Residual infectious risks in blood transfusion

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Rare transmission of hepatitis B by Dutch blood donors with occult infection

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ABSTRACT

Background
To reduce the rate of transfusion transmitted hepatitis B virus (HBV), HBV DNA testing was introduced for all Dutch blood donations in 2008, in addition to the existing screening for HBV surface antigen (HBsAg). This study describes the lookback results for repeat donors with an “HBV DNA-only” test result (HBV DNA-positive and HBsAg-negative).

Study design and methods
From November 2008 until June 2011, a total of 2.3 million blood donations were tested for HBV DNA and 22 donors showed the HBV DNA-only profile. Four donors had early preseroconversion HBV infection, two showed suppressed infection after vaccination, and 16 donors had occult chronic HBV infection (OBI). Potentially infectious donations were traced back as far as 1992. If possible the recipients were tested for current and past HBV infection.

Results
A total of 416 implicated donations could be traced in blood bank records, involving 448 issued blood products. For 170 (38%) of the recipients no information was obtained from the hospitals; 196 (44%) recipients had died, and 82 (18%) were tested for HBV. Six recipients tested positive for current (n=4) or past (n=2) HBV infection. For two recipients transfusion was ruled out as the source of infection. Three patients showed HBV DNA sequences matching with the HBV in one common OBI donor. Overall, in four of 82 tested recipients (5%) HBV transmission was likely.

Conclusion
In our lookback study HBV testing was possible in only a minority (18%) of potentially exposed recipients. A low transmission rate (5%) was observed in recipients of blood components from donors with OBI.
INTRODUCTION

Since 1973 all blood donations in the Netherlands have been tested for presence of hepatitis B virus (HBV) surface antigen (HBsAg). To further reduce transfusion transmitted HBV infection, additional HBV DNA nucleic acid amplification testing (NAT) was introduced in November 2008 for all blood donations. HBV NAT enables the detection of HBsAg-negative stages of HBV infection, including the pre- and posteroconversion stage of acute HBV infection; suppressed HBV infection after HBV vaccination; HBV Genotype G monoinfection; chronic occult HBV infection; and HBV infection with HBsAg mutations, missed by HBsAg assays [1-5].

The estimated anti-hepatitis B core antigen (HBc) prevalence in the general population, including immigrants, in the Netherlands is 3.5% (95% confidence interval [CI], 2.2-5.5), but it reaches 28.7% among first-generation immigrants [6]. The HBsAg prevalence is 0.2% (95% CI, 0.1-0.4), and first-generation immigrants account for 70% of the prevalent infections [6]. New HBV infections acquired in adults in low-prevalence countries occur mainly via sexual contact. The reported acute hepatitis B incidence in the Netherlands was 0.8 per 100,000 in 2013 [7]. The anti-HBc prevalence in HBsAg-negative Dutch donors in the first 2 years of anti-HBc screening was found to be 0.76% [8]. In the period 2007 to 2013, the mean number of HBV infections among new Dutch donors was stable at 47 per 100,000, while among repeat donors the incidence of HBV infection was stable at 1.7 per 100,000 donors per year.

As reported previously, most Dutch donors with an HBV DNA-only donation were classified as having chronic occult HBV infection (OBI) [1]. Previous donations of these donors may have infected the recipients. Lookback procedures seem indicated to trace and test the recipients for HBV infection [3]. But the effectivity of this policy is unclear, especially because the infectivity of OBI donations seems limited. In the Netherlands it is mandatory to notify the hospitals of (possibly) infectious issued blood components. Triggers for starting lookback are 1) donors testing positive in routine screening (HIV, HBV, HCV, and Lues), with potential previous window period donations; 2) donors found positive after introduction of a new screening test, who donated previously; and 3) infected donors identified through reports of posttransfusion infection in recipients. The lookback period is determined after a risk analysis, taking into account the infectious agent, the stage of infection, and the test results of archived donor samples. This study describes the lookback results for the 22 Dutch donors who showed an HBV DNA-only test result, since the introduction of HBV NAT in November 2008, until the introduction of anti-HBc screening in June 2011 [1].

