Transmission of human immunodeficiency virus Type-1 by fresh-frozen plasma treated with methylene blue and light

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BACKGROUND: The risk of transfusion-transmitted infection (TTI) has been minimized by introduction of nucleic acid testing (NAT) and pathogen inactivation (PI). This case report describes transmission of human immunodeficiency virus Type 1 (HIV-1) to two recipients despite these measures.

STUDY DESIGN AND METHODS: In March 2009 a possible TTI of HIV-1 was identified in a patient that had received pooled buffy coat platelet concentrate (BC-PLT) in November 2005. The subsequent lookback study found two more patients who had received methylene blue (MB)-treated fresh-frozen plasma (FFP) and red blood cells (RBCs) from the same donation. In November 2005 the donor had tested negative for both HIV antibodies and HIV-1 RNA by 44 minipool (44 MP) NAT. Repository samples of this donation and samples from the recipients were used for viral load (VL) and sequence analysis.

RESULTS: HIV-1 RNA was detectable by individual donation (ID)-NAT in the repository sample from the 2005 window period donation and a VL of 135 copies/mL was measured. HIV-1 infection was confirmed in both recipients of both BC-PLT (65 mL of plasma) and MB-FFP (261 mL of plasma), but not in the patient that had received 4-week-old RBCs (20 mL of plasma). The sequence analysis revealed a close phylogenetic relationship between the virus strains isolated from the donor and recipients, compatible with TTI.

CONCLUSIONS: Approximately 17,600 and 4400 virions in the MB-FFP and BC-PLT were infectious, but 1350 virions in the RBCs were not. ID-NAT would have prevented this transmission, but the combination of MP-NAT and MB-PI did not.

The main cause of transfusion-transmitted infections (TTIs) are incident window period (WP) infections in donors that are not detected by the screening tests.[1] It is estimated that a validated donor selection policy can eliminate 86% to 90% of this risk.[2] The length of the WP for human immunodeficiency virus Type-1 (HIV-1) has decreased from 56 days, with first-generation serologic tests,[1] to approximately 19 and 15 days with the third- and fourth-generation serologic assays, respectively.[3] The introduction of nucleic acid amplification technology (NAT) has further reduced the diagnostic WP. The length of the infectious WP depends on the sensitivity of the NAT method, the minipool (MP) size, and the transfusion plasma volume.[4] In addition, the first-generation polymerase chain reaction (PCR) assays

ABBREVIATIONS: BC-PLT = buffy coat platelet concentrate; ID = individual donation; LOD(s) = limit(s) of detection; MB = methylene blue; MID50 = 50% minimum infectious dose; MP = minipool; MSM = man practicing sex with men; PI = pathogen inactivation; TMA = transcription-mediated amplification; TTI(s) = transfusion-transmitted infection(s); VL(s) = viral load(s); WP = window period.

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are known to have caused detection failures due to mismatches in primers or probes.[5-7]

In July 1999, hepatitis C virus (HCV) NAT was introduced in the Valencian Regional Blood Transfusion Center (Valencia, Spain) in minipools of 44 donations (44 MP-NAT).[8] A few years later, in February 2004, HIV-1 RNA screening was incorporated in this 44 MP-NAT procedure. Later, in July 2006, individual-donation (ID) NAT was introduced in our center, which reduced the infectious WP to 1 to 4 days depending on the estimates of the 50% minimum infectious dose (MID50).[3,4] In addition, since November 1997 pathogen inactivation (PI) has been performed for fresh-frozen plasma (FFP) by adding methylene blue (MB) and subjecting the plasma bags to a visible light source. Validation studies claimed more than 6 logs reduction of infectivity of HIV model virus in tissue culture by this PI procedure.[9] Hence, MP-NAT screened MB-FFP units are believed to be safe with regard to HIV-1 transmission. This report calls this in question.

