Prevalence, incidence and residual risk of transfusion-transmitted hepatitis B virus infection in Italy from 2009 to 2018

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Background. In Italy, the use of nucleic acid testing for hepatitis B virus (HBV) in donor screening has allowed the detection of infections in the window phase, as well as the presence of occult infections which could potentially be transmitted. The aim of this study was to analyse the trends of epidemiological data focused on HBV infection in blood donors and to estimate the residual risk of transmitting HBV from both the window phase and occult infection over a 10-year period in Italy.

Materials and methods. Data were obtained from the Italian Haemovigilance System which includes the results of screening tests for transfusion transmissible infections. During the period of this survey (2009-2018), the molecular methods used for HBV screening were transcription-mediated amplification and polymerase chain reaction tests. Prevalence and incidence were calculated. The residual risk was estimated by applying the incidence-window period model for acute cases and a more recently reported model for estimating the risk due to occult infections.

Results. A total of 17,424,535 blood donors and 30,842,794 donations were tested for HBV. Altogether, 6,250 donors tested positive for HBV markers: 4,782 (175.6 × 10^5) were first time donors and 1,468 (10.0 × 10^5) were repeat donors. The prevalence of HBV markers in first time donors was 275.9 × 10^5 in 2009, declining to 143.6 × 10^5 in 2018. The incidence of new infections was 3.37 × 10^5 in 2009 and 2.17 × 10^5 in 2018. The overall residual risk for HBV amounted to 1 in 2,566,854 donations calculated as the sum of risks of both acute infections in the window period (1 in 5,835,306 donations) and occult infections (1 in 4,582,270 blood units).

Discussion. In Italy, the residual risk of transfusing a blood unit infected with HBV, both from window phase and occult infections, is currently very low, amounting to levels that can be considered tolerable.

Keywords: HBV, residual risk, incidence, prevalence, haemovigilance.

Introduction

In Italy, the safety of blood and blood products for transfusion has dramatically increased over the last decades thanks to the adoption of strict criteria for blood donor selection, screening of all blood units for transfusion-transmissible infections (TTIs), and the rational use of blood to avoid unnecessary transfusions. Following these measures, risk that infectious donations with hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV) can enter into the blood supply has incrementally declined, becoming too low for direct assessment through prospective follow up and look-back of blood recipients. Therefore, mathematical models have been developed to estimate the residual risk (RR) which still persists despite the combined implementation of highly sensitive antigen/antibody-based assays and nucleic acid testing (NAT) for donor screening1-4. In this regard, the risk of collecting an infectious donation that results undetectable by currently used assays depends on the incidence of the infection in blood donors and on the length of the window period (WP) of the viral infection (i.e., the time that elapses between infectious viremia and detection). Thus, the magnitude of RR for TTIs is strictly related to the country-endemicity of HBV, HCV, HIV and to the sensitivity of the assays used for donor screening5-7.

We have recently reported that the risk of releasing a potentially infectious HCV or HIV donation missed by currently available assays (NAT and serology) into the blood supply is extremely low8, if compared with the scale of ‘real-life’ situations9. In Italy, there are few data related to the RR for HBV10 and these need to be revised with consideration given to the current incidence of infection in donors, the implementation of
advanced technologies, and updated case definitions. In Italy, to reduce the risk of transfusion-transmitted HBV, blood screening includes detection of hepatitis B surface antigen (HBsAg, in place since 1971), using highly sensitive last generation assays, together with NAT (in place since 2008). On the other hand, universal antibody to core antigen (anti-HBc) screening has not been implemented due to the high prevalence of this marker in the Italian population. The implementation of donor screening by HBV NAT has allowed the identification of new HBV infections in the very early acute phase (incident cases) as well as the presence of occult HBV infections (OBI) which are chronic infections characterised by low, often transient, levels of circulating HBV DNA, undetectable HBsAg, with or without other HBV markers (anti-HBc and/or anti-HBs). Though infectivity of such donations seems to be lower than that from donors in the WP of the acute phase of infection, evidence shows that blood donations collected from OBI donors can transmit HBV to the recipients. Thus, the total residual risk associated with HBV is the sum of risks associated with both acute infections detected in the WP and OBI.

This study was designed to assess the prevalence and incidence of HBV infection among Italian blood donors, and to estimate the RR of transfusing an HBV-infected blood unit over a decade, from 2009 to 2018. The methodology used for calculating HBV RR includes separate estimates of the risk due to acute NAT WP infections according to the Bush model and of the risk due to OBI according to the refined model proposed by Seed et al.

Materials and methods

In Italy, haemovigilance data are collected and processed through the web-based national blood surveillance system (SISTRA, Sistema Informativo dei Servizi Trasfusionali) implemented in 2007 by the National Blood Centre, that is in charge of managing all information related to blood activities carried out nationwide, including data on demographic characteristics and risk factors of donors found positive to the HBV screening.