MATERIALS AND METHODS

Definitions
For this study the following definitions were used. A newly registered donor is someone who stated his/her wish to donate blood or blood components, but has not yet donated
blood for transfusion purposes [9]. In the Netherlands this donor is invited to visit the blood bank for predonation screening only. A first-time donor has undergone predonation screening, at least 14 days previously, and returns to the blood bank as an eligible donor to give his/her first donation for transfusion purposes. A repeat donor is a donor who has donated more than once.

Study population and testing
All Dutch donations are routinely screened for HBsAg since 1973. For this study we included all blood donations that tested HBsAg negative (PRISM, Abbott Laboratories, Chicago, IL), but HBV DNA positive in the period November 2008 until the introduction of anti-HBc screening in June 2011. A total of 2,332,986 blood donations (including predonation samples from newly registered donors) were tested for HBV DNA in pools of 6, using a fully automated NAT system (COBAS TaqScreen MPX test on the S201 platform, Roche Molecular Diagnostics, Pleasanton, CA; 95% lower limit of detection, 23 IU/mL). Reactive pools were deconstructed to identify individual HBV DNA reactive donations and to enable confirmatory testing on the individual donation. The confirmatory testing consisted of repeated HBsAg testing (AxSYM, Abbott Laboratories), HBV DNA PCR (COBAS AmpliScreen, Roche Molecular Diagnostics; 95% lower limit of detection, 5.2 IU/mL), and anti-HBc testing (Architect, Abbott Laboratories). If sufficient material was available additional testing was performed for anti-HBs, anti-HBe, and HBeAg (Architect, Abbott Laboratories). Subsequently, the donor was invited for counseling and follow-up testing. For each Dutch blood donation, a repository sample (0.8 mL) is kept in storage until 2 years after the donation. If available, repository samples were tested for anti-HBc and for HBV DNA.

Characterization of HBV DNA-only donors
Twenty-two HBV DNA-only donors were identified and characterized as summarized in Table 1: four donors were in the early preseroconversion stage of HBV infection, two donors showed suppressed acute HBV infection after vaccination, and 15 donors were characterized as OBI. HBV DNA sequencing and phylogenetic analysis succeeded in 14 donors as reported previously [1]. All donors returned for counseling and for follow-up testing, as reported in Table 1.

In addition to blood donor screening, Sanquin screens tissue donations for various organizations. During the study period a postmortem tissue donor was found to be HBV DNA-only positive and was characterized as OBI. The HBV DNA sequencing for phylogenetic analysis of the postmortem sample did not succeed. It appeared that in the past this person was a blood donor, who had donated 50 times. Therefore, this donor was included in the lookback exercise.

Lookback procedures
Previous donations of HBV DNA-positive and HBsAg negative blood donors were traced and analyzed as follows. Regarding the four HBV preseroconversion donors and the two vaccine-suppressed donors, issued blood components derived from the last HBV-nega-
Table 1. Characteristics of HBV DNA positive and HBsAg negative donors.