CASE REPORT

In March 2009, a Valencian hospital identified HIV-1 seroconversion in a 24-year-old patient who was diagnosed with T-cell acute lymphoblastic leukemia. He had received blood components from 77 donors from September 2005 to April 2006. During the traceback process, 63 donations were identified from returning donors who had negative serologic results. Hence, they were excluded from being the source of transmission. The remaining 14 donors were called back for a follow-up sample. Twelve donors returned, of whom 11 tested HIV-1 negative. The remaining donor tested positive for HIV-1 antibodies and RNA by ID-NAT in April 2009. The donor acknowledged being an HIV carrier since May 2006. His last blood donation was on November 23, 2005, from which red blood cells (RBCs), MB-FFP, and buffy coat platelet concentrate (BC-PLT) had been prepared. The buffy-coat had been included in a platelet (PLT) pool transfused to the recipient who subsequently had seroconverted to anti-HIV, as confirmed in a sample taken in 2009. At the time of donation, all mandatory screening assays were negative, including HIV-1 RNA by reverse transcription–PCR (RT-PCR) in 44 MP format. The cellular components from this donation had been leukoreduced. The lookback identified another HIV-1–infected recipient who had received MB-FFP in the context of a liver transplant. The other patient, who had received the RBC unit, was not infected, according to anti-HIV test, 23 months after transfusion. This could not be confirmed in 2009, because she had died earlier by a cause not related to transfusion.

MATERIALS AND METHODS

Routine tests

All blood products transfused were screened using a chemiluminescent immunoassay for anti-HIV-1 and HIV-2 antibodies (Abbott PRISM anti-HIV-1/2 assay, Abbott Laboratories, North Chicago, IL) and HIV-1 RNA in 44 MP-NAT using PCR (COBAS AmpliScreen HIV-1 test, Version 1.5, Roche Molecular System, Branchburg, NJ). For each donor, plasma containing EDTA was centrifuged at 1750 $\times$ *g* and held at 4°C until processed. An application for the Hamilton dispenser designed in the Galicia Blood Transfusion Center was used for pooling. The final volume of each 44-member MP was 1 mL. To minimize dilution effects, each MP was centrifuged at 23,600 $\times$ *g* for 60 minutes at 2 to 8°C. Then 900 μL of supernatant was discarded, and the 100 μL of RNA pellet was extracted.[8] The 95 and 50% lower limit of detection (LOD) of HIV-1 RNA in the multiprep Ampliscreen method was 78 (60-137) and 22 (13-29) IU/mL, respectively, according to analytical sensitivity studies on the WHO 97/656 International Standard reported by the manufacturer in the package insert. Recalled donors in April 2009 were also tested by ID-NAT by transcription-mediated amplification (TMA; Procleix Ultrio Assay on Tigris System, Chiron/Novartis, Emeryville, CA). The 95 and 50% LOD of HIV-1 RNA on the WHO International Standard 97/650 in the Ultrio assay was reported to be 20.3 (18.1-23.1) and 4.5 (3.5-5.9) IU/mL.[10] The serum sample from the HIV-1–infected donor obtained in 2009 tested anti-HIV positive repeat reactive and confirmed by immunoblot assay (INNOLIA HIV I/II Score, INNOGENETICS N.V., Gent, Belgium). The donor sample was also ID-NAT reactive and was identified as HIV-1 RNA positive by TMA discriminatory testing. Viral loads (VLs) were determined using the Roche COBAS Amplicor HIV-1 Monitor Test Version 1.5 (Roche Molecular Systems). Recipient plasma samples were obtained from the hospitals where they were receiving treatment; each one was frozen and sent to the reference center for amplification, sequencing, and phylogenetic analysis.

Phylogenetic analysis

Two plasma samples of the donor were available for sequence analysis, one from the donors' repository sample of 2005 and the other obtained in 2009. Plasma samples from the infected recipients were obtained in 2009. Plasma from 37 newly HIV-1 diagnosed persons obtained in the same city between 2004 and 2009 were used as local control samples. Specimens from the donor, the infected recipients, and the local controls underwent HIV RNA purification followed by RT-PCR and direct sequencing using procedures described elsewhere with minor modifications.[11] Sequences were obtained for a 728-nucleotide-long region from the *pol* gene, comprising partial protease (Codons 10 to 99) and reverse transcriptase (Codons 1 to