According to Italian law, only voluntary, non-remunerated donors can donate in our country. Their classification as first-time (FT) or repeat (RP) donors used in this study has been recently described. Very briefly, FT donors are individuals tested for the first time for markers of TTIs or with a prior testing more than 24 months before, while definition of RP donors includes subjects who donated blood after clinical evaluation and screening for TTIs with previous donation(s) found negative within the last 24 months, and subjects who donated for the first time after a pre-donation screening (without donation) whose clinical evaluation and testing for TTIs markers resulted negative.

All donations reviewed in this study were tested for HBsAg and HBV DNA, and those found positive were further tested for both IgM and total anti-HBc and for the antibody to hepatitis B surface antigen (anti-HBs) on the same sample, and, if antibody negative, on consecutive samples collected during the post-donation follow up to see whether seroconversion had occurred or not.

During the 10-year period covered by this study, Blood Establishments (BEs) performed serological HBsAg testing using automated analysers, based on the chemiluminescence immunoassay principle (CLIA) provided by different manufacturers and able to detect at least 0.13 IU/mL, in compliance with the standard reported in the European Directorate for the Quality of Medicines & Health Care (EDQM) Guide and CE licensed.

For NAT testing, two main amplification methods were used: the transcription-mediated amplification (TMA, for testing individual donors [ID]; PROCLEIX Ulitro and PROCLEIX Ulitro Plus on Tigris platform, and Ulitro Elite on Panther platform; Grifols, International S.A: Sant Cugat del Vallès, Barcelona, Spain); the polymerase chain reaction (PCR) for mini pool (MP) testing of 6 donors (TaqScreen MPX and TaqScreen v2; Roche Molecular System, Branchburg, NJ, USA). In addition, at the beginning of the study period, PCR COBAS Ampliscreen (Roche Diagnostics, Branchburg, NJ, USA) technology in pools of 10-24 samples was used; this method was then discontinued at the end of 2012. Since 2016, ID PCR Roche MPX Test (Cobas 6800/8800 Systems) has been progressively introduced in place of the minipool of 6-sample testing. The blood units tested by ID Cobas 6800/8800 Systems account for 8.0% of the total number of tested donations.

In case of repeatedly reactive samples, confirmatory and/or supplemental tests were performed according to the manufacturer’s instructions and following the national algorithm.

Serology for anti-HBc (IgM and total) and anti-HBs was carried out using immunoassays from different manufacturers.

Definition of acute and occult HBV infections

For this study, an HBV infection was considered acute when the donor resulted HBsAg and/or HBV DNA confirmed positive in the presence of IgM anti-HBc detected on the same sample or after seroconversion at the serological follow up on recalling the donors (1-3 month after donation). An HBV infection was considered occult (OBI) when the donor showed an HBV DNA positive test with negative HBsAg. Based on the HBV-specific antibody
HBsAg and HBV DNA prevalence and incidence

In the population of FT donors, prevalence was calculated as the rate between the number of HBsAg and/or HBV DNA positive FT donors per 100,000 FT donors.

In the population of RP donors, incidence was calculated as the number of positive subjects having a previous (within the last two years) negative donation or negative testing divided by the product of the total number of donations from RP donors in the study period and the mean inter-donation interval (IDI) expressed in years (=person-years at risk). Incidence is expressed as the number of new infections per 100,000 person-years at-risk.

The 95% confidence intervals (95% CI) for estimated prevalence and incidence rates were calculated assuming a Poisson distribution of the observed cases. A trend analysis was performed (Poisson regression analysis) to evaluate the changes in prevalence and incidence over time.

Residual risk calculation

Residual risk can be defined as the probability that an infected donation resulting negative at the screening test in use (WP at risk) could be transfused to the recipient. In the case of the HBV infection, the RR calculation is quite complex because, in addition to the incidence rate and the WP length, mathematical models must also take into account the number of OBI positive blood donors able to transmit the infection, as well as the probability that the recipient can be infected. In this study, we adopted the incidence ratio/window period (IR/WP) mathematical model5-7,19 to evaluate the RR due to WP infections and the more recently reported model16,20 for estimating the risk due to OBI. Briefly, the RR for the acute infection was estimated in RP donors multiplying the ratio between the number of NAT-only positive cases and person-years by the WP of NAT tests in use in Italy, as a fraction of a year. The RR for FT donors was calculated by multiplying the ratio between NAT-only positive cases and the total number of FT donors by the NAT WP. Due to the transient nature of HBsAg and HBV DNA, an adjustment factor was finally applied to those with a probability of having an undetected viral load (pNAT non-detection). This rate is multiplied by the probability that an OBI unit would be able to transmit the infection (p-transmission); Following a large Australian look-back study, in their model, Seed et al. assumed that only OBI donors with anti-HBs concentrations below 10 mIU/mL were potentially able to transmit HBV infection, estimating the probability of transmission to be around 1.81%15,23.

