Prevalence, incidence and residual risk of transfusion-transmitted hepatitis C virus and human immunodeficiency virus after the implementation of nucleic acid testing in Italy: a 7-year (2009-2015) survey

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Background. In Italy nucleic acid testing (NAT) became mandatory for hepatitis C virus (HCV) in 2002 and for human immunodeficiency virus (HIV) and hepatitis B virus in 2008. The aim of this study was to monitor the incidence and prevalence of HIV and HCV infections in Italian blood donors and the current residual risk of these infections after the introduction of NAT.

Materials and methods. The Italian national blood surveillance system includes data from tests used to screen for transfusion-transmissible infections. During the period of this survey (2009-2015), the NAT methods used were the transcription-mediated amplification test, for individual donor testing, and polymerase chain reaction analysis, mainly for pools of six donors. Prevalence and incidence were calculated. Three published formulae were applied to estimate the residual risk (the window period ratio model and the formulae recommended by the European Medicines Agency and the World Health Organization).

Results. Overall, 12,258,587 blood donors and 21,808,352 donations were tested for HCV and HIV. The prevalence of HCV decreased from 110.3×10^5 to 58.9×10^5 in years 2009 and 2015, respectively, while that of HIV remained stable over time (15.5×10^5 *vs* 15.4×10^5). The incidence of HCV decreased from 3.19×10^5 in 2009 to 1.58×10^5 in 2015, while the incidence of HIV did not show any significant fluctuations (average incidence 4.39×10^5). The residual risk of a viraemic unit entering the blood supply was estimated to be 0.077×10^6 or 1 in 12,979,949 donations for HCV and 0.521×10^6 or 1 in 1,917,250 for HIV, according to the window period ratio model, and lower with the other two formulae.

Discussion. HCV infection has declined over time in both first-time and repeat donors, while the data for HIV infection are stable. All three methods employed in this study showed that the residual risk of transmitting HCV or HIV through an infected blood unit is currently very low in Italy, but there are considerable differences in estimates between methods. Thus, harmonisation of these methods is advisable.

Keywords: HCV, HIV, incidence, prevalence, transfusional residual risk.

Introduction

The risk of acquiring hepatitis C virus (HCV) or human immunodeficiency virus (HIV) through blood transfusion has drastically decreased since the introduction of nucleic acid testing (NAT) along with the previously implemented antigen/antibody-based assays for blood screening and the adoption of restrictive criteria for donor selection. Following the serological and molecular screening of blood and blood components, in most countries the residual risk of transfusion-transmitted HCV and HIV infections has become too low for direct assessment through conventional approaches, such as prospective follow-up and retrospective look-back studies of blood recipients[1,2]. The risk of collecting an infected donation that is undetectable by screening tests is, therefore, presently calculated through mathematical modelling based on the incidence rate of HCV and HIV infections among donors and on the length of the window period of the viral infection, which is the temporal gap spanning from the time point of donor infection to the first detectability of specific viral markers[3-7]. Importantly, this diagnostic window period consists of two phases: the so-called eclipse period during which the virus has already

infected the donor but is not yet detected in blood even by highly sensitive NAT, and the ramp-up phase during which the viral concentration increases exponentially in the blood (i.e., viraemic phase) until a peak/plateau is reached. Blood collected in the viraemic phase of the diagnostic window can potentially transmit infection to the transfused recipient if the circulating viral load is below the diagnostic sensitivity of the screening assay. Hence, the sensitivity of the screening test being used is crucial in determining the length of the window period and, consequently, in calculating the residual risk.

Monitoring the residual risk of transfusion-transmitted infections (TTI) of a specific donor population through the collection of epidemiological data is a crucial step in assessing the preventive measures taken by national blood systems to assure the safety of their blood supplies. Thus, most industrialised countries maintain dedicated surveillance programmes on this issue. In this context, the European Medicines Agency (EMA) requires manufacturers of plasma-derived medicinal products to collect data on HCV and HIV, as well as hepatitis B virus (HBV), infections in order to ensure an adequate selection of donors of blood and plasma[8]. In addition, Blood Establishments which provide the raw material for industrial fractionation must comply with EMA requirements through continuous epidemiological surveillance, reporting and annual updating of the assessment of the residual risk of TTI in their donor populations. The World Health Organization (WHO) has also recently edited guidelines on the estimation of residual risk of transmitting infections by transfusion[9]. Both documents (issued by the EMA and WHO) provide simplified formulae to estimate the risk with any kind of screening methodology used.

In Italy, previous studies aimed at assessing the residual risk of TTI were either carried out before the implementation of NAT or did not include data of the nation's entire blood supply. Between 1999 and 2001, Gonzalez *et al.*[10] showed, using the incidence rate window period model in the pre-NAT era that the residual risk, estimated on nearly 90% of Italy's blood supply, was 1.9 infections per million donations for HIV, but higher for HCV (16.7 per million donations) and HBV (69.2 per million donations). More recently, Velati *et al.*[11] published the results of a survey carried out between 2001 and 2006 estimating that the introduction of NAT had improved the safety of blood remarkably, reducing the risk to 2.5 for HCV, 1.8 for HIV and 57.8 for HBV infectious blood units per million donations in the blood supply. For the same period the residual risk, calculated by the incidence/window period model, was 0.1×10^6 for HCV and 0.8×10^6 for HIV[12].

This study was undertaken with the aim of monitoring the prevalence and incidence of HCV and HIV infections among Italian blood donors and of estimating the trend of residual risk of these TTI over a 7-year period, from 2009 to 2015, following implementation of mandatory NAT.

Materials and methods

In Italy, haemovigilance data are collected and processed through a web-based national blood surveillance system (SISTRA, *Sistema Informativo dei Servizi Trasfusionali*). SISTRA, issued in 2007 by the Italian competent authority, the National Blood Centre (*Centro Nazionale Sangue*, CNS) of the Ministry of Health, is the system that manages all information related to blood activities carried out nationwide, with a specific section dedicated to epidemiological surveillance of donors: this section contains records concerning donors found to be positive for TTI by routine screening, as well as their demographic characteristics and risk factors.

