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Prevalence of Serological Markers for Hepatitis B and C Viruses in Brazilian Blood Donors, and Incidence and Residual Risk of Transfusion-Transmission of Hepatitis C Virus

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Abstract

Background—We evaluate the current prevalence of serological markers for HBV and HCV in blood donors and estimated HCV incidence and residual transfusion-transmitted risk at three large Brazilian blood centers.

Material and Methods—Data on whole blood and platelet donations were collected from January through December 2007 and analyzed by center, donor type (replacement vs. community), age, sex, donation status (first-time vs. repeat), and serological results for HBsAg, anti-HBc and anti-HCV. HBV (HBsAg+/anti-HBc+) and HCV (anti-HCV) prevalence rates were calculated for all first time donations. HCV incidence was derived including inter-donation intervals that preceded first repeat donations given during the study and HCV residual risk was estimated for transfusions derived from repeat donors.

Results—There were 307,354 donations from January through December 2007. Overall prevalence of concordant HBsAg and anti-HBc reactivity was 289 per 100,000 donations and of anti-HCV confirmed reactivity 191 per 100,000 donations. There were significant associations between older age and hepatitis markers, especially for HCV. HCV incidence was 3.11 (95% CI 0.77-7.03) per 100,000 person-years, and residual risk of HCV window-phase infections was estimated at 5.0 per million units transfused.

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Conclusion—Improvement in blood donor selection, socioeconomic conditions and preventive measures, implemented over time, may have helped to decrease prevalence of hepatitis B and C viruses, relative to previous reports. Incidence and residual risk of HCV are also diminishing. Ongoing monitoring of hepatitis B and C viral markers among Brazilian blood donors should help guide improved recruitment procedures, donor selection, laboratory screening methods and counseling strategies.

Keywords

Blood donors; Brazil; Residual Risk; Hepatitis B; Hepatitis C; Prevalence; Incidence

INTRODUCTION

Donor selection, serologic and molecular screening tests and pathogen inactivation are measures applied to decrease transfusion-transmitted diseases (TTDs). The implementation of Nucleic Acid Technology (NAT) has dramatically reduced the residual risk of transfusion-transmitted HIV and hepatitis B (HBV) and C viruses in developed countries.^{1,2} However, NAT tests are not available all over the world and residual risk of infection transmission persists mainly due to the window period of infectious agents and the high prevalence of disease in the source population.³ A better understanding of the prevalence and incidence of hepatitis virus infections among blood donors is needed to monitor blood safety and identify and establish measures to minimize transfusion risk, as well as to inform public health policies focused not only on blood donors but on the general community. In Brazil, which has an area of approximately 8,500,000 Km² and 190,000,000 inhabitants, Hepatitis B and C infection rates vary by geographical regions. Previous studies have reported low prevalence of HBsAg positivity (chronic carriers) in the South of the country, intermediate rates in Northeast and Southeast, and the highest prevalence in the Amazon region (up to 9.7%).^{4,5} It is been estimated that around 1.5% of the Brazilian population is HCV-positive.^{6,7} Higher HCV prevalence rates can be found in the Southeast and South, with lower rates in the North of Brazil. Additionally, the Brazilian Ministry of Health estimates that death rates as a primary consequence of HBV and HCV increased 4.7% from 2009 to 2010.8

Data on prevalence and incidence of HBV and HCV among blood donors in Brazil are very limited. The aims of this study were to evaluate the prevalence of serologic markers for HBV, prevalence and incidence of serological markers for HCV in blood donors, and to estimate residual risk of window phase infections for HCV and associated residual risk of transfusion-transmitted HCV at the three Brazilian REDS-II International Program participating centers.

MATERIALS AND METHODS

Data on whole blood and platelet donation type (replacement vs. community), age, sex, donation status (first-time vs. repeat), and serological results for HBV and HCV markers were collected from January 2007 through December 2007, among the main sites of each Brazilian REDS-II participating center, located in the cities of São Paulo, Belo Horizonte and Recife. The international REDS-II study in Brazil includes three public blood centers, two in the Southeast of Brazil (São Paulo and Belo Horizonte) and one, in the Northeast (Recife). Together these three blood centers collect almost 8% of all donated blood per year in Brazil. The study was approved by the Brazilian national ethics committee as part of the REDS-II International Program.