Results

From January 2009 to December 2018, a total of 17,424,535 blood donors were tested for HBV (Table I). Of these, 84.4% were RP donors and 15.6% were FT donors; the average ratio between RP and FT donors was 5.4. The total number of screened donations was reported by Galel et al.22 and, for the methods not included in the Galel's manuscript, the WP lengths reported by the manufacturers (see the Appendix). The weighted average pre-NAT infectious WP for the entire period of observation was estimated to be 17.3 days or 0.047 years.

Finally, the RR of the overall donor population for the acute HBV infection was estimated adjusting the incidences for RP and FT donors as follows: (FT%×FT rate or incidence) + (RP%×RP rate or incidence). The RR is expressed per million donations.

According to the refined model proposed by Seed et al.15, the RR for OBI was estimated as the rate between the number of blood units donated during the study period by OBI donors before the occurrence of HBV DNA NAT reactivity and the total number of blood units tested in the same period: in fact, these units are those with a probability of having an undetected viral load (pNAT non-detection). This rate is multiplied by the probability that an OBI unit would be able to transmit the infection (p-transmission); Following a large Australian look-back study, in their model, Seed et al. assumed that only OBI donors with anti-HBs concentrations below 10 mIU/mL were potentially able to transmit HBV infection, estimating the probability of transmission to be around 1.81%15,23.

Table I - Blood donors and donations tested for HBV in Italy, 2009-2018.

<table>
<thead>
<tr>
<th>Total n. of donors tested</th>
<th>17,424,535</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: range</td>
<td>18-70 years</td>
</tr>
<tr>
<td>M/F ratio</td>
<td>2.2</td>
</tr>
<tr>
<td>N of FT donors tested</td>
<td>2,723,639 (15.6%)</td>
</tr>
<tr>
<td>M/F ratio</td>
<td>1.6</td>
</tr>
<tr>
<td>N of RP donors tested</td>
<td>14,700,896 (84.4%)</td>
</tr>
<tr>
<td>M/F ratio</td>
<td>2.4</td>
</tr>
<tr>
<td>RP/FT donors ratio:</td>
<td>5.4</td>
</tr>
<tr>
<td>Total n. of blood donations tested</td>
<td>30,842,794</td>
</tr>
<tr>
<td>n. of blood donations from FT donors</td>
<td>2,723,639 (8.8%)</td>
</tr>
<tr>
<td>n. of blood donations from RP donors</td>
<td>28,119,155 (91.2%)</td>
</tr>
<tr>
<td>RP donation index (mean donations per year)</td>
<td>1.91</td>
</tr>
<tr>
<td>Interdonation interval (IDI)</td>
<td>191.1 days or 0.52 years</td>
</tr>
<tr>
<td>N. donations tested by:</td>
<td></td>
</tr>
<tr>
<td>NAT PCR</td>
<td>16,038,253 (52%)</td>
</tr>
<tr>
<td>NAT TMA</td>
<td>14,804,541 (48%)</td>
</tr>
</tbody>
</table>

HBV: hepatitis B virus; FT: first-time; RP: repeat; M/F: male/female; NAT: nucleic acid testing; PCR: polymerase chain reaction; TMA: transcription-mediated amplification.
30,842,794 (91.2% from RP donors; 8.8% from FT donors) with an overall average of 1.8 units per donor per year (total donation index). The same index, restricted to RP donors, amounted to 1.91 donations per year, with an inter-donation interval (IDI=365/1.91) of 191.1 days (0.52 years). Fifty-two percent of donations were tested by NAT PCR and 48% by NAT TMA.

Table II reports the total number of HBsAg and/or HBV DNA positive cases (either acute or OBI) grouped by FT and RP donors, age, and year of data collection. Altogether, 6,250 donors tested positive for HBV markers; 4,782 (175.6×10^5) FT donors and 1,468 (10.0×10^5) RP donors, with a frequency of positivity 17.5-fold higher among FT than among RP donors. In both FT and RP donors, the frequency of HBV markers was found to increase with older age, peaking in the 56-65 years age group.

