Evolution of the residual risk of HBV, HCV and HIV transmission through blood transfusion in the Region of Valencia, Spain, during a 15-year period (2003-2017)

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Background. Towards the end of the 20th century, transfusion-transmitted viral infections (TTVI) represented a huge problem for public health. From the beginning of the screening of blood donations, this risk has decreased to the point that it is no longer possible to measure it directly and it is necessary to use mathematical models. Using one of these models, the aim of this study was to analyse the evolution of the residual risk of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) transmission through blood transfusion from 2003 to 2017 in the Region of Valencia, Spain.

Materials and methods. Using data from the Blood Transfusion Centre of the Valencian Community, the incidence rate in donors and the residual risk were calculated for each agent and year by applying the most updated version of the incidence/window period model. For the set of the three viruses, these magnitudes were calculated as the algebraic sum of the specifics ones for each of them. The evolution of both magnitudes was analysed by the Mann-Kendall trend test and the Sen estimation of trend slope.

Results. The residual risks obtained vary depending on the agent and the year. Considering the three viruses jointly, they range from 1 per 360,380 to 1 per 44,715 donations. During the study period, there was a statistically significant downward trend in the incidence rate of HBV ($p<0.05$, trend slope −0.88), the residual risk of HBV ($p<0.0005$, slope −0.98), and the joint residual risk for the three viruses ($p<0.0001$, slope −0.99).

Discussion. The current risk of TTVI is very low in the Region of Valencia. In the last 15 years, there has been a reduction in the donor incidence rate and in the residual risk for the case of HBV; such a reduction cannot be confirmed for HCV and HIV. Consideration of the three viruses jointly confirms a reduction in the residual risk; we are unable to establish whether the evolution of the joint incidence rate has contributed to this reduction or whether it is due only to the shortening of window periods.

Keywords: blood transfusion, risk assessment, hepatitis B virus, hepatitis C virus, HIV.

Introduction

In the field of transfusion medicine, transfusion-transmitted viral infections (TTVI) have historically been the cause of greatest concern to medical professionals and society in general. Although it is difficult to know the exact magnitude of this problem, it has been estimated that since the onset of the epidemic of acquired immunodeficiency syndrome (AIDS) in 1981, until the introduction of the screening of donations for the human immunodeficiency virus (HIV) in 1985, transfusion would have been responsible for approximately 15,000 cases of HIV alone in United States[1]. On the other hand, it has been observed that blood transfusion was the second major risk factor for infection with hepatitis C virus (HCV) before the introduction of screening, second only to intravenous drug use[2]; for example, 9.6% of the recipients of transfusions in Barcelona contracted this infection in 1989[3].

The selection of donors and the introduction and progressive improvement of screening tests for virus detection in donations have reduced the risk of TTVI to the point that it is no longer possible to measure it directly and it is necessary to resort to mathematical models[4-10].

However, the risk continues to exist even in countries where more resources are allocated to its prevention. A particularly illustrative example is found in Valencia. In 2009, two cases of transfusion-transmitted HIV infection were reported in receptors of a platelet concentrate and a

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unit of fresh frozen plasma from the same donation made in 2005. This was in spite of the fact that the donation had passed all screening tests (including HIV-1 ribonucleic acid [RNA] detection) and that the fresh frozen plasma had been processed to reduce the viral load[11].

It is a sign that, even though the risk today is very low, TTVI should be continually monitored. The easiest and most straightforward way to calculate the risk would be to study the rate of infection among blood transfusion receptors. However, given the current low risk, this method would require an excessively large sample size, which in practice is unfeasible[4,5].

The alternative solution is to use mathematical models that can estimate the residual risk of TTVI, which is defined as the probability of accepting an infected donation from a donor whose infection is not detectable by screening tests[12].

The main cause of this residual risk, which is considered to be mainly responsible for all the current cases of TTVI, is the donation of blood by infected individuals who are in the diagnostic window period (DWP) during which laboratory tests are not able to detect the infection[4,10-13]. Therefore, the most widely accepted method for calculating residual risk currently in use is based on the combination of the incidence rate of seroconversions and conversions for nucleic acid amplification techniques (NAT) among repeat donors, with the probability that the donation prior to conversion (which exceeded screening and was accepted) took place during the DWP[4,10,12]. By applying this method, our aim was to analyse the annual residual risk of hepatitis B virus (HBV), HCV, and HIV infections in the Region of Valencia and its evolution over a period of 15 years (2003-2017) in order to find out if there has been a downward trend in the residual risk for any of these viruses or for the set of the three and, if so, how the evolution of the DWP and the incidence rate among donors have contributed to that trend.

Materials and methods

We conducted a retrospective study based on the archives of the Blood Transfusion Centre of the Valencian Community (CTCV) from which we obtained data on the number of donors, donations and positive results in the screening and confirmatory tests for HBV, HCV, and HIV during the period from 1st January 2003 to 31st December 2017.

All donations during this period were analysed using third-generation chemiluminescence immunoassays (ChLIA) for the detection of hepatitis B surface antigen (HBsAg), anti-HCV and anti-HIV-1/2 antibodies. In addition, donations made up until 30th June 2006 were analysed by polymerase chain reaction (PCR) in a mini-pool of 44 donations (44MP-NAT) for the detection of HCV-RNA. Those made between 24th February 2004 and 30th June 2006 were also analysed by 44MP-NAT for the detection of HIV-RNA. Finally, all donations made from 1st July 2006 were analysed by transcription-mediated amplification (TMA) for the detection of HCV-RNA, HIV-RNA and deoxyribonucleic acid (DNA) of HBV in individual donation (ID-NAT). Details of the screening tests used are available in Table I.