<table>
<thead>
<tr>
<th>Nature of HBV infection</th>
<th>Donor</th>
<th>M/F</th>
<th>Age</th>
<th>Donation type</th>
<th>HBV DNA load (IU/ml)</th>
<th>Index confirmation anti-HBs (IU/L)/ anti-HBc</th>
<th>Follow-up HBsAg/ anti-HBs (IU/L)/anti-HBc</th>
<th>Repository sample anti-HBc/ HBV-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early pre-seroconversion infection</td>
<td>D1</td>
<td>M</td>
<td>30</td>
<td>repeat</td>
<td>2870</td>
<td>neg/ neg</td>
<td>pos/ pos (105)/ pos</td>
<td>neg/ neg</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>F</td>
<td>27</td>
<td>new</td>
<td>nd</td>
<td>neg/ neg</td>
<td>pos/ neg/ neg</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td>M</td>
<td>33</td>
<td>new</td>
<td>205</td>
<td>neg/ neg</td>
<td>pos/ neg/ neg</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>D4</td>
<td>M</td>
<td>41</td>
<td>new</td>
<td>66</td>
<td>neg/ neg</td>
<td>pos/ neg/ pos</td>
<td>na</td>
</tr>
<tr>
<td>Suppressed infection after HBV vaccination</td>
<td>D5</td>
<td>M</td>
<td>43</td>
<td>repeat</td>
<td>26</td>
<td>pos (90)/ neg</td>
<td>neg/ pos (122)/ neg</td>
<td>nd* neg</td>
</tr>
<tr>
<td></td>
<td>D6</td>
<td>F</td>
<td>54</td>
<td>repeat</td>
<td>201</td>
<td>neg/ neg</td>
<td>neg/ pos (570)/ pos</td>
<td>neg/ neg</td>
</tr>
<tr>
<td>Occult chronic HBV infection</td>
<td>D7</td>
<td>M</td>
<td>46</td>
<td>repeat</td>
<td>&lt;12</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D8</td>
<td>F</td>
<td>63</td>
<td>repeat</td>
<td>220</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>pos/ pos</td>
</tr>
<tr>
<td></td>
<td>D9</td>
<td>F</td>
<td>61</td>
<td>repeat</td>
<td>&lt;12</td>
<td>pos (31)/ pos</td>
<td>neg/ pos (78)/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D10</td>
<td>M</td>
<td>62</td>
<td>repeat</td>
<td>&lt;12</td>
<td>pos (86)/ pos</td>
<td>neg/ pos (141)/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D11</td>
<td>M</td>
<td>64</td>
<td>repeat</td>
<td>&lt;12</td>
<td>pos (14)/ pos</td>
<td>neg/ pos (19)/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D12</td>
<td>M</td>
<td>58</td>
<td>first donation</td>
<td>&lt;12</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>D13</td>
<td>M</td>
<td>67</td>
<td>repeat</td>
<td>20</td>
<td>pos (23)/ pos</td>
<td>neg/ pos (25)/ pos</td>
<td>pos/ pos</td>
</tr>
<tr>
<td></td>
<td>D14</td>
<td>M</td>
<td>58</td>
<td>repeat</td>
<td>nd</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D15</td>
<td>F</td>
<td>43</td>
<td>repeat</td>
<td>&lt;12</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>D16</td>
<td>M</td>
<td>53</td>
<td>repeat</td>
<td>&lt;20</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D17</td>
<td>F</td>
<td>46</td>
<td>repeat</td>
<td>&lt;12</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D18</td>
<td>F</td>
<td>53</td>
<td>new</td>
<td>&lt;12</td>
<td>pos (32)/ pos</td>
<td>neg/ pos (32)/ pos</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>D19</td>
<td>F</td>
<td>60</td>
<td>repeat</td>
<td>33</td>
<td>pos (65)/ pos</td>
<td>neg/ pos (96)/ pos</td>
<td>pos/ neg</td>
</tr>
<tr>
<td></td>
<td>D20</td>
<td>M</td>
<td>51</td>
<td>repeat</td>
<td>&lt;20</td>
<td>pos (13)/ pos</td>
<td>neg/ pos (14)/ pos</td>
<td>pos/ nd</td>
</tr>
<tr>
<td></td>
<td>D21</td>
<td>M</td>
<td>31</td>
<td>repeat</td>
<td>23</td>
<td>neg/ pos</td>
<td>neg/ neg/ pos</td>
<td>pos/ nd</td>
</tr>
<tr>
<td></td>
<td>D22</td>
<td>M</td>
<td>66</td>
<td>tissue</td>
<td>130</td>
<td>pos (58)/ pos</td>
<td>na/ na/ na</td>
<td>pos/ nd</td>
</tr>
</tbody>
</table>

nd = not determined, due to lack of material; na = not applicable; *Repository sample D5 was not tested for anti-HBc. Anti-HBs was neg.
tive donation and from donations in the 6 months preceding the last HBV-negative do-
nation were traced. For the 16 OBI donors, lookback procedures were performed for all
donations that could be traced back as far as 1992. (For the donations before 1992 in-
sufficient paper and computerized records were available.) Fractionated plasma products
were not included for this lookback study.