Among HBV positive donors (Table III), 73.4% were positive for both HBsAg and HBV DNA, 22.1% were positive for HBV DNA and negative for HBsAg, and 4.5% were positive for HBsAg alone. As for risk factors, 74.1% denied any known risk factor, while the remaining 25.9% reported one or more risk. Of the latter,

### Table II - Donors with positive tests for HBV in FT and RP donors by age group in Italy, 2009-2018.

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>FT</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>18-25</td>
<td>76</td>
<td>57</td>
<td>54</td>
<td>50</td>
<td>38</td>
<td>24</td>
<td>29</td>
<td>16</td>
<td>20</td>
<td>14</td>
<td>378 (7.9)</td>
<td>668,124</td>
<td>56.6</td>
</tr>
<tr>
<td>26-35</td>
<td>183</td>
<td>123</td>
<td>109</td>
<td>120</td>
<td>100</td>
<td>90</td>
<td>89</td>
<td>78</td>
<td>66</td>
<td>55</td>
<td>1,013 (21.2)</td>
<td>628,780</td>
<td>161.1</td>
</tr>
<tr>
<td>36-45</td>
<td>253</td>
<td>181</td>
<td>169</td>
<td>148</td>
<td>149</td>
<td>115</td>
<td>113</td>
<td>141</td>
<td>117</td>
<td>83</td>
<td>1,469 (30.7)</td>
<td>705,521</td>
<td>208.2</td>
</tr>
<tr>
<td>46-55</td>
<td>164</td>
<td>144</td>
<td>151</td>
<td>158</td>
<td>120</td>
<td>130</td>
<td>107</td>
<td>148</td>
<td>143</td>
<td>133</td>
<td>1,398 (29.2)</td>
<td>536,421</td>
<td>260.6</td>
</tr>
<tr>
<td>56-65</td>
<td>52</td>
<td>43</td>
<td>48</td>
<td>58</td>
<td>33</td>
<td>39</td>
<td>39</td>
<td>66</td>
<td>70</td>
<td>69</td>
<td>517 (10.8)</td>
<td>181,017</td>
<td>285.6</td>
</tr>
<tr>
<td>over 65</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7 (0.1)</td>
<td>3,777</td>
<td>185.3</td>
</tr>
<tr>
<td>Total</td>
<td>730</td>
<td>548</td>
<td>531</td>
<td>534</td>
<td>441</td>
<td>398</td>
<td>378</td>
<td>450</td>
<td>417</td>
<td>355</td>
<td>4,782</td>
<td>2,723,639</td>
<td>175.6</td>
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<tr>
<td>RP</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>24 (1.6)</td>
<td>1,667,705</td>
<td>1.4</td>
</tr>
<tr>
<td>26-35</td>
<td>9</td>
<td>14</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>71 (4.8)</td>
<td>2,626,374</td>
<td>2.7</td>
</tr>
<tr>
<td>36-45</td>
<td>29</td>
<td>25</td>
<td>26</td>
<td>24</td>
<td>35</td>
<td>23</td>
<td>21</td>
<td>24</td>
<td>21</td>
<td>19</td>
<td>247 (16.8)</td>
<td>4,151,313</td>
<td>5.9</td>
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<tr>
<td>46-55</td>
<td>43</td>
<td>41</td>
<td>46</td>
<td>46</td>
<td>48</td>
<td>42</td>
<td>45</td>
<td>59</td>
<td>58</td>
<td>64</td>
<td>492 (35.5)</td>
<td>4,135,729</td>
<td>11.9</td>
</tr>
<tr>
<td>56-65</td>
<td>47</td>
<td>46</td>
<td>47</td>
<td>63</td>
<td>55</td>
<td>52</td>
<td>48</td>
<td>76</td>
<td>89</td>
<td>74</td>
<td>597 (40.7)</td>
<td>1,994,020</td>
<td>29.9</td>
</tr>
<tr>
<td>over 65</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>9</td>
<td>3</td>
<td>37 (2.5)</td>
<td>125,754</td>
<td>29.4</td>
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<tr>
<td>Total</td>
<td>133</td>
<td>132</td>
<td>134</td>
<td>152</td>
<td>153</td>
<td>127</td>
<td>123</td>
<td>166</td>
<td>182</td>
<td>166</td>
<td>1,468</td>
<td>14,700,896</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>863</td>
<td>680</td>
<td>665</td>
<td>686</td>
<td>594</td>
<td>525</td>
<td>501</td>
<td>616</td>
<td>599</td>
<td>521</td>
<td>6,250</td>
<td>17,424,535</td>
<td>35.9</td>
</tr>
</tbody>
</table>

HBV: hepatitis B virus; FT: first-time; RP: repeat.

