54	
9	2015–2	–		0.2	0.1	12	46	
10	2015–1	–		0.3	0.1	0.5	54	–
11	2014–2	–		0.2	0.2	0.6	71	–
12	2014–1	–[f]	–	0.2	0.1	0.3	52	–
13	2013–3	–[f]	–	0.2	0.1	0.3	50	–
14	2013–2	–[f]	–	0.2	0.1	0.7	53	–
15	2013–1	NT[g]	–	0.2	0.1	0.1	117	–
16	2012	NT	–	0.2	0.1	0.1	78	–
17	2011–3	NT	–	0.2	0.1	0.1	142	–
18	2011–2	NT	–	0.2	0.1	0.2	85	–
19	2011–1	NT	–	0.2	0.1	0.3	126	

[a]Individual donation NAT; 95% limit of detection, 3.1 IU/mL.
[b]Sensitivity: 0.05 IU/mL; cutoff: 1.0; range: 0.05–250 IU/mL.
[c]Sensitivity: 0.4–0.5 PEI U/mL; cutoff: 1.0; reactive: S/CO≥1.0.
[d]Range: 0–1000 mIU/mL (neat), 1001–1.5 million mIU/mL (diluted).
[e]Reactive: ≥5.0 mIU/mL; range: 5.0–150,000 mIU/mL.
[f]NAT performed using a 20 sample-mini-pool format; 95% limit of detection, 64 IU/mL (s-401, Roche Diagnostics).
[g]NT; NAT not performed because of high ALT level. Until 2016, ALT had been used as a surrogate marker for viral hepatitis and blood with ALT ≥61 U/L had not been subjected to NAT.

aminotransferase levels and positivity for HBsAg. Anti-HBc also turned positive. The 24 blood components transfused to the patient included fresh frozen plasma that had been donated by a male donor in his 40s. This donated blood had qualified with negative results for HBV ID-NAT, HBsAg, and anti-HBc. A subsequent donation 84 days after the index donation showed positive results only for ID-NAT, with the viral load below the quantifiable level. Part of the HBV DNA was successfully sequenced and verified to be identical to that of the HBV isolated from the patient's blood on day 122 post-transfusion over 1556 bp (nt 2333–667) in the P region and 223 bp (nt 1699–1921) in the PreC/CP region. The patient received anti-viral therapy including entecavir and became negative for HBV-DNA 6 months later.

3 | RESULTS

The donor related to the TT-HBV case had repeatedly donated blood since 1994. HBV-related screening test results for this donor are summarized in Table 1. TT-HBV-related blood donated in December 2019 (day 0) was all negative for ID-NAT, HBsAg and anti-HBc, but marginally positive for anti-HBs (10.7 mIU/mL). The 95% limit of HBV detection of ID-NAT used was 3.1 IU/mL. The blood was also negative in highly sensitive HBsAg testing with a sensitivity of 5.0 mIU/mL (Lumipulse HBsAg-HQ; Fujirebio) (Table 1). In the subsequent donation on day 84, anti-HBs titer had risen to 95.8 mIU/mL, with HBsAg and anti-HBc remaining negative. This donor exhibited fluctuating anti-HBs throughout the repeated donations with weak positive results in 2015 and 2016, whereas anti-HBc constantly showed negative results.

Using the current ID-NAT system, we retested repository blood samples from the donor obtained during the mini-pool era (Nos. 12–19 from 2011 to 2014 in Table 1). Although all samples were negative for HBV ID-NAT, sample 19 (2011) showed a result of 0.94 S/CO (cutoff, 1.0).

The transmitted HBV was genotype A2. The amino acid sequence for the whole PreC/Core region of the patient-derived HBV was identical to that of a reference strain (accession no. KP234051, data not shown) that is not uncommon in Japan. The DNA sequence of the major hydrophilic region of the S region was wild type and did not contain any special DNA sequences including vaccine escape mutants such as G145R, I/T126S/N, Q129H, M133L, K/G141E, P142S/L, or D144A. Patient HBV included spliced variant(s) for which nt 2454–488 and nt 1590–1823 were deleted by 22% and 28% in coverage, respectively, as verified using a next-generation sequencer. No such variants were detected in the donor HBV. Primers used for amplification did not include any part of the deleted region; nested PCR was conducted using a primer set of nt 2297–2317 and 685–704 for the first-round PCR and a primer set of nt 2310–2332 and 668–687 for the second-round PCR. The deletion was therefore considered unlikely to have affected sequence amplification, which might have resulted in undetectable splice mutants in the donor. However, one possibility is that the spliced variant was not amplified because of the extremely low HBV concentration (<20 IU/mL) in the donor's blood.

4 | DISCUSSION

We encountered a case of TT-HBV caused by blood negative for all of ID-NAT, HBsAg, and anti-HBc, but positive for anti-HBs. The donor exhibited an approximately 9-fold increase in anti-HBs titer without anti-HBc response after index donation. Such anti-HBs and anti-HBc kinetics suggest a second antibody response to a new HBV challenge, representing a vaccine breakthrough case. In fact, the donor showed periods of weak anti-HBs positivity in 2015 and 2016 (Table 1). Unfortunately, whether the donor had received HBV vaccination was unknown, as the individual refused any attempts at follow-up contact by JRC staff. Nevertheless, this case suggests that blood undergoing vaccine breakthrough could transmit HBV to the blood recipient.