Any positive results in serological screening tests subsequently underwent confirmatory tests, as follows. For HBsAg: Abbott IMx® HBsAg Confirmatory (Abbott Laboratories, North Chicago, IL, USA) from 01/01/2003 to 30/04/2009, Abbott AxSYM® HBsAg Confirmatory (Abbott Laboratories) from 01/05/2009 to 30/09/2012, VITROS® HBsAg Confirmatory Kit (Ortho Clinical Diagnostics, Rochester, NY, USA) from 01/10/2012 to 23/08/2017, and ARCHITECT® HBsAg Confirmatory Kit (Abbott Laboratories) from 24/08/2017 to 31/12/2017. For anti-HCV: CHIRON RIBA® HCV 3.0 Strip Immunoblot Assay (Chiron Corporation, Emeryville, CA, USA) from 01/01/2003 to 16/05/2006, and INNO-LIA® HCV Score (Fujirebio Europe NV, Ghent, Belgium) from 17/05/2006 to 31/12/2017. For anti-HIV-1/2: New LAV BLOT® I and II assays (Sanofi Pasteur, Lyon, France) from 01/01/2003 to 27/03/2006, and INNO-LIA® HIV I/II Assay (Fujirebio Europe NV) from 28/03/2006 to 31/12/2017. All of the repeatedly positive donations by TMA were given a discriminatory test to determine which of the three viruses was the cause of genomic detection.

For the calculation of incidence and residual risk rates, two groups of donors were defined: those who had made at least one donation previously (not necessarily in the same year) were considered repeat donors, while those who had not made any previous donation were considered first-time donors. Among the repeat donors, those who tested positive in any screening test after having passed it in their previous donation were called converting donors. The viraemic phase of the DWP (vDWP) was defined as that which extends from the moment from which a viral particle appears in a blood component until the time when the infection can be detected by screening techniques[12,14].

The incidence rate for each virus and year in repeat donors was calculated by dividing the number of converting donors among the total of repeat donors throughout the year. A converting repeat donor was defined as one who had a positive result in any screening test (serological or NAT-based) and that was later confirmed in a confirmatory test. For first-time donors, it is not possible to calculate the incidence rate by this method, since it is not possible to know if a positive result corresponds to an incident case or a prevalent case. Therefore, following the recommendations of

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Table I - Blood-screening assays used for the detection of HBV, HCV and HIV in donations made in the Region of Valencia from 01/01/2003 to 31/12/2017.

Agent	Period of time	Assays used	Manufacturer
HBV	01/01/2003-30/06/2006	ChLIA (HBsAg)	Abbott PRISM® HBsAg[a]
	01/07/2006-31/12/2017	ChLIA (HBsAg)	Abbot PRISMv HBsAg[a]: from 01/07/2006 to 30/09/2012 VITROS® HBsAg[b]: from 01/10/2012 to 23/08/2017 ARCHITECT® HBsAg[a]: from 24/08/2017 to 31/12/2017
		ID-NAT (HBV-DNA)	Procleix® Ultrio Assay[c]: from 01/07/2006 to 24/01/2011 Procleix® Ultrio Plus Assay[c]: from 25/01/2011 to 30/11/2014 Procleix® Ultrio Elite Assay[c]: from 01/12/2014 to 31/12/2017
HCV	01/01/2003-30/06/2006	ChLIA (anti-HCV)	Abbott PRISM® HCV[a]
		44MP-NAT (HCV-RNA)	COBAS® AmpliScreen HCV Test, v2.0[d]
	01/07/2006-31/12/2017	ChLIA (anti-HCV)	Abbott PRISM® HCV[a]: from 01/07/2006 to 30/09/2012 VITROS® Anti-HCV[b]: from 01/10/2012 to 23/08/2017 ARCHITECT® Anti-HCV assay[a]: from 24/08/2017 to 31/12/2017
		ID-NAT (HCV-RNA)	Procleix® Ultrio Assay[c]: from 01/07/2006 to 24/01/2011 Procleix® Ultrio Plus Assay[c]: from 25/01/2011 to 30/11/2014 Procleix® Ultrio Elite Assay[c]: from 01/12/2014 to 31/12/2017
HIV	01/01/2003-23/02/2004	ChLIA (anti-HIV-1/2)	Abbott PRISM® HIV O Plus[a]
	24/02/2004-30/06/2006	ChLIA (anti-HIV-1/2)	Abbott PRISM® HIV O Plus[a]
		44MP-NAT (HIV-RNA)	COBAS® AmpliScreen HIV-1 Test, v1.5[d]
	01/07/2006-31/12/2017	ChLIA (anti-HIV-1/2)	Abbott PRISM® HIV O Plus assay[a]: from 01/07/2006 to 30/09/2012 VITROS® Anti HIV 1+2[b]: from 01/10/2012 to 23/08/2017 ARCHITECT® HIV Ag/Ab Combo[a]: from 24/08/2017 to 31/12/2017
		ID-NAT (HIV-RNA)	Procleix® Ultrio[c]: from 01/07/2006 to 24/01/2011 Procleix® Ultrio Plus assay[c]: from 25/01/2011 to 30/11/2014 Procleix® Ultrio Elite assay[c]: from 01/12/2014 to 31/12/2017

[a]Abbott Laboratories, North Chicago, IL, USA; [b]Ortho Clinical Diagnostics, Rochester, NY, USA; [c]Novartis, Basel, Switzerland; [d]Hoffmann-La Roche, Basel, Switzerland; ChLIA: chemiluminescence immunoassays; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

the World Health Organization (WHO), we obtained the incidence rate in first-time donors by multiplying that of the repeat donors by an adjustment factor of 3[12]. The overall incidence rate was calculated as the mean between the incidence rates of both groups, weighted by their relative weight (as a fraction of the total number of donors represented by that group).