In agreement with Italian law[13], all donors are voluntary, unpaid donors and are classified as:

1. *first-time donors*: individuals whose blood/plasma is tested for the first time for markers of TTI without evidence of prior testing in the Italian blood system. These donors include two subsets:
   - 1.1 subjects considered in good health after clinical evaluation who were tested for TTI markers and donated blood (standard selection procedure);
   - 1.2 subjects clinically evaluated and tested for TTI markers, but who did not give blood and were allowed to donate only after an established period (pre-donation screening).
2. *Repeat donors*, including:
   - 2.1 individuals who donated blood, after clinical evaluation and screening for TTI, for whom previous donation(s) within the last 24 months had been found to be negative;
   - 2.2 subjects evaluated clinically and tested for TTI markers who donated for the first time after pre-donation screening.

For the purpose of epidemiological surveillance in this study, only the first-time donors of subgroup 1.1 were considered for calculation. Subjects in subgroup 1.2 were excluded because TTI markers always included serological testing, but not necessarily NAT, since no blood units were collected.

The ratio between first time and repeat donors was assessed on a regional basis to detect possible inter-regional variations in the epidemiological indices. Regions were grouped into three macro geographic areas: northern, central and southern/islands.

All donations reviewed in this study were tested for antibodies to HCV (anti-HCV), HCV RNA and HIV RNA. Approximately 80% of the donations were also tested for antibodies to HIV (anti-HIV1/2)/HIV antigen (Combo test) while the remaining 20% were tested for

anti-HIV1/2 only. Serological testing for anti-HCV, anti-HIV1/2 and HIV1/2 antibodies/antigen was carried out using third- or fourth-generation immunoassays from different manufacturers, approved as meeting European Community requisites. During the 7-year period covered by this study, the Blood Establishments employed automated analysers, based on the chemiluminescence immunoassay principle, for the tests. Different amplification methods were used for NAT: transcription-mediated amplification (Grifols, International S.A: Sant Cugat del Vallès, Barcelona, Spain; formerly Novartis Vaccines & Diagnostics) for testing individual donors (PROCLEIX Ultrio and PROCLEIX Ultrio Plus on Tigris platform, and Ultrio Elite on Panther platform) and polymerase chain reaction (Roche Molecular System, Branchburg, USA) for mini-pool testing of six donors (TaqScreen MPX and TaqScreen v2). During the years 2009-2012, nearly 3.7% of donations were examined by polymerase chain reaction COBAS Ampliscreen (Roche Diagnostics, Branchburg, USA) in pools of 10-24 samples. This method was abandoned in 2013. Confirmatory and/or supplemental testing was performed on all repeatedly reactive samples following a previously described algorithm[11].

Donors considered positive for HCV or HIV included: (i) those found to be positive for both viral serology (i.e., anti-HCV, anti-HIV) and NAT, (ii) those who were NAT positive in the absence of serological markers and (iii) those who were NAT negative but with positive serology.

Risk factors

Donors who resulted positive for TTI markers were recalled by the Transfusion Service where they had donated in order to undergo repeat testing and a post-donation interview with a physician who asked specific questions about risk factors. All these data were then reported in the web-based national blood surveillance system.

Prevalence and incidence

Prevalence is the proportion of infections (both recent and past) identified at a specified point in time or over a specified time period in a defined population. Incidence is the rate of newly acquired infections over a specified time period in a defined population[8]. In this study, prevalence was calculated in the population of first-time donors as the rate between the number of positive donors on the total number of this population in the same period of time per 100,000 donors[7]. Concerning the incidence, literature reports different ways[14] to calculate rates. For the epidemiological purpose of this study, incidence was calculated in the population of repeat donors as the number of positive subjects who had a previous negative donation or negative testing within the preceding 2 years, divided by the total number of donations from repeat donors in the study period per mean inter-donation interval expressed in years (= person-years at risk). Incidence is expressed as the number of new infected cases × 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.

Residual risk calculation

Residual risk for each TTI infection or risk that an infected donor would give a donation that tested negative (window period risk) was evaluated using the incidence rate window period model, which is the model most applied in different blood donor populations in the world[6,7,15-23]. After the mathematical models initially proposed by Lackriz, Schreiber and Busch[3,4,7], other authors introduced some risk model refinements[24] and two authoritative, international institutions have proposed simplified algorithms to evaluate the residual risk of transmitting an infection by transfusion therapy: the EMA[8] and the WHO[9]. Thus, we decided to apply all these three models to the Italian data and compare the results.

In this study, the "window period ratio method" was considered the reference method since we had used it in the past, making comparisons with previous data easier. Briefly, the residual risk is estimated, in first-time and repeat donors, multiplying the NAT yield rate (i.e. number of NAT-only positive cases/number donations screened) by the ratio between the infectious pre-NAT window period and that estimated as the time elapsing from NAT detectability and serological detectability.

Since different NAT screening methods have been employed in Italy during the 7-year period of observation, we calculated the duration of the NAT window period using data collected from our national inter-laboratory quality programme. Very briefly, every year, participants are asked to complete a questionnaire regarding the serological and NAT methods in use in each Blood Establishment. These data, integrated with those obtained through the National Haemovigilance System, allowed us to record, per year and for each Blood Establishment, the NAT method used, the number of units of blood tested and the positive results, subdivided by first-time and repeat donors. The weighted average pre-NAT infectious window period for the entire period of observation was estimated to be 4.74 days for HCV and 7.0 days for HIV. Thus, according to Busch *et al.*[7] the ratios between the two window periods of interest were 4.74/53.6 days for HCV and 7.0/8.0 days for HIV, respectively.

On the other hand, the EMA and WHO suggest estimating the residual risk as the product of the incidence and the window period (expressed in years) in which a new infection would be undetected, per million donations. The incidence is calculated as the number of new infections (NAT-only positives) among the repeat donors in the study period with a previous negative donation on the total number of repeat donors in the same study period. As far as the window period concerns, the EMA indicates that this is 8 days for HCV and 15 days for HIV, while the WHO indicates 5 days for HCV and 8 days for HIV.

