Italian blood donors with anti-HBc and occult hepatitis B virus infection

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ABSTRACT

Background and Objectives

Occult hepatitis B virus (HBV) infection might allow the release of viremic units into the blood supply network if blood is tested only for hepatitis B surface antigen (HBsAg). The aim of our study was to evaluate the actual prevalence, viral load and genotype of occult HBV infections among first-time blood donors in north-western Italy and to suggest a way to minimize risks of transmission of this infection.

Design and Methods

We assayed 6313 consecutive blood donors for antibodies to HBV core antigen (anti-HBc) in addition to mandatory screening. HBsAg-negative/anti-HBc-positive donors were assayed for antibodies to HBsAg (anti-HBs) and for HBV-DNA using COBAS Ampliscreen HBV (Roche™) on individual donations. All HBV-DNA-positive samples underwent confirmatory testing with additional polymerase chain reaction-based assays.

Results

The prevalence of anti-HBc positive subjects was 4.85%. Fourteen out of 288 blood donors (4.86%) were confirmed to have circulating HBV-DNA at a low level (range 8-108 IU/mL). All viremic donors were also anti-HBs-positive.

Interpretation and Conclusions

We estimate that in north-western Italy up to 2298 units per million donated units from first-time donors may contain HBV-DNA. The risk of an HBV-DNA positive unit from an occult carrier being released into the blood supply is more than 100 times higher than the estimated residual risk related to the window phase of HBV infection in our country. The potential infectivity of these units is debated, but their use cannot be considered safe at least in immunocompromised patients.

Key words: hepatitis B virus, HBV, anti-HBc, occult HBV infection, HBV-DNA$^+$ blood units, blood donors.

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Current serological screening for blood-borne hepatitis viruses has reduced the risk of post-transfusion hepatitis dramatically. Among the three viral infections routinely tested in blood, hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV), the residual transmission risk is highest for HBV;[1,2] this is attributed to the interval between initial HBV infection and the detection of hepatitis B surface antigen (HBsAg), resulting in a long window phase during which the virus is transmissible.[3] In a recent evaluation of the impact of the seronegative window phase on the residual risk of transmitting the three infections through blood transfusion in Italy, this was estimated to be 15.78 units per million for HBV compared to 2.45 and 4.45 units per million for HIV and HCV, respectively.[4] In the last 2 years, the risks of HCV and HIV transmission were further reduced to 0.5 and 1.1 units per million transfusions by the introduction of nucleic acid amplification technology (NAT) testing.[5] After the implementation of HBsAg screening in the 1970s, there have been no further measures in Italy to decrease the residual risk of HBV transmission, other than improving the sensitivity of the HBsAg assay. With the development of sensitive assays to detect HBV-DNA it was shown that healthy HBsAg-negative donors who have antibodies to HBV core antigen (anti-HBc) may harbor an occult HBV infection and maintain HBV-DNA sequences in their liver and blood, thus representing potential sources of HBV transmission.[6-17] Anti-HBc screening of blood donations is controversial and variably performed in different countries. Currently it is limited to areas where the seroprevalence of HBV is low (generally <2%), while it is not performed in areas with a high HBV seroprevalence because the impact of the deferral of anti-HBc-positive donors is considered not sustainable. However, the prevalence of occult HBV infection is higher in areas in which HBV infection itself is more frequent; therefore, in some of those areas an algorithm was introduced which allows the transfusion of units with low titer anti-HBc/high titer antibodies to HBsAg (anti-HBs) to minimize the risk of transmission. The safety of this measure is currently being debated.[17] To assess the risk of releasing blood units which contain occult HBV-DNA into the blood supply, we determined the current prevalence of anti-HBc positivity among first-time donors in the Region of Piedmont (north-western Italy) and the proportion of these donors who have HBV-DNA and anti-HBs detectable in their serum.