The residual risk of transmission for each virus, understood to be the probability of collecting and accepting an infected donation, was estimated following a mathematical model proposed by the Retrovirus Epidemiology Donor Study (REDS) group in 1996, in its most up-to-date version proposed by the WHO in 2017[4,12].

- The residual risk from donations made by repeat donors was calculated, for each virus and year, as the product of the incident rate in repeat donors and the duration of the shortest vDWP of the screening tests used that year, expressed as a fraction of a year (Table II).

  The residual risk due to the donations of first-time donors was obtained by multiplying the derivative of the repeat donors by an adjustment factor of 3, according to the same criterion used for the incidence rates. It can also be calculated as the product of the incidence rate in first-time donors and the duration of vDWP.
- The overall residual risk for each agent and year was calculated as the mean of residual risks for both groups, weighted by their relative weight (as a fraction of the total donations represented by that group).

In the case of HBV, to the incidence rates, we applied adjustment factors that take into account the undetected cases due to the transient nature of the antigenaemia and viraemia of this agent[15,16].

Finally, the incidence rate in donors and the residual risk of transmission considering the three viruses together were obtained by the algebraic sum of the specific incidence rates and residual risks for each one of them (using the adjusted values in the case of HBV).

The aforementioned methods for calculating the incidence rates among donors and the residual risks

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Table II - Length of the vDWP used to estimate the residual risk of transmission of each agent for each year of the study period.

Agent	Years	Screening assay[a]	Length of vDWP (days)[b]
HBV	2003-2006[c]	ChLIA (HBsAg)	42
	2007-2017	ID-NAT (HBV-DNA)	17
HCV	2003-2006[c]	44MP-NAT (HCV-RNA)	5[e]
	2007-2017	ID-NAT (HCV-RNA)	3
HIV	2003	ChLIA (anti-HIV-1/2)	21
	2004-2006[c,d]	44MP-NAT (HIV-RNA)	7[e]
	2007-2017	ID-NAT (HIV-RNA)	4

[a]For the years in which a serological and a NAT-based screening test were simultaneously used, every donor who obtained a positive result in the serological assay also obtained it in the NAT-based one. Given that NAT techniques detect the infection earlier and that the number of NAT-converting donors coincides with the number of total converting donors, only NAT-based assays are considered for the calculation of residual risk in these years.
[b]For a worst-case assumption, the vDWP is considered to start at the point at which the viral concentration reaches one virus particle in 20 mL of plasma (the volume co-transfused with a red blood cell unit)[12,14]. The vDWP used are the ones proposed by the WHO in its guidelines[12].
[c]The TMA in individual donation (ID-NAT) was introduced for the three viruses on 1st July 2006. Consequently, the assays used in the first and second semester of that year were different. When calculating the residual risk for that year, the length of the longest vDWP (the one of the assays used in the first semester) has been used for a worst-case assumption.
[d]The NAT for HIV was not introduced until 24th February 2004. Since most of the donations made in that year were analysed by NAT, when calculating the residual risk it has been overlooked that a few of them were analysed by ChLIA, and the vDWP used for that year was the one of the NAT.
[e]The vDWP of the MP44-NAT is not avaliable in the literature. Consequently, to calculate the residual risk for the cases of HCV and HIV in the years in which 44MP-NAT was the assay with the shortest vDWP, it was used the vDWP of the 16MP-NAT. Since the window period is considered to be shorter the smaller the minipool size is, using the one of the 16MP-NAT implies that the residual risk for the HCV during the period 2003-2006 and for the HIV during the period 2004-2006 could have been slightly underestimated. vDWP: viraemic phase of the diagnostic window period; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus. ChLIA: chemiluminescence immunoassays; HBsAg: hepatitis B surface antigen;

for each virus and for the set of the three are shown schematically in *Online Supplementary Figure S1*.

For the calculation of the 95% confidence intervals (95% CI), we used the table for variables with Poisson distribution as proposed by Haenszel *et al.*[17]. For statistical analysis, we used the Mann-Kendall trend test complemented with the Sen estimation of trend slope, applying them to the incidence rates and residual risks obtained for each virus and for the set of the three during the 15 years of study. We assumed a level of statistical significance of $p<0.05$. Data management was carried out on a spreadsheet of Microsoft Excel® 2011, version 14.7.1 (Microsoft Corporation, Redmon, WA, USA), and the statistical analysis was performed using the statistical Software XLSTAT®, version 2018.5 (Addinsoft, Paris, France).

Results

Between 1st January 2003 and 31st December 2017, a total of 2,684,206 allogeneic blood donations were analysed at the CTCV headquarters. Of these, 2,308,933 were made by repeat donors, while the remaining 375,173 were made by first-time donors. During this period, 874 cases of HBV infection, 642 cases of HCV infection, and 219 of HIV infection were detected among the donations made, of which 40 cases of HBV, 45 of HCV, and 130 of HIV were incident cases involving repeat donors. These and other data related to donation in the Region of Valencia during this period are shown in *Online Supplementary Table SI*.

The highest residual risk of TTVI was recorded in the year 2003, coinciding with the beginning of the study period. The residual risk in that year was 1 per 72,865 units donated in the case of HBV, 1 per 844,922 for HCV, and 1 per 134,115 for HIV. Considering the three viruses jointly, the risk of accepting an infected donation in 2003 was 1 per 44,715 units (Table III).

For the last year of the period studied (2017), the values obtained were much lower: 1 per 346,101 units for HBV, 1 per 4,727,119 for HCV, 1 per 1,418,136 for HIV, and 1 per 262,741 considering the three viruses jointly (Table III).