Donation is allowed for persons 18 to 65 years of age. Paying donors is forbidden in Brazil. Donors can give either at the request of or on behalf of a specific recipient to replace units in the overall supply (defined as replacement donations) or to the general blood supply (defined as community donations). Replacement donors are generally recruited among friends and relatives of hospitalized patients and community donors through media campaigns. First-time donation was defined as a donation originated from a donor who had given blood for the first-time in each blood center in 2007 and had no previous history or record of blood donation.

Serological tests were performed according to the standard operational procedures of each center and included tests to antibodies for hepatitis B surface antigen (HBsAg); anti-HBc, anti-HIV-1/2, anti-HTLV-1/2; and one screening test for syphilis. Laboratory screening was performed in the study centers using kits approved by the Brazilian Ministry of Health, each having sensitivity higher than 99% as described in the manufacturer's package insert information. The kits used in each center were not necessarily the same and some centers discarded units and referred samples for supplemental testing that had borderline or gray zone reactivity (sample to cut-off ratio 0.9 zone and 1). All samples that tested repeatedly reactive for anti-HCV or HBsAg testing were retested at the Serology Division of Fundação Pró-Sangue - Blood Center of São Paulo (FPS-HSP), with a second EIA kit that was not utilized by any of the three centers, with comparable or higher sensitivity. False-positive results were notified to the Serology Department of each blood center. For the purpose of this study, donations were considered HBV positive if repeat-reactive for both HBsAg (EIA) and anti-HBc (EIA) and HCV positive if anti-HCV (EIA) and immunoblot tests were reactive either on the donation or in a follow-up sample collected at the time of counseling. HBsAg non reactive and anti-HBc reactive donations were also recorded.

Prevalence was calculated for all first time donations only since detection of viral markers in repeat donor represent incident infections. Incidence for repeat donations was derived using interdonation intervals that preceded the first repeat donation given during the study. Prevalence was defined as number of seropositive first-time donations divided by total number of first-time donations in 2007. Incidence for HCV was calculated restricting to the total person-years at risk during 2007, and considering all HCV confirmed positive blood donations by repeat donors observed during the study period that were negative in a previous donation (collected either during or prior to the study period), then estimating the subset of incidents that occurred in 2007. For each repeat donation during the study period there was an associated interdonation interval, then the person-time was equal to the interdonation interval, and if the donation was confirmed positive, then an incident infection within the study period was known to occur. If the donation was confirmed positive during the study period and was negative in the previous donation, then an incident infection was known to have occurred within the interdonation interval. Assuming that the incident infection event was equally likely to occur in any date within the interdonation interval, the probability that it occurred within the study period can be estimated as a fraction of time (i.e., person-time/interdonation interval). The study period incidence was so estimated as the sum of incident infections and fractional incidents infections divided by the sum of persontime as already described.9

Residual risk for HCV was calculated as incidence (100,000 donations) x window phase (days)/365 days using infectious window phase estimates of 58.3 days pre-anti-HCV seroconversion.

Demographic characteristics, first time vs. repeat donor status, and community vs. replacement donation type associated with all donations were tabulated by blood center, Prevalence and 95% confidence intervals of HBV and HCV as well as number and

percentage of anti-HBc positive donations among first time donors were tabulated by blood center, age group, gender, and type of donations. Multivariable logistic regression analyses were conducted to examine the correlates of HBV and HCV prevalence. A p-value less than 0.01 was defined as statistically significant to account for multiple comparisons (Bonferroni adjustment). All analyses were conducted using SASTM Windows 9.2 software (SAS Institute, 2008).

RESULTS

There were 307,354 donations from January through December 2007 among the three Brazilian REDS-II participating centers, including 137,630 (44.8%) in São Paulo, 99,789 (32.5%) in Recife and 69,935 (22.7%) in Belo Horizonte. Most of the donations came from male (69.6%), young (under 35 years-old, 60.4%), repeat (69.0%) and community (66.5%) donors (Table 1).

