

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 up-dated case definitions¹¹. 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 population¹⁰. 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)¹¹. 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 recipients¹²⁻¹⁴. 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 *et al.*⁵ model and of the risk due to the OBI according to the refined model proposed by Seed *et al.*¹⁵.

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 law¹⁶, 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 described⁸. 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) Guide¹⁷ and CE licensed.

For NAT testing, two main amplification methods were used: the transcription-mediated amplification (TMA, for testing individual donors [ID]; PROCLEIX Ultrio and PROCLEIX Ultrio Plus on Tigris platform, and Ultrio 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 algorithm¹⁸.

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

profile, OBI were further classified as seropositive OBI (i.e., anti-HBc and/or anti-HBs positive) or seronegative OBI (i.e., anti-HBc and anti-HBs negative)¹¹.

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 model^{5-7,19} to evaluate the RR due to WP infections and the more recently reported model^{16,20} 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 incidence following the assumption used by Korelitz¹ and updated by O'Brien²¹.

In this study, the NAT-WP length was calculated using data collected from the Italian inter-laboratory quality programme regarding serological and NAT methods in use in each BE. These data allowed us to quantify the number of blood units tested with each NAT method in use during the 10-year period of observation and for each BE. For each method, we adopted the WP lengths

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}.

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

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

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

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.

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

Group of age	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total n. of positives donors (%)	Total donors	Frequency×10 ⁵	
18-25	76	57	54	50	38	24	29	16	20	14	378 (7.9)	668,124	56.6	
26-35	183	123	109	120	100	90	89	78	66	55	1,013 (21.2)	628,780	161.1	
36-45	253	181	169	148	149	115	113	141	117	83	1,469 (30.7)	705,521	208.2	
FT	46-55	164	144	151	158	120	130	107	148	143	133	1,398 (29.2)	536,421	260.6
	56-65	52	43	48	58	33	39	39	66	70	69	517 (10.8)	181,017	285.6
	over 65	2	-	-	-	1	-	1	1	1	1	7 (0.1)	3,777	185.3
	Total	730	548	531	534	441	398	378	450	417	355	4,782	2,723,639	175.6
RP	18-25	2	-	1	7	4	2	1	2	3	2	24 (1.6)	1,667,705	1.4
	26-35	9	14	11	10	9	5	4	3	2	4	71 (4.8)	2,626,374	2.7
	36-45	29	25	26	24	35	23	21	24	21	19	247 (16.8)	4,151,313	5.9
	46-55	43	41	46	46	48	42	45	59	58	64	492 (33.5)	4,135,729	11.9
	56-65	47	46	47	63	55	52	48	76	89	74	597 (40.7)	1,994,020	29.9
	over 65	3	6	3	2	2	3	4	2	9	3	37 (2.5)	125,754	29.4
	Total	133	132	134	152	153	127	123	166	182	166	1,468	14,700,896	10.0
Total		863	680	665	686	594	525	501	616	599	521	6,250	17,424,535	35.9

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

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 (175.6×10⁵) FT donors and 1,468 (10.0×10⁵) 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 III - Features of 6,250 blood donors tested positive for HBV marker in Italy, 2009-2018.

	N. of donors	%
HBV markers:		
HBsAg+ and HBV DNA+	4,588	73.4%
HBsAg- and HBV DNA+	1,378	22.1%
HBsAg+ and HBV DNA-	284	4.5%
Risk factors*:		
Unknown	5,066	74.1%
Known	1,770	25.9%
<i>of whom:</i>		
parenteral	1,348	76.1%
sexual behavior	309	17.5%
household contact of HBV carrier	113	6.4%

*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 175.6×10^5 , significantly ($p < 0.01$) decreasing over time from 275.9×10^5 in 2009 to 143.6×10^5 in 2018 (Table IV). In addition, the incidence of HBV new infections during the same 10-year study period was 2.68×10^5 , decreasing ($p < 0.05$) from 3.37×10^5 in 2009 to 2.17×10^5 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), while there were no such data for the remaining 304 (73 FT and 231 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 (20.3% had anti-HBc alone and 71.6% had both

anti-HBc and anti-HBs), 4.6% were anti-HBs alone, and 3.5% were HBV DNA only positive without any other serological marker (i.e., seronegative OBI).

In order 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.

To estimate the share of RR due to OBI, we assumed, in agreement with Seed *et al.*¹⁵, that only OBI RP donors with anti-HBs < 10 mIU/mL were potentially at risk of transmitting HBV to the recipients. In our data base, antibody titres were reported in 684 of such donors. Of these, 219 (32%) had antibody concentrations below 10 mIU/mL, 323 (47.2%) between 10 and 100 mIU/mL, and 142 (20.8%) over 100 mIU/mL.

The same adjustment adopted for acute cases was then carried out to assess the number of OBI RP donors with anti-HBs concentrations < 10 mIU/mL; we considered 349 donors for the final number OBI RR calculation.

During the years 2009–2018, the RR for HBV due to NAT WP infections was estimated to be 0.169748×10^6

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

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

*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 (0.1933446×10^6 or 1 in 5,169,390 units of blood collected from RP donors and 0.051769×10^6 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 0.218211×10^6 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 0.387959×10^6 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 donors¹⁰. 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 ^a	Person-years ^b	Incidence $\times 10^5$ (95% CI)	Adjusted incidence $\times 10^{5c}$ (95% CI)	RR $\times 10^6$ units (95% CI)	1: n. units
RP	40	14,700,896	0.272092 (0.19-0.37)	0.408138 (0.28-0.55)	0.193446 (0.14-0.26)	5,169,390
FT	2	2,723,639	0.073431 (0.01-0.26)	0.110146 (0.01-0.39)	0.051769 (0.004-0.18)	19,316,579
Total RR					0.169748 (0.11-0.25)	5,891,086
2. RR derived from OBI RP donors						
	Estimated n. of risk cases ^d	Total n. of blood donations	p(NAT non-detection) ^e $\times 10^5$	p(transmission) ^f	RR ^g $\times 10^6$ units (95% CI)	1: n. units
	339	28,119,155	1.205584 (1.08-1.34)	1.81%	0.218211 (0.19-0.24)	4,582,270
Overall HBV RR (1 + 2)					0.387959 (0.30-0.49)	2,577,592

^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 study¹⁵.

^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)^{24,25}. 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 studies^{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 reported³¹, 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 systems^{32,33} 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.

Acknowledgements

The Authors thank Professor Marilyn Scopes (Italian Foundation for Research on Anaemia and Haemoglobinopathies, Genoa, Italy) for her precious assistance with language editing and proofreading.

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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Arrived: 11 October 2019 - Revision accepted: 25 November 2019

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

Method	Percentage of blood units tested with the method	WP days#	Pool (size) or single test	Weighted WP
COBAS Ampliscreen	2,51	27,9	Pool (20-24)	0,6975
Cobas Taq Screen MPX Test (Cobas s 201 system)	20,15	22,8	Pool (6)	4,5942
Cobas Taq Screen MPX Test v 2.0 (Cobas s 201 system)	20,90	16,7	Pool (6)	3,4903
Cobas MPX Test (Cobas 6800/8800 systems)	8,03	8,6	single	0,6906
Procleix Ultrio Assay Tigris	19,75	22,35	single	4,4141
Procleix Ultrio Elite Panther	16,97	12,5	single	2,1212
Procleix Ultrio Plus Assay Tigris	11,69	11,1	single	1,2976
Total	100			17, 3055

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.