Design and Methods

From March 2005 to November 2005 all unpaid first time donors attending seven Blood Departments in the Region of Piedmont in north-western Italy were enrolled in the study; written consent was obtained at the time of sampling. The donors were asked to give their consent to anti-HBc, anti-HBs and HBV-DNA testing. First-time donors who were positive for anti-HCV and/or anti-HIV were excluded. A total of 6313 blood donors were enrolled. Within this cohort 1251 (19,8%) donors had been vaccinated against HBV: 1215 Italian donors had undergone mandatory vaccination at the age of 11 and 36 donors had spontaneously decided to be vaccinated against this virus. All donors were tested for anti-HBc using a microparticle enzyme immunoassay (MEIA) AxSYM CORE (Abbott™) in addition to routine viral screening. HBsAg-negative/anti-HBc-positive sera were re-tested with the same assay and by chemiluminescent microparticle immunoassay (ChLIA) PRISM HBc (Abbott™). Reactivity for anti-HBc was considered confirmed only if two positive results were obtained. All anti-HBc-positive sera were further tested for the antibody to HBsAg (anti-HBs) by the MEIA AxSYM AUSAB (Abbott™). Anti-HBc-positive confirmed samples were tested for HBV-DNA with the polymerase chain reaction (PCR)-based assay COBAS Ampliscreen HBV (Roche™); the assay was performed on single unit specimen. To confirm the presence of the viral genome, determine the HBV genotype and quantify viremia, serum samples that were found to be HBV-DNA-positive were further analyzed using four independent PCR-based assays: (i) an in-house nested PCR for precore-core region (nt 1604-2076); (ii) an in-house nested PCR for polymerase/surface region (nt 455-704); (iii) an in-house nested PCR for polymerase/surface region for genotyping (nt 2823-704); (iv) real-time quantitative PCR (RealART™ HBV RG PCR kit).

DNA from plasma specimens was prepared according to different downstream procedures: (i) for the COBAS Ampliscreen HBV test, HBV particles were pelleted from 1 mL of a sample of plasma by high speed centrifugation; after removal of 900 μL of supernatant, the pelleted virus was lysed with a chaotropic agent and the DNA precipitated with alcohol. The extracted DNA was then resuspended in 200 μL diluent; (ii) for amplification by in-house nested PCR, DNA was prepared from 0.1 mL of plasma following standard proteinase K digestion and phenol/chloroform extraction; (iii) for HBV DNA real-time quantitative PCR detection, HBV-DNA was extracted from a 0.5 mL plasma specimen using a QIAGEN DSP Virus kit (Qiagen™) and eluted in 26 μL elution buffer.

Amplification with the Ampliscreen HBV PCR kit was performed on the COBAS AMPLICOR Analyzer (Roche™) according to manufacturer's protocol, and was followed by the hybridization and detection reactions. The 97% detection limit of this assay is 5 IU/mL on the WHO international standard (97/746) or 30 copies/mL on the HBV NIBSC working reagent (98/780). Nested PCR for the HBV precore/core region was performed using the primers listed in Table 1.[18] The expected 322 bp PCR products were resolved on 2% agarose gel. Genotyping was assessed by PCR using type-specific primers (Table 1).[19] The genotype-specific DNA bands were resolved on 3% Metaphore (FMC Bioproducts™) agarose gel. A semi-nested PCR for polymerase/S region was performed using 2 μL

Table 1. Primers used for the in-house HBV PCR amplification.