TABLE 1. First HIV-1–infected patient results: traceback study

Subject	Diagnosis for transfusion	Time of storage (days)	Day of transfusion	Date of blood drawing	Viral testing results				
					NAT (TMA)	HBsAg	Anti-HCV	Anti-HIV	HIV Imblot
BC-PLT recipient	T-cell acute lymphoblastic leukemia	2	Nov 25, 2005	Oct 14, 2005	ND	Neg	Neg	Neg	NA
		NA	NA	Feb 06, 2009	ND	Neg	Neg	Pos	ND

Imblot = immunoblot; NA = not applicable; ND = not done or not reported; Neg = negative; Pos = positive.

TABLE 2. Subsequent lookback study

Subject	Diagnosis for transfusion	Time of storage (days)	Day of transfusion	Date of blood drawing	Viral testing results				
					NAT (TMA)	HBsAg	Anti-HCV	Anti-HIV	HIV Imblot
Donor	NA	NA	NA	Nov 23, 2005	Pos*	Neg	Neg	Neg	NA
	NA	NA	NA	Apr 28, 2009	Pos	Neg	Neg	Pos	Pos
MB-FFP recipient	In the context of liver transplant	15	Dec 8, 2005	May 24, 2009	ND	Neg	Neg	Pos	ND
RBC recipient	Anemia of chronic disease	27	Dec 20, 2005	Nov 15, 2007	ND	Neg	Neg	Neg	ND

* In 2005 NAT tested negative in 44 MP-NAT using PCR. This same sample, from the repository, tested positive in 2009 by ID-NAT using TMA.
Imblot = immunoblot; NA = not applicable; Neg = negative; ND = not done or not reported; Pos = positive.

152) genes. To establish the relationship between viruses obtained from the donor and the infected recipients, a phylogenetic analysis was carried out as described before.[12]

Pathogen inactivation

Plasma inactivation was performed using MB-Plasma Theraflex (MacoPharma, Tourcoing, France), following the manufacturer's instructions.

RESULTS

On November 23, 2005, a donation that transmitted HIV to two recipients tested negative for anti-HIV and HIV-1 RNA by NAT in 44-member MP and all other required screening tests. In March 2009, HIV seroconversion was detected in the BC-PLT and MB-FFP recipients. The RBC recipient remained healthy and anti-HIV negative 23 months after transfusion. In April 2009, a repository sample collected in 2005 from the donor tested HIV-1 RNA positive, with a VL of 135 copies/mL. The infectious blood was donated by a man practicing sex with men (MSM) aged 42 who knew of his HIV infection in May 2006, although he did not report this information to the blood center. A more recent blood sample from this donor, collected in April 2009, tested positive for anti-HIV by chemiluminescent immunoassay, immunoblot assay, and HIV-1 RNA by TMA (Tables 1 and 2).

Viral RNA was isolated from two donors' samples (the 2005 repository and 2009 blood drawing) and from each recipient's samples. HIV-1 RNA could not be isolated by the RT-PCR procedure in the reference laboratory from the 2005 repository sample probably due to its low VL and therefore could not be sequenced. The HIV RNA from the second donor sample and the samples from the MB-FFP recipient and the BC-PLT recipient, all collected in 2009, was successfully amplified and sequenced. Using different amplification strategies, two closely related nucleotide sequences were obtained from the sample corresponding to the MB-FFP's recipient. The four sequences corresponded to HIV-1 subtype B and they grouped in a well-supported monophyletic clade when compared with local reference sequences for this subtype (Fig. 1). The mean nucleotide identity for these sequences (excluding one redundant sequence from the MB-FFP's recipient) was 99.3% (range, 99.0%-99.7%) whereas the mean nucleotide identity to unrelated control sequences was 94.2% (range, 91.9%-96.8%). This type of phylogenetic grouping and high genetic identity are indicative of samples sharing a relatively recent common origin, compatible with a TTI from blood components from a single donor.