The year in which a lower residual risk was recorded was 2015 for HBV (1 per 700,421), 2010 for HCV (1 per 9,767,831), and 2004 for HIV (1 per 1,923,493). The lowest residual risk of transmission of any virus was recorded in the year 2015, with 1 per 360,380 donated

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Table III - Annual incidence rates in donors and residual risks of transmission of HBV, HCV and HIV.

Agent	Year	IR per 100,000 donor-years (95% CI)	RR per million donations (95% CI)	RR as 1 per number of donations (95% CI)
HBV	2003	13.32 (1.61-48.09)	13.72 (1.66-49.54)	72,865 (20,184-602,194)
	2004	12.88 (1.56-46.48)	13.40 (1.62-48.38)	74,617 (20,669-616,666)
	2005	13.11 (1.59-47.32)	13.59 (1.64-49.06)	73,576 (20,381-608,069)
	2006[a]	-	-	-
	2007	21.08 (7.74-45.96)	8.90 (3.27-19.40)	112,345 (51,534-306,117)
	2008	19.96 (7.32-43.51)	8.44 (3.10-18.40)	118,451 (54,335-322,755)
	2009	10.25 (2.11-29.93)	4.31 (0.89-12.57)	232,245 (79,536-1,127,401)
	2010	16.29 (5.28-37.96)	6.90 (2.24-16.08)	144,928 (62,201-447,309)
	2011	13.34 (3.63-34.16)	5.67 (1.54-14.50)	176,496 (68,944-648,883)
	2012	10.08 (2.08-29.44)	4.25 (0.88-12.42)	235,111 (80,517- 1,141,313)
	2013	3.62 (0.09-20.16)	1.54 (0.04-8.57)	649,695 (116,642-25,679,651)
	2014	3.37 0.09-18.80)	1.44 0.04-8.03)	693,921 (124,582-27,427,707)
	2015	3.35 (0.08-18.66)	1.43 (0.04-7.95)	700,421 (125,749-27,684,630)
	2016	6.57 (0.79-23.71)	2.81 (0.34-10.15)	355,506 (98,478-2,938,064)
	2017	6.77 (0.82-24.42)	2.89 (0.35-10.43)	346,101 (95,873-2,860,342)
HCV	2003	9.65 (3.54-21.04)	1.18 (0.43-2.58)	844,922 (387,579-2,302,239)
	2004	3.00 (0.36-10.82)	0.37 (0.04-1.34)	2,692,890 (745,953-22,255,286)
	2005	4.67 (0.96-13.63)	0.58 (0.12-1.68)	1,735,872 (594,477-8,426,564)
	2006	3.09 (0.37-11.14)	0.38 (0.05-1.38)	2,617,245 (724,999-21,630,128)
	2007	2.98 (0.36-10.77)	0.22 (0.03-0.80)	4,500,111 (1,246,568-37,191,002)
	2008	5.66 (1.54-14.49)	0.42 (0.11-1.08)	2,366,200 (924,297-8,699,263)
	2009	5.79 (1.58-14.83)	0.43 (0.12-1.10)	2,328,294 (909,490-8,559,906)
	2010	1.37 (0.03-7.63)	0.10 (0.00-0.57)	9,767,831 (1,753,650-386,080,270)
	2011	6.93 (2.25-16.15)	0.52 (0.17-1.21)	1,925,805 (826,526-5,943,842)
	2012	4.23 (0.87-12.36)	0.32 (0.06-0.92)	3,174,204 (1,087,056-15,408,759)
	2013	2.92 (0.35-10.55)	0.22 (0.03-0.79)	4,560,269 (1,263,232-37,688,174)
	2014	8.33 (3.06-18.15)	0.63 (0.23-1.37)	1,593,837 (731,118-4,342,880)
	2015	1.38 (0.03-7.67)	0.10 (0.00-0.58)	9,650,126 (1,732,518-381,427,886)
	2016	2.69 (0.33-9.70)	0.20 (0.02-0.73)	4,923,432 (1,363,832-40,689,524)
	2017	2.81 (0.34-10.13)	0.21 (0.03-0.76)	4,727,119 (1,309,451-39,067,099)
HIV	2003	14.48 (6.63-27.50)	7.46 (3.41-14.17)	134,115 (70,587-292,827)
	2004	3.00 (0.36-10.82)	0.52 (0.06-1.88)	1,923,493 (532,823-15,896,633)
	2005	6.22 (1.69-15.93)	1.08 (0.29-2.75)	929,932 (363,255-3,418,866)
	2006	7.72 (2.50-17.98)	1.34 (0.43-3.12)	747,784 (320,938-2,307,977)
	2007	8.95 (3.28-19.50)	0.89 (0.33-1.94)	1,125,028 (516,068-3,065,471)
	2008	9.91 (3.97-20.41)	0.99 (0.40-2.03)	1,014,086 (492,275-2,528,892)
	2009	11.59 (4.99-22.83)	1.15 (0.49-2.26)	873,110 (443,203-2,025,778)
	2010	13.70 (6.58-25.21)	1.37 (0.66-2.51)	732,587 (398,145-1,526,224)
	2011	19.40 (10.60-33.96)	1.94 (1.06-3.39)	515,841 (294,766-944,763)
	2012	23.98 (13.98-38.37)	2.38 (1.39-3.81)	420,115 (262,572-720,609)
	2013	26.29 (15.59-41.54)	2.63 (1.56-4.16)	380,022 (240,521-640,847)
	2014	12.49 (5.72-23.73)	1.25 (0.57-2.38)	796,918 (419,431-1,739,997)
	2015	12.40 (5.68-23.56)	1.24 (0.57-2.36)	804,177 (423,251-1,755,845)
	2016	9.41 (3.77-19.38)	0.95 (0.38-1.95)	1,055,021 (512,146-2,630,976)
	2017	7.02 (2.27-16.35)	0.71 (0.23-1.64)	1,418,136 (608,642-4,376,962)
Total[b]	2003	37.45 (21.83-59.92)	22.36 (13.04-35.78)	44,715 (27,947-76,698)
	2004	18.87 (6.93-41.14)	14.29 (5.25-31.16)	69,964 (32,094-190,637)
	2005	24.00 (10.99-45.60)	15.24 (6.98-28.96)	65,605 (34,529-143,242)
	2006[a]	-	-	-
	2007	33.01 (18.02-55.46)	10.01 (5.47-16.82)	99,878 (59,451-182,926)
	2008	35.53 (20.71-56.84)	9.85 (5.74-15.76)	101,512 (63,445-174,120)
	2009	27.63 (15.47-45.59)	5.88 (3.29-9.70)	170,050 (103,060-303,660)
	2010	31.36 (17.94-50.81)	8.37 (4.79-13.56)	119,512 (73,773-208,937)
	2011	39.68 (25.16-59.52)	8.12 (5.15-12.19)	123,097 (82,064-194,159)
	2012	38.30 (24.28-57.45)	6.95 (4.41-10.42)	143,913 (95,942-226,992)
	2013	32.83 (20.32-50.24)	4.39 (2.72-6.72)	227,796 (148,886-368,006)
	2014	24.19 (13.84-39.19)	3.32 (1.90-5.38)	300,902 (185,742-526,053)
	2015	17.13 (8.55-30.66)	2.77 (1.38-4.97)	360,380 (201,330-722,205)
	2016	18.66 (9.31-33.40)	3.96 (1.98-7.10)	252,280 (140,938-505,571)
	2017	16.59 (7.60-31.52)	3.81 (1.74-7.23)	262,741 (138,285-573,671)