Since the NAT-only rates for both first-time and repeat donors are available in Italy, the overall donor population residual risk was estimated as follows: (% first-time donors × first-time donor rate or incidence) + (% repeat donors × repeat donor rate or incidence). The residual risk is expressed per million donations.

The 95% CI for residual risk were calculated applying the described formulae to the extreme values of incidence 95% CI.

Results

Donors and donations

From January 2009 to December 2015, a total of 12,258,587 blood donors (age range: 18-70 years; male to female ratio: 2.3) were tested for markers of HCV and HIV infection (Table I). Of these donors, 15.8% were first-time donors (male to female ratio: 1.7), and 84.2% were repeat donors (male to female ratio: 2.4).

During the study period, the average ratio between repeat and first-time donors was 5.3 and no significant changes in the yearly repeat/first-time donor ratios were seen during the study period (*data not shown*). There were some inter-regional differences, with the proportions of RP donors being higher in northern regions of Italy than in southern regions (14.2 *vs* 3.0).

As reported in Table I, of the 21,808,352 blood donations tested for both HCV and HIV, 8.9% were given by first-time donors while 91.1% were given by repeat donors, with a general average of 1.78 units per donor per year. Repeat donors gave a mean number of 1.92 donations per year (donation index) with an inter-donation interval of 0.52 years. Fifty-four percent of the donations were tested using NAT polymerase chain reaction technology while the remaining 46% were tested by NAT transcription-mediated amplification technology.

Overall, a total of 2,641 donors tested positive for viral markers. Of these, 1,949 were found among the 1,934,612 (100.7×10^5) first-time donors and the remaining 692 were found among the 10,323,975 repeat donors (6.7×10^5). Thus, the frequency of positivity for viral markers was 15-fold higher among first-time donors than among repeat donors.

Table I - Blood donors and donations tested for hepatitis C virus and human immunodeficiency virus in Italy, 2009-2015.

Total n. of donors tested	12,258,587		
Age (range)	18-70 years		
Age and gender distribution	*Age groups*	*Male (%)*	*Female (%)*
	18-25	11.3	18.9
	26-35	18.9	20.2
	36-45	30.0	26.5
	46-55	26.8	23.5
	56-65	12.3	10.5
	>65	0.7	0.4
Male to female ratio	2.3		
N. of FT donors tested	1,934,612 (15.8%)		
Age and gender distribution	*Age groups*	*Male (%)*	*Female (%)*
	18-25	23.6	28.5
	26-35	24.2	23.2
	36-45	27.0	25.0
	46-55	18.8	18.0
	56-65	6.3	5.2
	>65	0.1	0.1
Male to female ratio	1.7		
N. of RP donors tested	10,323,975 (84.2%)		
Age and gender distribution	*Age groups*	*Male (%)*	*Female (%)*
	18-25	9.4	16.6
	26-35	18.0	19.6
	36-45	30.4	26.8
	46-55	28.1	24.8
	56-65	13.3	11.7
	>65	0.8	0.5
Male to female ratio	2.4		
RP/FT donor ratio			
North	14.2		
Centre	3.4		
South/Isles	3.0		
Italy	5.3		
Total n. of blood donations tested	21,808,352		
N. of blood donations from FT donors	1,934,612 (8.9%)		
N. of blood donations from RP donors	19,873,740 (91.1%)		
Donation index (mean donations per year)	1.78		
RP donation index (mean donations per year)	1.92		
Inter-donation interval	0.52 years		
N. of donations tested by:			
NAT PCR	11,771,573 (54%)		
NAT TMA	10,036,779 (46%)		

FT: first-time; RP: repeat; NAT: nucleic acid testing; PCR: polymerase chain reaction; TMA: transcription-mediated amplification.

Prevalence of markers of hepatitis C virus and human immunodeficiency virus in first-time donors

During the study period the average prevalence of HCV and HIV markers in FT donors was 85.7×10^5 and 15.0×10^5, respectively (Table II). The prevalence of HCV decreased from the year 2009 to 2015 from 110.3×10^5 to 58.9×10^5 while the prevalence of HIV remained stable over time (15.5×10^5 in 2009 *vs* 15.4×10^5 in 2015). The prevalence of both HCV and HIV infections showed inter-regional differences (Online supplementary content, Table SI). As for HCV, rates of infection were significantly lower in northern and central Italy compared with those reported in southern Italy, while HIV rates were somewhat higher in central Italy compared to those reported in the northern and southern regions, but the differences were not statistically significant.

As shown in Table III, most HCV- and HIV-positive first-time donors were males (66.7 and 79.7%, respectively), with the former more frequently present (34.0%) in the 36- to 45-year age group and the latter peaking (34.0%) in the 26- to 35-year age group.

With regards to HCV-related risk factors, the most reported (22.7%) were parenteral risk factors (i.e., dental treatment, surgery, tattooing, piercing, etc); 9.2% had sexual risks (of these, 77.2% reported unprotected heterosexual exposure to multiple, occasional partners and 22.8% reported having male-to-male sex); 67.1% denied having any known risk factor.

Sexual at-risk behaviours were most frequently (49.2%) reported among HIV-infected individuals (of these, 63.8% were heterosexuals and 36.2% were males who had sex with males), while no risk factors were identified in 39.6% of the positive first-time donors.

The age and gender of subjects with no risk factors for HCV and HIV did not differ significantly from the age and gender of those with risk factors.

Incidence of markers of hepatitis C virus and human immunodeficiency virus infection in repeat donors

As shown in Table IV, a total of 238 HCV-positive and 454 HIV-positive repeat donors were classified as seroconverters (incident cases) between 2009 and 2015. The average incidence of HCV during the 7-year study period was 2.30×10^5 and decreased from 3.19×10^5 in 2009 to 1.58×10^5 in 2015, while the average incidence of HIV in the same period was 4.39×10^5, ranging from 3.74×10^5 in 2010 and 5.29×10^5 in 2012, with no significant yearly fluctuations.

The incidence of both HCV and HIV showed some inter-regional differences (Online supplementary content, Table SII). Higher rates of HCV infection were found in southern regions than in central and northern regions, while rates of HIV infection were lower in the northern parts of the country.