Hospitals that received implicated blood components (red blood cells [RBCs], plate-
let [PLT] concentrates, and fresh-frozen plasma [FFP]) were informed about the possibil-
ity of transmission of HBV through the issued components. The hospitals were asked to
trace the issued material to specific recipients and to test the recipients for past and pres-
ent HBV infection, using HBsAg (or HBV NAT) and anti-HBc serology. In the Netherlands
the physician in charge of the patient is responsible for the decision to inform and test the
recipient in case of a possible transfusion transmitted infection. In case of possible HBV
transmission, analysis of HBV DNA sequences of the recipient was offered to confirm or
exclude donor to recipient transmission.

RESULTS

Lookback for donors with early preseroconversion HBV infection
Regarding the preseroconversion donors, three were newly registered donors who tested
positive at their first visit for predonation screening without donation. The fourth donor
was a repeat donor with three recent at-risk donations of which four blood components
were issued. Lookback for the four components was performed (see Fig. 1).

Two of the four recipients were tested. One recipient was found negative for HBsAg
and anti-HBc. For the other recipient who was reported as not being HBV infected, the
anti-HBc status is unknown. One recipient died and one recipient was not tested.

Lookback for donors with suppressed infection after HBV vaccination
Regarding the two repeat donors with vaccine-suppressed HBV infection one donor only
donated plasma for fractionation and no blood components were issued. The other donor
had one previous donation of which two blood components were issued. The two recipi-
ents were found to have died from non-liver-related cause; they were not tested for HBV.

Lookback for donors with chronic occult HBV infection
One donor was a newly registered donor, one donor was a first-time donor, and 14 do-
nors were repeat donors. According to historical records, the 14 repeat donors with OBI
donated at least 409 times since 1992. From 409 donations 442 blood components were
prepared and issued (302 RBC units, 134 PLT units, and 6 FFP units). The lookback
procedure for the 442 recipients of these components resulted in 80 (18%) tested recipi-
ents; 193 (44%) recipients who had died; and 169 (38%) untested recipients. Because
components were not traceable in the local hospital, components were not transfused, or
physicians decided not to test the recipient. The test results of the recipients are summa-
rized in Table 2. Four of the 80 recipients were found to be HBsAg positive. One recipient
was known to be chronically HBV infected before transfusion. Three recipients received blood components from Donor D8, as described below. Two of the 80 recipients tested negative for HBsAg but positive for anti-HBc. For one recipient the relation with the donor is less probable because the recipient originated from a highly endemic HBV area. One recipient received a blood component from Donor D15, as described below.

Of the 74 recipients who tested negative for HBsAg or HBV NAT, resolved HBV infection was excluded for 52 recipients (testing anti-HBc negative). Although we explicitly asked to determine the anti-HBc status, for 22 recipients the anti-HBc status was not reported unambiguously (nine were probably anti-HBc negative; 13 anti-HBc status unknown).
Three HBV-infected recipients associated with Donor D8

In 2009 OBI was detected in a female donor (D8, see Table 1). The HBsAg-negative index donation tested HBV DNA positive, with a viral load of 220 IU/mL. Additional testing showed a positive anti-HBc test, and negative test results for IgM anti-HBc, anti-HBs, HBeAg, and anti-HBe. The repository sample of the previous donation (4 months before the index donation) tested positive for HBV DNA and anti-HBc. Retrospectively, this donor had reported a history of acute hepatitis during her first visit, which was 25 years before the index donation and which was acceptable at that time. In 2008 a hospital had notified Sanquin blood bank of two recipients with hepatitis B after transfusion in 2007, upon the repository sample of that donation was tested for HBV DNA and found negative. While testing details are not available in detail, the failure to detect DNA might have been caused by limited sample volume or an HBV DNA level below the threshold of detection.

Donor D8 donated whole blood 47 times. Fifty-five blood components (32 RBC units, 20 PLT units, 3 FFP units), prepared from 34 donations, could be traced to various hospitals. The lookback results in the hospitals are depicted in Fig. 2: 27 recipients had died without signs or symptoms of acute hepatitis, and 15 recipients were tested. The test results of the tested recipients were as follows: eight recipients were HBsAg and anti-HBc negative; three recipients tested negative for HBsAg or HBV NAT, but anti-HBc is unknown; one recipient was already known with chronic HBV infection before the blood transfusion; and three patients (Recipients A, B, and C), tested using posttransfusion samples, were found to be HBV infected after blood transfusion.