### Table III - Features of 6,250 blood donors tested positive for HBV marker in Italy, 2009-2018.

<table>
<thead>
<tr>
<th>N. of donors</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBV markers:</strong></td>
<td></td>
</tr>
<tr>
<td>HBsAg+ and HBV DNA+</td>
<td>4,588</td>
</tr>
<tr>
<td>HBsAg− and HBV DNA+</td>
<td>1,378</td>
</tr>
<tr>
<td>HBsAg+ and HBV DNA−</td>
<td>284</td>
</tr>
<tr>
<td><strong>Risk factors:</strong></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5,066</td>
</tr>
<tr>
<td>Known</td>
<td>1,770</td>
</tr>
<tr>
<td>of whom:</td>
<td></td>
</tr>
<tr>
<td>parenteral</td>
<td>1,348</td>
</tr>
<tr>
<td>sexual behavior</td>
<td>309</td>
</tr>
<tr>
<td>household contact of HBV carrier</td>
<td>113</td>
</tr>
</tbody>
</table>

*Each case could report more than one risk factor(s). HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen.
76.1% had parenteral risk factors (i.e., dental treatments, surgery, tattooing, etc.), 17.5% declared sexual risk, and 6.4% were households of non-sexual relationships between HBV chronic carriers.

During the study period, the prevalence of HBV markers in FT donors was $175.6 \times 10^5$, significantly ($p<0.01$) decreasing over time from $275.9 \times 10^5$ in 2009 to $143.6 \times 10^5$ in 2018 (Table IV). In addition, the incidence of HBV new infections during the same 10-year study period was $2.68 \times 10^5$, decreasing ($p<0.05$) from $3.37 \times 10^5$ in 2009 to $2.17 \times 10^5$ in 2018.

Of the 1,378 donors found NAT positive but HBsAg negative (277 FT and 1,101 RP), sufficient serological data able to make a diagnosis of acute or OBI infection were available for 1,074 cases (204 FT and 870 RP) donors. Of these 1,074 cases, 33 (3.1%; 1 FT and 32 RP donors) were diagnosed as having acute HBV infection and 1,041 (96.9%; 203 FT and 838 RP donors) as having OBI. Donors of the former group were mainly males (90.9%) and more frequently belonged to the 46-55 years age group (48.5%), while donors of the latter were again mainly males (82.0%), but with a peak in the 56-65 years age group (48.3%).

Among the 1,041 OBI donors, 91.9% were anti-HBc positive (277 FT and 864 RP donors) and 8.1% were anti-HBs alone (8 FT and 244 RP donors). Donors of the former group were mainly males (92.6%) and more frequently belonged to the 56-65 years age group (47.3%), while donors of the latter were again mainly males (83.0%), but with a peak in the 46-55 years age group (44.9%).

To estimate the RR based on the whole number of the 1,378 NAT-only positive blood donors, we arbitrarily distributed the 304 unclassified cases among the groups of acute and OBI infections with the same proportion observed in the 1,074 known cases. After adjustment, a total of 42 (2 FT and 40 RP donors) acute cases and 1,336 (276 FT and 1,060 RP) OBI donors were considered for calculation.

Table IV - Prevalence and incidence of HBV in FT donors and in RP donors in Italy, 2009-2018.

<table>
<thead>
<tr>
<th>Year</th>
<th>N. FT donors</th>
<th>N. positives</th>
<th>Prevalence×10^5 (95% CI)</th>
<th>N. RP donations</th>
<th>Person-years</th>
<th>N. acute positive cases</th>
<th>Incidence×10^5 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>264,635</td>
<td>730</td>
<td>275.9 (256.2-296.6)</td>
<td>2,769,776</td>
<td>1,425,791</td>
<td>48</td>
<td>3.37 (2.48-4.46)</td>
</tr>
<tr>
<td>2010</td>
<td>281,153</td>
<td>548</td>
<td>194.9 (178.9-211.9)</td>
<td>2,824,685</td>
<td>1,441,350</td>
<td>38</td>
<td>2.64 (1.86-3.61)</td>
</tr>
<tr>
<td>2011</td>
<td>297,321</td>
<td>531</td>
<td>178.6 (163.7-194.5)</td>
<td>2,889,653</td>
<td>1,474,930</td>
<td>41</td>
<td>2.78 (1.99-3.77)</td>
</tr>
<tr>
<td>2012</td>
<td>287,380</td>
<td>534</td>
<td>185.8 (170.4-202.3)</td>
<td>2,905,769</td>
<td>1,501,319</td>
<td>54</td>
<td>3.60 (2.70-4.69)</td>
</tr>
<tr>
<td>2013</td>
<td>271,841</td>
<td>441</td>
<td>162.2 (147.4-178.1)</td>
<td>2,872,883</td>
<td>1,504,371</td>
<td>50</td>
<td>3.32 (2.46-4.38)</td>
</tr>
<tr>
<td>2014</td>
<td>265,543</td>
<td>398</td>
<td>149.9 (135.5-165.4)</td>
<td>2,816,234</td>
<td>1,487,313</td>
<td>31</td>
<td>2.08 (1.41-2.95)</td>
</tr>
<tr>
<td>2015</td>
<td>266,739</td>
<td>378</td>
<td>141.7 (127.8-156.7)</td>
<td>2,794,740</td>
<td>1,488,901</td>
<td>36</td>
<td>2.42 (1.69-3.35)</td>
</tr>
<tr>
<td>2016</td>
<td>276,151</td>
<td>450</td>
<td>162.9 (148.2-178.7)</td>
<td>2,760,483</td>
<td>1,451,854</td>
<td>31</td>
<td>2.14 (1.45-3.03)</td>
</tr>
<tr>
<td>2017</td>
<td>265,727</td>
<td>417</td>
<td>156.9 (142.2-172.7)</td>
<td>2,740,999</td>
<td>1,452,613</td>
<td>34</td>
<td>2.34 (1.62-3.27)</td>
</tr>
<tr>
<td>2018</td>
<td>247,169</td>
<td>355</td>
<td>143.6 (129.1-159.4)</td>
<td>2,743,933</td>
<td>1,472,454</td>
<td>32</td>
<td>2.17 (1.49-3.07)</td>
</tr>
<tr>
<td>Total</td>
<td>2,723,639</td>
<td>4,782*</td>
<td>175.6 (170.6-180.6)</td>
<td>28,119,155</td>
<td>14,700,896</td>
<td>394**</td>
<td>2.68 (2.42-2.96)</td>
</tr>
</tbody>
</table>