As shown in Table III, most (63%) HCV-infected repeat donors were males and belonged (31.5%) to the 36- to 45-year age group; those infected with HIV were mostly males (89.4%) and belonged (34.6%) to the 26- to 35-year age group. As far as risk factors concerns, 31.1% of HCV-infected repeat donors had

Table II - Prevalence of hepatitis C virus and human immunodeficiency virus infection among first-time donors in Italy, 2009-2015.

Year	N. of FT donors	HCV		HIV	
		N. of positives	*Prevalence ×10⁵ (95% CI)*	*N. of positives*	*Prevalence ×10⁵ (95% CI)*
2009	264,635	292	110.3 (98.0-123.7)	41	15.5 (11.1-21.0)
2010	281,153	303	107.8 (96.0-120.6)	33	11.7 (8.1-16.5)
2011	297,321	270	90.8 (80.3-102.3)	39	13.1 (9.3-17.9)
2012	287,380	231	80.4 (70.3-91.4)	43	15.0 (10.8-20.1)
2013	271,841	225	82.8 (72.3-94.3)	48	17.7 (13.0-23.4)
2014	265,543	180	67.8 (58.2-78.4)	46	17.3 (12.7-23.1)
2015	266,739	157	58.9 (50.0-68.8)	41	15.4 (11.0-20.8)
2009-2015	1,934,612	1,658*	85.7 (81.6-89.9)	291*	15.0 (13.4-16.9)

*21 subjects were HCV and HIV co-infected.
FT: first-time; HCV: hepatitis C virus; HIV: human immunodeficiency virus; 95% CI: 95% confidence interval.

Table III - Demographics characteristics and risk factors of first-time and repeat donors positive for markers of hepatitis C virus and human immunodeficiency virus in Italy, 2009-2015.

	FT donors		RP donors	
	HCV+ (n=1,658) N (%)	*HIV+ (n=291) N (%)*	*HCV (n=238) N (%)*	*HIV+ (n=454) N (%)*
Gender				
Male	1,106 (66.7)	232 (79.7)	150 (63)	406 (89.4)
Female	552 (33.3)	59 (20.3)	88 (37)	48 (10.6)
Age group				
18-25	122 (7.3)	54 (18.6)	15 (6.3)	60 (13.2)
26-35	306 (18.5)	99 (34.0)	56 (23.6)	157 (34.6)
36-45	564 (34.0)	85 (29.2)	75 (31.5)	141 (31.1)
46-55	500 (30.2)	44 (15.1)	71 (29.8)	76 (16.7)
56-65	163 (9.8)	9 (3.1)	20 (8.4)	20 (4.4)
>65	3 (0.2)	-	1 (0.4)	
*Risk factor**				
Sexual, of whom:	171 (9.2)	163 (49.2)	41 (14.3)	311 (60.6)
Heterosexual	132 (77.2)	104 (63.8)	38 (92.7)	193 (62.1)
MSM	39 (22.8)	59 (36.2)	3 (7.3)	118 (37.9)
Parenteral, of whom:	425 (22.7)	36 (10.9)	89 (31.1)	50 (9.7)
Intravenous drug use	28 (6.6)	1 (2.8)	2 (2.2)	-
Dental treatment	128 (30.1)	8 (22.2)	28 (31.5)	14 (28.0)
Surgery	124 (29.2)	2 (5.6)	25 (28.1)	13 (26.0)
Transfusion	37 (8.7)	11 (30.5)	2 (2.2)	-
Others (tattoo, piercing, etc.)	108 (25.4)	14 (38.9)	32 (36.0)	23 (46.0)
Household contact	19 (1.0)	1 (0.3)	4 (1.4)	4 (0.8)
*Not identified***	1,254 (67.1)	131 (39.6)	152 (53.2)	148 (28.9)

*Each case could report more than one risk factor. **Not identified includes subjects (nearly 50%) who didn't return to complete the post-donation interview.
FT: first-time; RP: repeat; HCV: hepatitis C virus; HIV: human immunodeficiency virus; MSM: males who have sex with males.

Table IV - Incidence of hepatitis C virus and human immunodeficiency virus infections among repeat donors in Italy, 2009-2015.

Year	N. of RP donors	N. of RP donations	Person-years*	HCV		HIV	
				N. of positives	*Incidence ×10⁵ (95% CI)*	*N. of positives*	*Incidence ×10⁵ (95% CI)*
2009	1,425,791	2,769,776	1,440,283	46	3.19 (2.34-4.26)	57	3.96 (3.00-5.13)
2010	1,441,350	2,824,685	1,468,836	37	2.52 (1.77-3.47)	55	3.74 (2.82-4.87)
2011	1,474,930	2,889,653	1,502,620	33	2.20 (1.51-3.08)	71	4.73 (3.69-5.96)
2012	1,501,319	2,905,769	1,511,000	34	2.25 (1.56-3.14)	80	5.29 (4.20-6.59)
2013	1,504,371	2,872,883	1,493,899	37	2.48 (1.74-3.41)	65	4.35 (3.36-5.55)
2014	1,487,313	2,816,234	1,453,265	28	1.93 (1.28-2.78)	62	4.27 (3.27-5.47)
2015	1,488,901	2,794,740	1,453,265	23	1.58 (1.00-2.37)	64	4.40 (3.39-5.62)
2009-2015	10,323,975	19,873,740	10,334,345	238	2.30 (2.02-2.6)	454	4.39 (4.00-4.82)

*We considered a donation index of 1.92 and an inter-donation interval of 0.52 years.
RP: repeat; FT: first-time; HCV: hepatitis C virus; HIV: human immunodeficiency virus; 95% CI: 95% confidence interval.

parenteral factors, 14.3% reported sexual exposure (of these 92.7% were heterosexuals and 7.3% were males who had sex with males), while 53.2% did not report any risk factor. Among HIV-positive repeat donors, the most frequently (60.6%) reported risk factor was sexual exposure (62.1% heterosexuals and 37.9% males who had sex with males), while no risk factors were identified in 28.9% of the donors.

