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ORIGINAL ARTICLES

Surveillance report

HUMAN IMMUNODEFICIENCY VIRUS, HEPATITIS C AND HEPATITIS B INFECTIONS AMONG BLOOD DONORS IN GERMANY 2000-2002: RISK OF VIRUS TRANSMISSION AND THE IMPACT OF NUCLEIC ACID AMPLIFICATION TESTING

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Blood and plasma donations in Germany are collected by several institutions, namely the German Red Cross, community and hospital-based blood services, private blood centres, commercial plasma donation sites and transfusion services of the army. All blood donation centres are required to report quarterly data on infection markers to the Robert Koch Institute, thus providing current and accurate epidemiological data. The prevalence and incidence of relevant viral infections are low in the blood donor population in Germany, with a decreasing trend for hepatitis C infections in new and repeat donors since 1997. The implementation of mandatory nucleic acid amplification technique (NAT) testing for hepatitis C virus (HCV) in 1999 has markedly improved transfusion safety. HIV-NAT became mandatory in 2004 but was done voluntarily by the majority of the blood donation services before then. The potential benefit of hepatitis B virus (HBV) minipool NAT is not as clear because chronic HBV carriers with very low virus levels might donate unidentified. The residual risk of an infectious window period donation inadvertently entering the blood supply can be estimated using a mathematic model which multiplies the incidence rate by the number of days during which an infection may be present but not detectable, i.e. the length of the window period. The risk of an undetected infection without NAT testing was estimated to be 1 in 2 770 000 for HIV, 1 in 670 000 for HCV and 1 in 230 000 for HBV in 2001/2002. This contrasts with 1 in 5 540 000 for HIV, 1 in 4 400 000 for HCV and 1 in 620 000 for HBV with minipool NAT testing. This demonstrates that NAT testing can further reduce the already very small risk of infectious donations entering the blood supply.

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Introduction

Protection of the blood supply from virus-infected donations has reached a very high level due to effective donor selection and testing with the latest techniques. The most sensitive diagnostic method suitable for donor screening, nucleic acid amplification technique (NAT) testing, has become mandatory for hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-1 in Germany in 1999 and 2004, respectively. Surveillance of infectious disease markers in the blood donor population is important in recognising trends in prevalence and incidence of transfusion related infections. It also provides an opportunity to estimate the risk of an infectious donation inadvertently entering the blood supply. Mathematic models applied to surveillance data help evaluate the potential benefit of new tests, like the introduction of minipool or individual donation NAT. Epidemiological data on HIV, HCV and hepatitis B virus (HBV) infections has been systematically analysed in Germany since 1996 and reporting of detected infections has become mandatory with the enactment of the Transfusion Act in July 1999. The Robert Koch-Institute (RKI) collects and analyses nationwide data. In Germany, more than 100 individual blood donation services collect several thousand to several hundred thousand donations per year. In this report we present data collected from 2000 to 2002, including residual risk estimates which are representative for all German blood donations.

Methods

Data were obtained from the RKI nationwide blood donation infection surveillance and included more than 99% of all donations in 2000 and 100% of all donations in 2001 and 2002. Blood and plasma donation centres reported aggregated data on number and

type of donations from new and repeat donors and the number of confirmed HIV, HCV and HBV infections. Detailed serological results from all positive donors were available. An infection was considered confirmed positive if a reactive screening result was verified by an appropriate supplementary test in a different test system and/or NAT. During the study period all blood donations were screened for anti-HIV 1/2, anti-HCV, HCV genome and hepatitis B surface antigen (HbsAg). A large number of donations was also screened with HIV-1 NAT, HBV NAT and to a lesser extent, tested for anti-HBc on a voluntary basis. A minimum sensitivity of 5000 IU/ml with respect to the individual donation was required for HCV-NAT testing. Sensitivity of NAT had to be validated with limiting dilutions followed by probit analysis as recommended by the German licensing agency, the Paul-Ehrlich-Institute [1]. The majority of donations were screened with an in-house Taqman PCR in minipools of up to 96 samples [2,3]. To a lesser extent, donations were tested using commercially available NAT tests or in-house NAT with small pool sizes or with individual donation-NAT. All NAT-only positive results had to be confirmed either by later seroconversion or by positive NAT from a second independently drawn blood sample.