[a]The HBV and total incidence rates and residual risks for the year 2006 could not be calculated as a consequence of the absence of converting donors for HBV in that year.
[b]"Total" refers to the joint incidence rate and residual risk of the three viruses when considered together.
IR: incidence rate; RR: residual risk; CI: confidence interval; HBV: hepatitis B virus HCV: hepatitis C virus HIV: human immunodeficiency virus.

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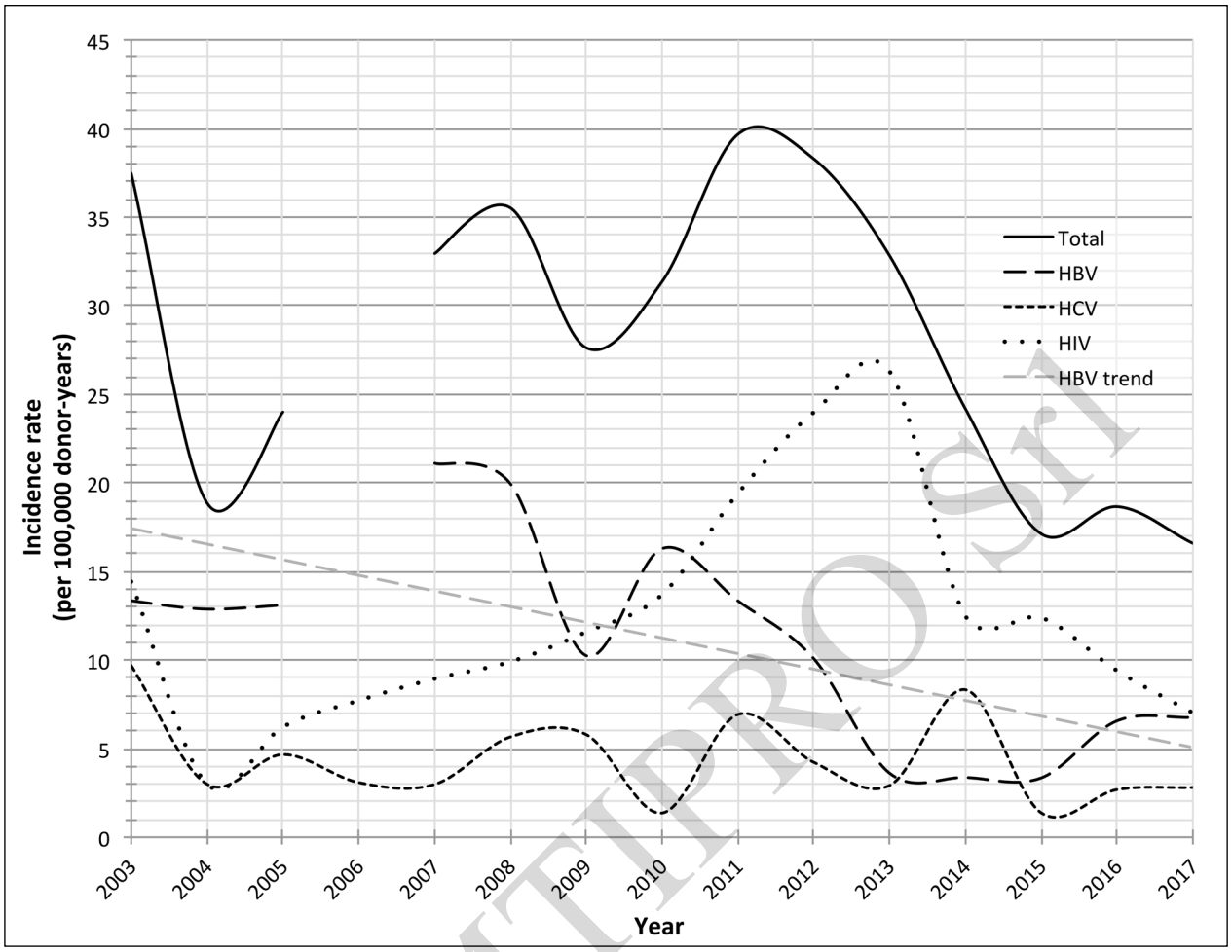