Recipients A and B received RBCs and PLTs from Donor D8 in 2007, and Recipient C received FFP from Donor D8 in 1993. Recipients A and B were known to be HBsAg and anti-HBc negative before transfusion. For Recipient C the HBV status before transfusion was unknown. To exclude or confirm the transmission of HBV from Donor D8 to Recipients A, B, and C, phylogenetic analysis was performed as follows. By HBV DNA sequencing a nearly complete genome was obtained of the HBV strain in Donor D8 (GenBank Accession Number JX310726), as reported previously [2]. This HBV DNA sequence was compared to HBV DNA sequences from Patients A, B, and C, obtained from blood samples drawn in 2009. For Patient A, a 656-nucleotide fragment of the HBV surface gene

Table 2. Test results of recipients of blood components from occult chronic HBV-infected donors.

<table>
<thead>
<tr>
<th>HBsAg positive (n=4)</th>
<th>HBsAg negative (n=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-HBc positive (n=2)</td>
</tr>
<tr>
<td>3 recipients, associated with D8</td>
<td>1 recipient from highly endemic HBV region, associated with D14</td>
</tr>
<tr>
<td>1 recipients HBV infected before transfusion, associated with D8</td>
<td>1 recipient, associated with D15</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*probably anti-HBc negative, because recipients were reported to have “negative hepatitis B serology”, and serology included anti-HBc testing.
Yield of lookback for HBV NAT only blood donors

and a 655-nucleotide fragment of the HBV core gene was obtained (GenBank Accession Numbers KP288874 and KP288876). For Patient B, a 656-nucleotide fragment of the HBV surface gene was obtained (GenBank Accession Number KP288873). For patient C a complete HBV genome was obtained (GenBank Accession Number KP288875).

Patients A and B received blood components from Donor D8 in 2007, and Patient C in 1993. Hence, regarding Donor D8 and Patients A and B, divergent viral evolution of HBV could take place during 2 years; divergent viral evolution of HBV in Donor D8 and Patient C could take place during 16 years. Comparing the S gene sequences of Donor D8 and Patients A, B, and C, Patients A and B were found to differ only one nucleotide from Donor D8. Patient C was found to differ five nucleotides from Donor D8. Comparing the core gene sequences of Donor D8 and Patients A and C, Donor D8 and Patient A showed an identical core sequence, while Patient C differed in 11 nucleotides from Donor D8. It is safe to conclude that Donor D8 and Patients A and B probably share a route of infection. At first sight the nucleotide differences between Patient C and Donor D8 seem significant, but it must be realized that if indeed Donor D8 infected Patient C, 16 years of divergent viral evolution took place before Donor D8 and Patient C were sampled. To further determine the likelihood of HBV transmission from Donor D8 to Patients A, B, and C, the late Dr Boot (National Institute for Public Health and the Environment, Bilthoven, the Netherlands) compared the HBV surface gene sequences from Donor D8 and Patients A, B, and C, to 375 HBV Genotype D surface gene sequences, obtained from notified

Figure 2. Lookback results for 55 recipients of Donor D8, including three transmissions of HBV. For the tested recipients the HBV status is indicated as follows: † = confirmed transmission of HBV; — — = HBsAg and anti-HBc negative; (—) = HBsAg negative, anti-HBc unknown; (+) = HBV infection was documented before transfusion.
cases of hepatitis B in the Netherlands, as depicted in the minimum spanning tree in Fig. 3A (based on 644 nucleotides in common). In addition, he compared the HBV core gene sequences from Donor D8 and Patients A and C to 86 HBV Genotype D core gene sequences, obtained from notified cases of hepatitis B in the Netherlands, as depicted in the minimum spanning tree in Fig. 3B (based on 654 nucleotides in common). Considering the clustering in Figs. 3A and 3B, it can be concluded that Donor D8 infected Patients A, B, and C. The clinical state of the recipients was as follows: Patient A cleared HBV infection; Patient B (with acute hepatitis B) died years later due to the underlying hematologic disease; and Patient C was found to have chronic HBV infection, 17 years after the infectious transfusion.