*co-infections: 2 HBV-HIV, 31 HBV-Treponema pallidum, 26 HBV-HCV, 2 HBV-HIV-Treponema pallidum and 2 HBV-HCV-HIV; **co-infections: 5 HBV-Treponema pallidum and 2 HBV-HIV.

HBV: hepatitis B virus; FT: first-time; RP: repeat; CI: confidence interval; HIV: human immunodeficiency virus.
or 1 in \(5,891,086\) donations (\(0.1933446 \times 10^6\) or 1 in \(5,169,390\) units of blood collected from RP donors and \(0.051769 \times 10^6\) or 1 in \(19,316,579\) units of FT donors, respectively) (Table V). In addition, the RR from the OBI RP donors was calculated to be \(0.218211 \times 10^6\) or 1 in \(4,582,270\) blood units. Thus, the overall RR of an HBV infectious unit entering the blood supply calculated as the sum of risks caused by both acute infections in the WP and OBI amounted to \(0.387959 \times 10^6\) or 1 in \(2,577,592\) donations.

**Discussion**

In Italy, to prevent and control transfusion-transmitted HBV infection, mandatory testing is in place for all blood donations for HBsAg and, starting from 2008, for HBV NAT. Anti-HBc testing is not universally applied because prevalence of this antibody is too high for screening, thus potentially excluding an unnecessary number of donors\(^{10}\). Evidence shows that anti-HBc is the most reliable serological marker detectable in individuals with OBI. Thus, while the risk of transmitting OBI has been almost entirely eliminated (or mitigated) in countries where screening to ensure virological blood safety relies on the detection of anti-HBc together with the detection of HBsAg and HBV NAT, such a risk could be of significant concern in those countries where anti-HBc is not performed, depending on the HBV endemicity and on the sensitivity of the HBV DNA assay used for screening. This is because viral DNA loads detectable in people with OBI are generally low and fluctuate around or below the lower limit of detection of the currently used assays, even when applied in to an individual donation. Since blood components from OBI donors may be infectious, the model for calculating the total HBV transfusion-transmission RR in countries with no universal anti-HBc testing, like Italy, should take into account the sum of both RR due to acute NAT WP infections and to OBI.

In this study, over 30 million donations (approximately 91% given by RP and 9% by FT donors) were screened for HBsAg and HBV DNA between 2009-2018. Both the average prevalence of HBV markers among FT donors and the incidence among RP donors showed a significant decreasing trend over time. Disturbingly, only 24% of positive donors reported HBV-associated risk factors (i.e., parenteral exposure such as tattooing, piercing, dental surgery; at-risk sexual behavior; and cohabitation with HBV carriers), while 76% reported

| Table V - Residual risk for HBV infection in Italy, 2009-2018. |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| **1. RR derived from acute HBV NAT-only positive incidence rate** | **Estimated n of incident cases** | **Person-years** | **Incidence\(\times 10^5\)** | **Adjusted incidence\(\times 10^5\)** | **RR\(\times 10^6\)** |
| | | | (95% CI) | (95% CI) | (95% CI) |
| RP | 40 | 14,700,896 | 0.272092 | 0.408138 | 0.193446 | 5,169,390 |
| FT | 2 | 2,723,639 | 0.073431 | 0.110146 | 0.051769 | 19,316,579 |
| Total RR | | | | | | |
| | | | | | 0.169748 | 5,891,086 |