HBV target gene	*Primer type*	*Primer name and sequence*	*Map position nt*
Precore-Core	(first PCR)		
	forward	P1 -5' TCGCATGGAGACCACCGTGA 3'	1604-1623
	reverse	P2 -5' ATAGCTTGCCTGAGTGC 3'	2076-2060
	nested PCR		
	forward	P3 -5' CATAAGAGGACTCTTGGACT 3'	1653-1672
	reverse	P4 -5' GGAAAGAAGTCAGAAGGC 3'	1974-1957
Polymerase/Surface	(first PCR)		
	forward	HB2823 -5' TCACCATATTCTTGGGAACAAGA 3'	2823-2845
	reverse	HB704 -5' CGAACCACTGAACAAATGGC 3'	685-704
(genotyping)	mix A		
	fw	B2 -5' GGCTCMAGTTCMGGAACAGT 3'	67-86
	rv	BA1R - 5' CTCGCGGAG ATTGACGAGATGT 3'	113-134
	rv	BB1R -5' CAGGTTGGTGAGTGACTGGAGA 3'	324-345
	rv	BC1R - 5' GGTCCTAGGAATCCTGATGTTG 3'	165-186
	mix B		
	fw	BD1 -5' GCCAACAAGGTAGGAGCT 3'	2979-2996
	fw	BE1 -5' CACCAGAAATCCAGATTGGGACCA 3'	2955-2978
	fw	BF1 -5' GYTACGGTCCAGGGTTCACA 3'	3032-3051
	rv	B2R -5' GGAGGCGGATYTGCTGGCAA 3'	3078-3097
Polymerase/Surface	(first PCR)		
	forward	HB2823 -5' TCACCATATTCTTGGGAACAAGA 3'	2823-2845
	reverse	HB704 -5' CGAACCACTGAACAAATGGC 3'	685-704
	semi-nested		
	forward	HB75 -5' CAAGGTATGTTGCCCGTTTGTCC 3'	455-477
	reverse	HB704 -5' CGAACCACTGAACAAATGGC 3'	685-704

of the first amplification performed for genotyping with the primers listed in Table 1. The expected 250 bp band in the polymerase/S region was resolved on 2% agarose gel. All in-house PCR amplifications were run on a Perkin Elmer/ABI thermal cycler 2400 or 9700 using HotStartTaq DNA polymerase (Qiagen™). HBV DNA quantification by real-time PCR was performed using a RealART HBV RG PCR kit (Artus™ GmbH, Hamburg Germany) on the iCycler iQ optical thermal cycler (BIO-RAD™). Reactions were set-up according to manufacturers' protocols. The 95% detection limit for the RealArt HBV RG PCR kit is 3.8 IU/mL (RotorGene2000/3000) assessed by the WHO international standard (97/746). To assay the sensitivity of the kit on our instrument, we tested serial 10-fold dilutions of the reference preparation HBV-DNA ISS 0501 with a nominal titer of 4900 IU/mL (determined versus the WHO 97/746 standard and provided by the Italian National Institute of Health). The sensitivity of the assay for HBV DNA obtained from 0.5 mL of plasma was 4.9 IU/mL. The samples were confirmed to contain HBV-DNA if positive with the Ampliscreen test and positive with at least one of the other four PCR-based assays performed.

Statistical analysis

Categorical data are reported as frequencies and in percentages. Continuous variables are expressed as the mean ± SD or median, when appropriate. The χ^2 test was used to compare differences in proportions; continuous variables were tested for normality and comparisons of differences in means between groups were made using the two-tailed Student's t test. Analyses were performed using the SPSS for Windows software package, version 10.0 (SPSS Inc., Chicago, USA). Test results were considered statistically significant when $p<0.05$. Differences in the prevalence values of anti-HBc positivity among different age groups and among donors of different ethnic origins were analyzed using the χ^2 test and when significant the relative risk (RR) is reported. The correlation coefficient r was computed with the linear and exponential regression model.

Results

We enrolled 6313 anti-HCV and anti-HIV negative first-time donors, of whom 3678 were males (58.3%) and 2635 females (41.7%); their mean age was 34.3±11.2 years (range, 18-69). Ninety-six percent of the donors were born in Italy while 3.8% were born in another country. The age distribution in the two different groups of donors (Italians and non-Italians) was similar (mean age 34.3±11.2 and 34.9±10.1 years, respectively, p=n.s.; range, 18-69 and 24-50 years, respectively). Twenty-six of the 6313 donors (0.4%) were HBsAg positive; of these, 18 were males and 8 females with a mean age of 42.8±10.3 years (range, 23-58). The country of birth and the prevalence of HBsAg found among donors are shown in Table 2. The overall

Table 2. Geographical origin of blood donors and prevalence of HBV markers in the different groups.