Notably, donor blood from day 84 after index donation still showed an extremely low viral concentration below the quantifiable level (<20 IU/mL). Considering the HBV doubling time in peripheral blood during the window period, such a slow tempo of replication of HBV is unusual even when a varied length of doubling times is considered.[5] In contrast, the transfused patient developed HB hepatitis with a typical clinical course with verified HBV-DNA positivity on day 72 and elevated ALT levels on day 121, suggesting that HBV from the donor possessed usual replication capability, at least in the body of the recipient patient.[6] The donor seems to have had weak but functional preexisting anti-HBs or memorized machinery of HBV elimination that might have somehow affected the replication of HBV. Without further information after the index donation, whether this individual ended up with abortive infection is unknown. Deng et al. described most NAT-positive results with isolated anti-HBs as representing vaccinated immunocompetent adults undergoing aborted infection after exposure to HBV.[7] This situation may not be rare, as a study in Taiwan for the presence of HBV viremia in 705 completely immunized, anti-HBc-negative individuals revealed HBV viremia in approximately 5% of these individuals.[8] Stramer et al. reported breakthrough subclinical infections among

blood donors who had been immunized with HBV genotype-mismatched vaccines.[9]

Another possibility for this case is that the donor had chronic OBI with isolated anti-HBs. Although the NAT result of sample 19 (2011) was negative, the S/CO value of 0.94 for the sample was very close to the cut off 1.0 and clearly higher than other values among negative samples, which ranged between 0.00 and 0.09. Although donor anti-HBs was positive in 2015 and 2016, the titers were low and seemed to fluctuate afterwards. These facts suggest the possibility of OBI for the donor who developed slight transient viremia around the donation in 2019, which eventually led to the TT-HBV case. Repeated ID-NAT was not done for the repository samples with ID-NAT-negative results at screening between 2014 and 2019. Lookback for patients who had been transfused with the blood donated by this donor prior to the index donation was also not conducted. Such studies might have provided a better insight into the possibility of chronic OBI for the donor.

Adults who have recovered from acute HBV infection exhibit gradually decreasing titers for anti-HBs and anti-HBc with age. Although anti-HBc usually persists throughout life, a proportion lose anti-HBc earlier than anti-HBs, with only anti-HBs remaining positive. A multi-regional study for different categories of HBV infection revealed that chronic infection with only anti-HBs positivity accounted for 4.4%,[10] or 11%[11] of OBI. In fact, HBV reactivation has been reported among cases with positive results for anti-HBs only, and individuals in this category have been listed in the Japanese guidelines for the management of HBV infection as those who need close observation for reactivation when immunosuppressive treatment regimens are to be started.[12]

Several mechanisms for the absence of anti-HBc in individuals with replication-potent HBV have been proposed, such as core region mutations including deletions that lead to impaired HBcAg expression;[6] immunosuppression; and immunotolerance of HBcAg. The first possibility of core region mutation is unlikely in the present case, because the patient-derived HBV included no special sequence in the core region and the patient developed anti-HBc as a result of transfusion, indicating that the donor blood included HBV clones expressing intact core antigen, although we failed to sequence the whole core region of the donor HBV. Inefficient immune response has been described as the most likely mechanism in some reports.[13,14] This, however, is also unlikely for the donor in this case, who had repeatedly donated blood over the course of more than a decade. The donor had declared no health problems at donation interviews. Immunotolerance for HBcAg has been reported in early infancy, but eventually wanes with age and anti-HBc formation.[15,16] A slight possibility of lower sensitivity for anti-HBc detection with the testing system used might remain,[17] although anti-HBc was also negative with another system (CL-4800; Fujirebio) until 2018.

If this case represents TT-HBV caused by vaccine breakthrough blood, a new TT-HBV risk must be considered; a proportion of HBV vaccinees with weak anti-HBs can no longer be relied upon as HBV-safe donors and may need to be considered as risk-containing donors. Moreover, weak anti-HBs may suppress HBV replication tempo under rechallenge with HBV, which would lengthen the period during which donated blood carries a risk of causing TT-HBV.

On the other hand, if this donor represents OBI without anti-HBc, we face great difficulty in the prevention of TT-HBV. The existence of a proportion of individuals with isolated anti-HBs has been reported among apparently healthy individuals with possible past HBV infection.[10,18] Such individuals cannot be distinguished from vaccinees without multiple point test results or a reliable vaccination history, but could have OBI with very low viral loads undetectable using ID-NAT, as illustrated in this case. Excluding all donors with weak anti-HBs as potential OBI donors is unfeasible as a preventive measure that would seriously impact the blood inventory; in the era of universal HBV vaccination, a large proportion of individuals would currently show weak anti-HBs.

In either case, selecting individuals harboring TT-HBV risk among individuals with isolated anti-HBs is difficult under current screening strategies. Blood donor qualification at donation sites relying on donor responses to questionnaires regarding vaccination and HBV infection history is unrealistic because of the uncertainty of donor responses. OBI harboring TT-HBV risk among isolated anti-HBs may be identified by increasing the sensitivity of anti-HBc. However, whether such an extremely low anti-HBc level generally applies to TT-HBV risk is uncertain. Notably, anti-HBc testing used to be notorious for the high rate of nonspecific reactions. Measurement of HBV core-related antigen that simultaneously detects core antigen, HBe antigen and p22cr antigen may be helpful to identify risk-harboring OBI donors, since measured values reportedly correlate with HBV cccDNA in the liver. However, the sensitivity of this system has yet to be improved.

5 | CONCLUSION

We have presented a case of TT-HBV in which transfused blood was derived from either vaccine breakthrough or anti-HBs-only positive chronic OBI. Whichever the case, donors with isolated weak anti-HBs may include a rare

population carrying a risk of causing TT-HBV. While we will continue surveying the occurrence of TT-HBV with isolated anti-HBs blood, the need for and characteristics of further initiatives are under consideration.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose in relation to this work.

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