Figure 1 - Evolution of the incidence rates among donors from 2003 to 2017.
Total: the joint incidence rate of the three viruses when considered together. HBV trend: $p<0.05$; *Sen* slope: −0.88 (95% CI: −0.96, −0.80).
HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

units (Table III). Table III also shows the estimated incident rates in donors for each agent and year.

The weighted average residual risks for the 15 years were as follows. For HBV, 6.36 per million donations (95% CI: 4.54-8.65) or 1 in 157,271 donated units. For HCV, 0.39 per million (95% CI: 0.28-0.52) or 1 in 2,557,987. For HIV, 1.72 per million (95% CI: 1.44-2.05) or 1 in 580,826.

The statistical analysis showed the following results.

- a decreasing trend in the adjusted incidence rate of HBV ($p<0.05$) and in the residual risk of transmission for that agent ($p<0.0005$), with Sen slopes of −0.88 and −0.98, respectively (Figures 1 and 2).
- A decreasing trend in the residual risk when the three viruses are considered jointly ($p<0.0001$), with a Sen slope of −0.99 (Figure 2).
- Results not statistically significant for the incidence rates and residual risks of HCV and HIV, and for the joint incidence rate of the three viruses.

Discussion

The risk of TTVI should be continuously monitored by mathematical models that allow its magnitude to be estimated. Among them, the incidence/window period model is currently the most widely used[10]. This method calculates the probability of accepting an infected donation (residual risk). We must bear in mind that each individual donation can be used to transfuse up to three blood components (erythrocyte concentrate, platelet concentrate, and fresh plasma), so there is no exact correlation between donated unit and transfused unit. However, if we assume that infected and non-infected donations have the same probability of being destined for between zero and three transfusions, the residual risk and the likelihood that the recipients will receive an infected component could be considered equivalent.

The probability of receiving an infected component does not necessarily coincide with the risk of infection, for which we should also consider the probability that the

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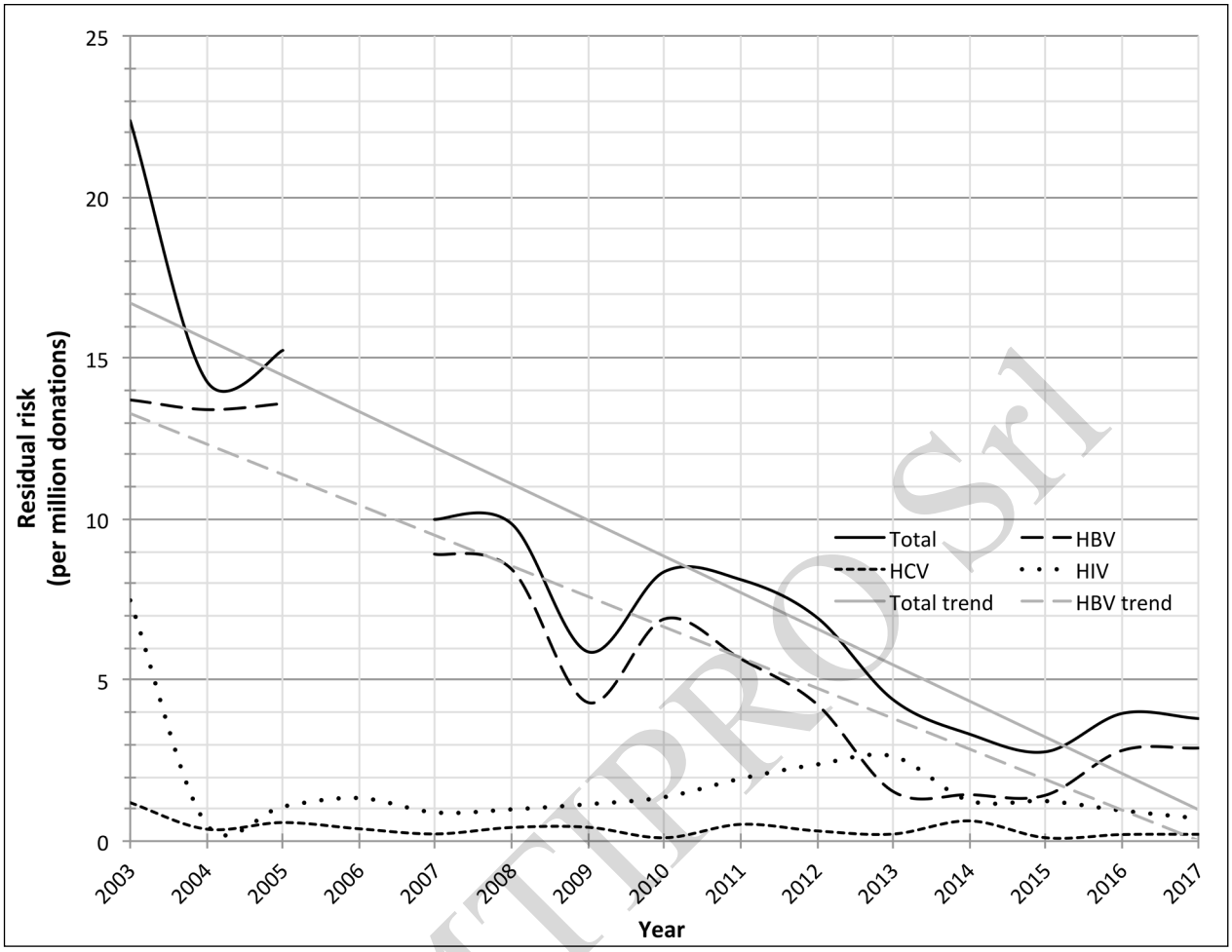