Geographical origin	Donors tested	HBsAg+ n.	HBsAg+ Prevalence (CI) %	HBsAg− n.	Anti-HBc+ n.	Anti-HBc+ Prevalence (CI) %
Italian	6071	21	0.35 (0.20-0.50)	6050	267	4.41 (3.89-4.93)
Non-Italian	242	5	2.07 (0.7-3.2)	237	38	16.03 (13.56-19.43)
North-West Europe/ North-America	*49*	–		*49*	*1*	*2.04**
Eastern-Europe§	*110*	*4*	*3.64 (0.14-7.14)*	*106*	*22*	*20.75 (13.03-28.47)*
Africa	*46*	*1*	*2.17**	*45*	*13*	*28.89 (15.65-42.13)*
South America	*24*	–		*24*	*1*	*4.17**
Asia	*13*	–		*13*	*1*	*7.69**
Total	6313	26	0.41 (0.25-0.57)	6287	305†	4.85 (4.32-5.38)

*§Romania, Albania, Fyrom, Moldavia; *CI not reported because of the low number of cases. †Number of confirmed anti-HBc-positive cases out of 312 initially reactive for anti-HBc.*

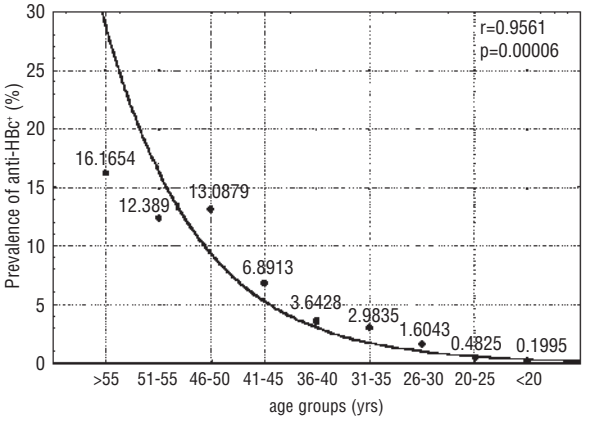

Figure 1. Prevalence of Italian anti-HBc-positive donors in different age groups.

prevalence of markers of HBV infection was higher in foreign donors than in Italians (2.07% *vs* 0.35%, respectively, for HBsAg+ and 16.03% *vs* 4.41%, respectively, for antiHBc+). Among foreign donors, only those born in Eastern European and African countries contributed to the high prevalence rate of HBV infection markers; therefore these two subgroups of donors were compared to Italians. The prevalence of HBsAg-positive donors born in East-Europe/Africa was significantly higher than that of HBsAg-positive Italian donors (3.64% and 2.17% *vs* 0.35%) (p<0·01). The mean age of the HBsAg-positive donors from Italy and from Eastern-Europe/Africa was 45.2±8.9 years (range, 25-58) and 32.8±10.4 years (range, 23-48), respectively (p<0.05).

When first tested with the MEIA AxSYM CORE, 312 out of 6287 HBsAg-negative donors were anti-HBc positive. All were retested using the same assay and also with the ChLIA PRISM HBc assay. In seven cases anti-HBc reactivity was not confirmed by the second assay and were, therefore, considered MEIA AxSYM CORE false positives. The other 305 donors were confirmed to be anti-HBc positive, giving an overall prevalence of 4.85% subjects who showed evidence of a past HBV infection (HBsAg-negative/anti-HBc-positive) among first-time donors. These 305 donors comprised 176 males and 129 females with a mean age of 44.4±9.8 years (range, 19-62), meaning that they were significantly older than the anti-HBc-negative donors (p<0.01). Two hundred and sixty-six of these donors (87.21%) were also positive for anti-HBs with the titer being greater than 100 IU/L in 68.5% of them. The prevalence of anti-HBc-positive donors among Italians and among donors from Eastern-Europe and Africa was 4.41% *vs* 20.75% and 28.89%, respectively (p<0.01). The prevalence of anti-HBc in Italian donors was directly correlated to age, with an exponential fit (Figure 1).