Yield of positive nucleic acid tests in Italy

During the 7 years of observation, 32 units of blood were identified as NAT-only positive: 19 units (8 collected from first-time and 11 from repeat donors) were found HCV RNA positive and anti-HCV antibody negative and 13 units (3 from first-time donors and 10 from repeat donors) were HIV RNA positive and HIV-antigen/antibody negative. Of the 19 HCV NAT-only positive subjects, 10 were males and 9 females (median age, 47 years), while of the 13 HIV NAT-only positive subjects 12 were males and 1 female with a median age of 32 years. All 32 infected donors detected as NAT-only cases seroconverted to anti-HCV and anti-HIV positivity during the follow up.

Estimated residual risk of hepatitis C virus and human immunodeficiency virus

The results of the evaluation of residual risk for HCV and HIV in Italy obtained with the three methods are reported in Tables V and VI.

As shown in Table V, the residual risk of a non-detected, but HCV viraemic unit was, for the entire examined period, 0.077×10^6 (or 1 in 12.98 million donations) according to the window period ratio model; 0.034×10^6 (or 1 in 29.44 million donations) according to the EMA method and 0.021×10^6 (or 1 in 47.10 million donations) with the WHO method.

In Table VI the same parameters are reported for HIV. In this case the window period ratio model gave a value of 0.52×10^6 (or 1 in 1.93 million units), while the EMA method gave a value of 0.043×10^6 (or 1 in 22.94 million units) and the WHO method resulted in a value of 0.023 (or 1 in 43.02 million units).

For both HCV and HIV infections, the residual risk values estimated by the EMA and WHO methods overlapped, but were significantly lower than the value obtained with Busch's method.

Discussion

Blood has never been safer but the achievement of zero-risk for TTI remains a goal to pursue.

In the current epidemiological situation, the risk of transmitting an infection to the transfused recipient or of contaminating plasma pools used for manufacturing blood products is mainly due to the collection of a blood donation during the viraemic phase of the diagnostic window, whose length depends on the screening marker, the category of screening assay (NAT *vs* antibody or antigen-based assays), the sensitivity of the assay used and the replication kinetics of the virus during the early phase of infection. In this context, the implementation of highly sensitive NAT together with antigen/antibody-based assays has provided an efficient, supplementary tool for further reducing the residual risk of transmitting HCV, HIV and HBV through blood transfusion.

HCV NAT screening of blood donations was implemented in Italy in 2002, while HIV NAT was first introduced in a few regions in 2002, and then became mandatory - together with HBV NAT - on a national scale in 2008. At present, according to the Italian law, all blood donations are tested for HBV DNA and HBsAg, HCV RNA and anti-HCV, HIV RNA and anti-HIV-1-2/HIV antigen, and syphilis. In addition, donations are tested for West Nile virus during the at-risk period of the year. Furthermore, test screening is preceded by restrictive donor selection based on a physician-collected clinical history, including confidential unit exclusion, and behavioural screening based on individual risk assessment.

From the data in this study, the general trends of HCV and HIV infections in the Italian blood population seem quite different. The rate of HCV infection has diminished in both first-time donors (prevalence from 110.0×10^5 in 2009 to 58.9×10^5 in 2015) and repeat donors (incidence from 3.19×10^5 in 2009 to 1.58×10^5 in 2015), while the rate of HIV infection remained stable over the years. Taken together, the frequency of viral positive markers was much higher (15-fold) among first-time donors than among repeat donors, a finding that is driving our National Blood Centre to progressive introduction of pre-donation screening and elimination of first-time donors who do not return to donate again.

The HCV- and HIV-infected donors show some demographic and risk behaviour differences, with the HCV-positive subjects being approximately 10 years older than those infected by HIV, a distribution that was observed in both first-time and repeat donors. Parenteral exposure (i.e., tattooing, piercing, dental treatments, surgery) was the main known risk factor reported in first-time donors (22.7%) as well as in repeat (31.1%) HCV-infected donors, followed by sexual at-risk behaviours (9.2 and 14.3%, respectively). It is worth noting that most first-time and repeat donors with sexual at-risk behaviours were heterosexuals who reported having unprotected exposure to multiple occasional partners (77.2 and 92.7%, respectively), while 22.8% of the former and 7.3% of the latter reported having male to male sex. Finally, it is of some concern that 67.1% of first-time donors and 53.2% of repeat donors reported no risk behaviours that, if detected

Table V - Residual risk of transfusing an infected blood unit collected during the window period of hepatitis C virus infection in Italy, 2009-2015.