Hepatitis B Seroprevalence in First Time and Seroconversions in Repeat Donors

Overall prevalence of HBsAg plus anti-HBc-reactivity among first-time donations (i.e., chronic HBV carriers) was 289 per 100,000 donations. Prevalence of these markers was almost twice as high in Recife (419 per 100,000) than in São Paulo (213 per 100,000), (OR = 1.73, CI: 1.31-2.28, p<0.001), while prevalence in Belo Horizonte (270 per 100,000) was not statistically different than in São Paulo. Donations from male donors had higher prevalence than those from female donors (359 vs. 179 per 100,000), (OR = 1.86, CI: 1.40-2.47, p < 0.001). Donors aged 25 or older showed higher prevalence of HBsAg plus anti-HBc than those younger than 25 years of age, OR ranging from 1.85-2.91, p<0.001. There was no difference in prevalence between replacement and community donation types, 345/100,000 vs. 238/100,000, respectively (OR = 0.84, CI: 0.65-1.07, p=0.16). During 2007 two repeat donors that were negative for anti-HBc and HBsAg, seroconverted for both markers. This low rate of HBV seroconversion and requirement for adjust factors to infer total HBV incidence from HBsAg seroconversion rates precluded calculation of HBV incidence from our dataset.

Anti-HBc reactivity with negative HBsAg screening EIA results was found in 3.6% of first time donations, with the highest prevalence in Recife (4.7%, vs. 2.8% in São Paulo; p<0.001), increasing prevalence with age (1.6% among donors under 25 years vs. 11% among donors aged 55 years-old or more; p<0.001) and higher rates of HBV exposure among men (4.0% men vs. 2.8% women; p<0.001) (Table 2). Among repeat donations 573 (0.27%) of 210,232 donations tested anti-HBc reactive, a markedly lower rate than observed in FT donors due to culling of the repeat donor pool as a result of routine anti-HBc screening in Brazil.

Hepatitis C seroprevalence, incidence and residual risk

Prevalence of RIBA-confirmed anti-HCV reactivity among first-time donors was 191 per 100,000 donations (Table 3). Prevalence of HCV antibody positivity was two-to-three fold higher in São Paulo (287 per 100,000) compared with Belo Horizonte (78 per 100,000 donations) and Recife (131 per 100,000 donations). A significant age effect on HCV prevalence was observed. Donations from donors aged 55 or older had almost 30 times higher prevalence of HCV positive markers than those from donors under 25 years. Donations from donors older than 25 and younger than 55 presented 8-23 times higher prevalence than those younger than 25, p <0 .001. Donations from community donors also presented higher prevalence of HCV antibody when compared to replacement donors. There was no statistical difference in the prevalence of HCV among men and women (199 vs. 179 per 100,000 donations, respectively).

During the one year prospective study period, we observed only one HCV seroconverter, i.e, a donor who made one HCV negative donation in 2007 prior to making a HCV confirmed positive donation in the same year. By including historical data prior to 2007 for repeat donors who presented in 2007, an additional nine donations from repeat donors were HCV positive; on detailed review of prior donation data two of these donors had reactive anti-HCV screening results on their previous donations and therefore were removed from the incidence analysis. The other seven repeat donors all made HCV negative donations prior to their positive donations in the study period, which in addition to the seroconverter indentified in 2007 yielded a total of eight incident HCV cases, from which we estimated 2.87 incidents occurred in 2007⁹. We calculated that the total person time during 2007 among all repeat donors as 92,095 person-years, yielding an HCV incidence rate estimate of 3.11 (95% CI 0.77-7.03) per 100,000 person-years⁹. Using 58.3 days¹⁰ for the infectious pre-antibody window phase, the residual risk of transfusion-transmitted HCV was estimated as 5.0 per million donations (95% CI 1.2, 11.3 per million donations) or 1:201,000 donations. Further, Busch et al. 10 estimated the window phase could be reduced by 51 days using NAT testing, to an infectious pre-NAT window phase of 7 days. Hence the yield of NAT HCV was estimated as 4.3 per million donations (95% CI 1.1, 9.8 per million donations) or 1:230,000 donations and the NAT residual risk of transfusion-transmitted HCV was estimated as 0.6 per million donations (95% CI 0.2, 1.2 per million donations) or 1:1,585,000 donations.