Two hundred and eighty-eight anti-HBc-positive plasma samples were tested for HBV-DNA; ten out of the 305 anti-HBc-positive donors refused consent to HBV-DNA testing and in seven cases there was not enough plasma to perform these additional assays. Out of the 288 anti-HBc-positive samples tested for HBV-DNA, 242 were anti-HBs-positive (84.0%), and 165 had an antibody titer greater than 100 IU/L (68.2%). Sixteen samples (5.55%) were HBV-DNA positive by the COBAS Ampliscreen HBV assay; these were from eight males and eight females with a mean age of 42.18 years (range, 30-59). Place of birth, anti-HBs titer and HBV-DNA results of this group of donors are shown in Table 3. Fourteen samples were positive with the quantitative real-time PCR so the prevalence of confirmed viremic donors among the 288 anti-HBc-positive tested was 4.86% (14/288) (CI:2.38%-7.34%); these 14 donors comprised eight males and six females with a mean age of 42.21 years (range, 30-59), all had anti-HBs with titers ranging from 12 IU/L to >1000 IU/L. We found no viremic subjects positive for anti-HBc antibody only. Levels of viremia were low: only one sample contained slightly more than 100 IU/mL (#10). Genotyping was possible for only six of the 14 confirmed HBV-DNA-positive samples (Table 3); in four cases there was insufficient plasma to perform the test and in the other four cases no band was observed after the second amplification step. The identified genotype was type D for all of the six positive

results. None of the viremic donors had been previously submitted to HBV immunization.

We estimated that 0.23% of released units (312 anti-HBc-positive out of 6313 tested donors times 305 confirmed anti-HBc-positive out of 312 anti-HBc-initially-reactive times 14 HBV-DNA-positive out of 288 anti-HBc-confirmed-positive tested for viremia) could have been viremic and otherwise transfusable. This is equalent to the release of one unit containing HBV-DNA in 435 allogeneic donations or 2298 in one million units eligible for transfusion. We assigned to repeat blood-donors the same prevalence of anti-HBc as that in first-time donors; this extrapolation, however, may underestimate the percentage of HBsAg-negative/anti-HBc-positive units, since repeat donors are older than first-time donors and the prevalence of anti-HBc increases distinctly with age in our population.

Discussion

This study has shown that in Piedmont, a region in north-western Italy, the prevalence of anti-HBc antibody among HBsAg-negative first-time blood donors is not negligible: 4.85% were positive and 87.2% of these also had anti-HBs antibodies. This figure compares with anti-HBc prevalence rates among HBsAg-negative blood donors that vary from 0.56% in the United Kingdom,[20] 0.84% in United States,[21] 1.4% in Germany,[11] 15.03% in Greece,[22] 16.4% in Saudi Arabia[23] to 76% in Ghana.[20] In our area the current HBsAg-negative/anti-HBc-positive donor population is made up of two groups: one of native Italians and another, on the increase, of foreign donors belonging to immigrant fluxes from East-European and African countries. The prevalence rates of anti-HBc antibody and HBsAg were distinctly higher in immigrants than in natives; furthermore, anti-HBc-positive donors born in Italy were significantly older than those born abroad. The increase of anti-HBc positivity with older age in the Italian donors mirrors a cohort effect of HBV infections acquired decades ago when HBV was highly endemic in Italy. While, in recent years, the circulation of HBV has decreased in Italy, the overall rate of HBV infection among blood donors could remain considerable. This is because of the significant, high prevalence of HBV markers in younger migratory populations that increasingly contribute to the blood supply.

The isolated finding of anti-HBc antibody in HBsAg-negative subjects was considered a marker of past exposure to HBV and of resolved infection. However the use of sensitive techniques to detect HBV-DNA has shown that low levels of viremia are detectable in 1.6% to 38% of HBsAg-negative/anti-HBc-positive donors.[10-12] In this study we detected HBV-DNA in the serum of 14 out of 288 HBsAg-negative/anti-HBc-positive blood donors (4.86%).