Prevalence was calculated as number of infections in all individuals who presented at the blood donation centre for the first time (new donors). Seroconversions refer to all confirmed infections found in donations from repeat donors. Infection rates were compared to data from previous nationwide studies on infectious disease markers in blood donors [4,5,6]. Trends were calculated using a Chi Square test for linear trends, 95% confidence intervals (CI) were determined using a binomial distribution. Additional data on NAT-only positives from the NAT-study of the German Red Cross (GRC) blood donor service were included (Roth, written communication). Residual risk calculations were performed using a modified incidence rate/window period model [7,8]: Briefly, the residual risk attributable to window period donations was calculated as

(window period) x(adjusted incidence/person years at risk).

Window periods for testing procedures were derived from the literature [7,9]. Incidence was calculated as number of seroconversions for HIV, HCV and HBV reported to the RKI in the study period, respectively ("crude incidence"). Donations which would not have entered the blood supply due to an additional positive test result (ALT, syphilis) or a confidential self exclusion were subtracted from the number of seroconversions to calculate the "adjusted incidence" used in the model. For HBsAg, risk was calculated both with and without the correction factor to compensate for the transient nature of HBsAg [10]. The correction factor was determined to be 2.73 calculated from the individual interdonation intervals of the HBV-positive donations from German blood donors. Person years at risk were derived from the number of repeat whole blood donations from donors who had given at least 2 donations within the 2 year study periods ("regular donors") between 2000/2001 and 2001/2002 divided by the mean interdonation interval length (0.52 years).The window period for HBsAg was reduced by 9 days to account for the higher sensitivity of HBsAg tests used in Germany compared with FDA licensed tests commonly used for the determination of the window period [11]. Residual risks were calculated for the 2 overlapping periods 2000/2001 and 2001/2002.

Results

German blood donation services tested 17 925 610 donations during the 3 year study period from 2000 to 2002. Of these, 91.2% were donations from repeat donors. The proportion of whole blood donations was 77.9%. Test results from new donors and repeat donors respectively are given in Table 1 including data from previous studies [5,6].

Comparing the results of blood donor screening in Germany from 1997 to 2002 the prevalence of HBV infections remains relatively stable whereas HIV prevalence increased in 2002. Seroconversion rates for both infections did not change significantly over time. HCV infections, however, demonstrate a significant decrease since 1997, both for prevalence (from 148.8 to 97.4 infections/105 new donors, $p<0.000$) and for the rate of seroconversions (from 2.6 to 1.5 infections/105 donations from repeat donors, $p<0.000$).

From 2000 to 2002, more than 17 million donations were reported to the RKI representing > 99 % of all collected donations including those of the GRC. All donations were tested with HCV NAT. With HIV-1 and HBV NAT not being mandatory in the study period, the proportion of donations screened for HIV-1 and HBV genome could not be determined exactly but certainly exceeded 60%.

The GRC blood donor service collects about 75 % of all whole blood donations in Germany and implemented NAT testing as early as 1996 in some centres for all three viruses [12]. The NAT study of the GRC included more than 21 million donations from January 1997 to October 2003. The number of NAT-only positive donations for both studies is given in Table 2.

Table 1

Prevalence and seroconversions of confirmed HIV, HCV and HBV infections in blood donations in Germany, 1997-2002