DISCUSSION

This article describes the prevalence of HBV and HCV serologic markers, and the incidence and residual risk of HCV among allogeneic donations in three large blood centers in Brazil. Serologic markers for both infections showed a low prevalence relative to general population estimates in the three geographically dispersed donor population. A higher prevalence of HBV markers was found in the Northeast while HCV prevalence was higher in the Southeast, especially in São Paulo. Additionally, an association with increasing age was observed for both anti-HCV and anti-HBc among donors in all three regions. HCV incidence was similar to the incidence rates reported in the US and Europe, and residual risk estimates were comparable to what existed before introduction of NAT in these countries. ¹¹⁻¹³

Most publications evaluating prevalence of HBV among Brazilian blood donors have separately estimated the positivity rates for HBsAg and anti-HBc, which have varied from 0.18% to 0.98% and from 4.31% to 9.4%, respectively. 4,14-16 The definition that we employed for an HBV positive donation, i.e. repeat-reactive serologic test results for both HBsAg and anti-HBc, was more stringent than used in most other studies, but we felt was warranted to avoid false positive HBsAg results which are increasingly recognized as problematic in studies involving donor populations.^{2,17} Overall, the prevalence of concordant HBV positive markers found in our study population was in the lower range of rates in earlier reports. These findings may indicate a true decline in HBV infection as a result of vaccination and other public health programs, different prevalence rates among different donor populations, or be a consequence of different criteria to define HBV prevalence. Publically financed immunization for HBV is currently provided in Brazil for all infants and for young adults until 24 years-old, as well as offered to blood donors, to health students and professionals, and to other persons at high risk of HBV infection such as household contacts and sexual partners of HBV infected persons. ¹⁸ This program started in the early 1990s in the North of Brazil and was extended all over the country over the subsequent years. The estimated coverage of hepatitis B vaccine varies from 56% for the population in the North to ~40% in the Southeast and 25% in the Northeast. 19 It is noteworthy that prevalence of HBV serologic markers was almost two fold higher in Recife

than in the São Paulo, still reflecting the historically higher rates of HBV and lower vaccination coverage in Northeastern Brazil.

Prevalence of HCV serologic markers among Brazilian blood donors has been reported to range from 0.32% in the Amazon in 2007 to 1.2% in the Southeast of the country in 1996 ^{14,15,20-22}. Similar to what we observed for HBV, we found a lower HCV prevalence than described earlier, probably because we employed an immunoblot as the confirmatory test to define HCV positivity, whereas most prior reported rates of EIA repeat reactivity due to cost considerations. The improved control of transfusion-transmitted HCV after anti-HCV screening of all blood donations and implementation of public health procedures such as needle exchange to reduce HIV transmission, may also explain the lower prevalence found in our study²³. A recently published phylogenetic analysis indicated that all major HCV clades started to circulate in Brazil during the second half of the 20th century, coinciding with the implementation of blood transfusions in the 1940s and 1950s and with the expansion of intravenous drug use from 1976 to 1986. The authors also reported a reduction in the growth of the HCV epidemic from 1990 to 1995 after the introduction of anti-HCV screening, suggesting that the expansion of HCV may have been effectively controlled in Brazil²⁴. The age association with prevalence of HCV markers may be a consequence of a birth cohort effect reflecting the past expansion and contemporary restraint of this infection. Oliveira et al.²⁵ detected almost 70% of anti-HCV among IV drug users in Rio de Janeiro. This marker was associated with needle sharing in the last 6 months and with longer duration of IV drug use. Additionally, the higher HCV prevalence found in the Southeast, mainly in São Paulo, may be correlated with both injection drug use (including Gluconergan, a non-illicit IV drug commonly used in the 1970s as a stimulant 23,24) and the intensive medical treatments implemented in that city, which has continuously been a national health care reference center since the 40s-50s.