This higher prevalence compared to that found in other European and American series[10-12,22] can be explained in part by the sensitivity of the assay used,

Table 3. Geographical origin, anti-HBs titer and HBV-DNA results of anti-HBc-positive subjects tested positive by the COBAS™ Ampliscreen HBV assay.

Pts. #	*Place of birth*	*Anti-HBs titer*	*Ampliscreen‡*	*In-house nested PCR*			*Real-time PCR§*
		IU/L	*HBV-DNA*	*preCORE*	*POLYM*	*Genotype*	*IU/mL*
1	Italy	12	POS	POS	NEG	not ampl†	23
2	Italy	1000	POS	POS	NEG	not ampl†	19
3	Italy	321	POS	NEG	POS	D	80
4	Italy	1000	POS	POS	NEG	n/a*	24
5	Italy	1000	POS	NEG	weakPOS	–	<5
6	Italy	198	POS	NEG	POS	not ampl†	64
7	Italy	49	POS	NEG	POS	D	48
8	Albania	>1000	POS	NEG	POS	D	49
9	Morocco	119	POS	NEG	POS	D	59
10	Italy	617	POS	POS	POS	D	108
11	Italy	534	POS	POS	POS	n/a*	23
12	Italy	107	POS	NEG	POS	not ampl†	8
13	Italy	362	POS	weakPOS	NEG	–	<5
14	Albania	260	POS	POS	POS	D	72
15	Italy	>1000	POS	POS	POS	n/a*	21
16	Italy	232	POS	POS	POS	n/a*	18

**n/a: sample not available; †not ampl: no band observed after the second amplification step. ‡97% detection limit of the Ampliscreen HBV assay: 5 IU/mL (WHO international standard) or 30 copies/mL (NIBSC working reagent). §95% detection limit of real-time quantitative PCR: 4.9 IU/mL (Italian National Institute of Health standard).*

which has a detection limit of 5 IU/mL or 30 copies/mL. Another point is that some HBV-DNA positive samples might have been not reactive for the HBsAg because of mutations in the "a" determinant of the surface antigen.[24] This is a possibility in a country such as Italy where the HBV vaccination was introduced in 1991 and since breakthrough HBV variants may have occurred. Nevertheless, in Italy only a minority of the population is vaccinated: subjects younger than 27 years submitted to mandatory immunization and a negligible number of subjects who spontaneously decided to undergo vaccination. In our cohort, less than 20% of the donors were vaccinated and 97.13% of them had undergo mandatory immunization at the age of 11.

Thus the role of HBV vaccination appears to be of limited relevance. Finally, contamination artefacts were excluded by confirming HBV-DNA positivity with other assays, performed in a different laboratory using independent HBV primer sets.

In our study we estimated that 2298 units per million donated units could potentially contain HBV-DNA; this figure represents a risk 100 times higher than the risk of transfusing an HBV-DNA-positive donation collected during the window phase of the infection (estimated to be 15.78 units per million donated units).[4] Whether HBsAg-negative/anti-HBc-positive units can transmit HBV infection to recipients is debated.[13-17,25-27] The low viremic content and often the concomitant presence of neutralizing anti-HBs antibodies has led several authors to conclude that blood from anti-HBc/anti-HBs-positive donors is safe; the English experience seems reassuring as no case of hepatitis B transmission was related to transfusion in a survey of 20000 blood units controlled only for HBsAg.[25] However, the prevalence of HBV is very low in Northern Europe and the number of units traced was relatively small, while the transfusion risk may be different in areas such as Italy, where HBV remains endemic. In four large studies of post-transfusion hepatitis performed in the 1970s in the United States, Australia and The Netherlands, the overall rate of HBV infection after receipt of blood tested for HBsAg only varied from 1 to 2.5%; however in all series, hepatitis B was more frequent after transfusion with anti-HBc-positive than with anti-HBc-negative blood, with the rate of transmission varying between 2% and 8.6% among recipients of anti-HBc-positive blood.[26-29] A number of other studies showed cases of HBV transmission after the transfusion of anti-HBc-positive blood.[13-16]

A recent study showed that up to 16% of anti-HBc/anti-HBs-positive donors have circulating HBV-DNA unbound to anti-HBs in their sera and thus in a potentially infective form.[10] In our study, the prevalence of occult HBV was also high in donors with high anti-HBs titers; HBV-DNA was detected in 12 out of 165 donors with an anti-HBs titer >100 IU/L and 2 out of 77 with an anti-HBs titer <100 IU/L. While countries such as Germany, Austria and Japan allow transfusion of units with anti-HBs titers higher than 100 IU/L, our findings raise doubt about whether high titer anti-HBs blood may guarantee against HBV transmission.