		Year							
		2009	***2010***	***2011***	***2012***	***2013***	***2014***	***2015***	***2009-2015***
Method 1 (Busch *et al.*, 2005)	RP positive donations/donations tested	1/2,769,776	1/2,824,685	0/2,889,653	1/2,905,769	3/2,872,883	2/2,816,234	3/2,794,740	11/19,873,740
	RP RR ×10^6 (95% CI)	0.032 (0.008-0.178)	0.031 (0.009-1.972)	-	0.030 (0.009-1.917)	0.092 (0.215-3.052)	0.063 (0.086-2.565)	0.095 (0.221-3.137)	0.049 (0.024-0.088) or 1 in 20,430,235
	FT positive donations/donations tested	1/264,635	3/281,153	2/297,321	1/287,380	0/271,841	1/265,543	0/266,739	8/1,934,612
	FT RR ×10^6 (95% CI)	0.334 (0.096-21.053)	0.944 (2.2-31.183)	0.595 (0.815-24.299)	0.308 (0.088-19.388)	-	0.333 (0.095-20.982)	-	0.366 (0.158-0.721) or 1 in 2,734,578
	Total RR ×10^6 (95% CI)	0.059 (0.017-3.070)	0.112 (0.203-4.563)	0.055 (0.072-3.319)	0.055 (0.001-0.307)	0.084 (0.196-3.985)	0.087 (0.087-4.199)	0.087 (0.202-4.086)	0.077 (0.036-0.144) or 1 in 12,979,949
Method 2 (EMA, 2016)	RP positive donors/donors tested	1/1,425,791	1/1,441,350	0/1,474,930	1/1,501,319	3/1,504,371	2/1,487,313	3/1,488,901	11/10,323,975
	RP RR ×10^6 (95% CI)	0.015 (0.0004-0.086)	0.015 (0.0004-0.085)	-	0.015 (0.0004-0.081)	0.044 (0.009-0.128)	0.029 (0.004-0.106)	0.044 (0.009-0.129)	0.023 (0.012-0.042) or 1 in 42,821,033
	FT positive donors/donors tested	1/264,635	3/281,153	2/297,321	1/287,380	0/271,841	1/265,543	0/266,739	8/1,934,612
	FT RR ×10^6 (95% CI)	0.082 (0.002-0.461)	0.234 (0.048-0.683)	0.147 (0.018-0.532)	0.076 (0.002-0.425)	-	0.082 (0.002-0.460)	-	0.091 (0.039-0.179) or 1 in 11,033,334
	Total RR ×10^6 (95% CI)	0.026 (0.0004-0.091)	0.050 (0.005-0.112)	0.025 (0.002-0.081)	0.024 (0.0004-0.085)	0.037 (0.005-0.097)	0.038 (0.002-0.101)	0.037 (0.005-0.098)	0.034 (0.01-0.040) or 1 in 29,437,723
Method 3 (WHO, 2017)	RP positive donors/donors tested	1/1,425,791	1/1,441,350	0/1,474,930	1/1,501,319	3/1,504,371	2/1,487,313	3/1,488,901	11/10,323,975
	RP RR ×10^6 (95% CI)	0.010 (0.0002-0.054)	0.009 (0.0002-0.053)	-	0.009 (0.0002-0.051)	0.027 (0.006-0.080)	0.018 (0.002-0.067)	0.028 (0.006-0.081)	0.015 (0.007-0.026) or 1 in 68,513,652
	FT positive donors/donors tested	1/264,635	3/281,153	2/297,321	1/287,380	0/271,841	1/265,543	0/266,739	8/1,934,612
	FT RR ×10^6 (95% CI)	0.052 (0.001-0.288)	0.146 (0.03-0.427)	0.092 (0.011-0.333)	0.048 (0.001-0.266)	-	0.052 (0.001-0.287)	-	0.057 (0.024-0.112) or 1 in 17,653,335
	Total RR ×10^6 (95% CI)	0.016 (0.0004-0.091)	0.031 (0.005-0.112)	0.015 (0.002-0.081)	0.015 (0.0004-0.085)	0.023 (0.05-0.097)	0.024 (0.002-0.101)	0.023 (0.005-0.098)	0.021 (0.01-0.04) or 1 in 47,100,357

The RR is expressed as units ×10^6 and, for the full period, also as 1 unit in n million units tested.
RP: repeat; RR: residual risk; FT: first-time; 95% CI: 95% confidence interval; EMA: European Medicines Agency; WHO: World Health Organization.

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Table VI - Residual risk of transfusing an infected blood unit collected during the window period of the human immunodeficiency virus infection in Italy, 2009-2015.

		Year							
		2009	***2010***	***2011***	***2012***	***2013***	***2014***	***2015***	***2009-2015***
Method 1 (Busch *et al.*, 2005)	RP positive donations/donations tested	3 /2,769,776	0/2,824,685	1/2,889,653	4 /2,905,769	1/ 2,872,883	1/ 2,816,234	0/2,794,740	10/19,873,740
	RP RR ×10^6 (95% CI)	0.948 (0.195-2.770)	0.153 (0.004-0.646)		1.20 (0.375-3.525)	0.305 (0.009-1.939)	0.311 (0.009-1.978)	-	0.440 (0.241-0.925) or 1 in 2,271,285
	FT positive donation/donations tested	0/264,635	0/281,153	0/297,321	0/287,380	2/271,841	0/265,543	1/266,739	3/1,934,612
	FT RR ×10^6 (95% CI)	-	-		-	6.438 (0.891-26.577)	-	3.280 (0.095-20.888)	1.357 (0.320-4.532) or 1 in 736,995
	Total RR ×10^6 (95% CI)	0.865 (0.204-4.121)	0.139 (0.004-0.586)		1.096 (0.341-1.155)	0.849 (0.087-4.125)	0.284 (0.08-1.197)	0.286 (0.008-1.820)	0.521 (0.248-1.245) or 1 in 1,917,250
Method 2 (EMA, 2016)	RP positive donors/donors tested	3/1,425,791	0/1,441,350	1/1,474,930	4/1,501,319	1/1,504,371	1/1,487,13	0/1,488,901	10/10,323,975
	RP RR ×10^6 (95% CI)	0.086 (0.018-0.253)	0.014 (0.0004-0.052)		0.109 (0.030-0.280)	0.027 (0.0007-0.152)	0.028 (0.0007-0.154)	-	0.040 (0.019-0.073) or 1 in 25,121,673
	FT positive donors/donors tested	0/264,635	0/281,153	0/297,321	0/287,380	2/271,841	0/265,543	1/266,739	3/1,934,612
	FT RR ×10^6 (95% CI)	-	-		-	0.302 (0.037-1.092)	-	0.154 (0.004-0.858)	0.064 (0.013-0.186) or 1 in 15,691,853
	Total RR ×10^6 (95% CI)	0.073 (0.015-0.213)	0.012 (0.0003-0.043)		0.092 (0.025-0.085)	0.071 (0.006-0.300)	0.023 (0.0006-0.104)	0.023 (0.0006-0.130)	0.043 (0.018-0.091) or 1 in 22,945,773
Method 3 (WHO, 2017)	RP positive donors/donors tested	3/1,425,791	0/1,441,350	1/1,474,930	4/1,501,319	1/1,504,371	1/1,487,13	0/1,488,901	10/10,323,975
	RP RR ×10^6 (95% CI)	0.046 (0.010-0.135)	0.007 (0.0002-0.028)		0.058 (0.016-0.150)	0.015 (0.0004-0.081)	0.015 (0.0004-0.082)	-	0.021 (0.010-0.039) or 1 in 47,103,136
	FT positive donors/donors tested	0/264,635	0/281,153	0/297,321	0/287,380	2/271,841	0/265,543	1/266,739	3/1,934,612
	FT RR ×10^6 (95% CI)	-	-		-	0.161 (0.020-0.583)	-	0.082 (0.002-0.458)	0.034 (0.007-0.099) or 1 in 29,422,224
	Total RR ×10^6 (95% CI)	0.0389 (0.008-0.048)	0.006 (0.0002-0.023)		0.049 (0.013-0.125)	0.038 (0.003-0.160)	0.012 (0.0003-0.070)	0.012 (0.0003-0.070)	0.023 (0.010-0.049) or 1 in 43,023,324