There are two previous studies that have estimated HCV incidence among Brazilian blood donors and calculated residual risk of hepatitis C infection from transfusions; these estimates, both from the State of Santa Catarina in the South of the country, were similar at 1:13,721 and 1:19,300 transfusion-transmitted infections per unit transfused^{3,26}. It is noteworthy that Santa Catarina showed twice to six time higher HCV prevalence than the sites we evaluated. Kupek²⁶ considered only screening tests to calculate HCV incidence and residual risk between 1991 and 1999. Moreover, Maresch et al.³ applied Bayesian methods to calculate incidence and residual risk between 1998 and 2002. We estimated hepatitis C residual risk from antibody screened blood to be only 5.0 per million, 10 to 13 times lower than earlier estimates from Brazil and comparable to the residual risk found in US before NAT introduction². Indeed, in European countries the residual risk for hepatitis C found was 16.7, 4.7 and 1.2 per million donations in Italy²⁷, Spain¹³ and France²⁸, respectively. In addition, NAT (in pools of six donations) for HCV and HIV has been implemented in some large Brazilian cities since 2011 and will be scaled-up throughout the country in the next few years. We estimate that universal HCV-NAT testing could reduce the residual risk of HCV by more than seven-fold. Thus in Brazil as in the US, the impact of NAT on HCV residual risk was much greater than its two-to three-fold reduction of HIV residual risk⁹. HBV and HCV prevalence were three and two times, respectively, higher than HIV prevalence as recently described by Sabino et al.⁹ in the same blood centers. Contrary to what many would assume, community donors have higher prevalence than replacement donors of syphilis²⁹, HIV^{9,30} and HCV among Brazilian blood centers. HIV test-seeking may explain the higher proportion of these serological markers among community donors.³¹ Hence, qualified replacement donors may be equally safe in Brazil.³² Considering HIV. HBV and HCV share major routes of transmission, the widespread of the HBV vaccination in the general population may explain the absence of association between HBV serological markers and donation type.

We recognize limitations in our study. First, in 2007 only two repeat donors seroconverted for HBsAg and anti-HBc. Hence we were not able to calculate incidence of HBV due to the low number of HBsAg seroconversions, as well as lack of data on the rate of vaccinated donors and the duration of the transient viremia among donors in Brazil. Second, HBV prevalence may have been underestimated. We considered donations positive for HBV only if they were concordant HBsAg-positive and anti-HBc reactive. We may have missed some donations from donors who tested only HBsAg positive, in the early phase of hepatitis B infection. Third, we were unfortunately not able to perform additional testing on the large number of HBsAg negative/anti-HBc reactive donations observed in the study. Anti-HBs testing would not be useful to confirm anti-HBc reactivity due to the widespread use of the HBV vaccine in Brazil. Moreover, none of the US studies conducted by REDS and American Red Cross (ARC) have performed confirmatory testing on anti-HBc reactive donations due to lack of a reliable confirmatory testing algorithm. Finally, for HCV prevalence we included only donors who tested anti-HCV EIA-reactive and immunoblot tests positive; currently or previously infected donors who did not test immunoblot positive or seroreverted would not been accounted for prevalence rates. On the other hand, the criteria adopted in our study were able to avoid false-positive results that result in overestimates of prevalence rates in low risk population studies.

In conclusion, we found low rates of hepatitis B and C viral infection among blood donors in Brazil, probably due to improvement in blood donor screening and use of more rigorous confirmatory assays and definitions than employed in previous studies. In addition socioeconomic conditions of Brazil's population have also improved and preventive measures have been implemented over time such as expanding HBV vaccination policies and practices. The incidence and residual risk of hepatitis C are likewise diminishing as a result of a decreased risk of infection over the past several generations. Continuous monitoring of the prevalence and incidence of hepatitis B and C among blood donors may help to improve blood safety by targeting recruitment to lower risk donors and driving policies to enhance laboratory screening methods including implementation of NAT screening. Moreover, for public health, these findings support the impact of preventive measures, such as promoting HBV vaccine effectiveness.

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Abbreviations

EIA enzyme immunoassay

HBV hepatitis B

HCV hepatitis C

NAT Nucleic Acid Technology

RIBA recombinant immunoblot assay

TTD transfusion-transmitted diseases

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Table 1

Total Number (%) of Donations Demographic Characteristics, First time vs. Repeat Donor Status, and Donation Type by Blood Centers.*