Year	Donations	HIV infections	HIV inf./ 105 donations	CI 95%	HCV infections	HCV inf./ 105 donations	CI 95%	HBV infections	HBV inf. / 105 donations	CI 95%
New donors										
1997	423 364	25	5.9	3.8-8.7	630	148.8	137.4-160.9	742	175.3	162.9-188.3
1998	452 820	21	4.6	2.9-7.1	503	111.1	101.6-121.2	749	165.4	153.8-177.7
1999	452 692	16	3.5	2.0-5.7	470	103.8	94.7-113.6	680	150.2	139.1-161.9
2000	478 263	17	3.6	2.1-5.7	465	97.2	88.6-106.5	702	146.8	136.1-158.0
2001	535 324	25	4.7	3.0-6.9	507	94.7	86.7-103.3	851	159.0	148.5-170.0
2002	576 979	43	7.5	5.4-10.0	562	97.4	89.5-105.8	947	164.1	153.9-174.9
Repeat donors										
1997	4 657 843	34	0.7	0.5-1.0	121	2.6	2.2-3.1	65	1.4	1.1-1.8
1998	4 859 415	23	0.5	0.3-0.7	131	2.7	2.3-3.2	74	1.5	1.2-1.9
1999	4 979 349	28	0.6	0.4-0.8	113*	2.7	2.2-3.2	69	1.4	1.1-1.8
2000	5 105 247	35	0.7	0.5-1.0	165	3.2	2.8-3.8	55	1.1	0.8-1.4
2001	5 174 342	27	0.5	0.3-0.8	83	1.6	1.3-2.0	74	1.4	1.1-1.8
2002	6 055 455	43	0.7	0.5-1.0	93	1.5	1.2-1.9	72	1.2	0.9-1.5

* refers to 4 254 364 donations [3]

TABLE 2

HIV, HCV and HBV NAT-only positive donations reported to the RKI or from the NAT-study of the GRC blood donor service, Germany, 1997-2003

Virus	Study	Period of observation	Donations tested	NAT-only positive	Incidence /10^5
HCV	RKI	2000-2002	17 925 610	11	0.061
	GRC	1997-Oct. 2003	23 702 392	16	0.068
HIV	RKI	2000-2002	n.a.	5	n.a
	GRC	1997- Oct. 2003	21 695 596	6	0.028
HBV	RKI	2000-2002	n.a.	3	n.a.
	GRC	1997- Oct. 2003	21 733 529	47	0.216

n.a. = not available

The residual risk of an infectious window period donation entering the blood supply unrecognised was calculated using the epidemiological data reported to the RKI. Data are shown for two overlapping two-year periods 2000/2001 and 2001/2002. With the same test systems in place the estimated window periods remained the same in both observation periods. The decrease of the adjusted incidence of HCV and to a lesser extend also of HIV lead to a reduction of the estimated residual risk of window period donations. In 2001/2002 it was calculated to be 1 in 2 770 000 for HIV, 1 in 670 000 for HCV and 1 in 230 000 for HBV (corrected) without NAT and 1 in 5 540 000 for HIV, 1 in 4 400 000 for HCV and 1 in 620 000 for HBV with minipool NAT. The risk of an undetected window period donation could be further reduced to 1 in 820,000 for HBV with ID NAT. The results are shown in Table 3.

Discussion

Infection rates among blood donors in Germany are low and since 1997, a significant decrease with regard to HCV infections among new and repeat donors has been observed. Similar trends were also found in other countries [13,14]. The recent rise in HIV prevalence has to be investigated carefully to reveal possible changes in donor characteristics. People seeking free-of-charge HIV tests results by donating blood might contribute to the observed rise in prevalence. Case control studies are necessary to verify this hypothesis.