Years 2010 and 2011 are calculated together since in 2010 no NAT only positive cases were identified. The RR is expressed as units ×10^6 and, for the full period, also as 1 unit in n million units tested.
RP: repeat; RR: residual risk; FT: first-time; 95% CI: 95% confidence interval; EMA: European Medicines Agency; WHO: World Health Organization.

when compiling the case history, would have led them to exclusion from blood donation.

As far as concerns HIV-infected donors, sexual behaviour was the most common risk factor: 49.2% in first-time donors and 60.6% in repeat donors with a heterosexual/males having sex with males ratio of 1.76 among first-time donors and of 1.64 in repeat donors. This picture in Italy, where the criteria for blood donor selection are the same for men who have sex with men and heterosexuals and are based on individual risk assessment, is similar to that observed in the Italian non-donor HIV-infected population[25,26], but differs from that reported in HIV-infected donors of a number of other countries[27-30].

Similarly to what was seen with HCV, a remarkable proportion of both first-time (39%) and repeat (29%) HIV-positive donors declared no known HIV-related risk factors. The large proportion of HCV- and HIV-infected donors who did not report any risk factor strongly indicates that the pre- and post-donation counselling procedures currently in place in several Blood Establishments need to be improved. Thus, a specific standardised questionnaire issued by our National Blood Centre has recently been mandated on a national scale together with the adoption of more stringent educational programmes targeted to convince at-risk donors to self-defer and to provide truthful responses to the health history questions.

Of the over 21.8 million blood donations tested between 2009 and 2015, 19 were found to be HCV NAT-only positive and 13 were found to be HIV NAT-only positive. All donors who donated these infected donations were identified in the viraemic window period since they seroconverted to anti-HCV or anti-HIV during the follow up. Thus, the use of NAT has improved blood safety by averting the risk of entering 0.87 (or 1 in 1,149,425) HCV-infected units and 0.59 (or 1 in 1,667,852) HIV-infected units per million donations tested into the blood supply. Of importance, in the absence of other serological markers, the rate of NAT positivity in blood units collected from first-time donors was 7.4 times higher than the rate among units collected from repeat donors for HCV and 3 times for HIV. This current yield in detecting infectious blood by NAT before transfusion is somewhat lower than that previously reported in a similar 6-year survey carried out in Italy between 2001 and 2006[11]. In particular, in the study by Velati *et al.* the NAT yield was estimated to be around 2.5 (or 1 in 450,000) HCV-infected units and 1.8 (or 1 in 555,555) HIV-infected units per million donations tested, which are values that are nearly 3 times higher than those detected in the current survey for both infections.

In this study, we estimated the residual risk of transmitting a unit of HIV- or HCV-infected blood using three models adopted by the scientific and professional community: the window period ratio method described by Busch *et al.*[7], and those reported by the EMA[8] and by the WHO Expert Committee on Biological Standardisation[9]. Our findings indicate that the current residual risks of HCV and HIV are lower than earlier estimates[11]. For example, according to the window period ratio method, the overall residual risk of HCV changed from 1 unit per 10 million tested in 2001-2006 to 1 unit per 12.98 million tested in 2009-2015, while for HIV the residual risk changed from 1 unit per 1.25 million tested to 1 unit per 1.9 million tested in the same two periods of time. Current estimates of residual risk for both HCV and HIV were much lower using the EMA method (1 unit per 29.4 million tested for HCV and 1 unit per 22.9 million tested for HIV) or WHO method (1 unit per 47.1 million tested for HCV and 1 unit per 43 million tested for HIV). Assuming the residual risk estimates obtained with Busch's model as the worst scenario, we can say that in Italy the present risk of releasing a potentially infectious HCV or HIV donation missed by screening with current assays (NAT and serology) into the blood supply is negligible, if compared to the risk of everyday living[31]. These findings are reassuring when residual risk estimates are used to counsel patients about the risk of transfusion.

Conclusions

In conclusion, blood transfusion is extremely safe in Italy, though a residual risk of TTI remains, mainly because of imperfect risk behaviour-based donor selection and the persistence of a diagnostic window period that cannot yet be closed. Continued effort is required to maintain and further improve the high level of blood safety.

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Authorship contributions

CV, AZ and LR processed the data and prepared the manuscript. GM, LC, VP, GF, SV and IP collected data from the National Haemovigilance System. MET performed the statistical analysis. SP, GG, MET and GML reviewed the manuscript.

Disclosure of conflicts of interest

GML is the Editor-in-Chief of Blood Transfusion and this manuscript has undergone additional external review as a result. The other Authors declared that they have no conflicts of interest.