	Recife N (%)	Belo Horizonte N (%)	Sao Paulo N (%)	N (%) of Donations
Age in Years				
<25	24,299 (24.4)	18,159(26.0)	31,502(22.9)	73,960 (24.1)
> 25 < 35	33,648 (33.7)	27,499 (39.3)	50,273 (36.5)	111,420(36.3)
> 35 < 45	27,170 (27.2)	16,861 (24.1)	35,314(25.7)	79,345 (25.8)
> 45 < 55	11,881 (11.9)	6,308 (9.0)	15,782(11.5)	33,971 (11.0)
> 55	2,759 (2.8)	1,108 (1.6)	4,755 (3.5)	8,622 (2.8)
Gender				
Female	16,919(16.9)	24,953 (35.7)	51,672(37.5)	93,544 (30.4)
Male	82,870 (83.1)	44,982 (64.3)	85,958 (62.5)	213,810(69.6)
Donor Status				
Repeat	70,966 (71.1)	47,489 (67.9)	93,731 (68.1)	212,186 (69.0%)
First Time	28,823 (28.9)	22,446(32.1)	43,899(31.9)	95,168 (31.0%)
Donation Type				
Community	58,747 (58.9)	37,658 (53.9)	107,961 (78.4)	204,366 (66.5)
Replacement	41,034(41.1)	32,277(46.1)	29,669 (21.6)	102,980 (33.5)

^{*} Age and donation type missing for a few donations (hence totals by age and donation type do not add up to 307,354).

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Table 2

Prevalence of HBsAg-positive/anti-HBc-reactive and anti-HBc-reactive/HBsAg-negative donations among Brazilian First Time Donors in 2007 by Blood Center and Demographics*

	Number of Donations	Number of HBsAg plus anti- HBc positive donations	Prevalence/per 100,000 donations and 95% CI	Odds Ratios and 95% CI	Number (%) of anti-HBC only positive donations
Total	93,710	271	289 (255-324)	-	3,329 (3.6%)
Blood Center					
Recife	28,364	119	419 (344-495)	1.73 (1.31-2.28)	1,338 (4.7%)
Belo Horizonte	21,823	59	270 (201-339)	1.27 (0.91-1.76)	753 (3.4%)
São Paulo	43,523	93	213 (170-257)	1	1,238 (2.8%)
Age in Years					
<25	37,309	61	164 (123-205)	-	583 (1.6%)
25 <35	30,739	94	306 (244-368)	1.85 (1.34-2.56)	955 (3.1%)
35 < 45	16,818	83	493 (388-599)	2.91 (2.08-4.06)	980 (5.8%)
45 <55	7,058	26	368 (227-510)	2.25 (1.41-3.57)	617 (8.7%)
55	1,751	9	343 (69-616)	2.07 (0.89-4.80)	192 (11.0%)
Gender					
Female	36,396	99	179 (135-222)	1	1,027 (2.8%)
Male	57,314	206	359 (310-408)	1.86 (1.40-2.47)	2,302 (4.0%)
Donation Type					
Community	48,769	116	238 (195-281)	0.84 (0.65-1.07)	1,548 (3.2%)
Replacement	44,938	155	345 (291-399)	1	1,781 (4.0%)

* 93,710 of the 95,168 first time donations have HBV test results; age and donation type missing for a few donations (hence totals by age and donation type do not add up to 93,710).

Table 3

Prevalence of anti-HCV among First Time Brazilian Donors in 2007 by Demographics and Blood Center and Results of Logistic Regression Analysis*.

	Number of Donations	Number of anti- HCV Positive donations	Prevalence/per 100,000 donations and 95% CI	Odds Ratios and 95% CI			
Total	93,690	179	191 (163-219)				
Blood Center							
Recife	28,345	37	131 (89-173)	0.47 (0.32-0.68)			
Belo Horizonte	21,825	17	78 (41-115)	0.35 (0.21-0.58)			
Sao Paulo	43,520	125	287 (237-337)	1			
Age in Years							
<25	37,307	10	27 (10-44)	1			
25 <35	30,732	65	212 (161-263)	8.18 (4.20-15.94)			
35 < 45	15,719	42	267 (186-348)	10.71 (5.36-21.40)			
45 <55	7,748	45	581 (412-750)	22.97 (11.54-45.71)			
55	2,152	17	790 (416-1164)	29.72 (13.55-65.19)			
Gender							
Female	36,390	65	179 (136-222)	1			
Male	57,300	114	199 (163-235)	1.28 (0.94-1.75)			
Donation Type							
Community	48,754	111	228 (186-270)	1.70 (1.25-2.32)			
Replacement	44,933	68	151 (115-187)	1			

^{*93,690} of the 95,168 first time donations have HCV test results confirmed by RIBA; age and donation type missing for a few donations (hence totals by age and donationtype do not add up to 93,690).