<table>
<thead>
<tr>
<th><strong>2. RR derived from OBI RP donors</strong></th>
<th><strong>Estimated n. of risk cases</strong></th>
<th><strong>Total n. of blood donations</strong></th>
<th><strong>p(NAT non-detection)(\times 10^2)</strong></th>
<th><strong>p(transmission)</strong></th>
<th><strong>RR(\times 10^6)</strong></th>
<th><strong>1: n. units</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall HBV RR (1 + 2)</td>
<td>339</td>
<td>28,119,155</td>
<td>1.205584</td>
<td>1.81%</td>
<td>0.218211</td>
<td>4,582,270</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.08-1.34)</td>
<td>(0.19-0.24)</td>
</tr>
</tbody>
</table>

\(a\) Proportion between the documented cases and the total number of the NAT-only positives observed cases.

\(b\) Calculated as N donations\(\times\)IDI (0.52), for RP donors. In FT donors the denominator is the total number of FT donors.

\(c\) An adjustment factor of 1.5 has been used\(^{1,21}\).

\(d\) N of RP donations resulted HBV NAT negative from subjects subsequently identified as OBI. Since the IDI is 0.52, we assumed that each OBI donors gave previously one blood donation resulted HBV DNA negative during the year. Only OBI donations with anti-HBs <10 IU/L were considered. The number of risk cases is obtained by the proportion between the documented cases and the total number of the OBI RP donors observed.

\(e\) p(NAT non-detection) = n risk donations/total number of blood donations tested for HBV DNA.

\(f\) p(transmission) = the probability of an OBI blood unit to transmit the infection is indicated as the percentage of OBI units able to transmit HBV infection in the Australian follow up study\(^{15}\).

\(g\) OBI RR = p(NAT non-detection)\(\times\)p(transmission).

HBV: hepatitis B virus; RR: residual risk; FT: first-time; RP: repeat; CI: confidence interval; OBI: occult HBV infections.
no risks, and this remains a matter of some considerable
concern. This means that approximately 75% of donors
who were detected positive at the screening test were
not intercepted at the pre-donation anamnesis targeted at
excluding donations from donors at risk of transmitting
HBV. We believe that this apparent poor efficiency of
donor selection criteria, rather than any inaccuracy in
compiling the case history, could be due to the fact that
most (i.e., 97%) of our infected donors had OBI, likely
acquired in the past as an asymptomatic infection that
had gone unrecognised.

The finding that frequency of HBV markers in both
FT donors and in RP donors was found to be much lower
among those in the fourth decade of life is probably
due to the introduction of our mandatory programme of
universal anti-HBV vaccination in 1991 in infants and
12-years adolescents (for this latter group, restricted to
the first 12 years of application of the law)21,25. Thanks to
this vaccination policy, over 20 million Italians (almost
all of them under 40 years of age) have so far been
successfully vaccinated, and are now protected against
HBV. Consequently, a remarkable overall decline in
incidence of acute HBV (particularly striking among
children and young adults) was reported over the years
by the Italian National Surveillance System (SEIEVA)26.
Additionally, a generation of young people (those who
are currently <40 years of age) is emerging with almost
no markers of HBV infection, reflecting the decline of
the viral circulation in Italy. Thus, the priorities to be
addressed in order to prevent and control HBV should
be aimed at lowering the probability of acquiring HBV
through blood transfusion to a marginal RR through:
- maintaining mandatory vaccination in infants;
- increasing the rate of HBV vaccination coverage in
  high-risk groups;
- refining blood screening procedures;
- improving the criteria for donor selection.

In our study, to evaluate the RR of HBV, we
considered separately the estimates of the risk due
to acute NAT WP infections according to the model
previously reported by Bush et al.5 and of the risk due
to OBI according to the refined model proposed by Seed
et al.15. The overall RR was then calculated as the sum
of the risk associated with acute NAT WP infections and
the risk associated with chronic OBI. As for the latter,
several look-back studies2,12,14,23,27-30 aimed at estimating
the risk of receiving blood components from donors with
OBI have shown a wide range of HBV transmission
that depends on a number of factors, including plasma
volume transfused, viral load in the component, absence
of protective anti-HBs, and the immune status of the
recipient.

In Italy, we need to record the call-back of donors
found positive for TTI markers and the look-back of
patients transfused with blood units made by the donor
within the six months preceding the occurrence of
HBV DNA NAT reactivity. Storage of samples of all
donations and of all correspondent transfused patients,
though highly recommended, is not mandatory and is
not, therefore, performed on a national scale. Therefore,
lack of local look-back data drove us to consider the
presence of the anti-HBs concentration of 10 mIU/mL
as the antibody infectivity threshold able to distinguish
potentially infectious blood (<10 mIU/mL) from non-
infectious blood (≥10 mIU/mL) derived from OBI
donors. This assumption was made on the basis of the
evidence reported by a large Australian study23 showing
that no confirmed HBV transmission was detected in 578
recipients who were given OBI donations with anti-HBs
antibody above 10 mIU/mL.