Figure 2 - Evolution of the residual risks from 2003 to 2017.
"Total" refers to the joint residual risk of the three viruses when considered together.
HBV trend: p<0.0005; Sen slope: −0.98 (95% CI: −1.01, −0.94). Total trend: p<0.0001; Sen slope −0.99 (95% CI −1.03, −0.95). HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

recipient will develop the infection[12,18]. For the case of HIV, for example, it has been estimated that probability of this is 92.5%[18]. Nevertheless, since it is not possible to predict which receivers will develop the infection when receiving an infected unit and which ones will not, the aim must be to avoid transfusion of infected components. Therefore, the magnitude of the residual risk is the most important parameter for assessing the safety of the blood supply; even more important than risk of infection.

Hepatitis B virus was the agent for which a higher residual risk was recorded in all the years of the study period, except in 2006 (for which the residual risk of HBV could not be calculated due to the absence of converting repeat donors) and 2013, when there was a higher risk for HIV (Table III and Figure 2). The total residual risk runs almost perfectly parallel to that of HBV because its contribution to the joint risk is much higher than that of HCV and HIV (Figure 2).

The incidence rate of HBV among donors was higher in 2007 than in 2005. However, the residual risk was higher in 2005 (Figure 2). This is because the increase in incidence was offset by the introduction of a new screening test that considerably shortened the window period (Table II). It is an example of how the introduction of new techniques is able to reduce the risk of transmission regardless of the evolution of the incidence among donors.

The most remarkable fact in the case of HIV is the peak of incidence that took place in the period 2011-2013 (Figure 1), which was translated in turn into a peak of risk (Figure 2).

In the case of HCV, it is striking that the estimated risk has been virtually negligible in all of the years studied, and much lower than that of HBV and HIV (Figure 2). This shows that the selection of donors and screening of donations for the prevention of HCV transmission has reached a level of success above all

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expectations, if we consider that in the past it was the virus associated with the highest number of cases of transfusion transmission. However, it is important to note that the number of converting donors was small for the three viruses in all the years of the study period, which increases year-to-year variability in incidence rates and, consequently, in residual risks. Hence, the above considerations should be analysed with caution.

The results of the statistical analysis confirm a downward trend in the residual risk for the case of HBV and for the set of the three viruses, and in the incidence rate in donors for the case of HBV but not for the set of the three viruses. For HCV and HIV, we are not able to confirm any trend. For these two viruses, NAT techniques were used from the beginning of the study period (except in the year 2003 for HIV), so there was a slight shortening of the vDWP throughout that period. It is likely that more illustrative results would have been obtained if a period had been chosen which covered the transition from serological tests to NAT-based techniques (as has occurred in the case of HBV).

Probably the most interesting result of this study is the statistically significant decreasing trend in the residual risk of TTVI when we consider the three viruses jointly, in the absence of such a statistically significant trend for the joint incidence rate in donors. As HBV residual risk far exceeds HCV and HIV risks during most of the years of the study period, the downward trend when considering all three viruses is mostly driven by HBV. In any case, the conclusion to be drawn is that the decrease in risk is mostly a consequence of the progressive shortening of the DWPs. This highlights the importance that the introduction of increasingly sensitive screening techniques has had in the field of transfusion security.

It should be kept in mind that the mathematical model used, despite being the most standardised, has a number of important limitations.

- First, its reliability is subject to the accuracy of the estimation of the duration of the window periods[4]. This is a difficult magnitude to calculate, and can also present significant variations between the techniques of the same category produced by different manufacturers[12].
- We must also bear in mind that the residual risk comes from the penultimate donation made by a converting donor, because it is the one in which there is a certain probability that it is within the window period. For this reason, the WHO model assumes that all repeat donors have made at least two donations in the year for which the risk is calculated, which is not always true. A more accurate estimate would require the number of converting donors whose penultimate (and not last) donation was made in the year in question to be used in the numerator of the incidence rate in repeat donors. In the same way, the denominator would be represented by the number of repeat donors who made their penultimate donation in that year. However, this proceeding would imply an individual follow up to each donor on a sample of hundreds of thousands of them, greatly complicating the collection of data. Another option would be to include only those repeat donors who had made at least two donations in that year, but this would greatly reduce the size of the sample and the statistical power, while also assuming that the donors not included in the study have the same risk of being in the window period as those included. Thus, the method used does not seek the highest accuracy, but the best balance between accuracy and feasibility.
- Another limitation of this model is the fact that it does not allow the incidence rate in first-time donors to be directly calculated, so this is obtained by multiplying that of repeat donors by an adjustment factor that depends on the relative incidence between the two groups of donors. However, this relative incidence is unknown in most regions of the world, and results in forced extrapolation of data obtained from studies conducted in regions with different characteristics[12].
- On the other hand, large variations per year in the number of converting donors, which in turn are a source of variation in incidence rates and residual risks, could respond to a relatively small sample size for a variable with such a low incidence[12]. This problem could be solved by calculating the residual risk for periods exceeding one year, as performed in the original model proposed by the REDS[4]. However, this would mean totalling the intervals between donations from all donors to get the number of person-years at risk. Again, this would require individual monitoring of each donor, which is only possible with high-powered computer systems. It is a new example of how the WHO simplified model yields accuracy in favour of viability and simplicity.