DISCUSSION

To our knowledge this is the first case of HIV-1 transmission related to a blood component undergoing a PI process. It is difficult to imagine how MB-treated plasma that

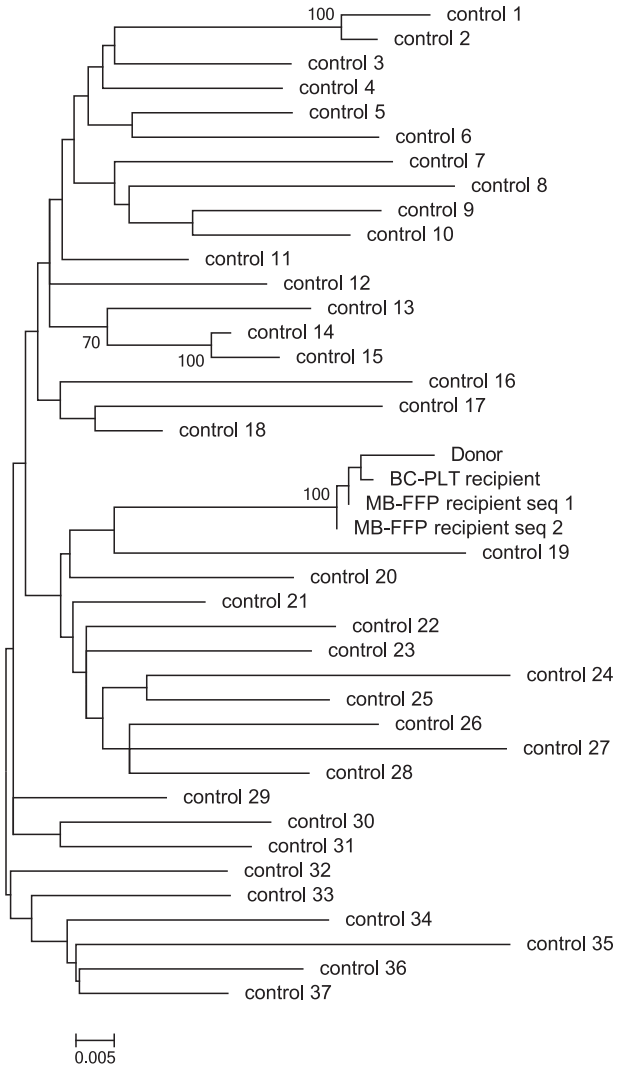

Fig. 1. Maximum likelihood phylogenetic tree for pol sequences (728 nucleotides) from blood donor, BC-PLT recipient, and MB-FFP recipient (two sequences) along with 37 local control sequences. The phylogenetic tree was built with RAxML with bootstrap support for branches after 1000 replicates. Only bootstrap values higher than 70% are shown. The scale bar indicates number of substitutions per nucleotide.

tested nonreactive in 44 MP-NAT (Ampliscreen) method used in 2005 might have transmitted HIV-1. One explanation is that the PI failure could be due to manufacturing or process errors, such as: 1) the Plasmaflex PLAS4 filter was defective, allowing the passage of residual HIV-infected white blood cells into the illumination bag. The inactivation of intracellular virus by MB is known to be inefficient;[9] 2) there was no MB pill in the bag system; 3) the MB tablet was not completely dissolved or homogeneously mixed over all compartments of the bag; 4) something had gone wrong with the illumination of the plasma bag (the inactivation is known to be limited without visible light); 5) standard operating procedures related to good manufacturing practices in the blood center were violated. Another explanation may be that viruses associated with human cell membranes or lipids in plasma are partially protected from inactivation by MB. The manufacturer found more than 6-log reduction of HIV-1 by MB treatment in tissue culture experiments but these may not be representative for the infectivity of HIV in the WP of plasma transfused to humans. Finally, it is theoretically possible that the viremia level (135 copies/mL) of the HIV-1 strain detected in the infectious donation was underquantified by the Amplicor Monitor PCR assay as a consequence of oligonucleotide mismatches. This, however, seems unlikely since the RBC unit did not transmit HIV infection and other differential transmission cases in which RBCs were not infectious were associated with even lower VL.[13]

The observed transmission of HIV-1 by MB-FFP and BC-PLT but not by the RBC unit is not surprising because:

1. RBCs contain approximately 20 mL plasma;[14] therefore, fewer virions than the BC-PLT and MB-FFP unit in which 65 and 261 mL of plasma was present.
2. The MB-FFP and BC-PLT recipients were receiving immunosuppressive therapy, whereas the RBC recipient was not.
3. The storage period of the RBCs before transfusion was 27 days (in contrast to only 2 days of the infectious BC-PLT) during which the infectivity of the viral particles has likely been more than 10-fold reduced as has been observed in tissue culture experiments.[15] Weusten and coauthors[4] constructed a probabilistic infectivity risk model for NAT-screened units donated within the WP. A major driver of the residual risk in this model is the MID50 that may lie between 1 and 10 virions in FFP units or BC-PLT but is likely much higher (between 100 and 1000 virions) in longer-stored RBC units as estimated from MP-NAT breakthrough transmission cases.[14]

Since plasma volume was 261 mL, the amount of virions in MB-FFP was calculated to be 17,618 particles (since one virion contains two RNA copies); likewise, 65 mL of BC-PLT contained 4388 virions and RBC carried 1350 virions (20 mL of plasma). One can imagine that the amount of infectious virus in the BC-PLT stored for 2 days was logarithmically higher than in the RBC unit, in which the infectivity of the virus had likely reduced during the long storage period of 4 weeks. For example, with the formulas of Weusten and colleagues,[4] it can be estimated that the probability of infectivity of 1350 virions in the RBC unit would be reduced from 100% to 25% if the MID50 had reduced 1000-fold (from 1-10 to 1000-10,000 copies). Finally, it must be emphasized that the RBC recipient was immunocompetent, while the others were under immunosuppressive therapy. If the MB-FFP unit (like the RBC

unit) had also been borderline infectious the immune status of the liver transplant patient could very well have contributed to the outcome of the infection. It is important to note that these transmission cases would not have occurred if ID-NAT had been in place in 2005. The 95 and 50% LODs of the Ultrio assay are estimated at 13.8 and 2.4 copies/mL, whereas for the multiprep Ampliscreen assay these were 135 and 38 copies/mL, respectively (calculated from the LODs reported in IU in methods with a conversion factor of 0.58).[10] With a MID50 of one virion and a doubling time of 0.85 days it can be calculated with the formulas given by Weusten and coworkers[4] that the lengths of the infectious WPs for RBC, BC-PLT, and FFP transfusion were 4.2, 5.7, and 7.4 days, respectively, and for the previously used 44 MP-NAT system, 12.2, 13.6, and 15.3 days. Hence the introduction of ID-NAT reduced the infectious WP by 8 days.[16]

Nevertheless, in Spain, HIV RNA blood donation screening is not mandatory nowadays. However, there is no doubt of the added value of NAT or additional sensitivity of ID-NAT compared to MP-NAT.[5,17] It should be recognized that all blood safety programs have limitations and that absolute safety, in terms of absence of infectious risk, cannot be guaranteed.[18] Careful donor selection remains critical, even in the era of application of both NAT and PI.[19] This is the first step to avoid individuals at risk of being in an early infection stage from donating. In our case, the donor did not admit MSM practices and risk for HIV infection when he donated in 2005. In Spain, 74% of HIV-positive blood donations are given by noncompliant MSM donors.[20]

In conclusion, the following lessons can be learned from these HIV-1 transmission cases: 1) PI methods may not always be sufficiently efficacious (as was recently also observed by transmission of hepatitis E virus by FFP treated with the Intercept method).[21] 2) Zero risk is not attainable even if a combination of MP-NAT and PI is used, but the residual risk may become negligible when ID-NAT is combined with PI. 3) Efficient quality control checks on proper performance of NAT and PI may need to be developed to guarantee consistent efficacy of these interventions. 4) Further improvements in the sensitivity of NAT screening tests and the effectiveness of PI are desirable, considering that, according to conservative estimates, one infectious virion in a blood component is enough to cause infection in a recipient. 5) Continuing attention should be given to the selection of safe blood donors[22] and finally 6) with limited resources currently available the cost-effectiveness of the applied blood screening methods and PI needs to be seriously monitored.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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