Our finding indicates that the RR for HBV due to
NAT WP infections, which has remarkably declined
from that previously reported11, currently amounts to 1
in 5,835,306 donations (1 in 5,169,390 units of blood
collected from RP donors and 1 in 19,316,979 units of
FT donors). The RR associated to blood collected from
RP donors is over three times higher than that associated
with blood collected from FT donors. This may be
surprising since both prevalence and incidence of HBV
markers are generally higher among FT than among RP
donors. A possible explanation for this discrepancy is
that most Italian FT donors (over 50%) include young
people under 40 years of age who have been vaccinated
against HBV, while RP donors are older and mostly
belong to cohorts born before the introduction of the
vaccination programme.

In addition, RR from the OBI RP donors was
calculated to be 1 in 4,582,270 blood units, i.e.,
approximately 22% higher than that due to NAT WP
infections.

In the refined model for OBI proposed by Seed
et al.15, plasma units used for manufactured plasma
products were excluded from RR calculation. This was
because the virucidal treatments in the manufacturing
process, remove the risk of viral transmission to the
recipients. A limitation of this study is that the donations
taken forward for fractionation were not excluded from
the RR calculation. Thus, all donations with anti-HBs
concentrations <10 mIU/mL were included in our
analysis regardless of whether they were used to make
fresh blood components or plasma-derived products, and
this probably led to an overestimation of our OBI RR.

Finally, the total HBV RR calculated as the sum of
risk caused by both acute infections in the WP and OBI
amounted to 1 in 2,566,854 donations, a threshold that
can be considered very low and deemed to be tolerable.
In this regard, the UK and Australian haemovigilance
systems32,33 indicate, for any kind of TTIs, a RR of
1/1,000,000 as the limit above which blood transfusion can be considered to be reasonably safe.

Conclusions
In Italy, the adoption of strict criteria for selection of non-remunerated and repeat blood donors, screening of all blood units for transfusion-transmissible infections with serological and molecular tests, the rational use of blood to avoid unnecessary transfusions, as well as the policy of universal anti-hepatitis B vaccination, have all reduced the RR of transfusing an HBV-infected unit to negligible values. Nevertheless, a RR persists, and it is essential that we do not lower our guard, and maintain and improve the safety of transfusion therapies.

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Authorship contributions
All Authors contributed to the collection, analysis, interpretation of data, and critical revision of the article. CV, AZ and LR designed the study, and wrote the final version of this paper.

Disclosure of conflicts of interest
GML is the Editor-in-Chief of Blood Transfusion. As a result, this manuscript was subjected to an additional external review. All other Authors declare that they have no conflict of interest.

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Appendix - Distribution of HBV NAT methods used in Italy during the 2009-2018 period.

<table>
<thead>
<tr>
<th>Method</th>
<th>Percentage of blood units tested with the method</th>
<th>WP days#</th>
<th>Pool (size) or single test</th>
<th>Weighted WP</th>
</tr>
</thead>
<tbody>
<tr>
<td>COBAS Ampliscreen</td>
<td>2,51</td>
<td>27.9</td>
<td>Pool (20-24)</td>
<td>0,6975</td>
</tr>
<tr>
<td>Cobas Taq Screen MPX Test (Cobas s 201 system)</td>
<td>20,15</td>
<td>22,8</td>
<td>Pool (6)</td>
<td>4,5942</td>
</tr>
<tr>
<td>Cobas Taq Screen MPX Test v 2.0 (Cobas s 201 system)</td>
<td>20,90</td>
<td>16,7</td>
<td>Pool (6)</td>
<td>3,4903</td>
</tr>
<tr>
<td>Cobas MPX Test (Cobas 6800/8800 systems)</td>
<td>8,03</td>
<td>8,6</td>
<td>single</td>
<td>0,6906</td>
</tr>
<tr>
<td>Procleix Ultra Assay Tigris</td>
<td>19,75</td>
<td>22,35</td>
<td>single</td>
<td>4,4141</td>
</tr>
<tr>
<td>Procleix Ultra Elite Panther</td>
<td>16,97</td>
<td>12,5</td>
<td>single</td>
<td>2,1212</td>
</tr>
<tr>
<td>Procleix Ultra Plus Assay Tigris</td>
<td>11,69</td>
<td>11,1</td>
<td>single</td>
<td>1,2976</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td></td>
<td>17,3055</td>
</tr>
</tbody>
</table>

WP: window period; # WP’s days referred to Galel et al.22 are reported in Arabic numbers; in Italics are reported the WP following the manufacturers’ indications.