For the aforementioned reasons, the data obtained cannot be assumed to be exact, but they do provide an approximate idea of the magnitude of the problem.

Throughout the 15 years of the study period, one case of HBV and two cases of HIV transfusion-transmitted infections were reported in the Region of Valencia[11,19]. Therefore, the observed risk of HBV infection was 1 per 2,684,206 donations (the total number of donations made in the study period). Given that the two cases of HIV transmission came from the same infected donor, the observed risk for HIV was also 1 per 2,684,206 donated units. This corresponds to a risk of 0.37 per million donations for both viruses (95% CI: 0.01-2.08). For HCV, no case of transmission was reported throughout the 15 years.

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If we compare these data with the estimated average risks for the 15 years (6.36 per million for the case of HBV and 1.72 per million for the case of HIV), we can see that the observed risks are much lower than the estimated ones. However, this discordance is to some extent expected if we consider the following.

- With the current small number of TTVI, the observed risk is not a precise measurement of real risk, and the wide confidence interval is good proof of this. In fact, in the case of HIV, the estimated risk is within the 95% CI of the observed risk. Therefore, in this case, the differences between the estimated and the observed risk could be due to chance.
- Since a large proportion of HBV and HCV infections remain asymptomatic, some recipients could have developed an infection that was not noticed.
- Moreover, a significant proportion of the recipients of donations are affected by serious diseases and die before a hypothetical transfusion-transmitted infection becomes manifest.
- Although trace-back programmes have been conducted in our region when an infection has been reported in a patient with antecedents of receiving a transfusion, and look-back programmes have been conducted whenever a repeat donor conversion has been detected in a screening test, not all the investigations in this respect have been successfully resolved.

All these considerations suggest that the real number of TTVI could be higher than that reported, which reinforces the idea that the observed risk is not a sufficiently good parameter to show the real risk and confirms that mathematical models are needed.

We compared the results of different studies conducted in Spain to estimate residual risk of transfusion-transmitted infections over 3-year periods[5,20,21] (Table IV) and added the results of our own study for the three years 2015-2017. Since these data have not been compared using a statistical model, they do not allow us to draw any definitive conclusions. Nevertheless, they do allow us to propose some hypotheses for future study.

Firstly, it seems that the residual risk of transmission of HBV and HCV infections has progressively decreased since the last years of the 20th century to the present day. If this turns out to be true, it would probably be mainly due to the reduction of the risk obtained with the transition from serological techniques to NAT-based techniques, which became general practice in Spain during the first decade of the 21st century. In the case of HIV, the risk may have remained relatively stable, with an important reduction only in recent years.

Secondly, residual risk seems to be practically negligible in Spain since the introduction of NAT-based screening assays, which would be consistent with our results in the Region of Valencia.

Conclusions

The risk of transmission of HBV, HCV, and HIV through blood transfusion in the Region of Valencia has been very low since the beginning of the study period, especially for HCV. In addition, in the last 15 years there has been a downward trend in the risk of HBV due to the decrease in the rate of incidence in donors and the shortening of the window period of screening tests. It cannot be ensured that such a reduction in the incidence rate in donors and the risk of transmission to recipients

Table IV - Comparison between different studies carried out in Spain for the estimation of the residual risk of transfusion-transmitted viral infections.

Study period	Study region[ref]	Method used (first-time donors inclusion: yes/no)	Estimated residual risk per million donations		
			HBV	*HCV*	*HIV*
1997-1999	Spain (7 centres)[20]	REDS (no)[a]	18.67	10.96	2.49
	Spain (22 centres)[21]	REDS (no)[a]	13.51	6.69	1.95
2000-2002	Spain (7 centres)[20]	REDS (no)[a]	9.78	3.94	2.48
2010-2012	Spain (all centres)[5]	NHLBI-REDS (yes)[b]	7.80	0.19	1.90
2015-2017	Region of Valencia[c]	WHO (yes)[d]	2.37[e]	0.17[e]	0.97[e]

[a]Original version of the incidence-window period model, proposed by Schreiber *et al.* for the Retrovirus Epidemiology Donor Study in 1996[4]. Given that the incidence rate is believed to be higher in first-time donors than in repeat donors, the exclusion of first-time donors may have led to an underestimation of the residual risk in these studies.[b]Alternative model proposed by Busch *et al.* for the National Heart, Lung and Blood Institute Recipient Epidemiology and Donor Evaluation Study in 2005[14]. [c]The Region of Valencia (Valencian Community) includes the provinces of Valencia, Alicante and Castellón. [d]Up-dated version of the incidence-window period model, proposed by the WHO Expert Committee on Biological Standardization in its 2017 guidelines[12]. [e]The results for the 2015-2017 triennium in the Region of Valencia are calculated as the weighted averages of the annual residual risks obtained for the three years included in that triennium. HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

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has occurred in the case of HCV and HIV. However, when the three viruses are considered together, we have observed a decreasing trend in the risk in which the shortening of the window periods seems to play an important role. It is not certain whether the evolution of the incidence rate in donors for the three viruses considered jointly has had an influence on that trend.

Authors' contributions

CL-M and MA designed the study. CL-M also performed the mathematical and statistical analysis of the data, interpreted the results, wrote the manuscript, and elaborated the graphic elements, while MA collected the data from the registries of the CTCV and revised the manuscript. PF designed the statistical analysis and revised the manuscript. MG translated the manuscript from the original version in Spanish and revised it. MIO-S and CA revised the manuscript.

The Authors declare no conflicts of interest.

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