One resolved HBV infection associated with Donor D15
The female whole blood Donor D15 was categorized as having OBI. The index donation showed a weak positive test result for HBV DNA (<12 IU/mL) and a negative test result for HBsAg. Confirmatory testing showed total anti-HBc positive and negative test results for IgM anti-HBc, anti-HBs, HBeAg, and anti-HBe. Repository samples were not available because the previous donations took place more than 2 years before. The donor was born in a country where HBV is highly endemic; she lived in the Netherlands for 21 years. The lookback exercise for Donor D15 involved three whole blood donations of which four blood components were issued (3 RBC units and 1 PLT unit). One recipient, who was tested 3 years after the implicated blood transfusion, showed serologic signs of cleared HBV infection (HBsAg negative, anti-HBs positive [titer 883 IU/L], anti-HBc total positive, IgM anti-HBc negative, HBeAg negative, anti-HBe positive). Anamnestic information from the recipient revealed no other risk factors for hepatitis than blood transfusion. The second recipient tested negative for HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe. Regarding the third recipient (82 years old, with no signs of hepatitis), the general practitioner decided not to test for HBV. The fourth recipient died of lung disease without symptoms of hepatitis.

One donor with occult HBV infection, identified postmortem (D22)
A male tissue donor was postmortem found to be HBV DNA positive (130 IU/mL) and was categorized as OBI. Because he was also a blood donor the blood bank was notified. The source of infection is unknown. He had donated 50 times, of which 35 donations were after 1991 and could be traced. Five of the most recent donations were all HBV DNA negative in the regular blood screening. The oldest repository sample available was anti-HBc and anti-HBs positive (anti-HBs titer 167 IU/L). Eight recipients tested negative for HBsAg (five anti-HBc negative, three anti-HBc unknown). Six tested recipients received blood components derived from recent donations, which were nonreactive in the HBV DNA donor screening. This case illustrates that OBI donors can donate for many years while HBV DNA remains undetectable for routine NAT screening.
Yield of lookback for HBV NAT only blood donors

DISCUSSION

A lookback exercise in the Netherlands for 22 HBV NAT only donors involved 448 blood products. Eventually only 82 (18%) potentially exposed recipients were tested for HBV infection. In four of these patients (5%) HBV infection could be attributed to administration of blood components from blood donors with occult HBV infection and low levels of viremia. The proportion of tested recipients was low because many recipients (44%) had died because of underlying diseases. Signs of HBV infection or other liver-related diseases were not reported for these cases. The survival rate of the recipients is comparable with survival rates in literature [9-12]. Data were not available for the remaining 38% because many transfusions occurred long ago, and imperfect administration in hospitals and local blood banks made recipients untraceable.

The HBV transmission rate via components from chronic occult blood donors in our study is comparable with other donor-triggered lookback studies. In Australia, Seed and colleagues [13] found a transmission rate of 7.75% unadjusted, and 0.85% (0-2.3%) adjusted for anti-HBc prevalence in the general population, of possible or probable HBV transmission associated with confirmed OBI donors. Confirmation of transmission with HBV DNA sequence analysis was not performed. A donor-triggered lookback program in Japan revealed a transfusion transmission rate of 3% (1/33) attributed to OBI [14]. Confirmation of transmission with HBV DNA sequence analysis was not performed. Yuen and coworkers [15] studied 49 recipients of OBI donations. Analyzing four recipients with active HBV infection, they concluded that one case probably acquired HBV infection from an OBI donor, based on HBV sequence analysis with a viral sequence homology of 95%, while for three recipients transfusion transmission could be excluded based on HBV DNA