This point is also questioned by a recent Japanese study in which 16 cases of HBV infections were found after the introduction of the Red Cross test algorithm allowing transfusion of blood units with low-titer anti-HBc/high-titer anti-HBs. Two out of the 16 cases were related to anti-HBc-positive donors with high titers of anti-HBs who were retrospectively found to be positive for HBV-DNA.[17] If indeed up to 1 in 435 Italian donations could have been infective, at least some of the regular donors involved should have left a trail of cases of hepatitis B. The reasons why no such phenomenon has been described in Italy could be the following: (i) the mean age of the population transfused in our region is 68.8 years and therefore the prevalence of protected recipients is high (i.e immunized anti-HBc-positive patients); in a recent survey more than 35% of blood recipients in our hospitals were positive for anti-HBc (*data not published*); (ii) at least 90% of adult patients infected by HBV develop an asymptomatic infection and about 95% of them clear the virus; (iii) no surveillance system has been enforced in Italy to evaluate the rate of seroconversion in patients submitted to transfusion, nor is a repository of frozen sera from patients submitted to transfusion and from blood donors available in our country. It seems reasonable to conclude that even if there is no evidence of a high rate of transfusion-related hepatitis B in Italian recipients of blood screened only for HBsAg, there is also no evidence to rule out the possibility that HBV infection may ultimately occur in recipients of blood from carriers of occult HBV. Factors other than the virologic characteristics of the donor may be involved in the risk of transmission of HBV infection, particularly the clinical situation of the recipient. Recipients who are immunocompromised either naturally or by therapy are notoriously at higher risk and their number is increasing due to the better survival of patients given immunosuppressive therapies.[30]

While anti-HBc-positive blood may be a potential source of HBV transmission, routine application of anti-HBc screening is not feasible in many regions, as it would seriously limit the blood supply. In Italy, a country that has not reached blood self-sufficiency, anti-HBc testing would cause the exclusion of a consistent number of donors, 95% of whom are HBV-DNA negative. Though the introduction of NAT testing for HBV is going to be enforced in some regions of Italy, this screening will not be effective if performed on pooled samples. None of our donors had a virus titer detectable with the COBAS Ampliscreen HBV test performed on 24-unit-pools (120 IU/mL lowest detectable viremia) and only 50% of viremic donors would have been detected using 8-unit-pools (40 IU/mL lowest detectable viremia). In this scenario, HBV-DNA NAT testing becomes effective to reduce the risk of HBV transmission only if performed on individual donations; however, the costs of such a strategy would be prohibitive until multiplex NAT testing for

blood-borne viruses is available everywhere. The introduction of anti-HBc antibody screening, at least in first time donors, will prevent subjects who have been previously in contact with HBV from being included in the donor pool. At present, an additional, albeit limited, option to avoid the potential risk of HBV transmission through transfusion from occult HBV donors could be the immunization against HBV of selected HBV-naïve patients, in particular those who are immunocompromised and who are likely to receive multiple transfusions.

Authors' Contributions

PMan: designed the research, collected and analyzed the data and wrote the paper; MG, RB, OG, RG, ML and DT: designed the research and performed tests; PG, MV, FD, AP, and CV: performed tests; FCa: designed the research, analyzed the data; FCu: designed the research; PMag: analyzed the data; MLA: performed tests, wrote the paper; AS: analyzed the data and wrote the paper; MR: wrote and revised the paper.

Conflict of Interest

The authors reported no potential conflicts of interest.

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