The implementation of HCV NAT has lead to the identification of 11 otherwise unrecognised HCV-positive donations as reported to the RKI between 2000 and 2002. The benefit of the introduction of HCV NAT was also reflected in the national haemovigilance report [15]. No HCV transmissions have been reported to the Paul-Ehrlich-Institute since HCV NAT testing became mandatory. The additional gain in safety achieved by introduction of HIV-1 NAT is not quite as marked due to the smaller reduction in the diagnostic window period compared with HCV NAT. Still, HIV-1 NAT did identify some otherwise undetected infectious donations which might have led to transmissions – an important result with respect to the severity of the disease. HBV NAT proved helpful in reducing HBV transmissions but this depends largely on the sensitivity of the NAT performed. With the highly sensitive PCR minipool testing following virus enrichment as performed by the GRC [2], 47 HBV NAT-only positive donations could be identified including preseroconversion donors as well as chronic HBsAg-negative HBV carriers. Still, some infectious are missed by minipool NAT after enrichment or even by individual donation NAT [1]. Compared to sensitive HBsAg tests standard minipool NAT can only add little to reduce the window period for HBV infections [16]. Due to the slow replication rate of HBV in the early phase of infection, only a very sensitive individual donation HBV NAT (e.g. with a detection limit of 50 copies/ml or less) would help to avoid a greater number of undetected infectious donations [17]. Another approach to reduce HBV-transmissions is to introduce additional anti-HBc testing to identify chronic HBV carriers with a very low viral load. There is evidence that blood components containing anti-HBc and anti-HBs do not transmit HBV [18]. Therefore re-entry of donors with anti-HBc and anti-HBs (>100 IU/l) who are negative in individual donation HBV NAT should be taken into consideration to minimise the prospective loss of donors if anti-HBc screening were introduced in Germany. Finally both measures, individual donation-NAT and anti-HBc testing should be carefully evaluated in terms of cost-benefit [19,20]. The observed difference between the RKI's reported numbers and GRC data with respect to HBV NAT-only donations can be explained by the fact that the reporting of an (initially non confirmed) NAT-only positive result is not yet mandatory in Germany. Obviously, these infections are mainly reported after follow up testing revealed seroconversion or presence of HBsAg.

The residual risk of infectious window period donations entering the blood supply in Germany is low. The implementation of HCV NAT and the significant decrease in HCV incidence among repeat donors has lead to a measurable fall in the estimated residual risk.

TABLE 3

Estimated risk of an undetected infectious donation entering the blood supply using a modified incidence/window period (WP-model), Germany, 2000-2002

Period of observation	Virus	Adjusted incidence/ 105 person years	Test	Window period (days)	Risk per 106 donations	Risk (Rate of undetected infectious donations)
2000-2001	HIV	0.72	anti-HIV 1/2	22	0.43	1:2 320 000
			anti-HIV 1/2, plus NAT	11	0.22	1:4 640 000
	HCV	1.34	anti-HCV	66	2.42	1:410 000
			anti-HCV, plus NAT	10	0.37	1:2 730 000
	HBV	1.22	HBsAg no correction	50	1.68	1:600 000
			HBsAg, corrected	50	4.08	1:250 000
			HBsAg, plus minipool NAT	45	1.51	1:660 000
			HBsAg plus single donation NAT	34	1.14	1:880 000
2001-2002	HIV	0.60	anti-HIV 1/2	22	0.36	1:2 770 000
			anti-HIV 1/2, plus NAT	11	0.18	1:5 540 000
	HCV	0.83	anti-HCV	66	1.50	1:670 000
			anti-HCV, plus NAT	10	0.23	1:4 400 000
	HBV	1.31	HBsAg no correction	50	1.80	1:560 000
			HBsAg, corrected	50	4.37	1:230,000
			HBsAg, plus minipool NAT	45	1.62	1:620 000
			HBsAg plus single donation NAT	34	1.22	1:820 000

Also the implementation of HIV-1 and HBV NAT has an impact on the risk of undetected infectious donations because of the shortening of the window period. Comparing risk estimates between countries remains difficult as the mathematical models used are commonly adapted to the specific national data characteristics leading to significant differences in risk estimates [21].

Residual risk estimates always have limitations. The determining factor in the equation is the length of the window period which may vary considerably depending on the specificity and sensitivity of the test used. This might also hold true for the German data with different NAT tests and different pool sizes or individual donation-NAT in place. The used window period derived from the literature reflect average sensitivity of minipool NAT which is higher in some blood donation services especially when individual donation NAT is performed and consequently leads to a smaller residual risk. Furthermore, in our model we considered all window period donations to be infectious although during the early ramp-up phase of viral replication, this might not be the case [22]. It must also be kept in mind that given risk estimates are derived from repeat whole blood donors only and might therefore underestimate the true number of undetected infectious donations, as it has been shown that new donors might pose a greater risk of infectious donations than repeat donors [23]. Also, other influencing factors such as test or process errors or mutant viruses that are not detected by blood donor screening are not considered in the model. Still, keeping those limitations in mind, the residual risk model was able to demonstrate the benefit of NAT techniques in reducing window period donations especially for HCV and HIV.

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