Figure 3. (A) Minimum spanning tree, showing HBV surface gene sequences of Donor D8 (red), Patient A (light green), Patient B (dark green), Patient C (blue), and 375 Dutch cases of hepatitis B. (B) Minimum spanning tree, showing HBV core gene sequences of Donor D8 (red), Patient A (green), Patient C (blue), and 86 Dutch cases of hepatitis B.
sequence analysis. For 16 of 49 recipients with signs of recovered HBV infection (HBsAg negative, anti-HBc positive, anti-HBs positive or negative, and HBV DNA negative) transmission through OBI donations could not be excluded or confirmed. It must be noted that it is more difficult to exclude or confirm HBV transmission through transfusion and to determine the transmission rate in countries highly endemic for HBV, where on one hand a large proportion of the population is vaccinated and on the other hand acquiring HBV from other sources is more likely [16]. A study from Allain and colleagues [17] estimated from lookback cases a high transmission rate of 22% for anti-HBs–negative blood from OBI donors in Europe. Pretransfusion samples were not available and molecular evidence was available in only 10% of the cases. It was reported that the transmission rate depended on the plasma volume transfused, since no transmissions occurred via RBC transfusion [17]. The four HBV transmissions that we observed were associated with the administration of RBCs, PLTs, and FFP (transmissions per implicated products: 1/1 FFP, 1/23 PLTs, and 2/56 RBCs). The transmission rate in our study may be slightly underestimated due to lack of information on resolved HBV infection in 22 of 76 recipients. Half of our OBI blood donors were anti-HBs positive with anti-HBs titers ranging from 13 to 167 IU/L. For none of these donors was HBV transmission found, supporting the conclusion of Allain and colleagues [17] that the presence of anti-HBs in OBI donors reduces the risk of transmission.

One donor with chronic occult HBV infection in our study strikingly demonstrated that OBI donors may only be intermittently infectious. This donor was found to be HBV DNA positive in 2009. In hindsight, the donor had already infected two recipients in 2007, respectively, via RBCs and via PLTs obtained from the same whole blood donation, as confirmed by later phylogenetic analysis of the HBV isolates involved. In this study, by means of lookback a third infected recipient was traced who was infected as a newborn in 1993 via FFP, 16 years before OBI was detected in the donor. Between the transmission incidents in 1993 and 2007, several recipients did not acquire HBV infection from the donor. Theoretically all these recipients may have been immune to HBV, but this is unlikely. Another striking observation is the lack of divergent viral evolution of the HBV strains in the donor and in the recipient, during the 16 years between the transmission event and the detection and sequencing of the viruses involved. The transmission from the donor to the recipient could be confirmed irrefutably after comparison with other Dutch HBV strains.

Lookback exercises are labor-intensive and the yield of identification of a transfusion-transmitted infection in recipients is low, but the benefits of antiviral treatment for a chronically HBV-infected recipient may be considerable [18]. An alternative approach can be considered for large lookback exercises. Tracing of recipients could be limited to the recipients more prone to a bad outcome of HBV infection, for whom medical intervention is expected to be beneficial. Recipients who are prone to develop chronic HBV infection include immunosuppressed patients and unvaccinated infants. (In the Netherlands, universal HBV vaccination of newborns was introduced as late as 2011.) The advantage of this approach is limited, because it still involves finding out the background of all recipients involved, and therefore blood banks still have to inform the hospitals of all issued blood components.
This study applies to the period before anti-HBc screening was introduced in the Netherlands. The introduction of anti-HBc screening eliminated donors at risk for OBI (anti-HBc positive and anti-HBs titer <200 IU/L), and indeed since the introduction of anti-HBc screening no OBI donors were identified. It can be expected that future lookback exercises for HBV will be recipient triggered or donor triggered because of window period donations.

Before this lookback study was started, it was expected that the yield of lookback for previous donations of HBV NAT-only donors (including OBI) would be very low, because of the practical problems concerning looking back in the far past, and the limited infectivity of OBI donors. Therefore, we questioned whether such extensive exercises are warranted. Yet, the yield of transfusion-transmitted HBV infection in at least 5% of tested recipients does not support the abolition of lookback exercises, even if the transfusions involved occurred long ago. Although in the majority of cases HBV infection in healthy adults resolves spontaneously, the failure to identify chronic HBV infection in an exposed recipient may have dramatic consequences for the recipient and his or her contacts.

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