References

1) Regan FA, Hewitt P, Barbara JA, Contreras M on behalf of the current TTI Study Group. Prospective investigation of transfusion transmitted infection in recipients of over 20,000 units of blood. BMJ 2000; **320**: 403-6.
2) Soldan K, Barbara JA, Ramsay ME, Hall AJ. Estimation of the risk of hepatitis B virus, hepatitis C virus and human immunodeficiency virus infectious donations entering the blood supply in England, 1993-2001. Vox Sang 2003; **84**: 274-86.
3) Lackritz EM, Satten GA, Aberle-Grasse J, et al. Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. N Engl J Med 1995; **333**: 1721-5.
4) Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ for the Retrovirus Epidemiology Donor Study. The risk of transfusion-transmitted viral infections. N Engl J Med 1996; **334**: 1685-90.
5) Korelitz JJ, Busch MP, Kleinman SH, et al. A method for estimating hepatitis B virus incidence rates in volunteer blood donors. Transfusion 1997; **37**: 634-40.
6) Glynn SA, Kleinman SH, Wright DJ, Busch MP. International application of the incidence rate/window period model. Transfusion 2002; **42**: 966-72.
7) Busch MP, Glynn SA, Stramer SL, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005; **45**: 254-64.
8) European Medicine Agency. Guideline on epidemiological data on blood transmissible infections. 25 February 2016 EMA/CHMP/BWP/548524/2008 rev 1 Committee for Medicinal Products for Human Use (CHMP). Available at: http://www.ema.europa.eu/docs/en_GB/ document_library/Scientific_guideline/2016/02/WC500202385.pdf. Accessed on 04/04/2018.
9) Annex 4: Guidelines on estimation of residual risk of HIV, HBV or HCV infections via cellular blood components and plasma. In: WHO Expert Committee on Biological Standardization. Sixty-seventh report. WHO technical report series; no. 1004. World Health Organization; 2017. p. 163-96. Available at: http://www.who.int/biologicals/WHO_TRS_1004_web.pdf. Accessed on 04/04/2018.
10) Gonzalez M, Regine V, Piccinini V, et al. Residual risk of transfusion-transmitted human immunodeficiency virus, hepatitis C virus, and hepatitis B virus infections in Italy. Transfusion 2005; **45**: 1670-5.
11) Velati C, Romanò L, Fomiatti L. et al. for the SIMTI Research Group. Impact of nucleic acid testing for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus on the safety of blood supply in Italy: a 6-year survey. Transfusion 2008; **48**: 2205-13.
12) Società Italiana di Medicina Trasfusionale. [Screening of blood donations with NAT techniques in Italy.] Available at: http://www.simti.it/progetto.aspx? id=4&area=1. Accessed on 28/03/2018. [In Italian.]
13) Ministry of Health. [Decree of 2 November, 2015. Provisions relating to the quality and safety requisites for blood and blood components. Official Gazette of the Italian Republic December 28, 2015]. [In Italian.]
14) Brambilla DJ, Busch MP, Dodd RY, et al for the National Heart, Lung, and Blood Institute Recipient Epidemiology and Donor Evaluation Study-III (REDS-III). A comparison of methods for estimating the incidence of human immunodeficiency virus infection in repeat blood donors. Transfusion 2017; **57**: 823-31.
15) Seed CR, Cheng A, Ismay SL, et al. Virology Subcommittee of the National Donor and Product Safety Committee, Australian Red Cross Blood Service. Assessing the accuracy of three viral risk models in predicting the outcome of implementing HIV and HCV NAT donor screening in Australia and the implications for future HBV NAT. Transfusion 2002; **42**: 1365-72.
16) Soldan K, Davison K, Dow B. Estimates of the frequency of HBV, HCV and HIV infectious donations entering the blood supply in the United Kingdom, 1996 to 2003. Viral safety of blood. Eurosurveillance 2005; **10**: 17-9.
17) Zou S, Fang CT, Dodd RY for the ARCNET Research Group and Jerome H. Holland Laboratory for Biomedical Sciences. A method for estimating incidence rate of infectious diseases among first-time blood donors. Transfusion 2008; **48**: 1827-32.
18) Lefrère JJ, Dahourouh H, Dokekias AE, et al. Estimate of the residual risk of transfusion-transmitted human immunodeficiency virus infection in sub-Saharan Africa: a multinational collaborative study. Transfusion 2011; **51**: 486-92.
19) Zou S, Stramer SL, Dodd RY. Donor testing and risk: current prevalence, incidence, and residual risk of transfusion-transmissible agents in US allogeneic donations. Transf Med Rev 2012; **26**: 119-28.
20) O'Brien SF, Yi QL, Fan W, et al. Current incidence and residual risk of HIV, HBV and HCV at Canadian Blood Services. Vox Sang 2012; **103**: 83-6.
21) de Almeida-Neto C, Sabino EC, Liu J, et al. 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. Transfusion 2013; **53**: 827-34.
22) Vieira PC, Lamarão LM, Amaral CE, et al. Residual risk of transmission of human immunodeficiency virus and hepatitis C virus infections by blood transfusion in northern Brazil. Transfusion 2017; **57**: 1968-76.
23) Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002; **42**: 975-9.
24) Weusten J, Vermeulen M, van Drimmelen H, Lelie N. Refinement of a viral transmission risk model for blood donations in seroconversion window phase screened by nucleic acid testing in different pool sizes and repeat test algorithms. Transfusion 2011; **51**: 203-15.
25) Suligoi B, Pupella S, Regine V, et al. Changing blood donor screening criteria from permanent deferral for men who have sex with men to individual sexual risk assessment: no evidence of a significant impact on the human immunodeficiency virus epidemic in Italy. Blood Transfus 2013; **11**: 441-8.
26) Regine V, Pugliese L, Boros S, et al. [Update of new diagnoses of HIV infection and AIDS cases in Italy at December 31, 2016.] Not Ist Super Sanità 2017; **29** (9 Suppl 1): 3-51. [In Italian.]
27) Pillonel J, Laperche S. Trends in risk of transfusion-transmitted viral infections (HIV, HCV, HBV) in France between 1992 and 2003 and impact of nucleic acid testing (NAT). Eurosurveill 2005; **10**: 5-8.
28) Custer B, Kessler D, Vahidnia F, et al. Risk factors for retrovirus and hepatitis virus infections in accepted blood donors. Transfusion 2015; **55**: 1098-107.
29) Goldman M, O'Brien SF. Donor deferral policies for men who have sex with men: where are we today? Curr Opin Hematol 2016; **23**: 568-72.
30) Bes M, Piron M, Casamitjana N, et al. Epidemiological trends of HIV-1 infection in blood donors from Catalonia, Spain (2005-2014). Transfusion 2017; **57**: 2164-73.
31) Calman K. Cancer: science and society and the communication of risk. BMJ 1996; **13**: 799-802.

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