Human immunodeficiency virus transfusion transmission despite nucleic acid testing

The residual risk of human immunodeficiency virus (HIV) transmission by blood transfusion in our blood center, Fundação Pró-Sangue (FPS), São Paulo, Brazil, was estimated to be approximately 1 per 100,000 donations by applying fourth-generation HIV antigen-antibody testing (Genscreen Ultra HIV Ag-Ab, Bio-Rad, Marnes La Coquette, France) and projected to be further reduced to 0.68 per 100,000 donations[1] after adoption of nucleic acid testing (NAT, Bio-Manguinhos, Fiocruz, Brazil) on minipools of six donations in May 2011.

In November 2012, a leukemia patient was submitted to serologic and nucleic acid testing (NAT). Anti-HIV reactivity (CMIA HIV Ag/Ab Combo Architect System, Abbott, Wiesbaden, Germany) was observed with a concomitant nonreactive Western blot (WB HIV Blot 2.2 Western blot assay, MP Diagnostics, Singapore) and HIV RNA was detected by NAT. This patient was previously tested for anti-HIV in July 2012 with nonreactive results. Between August and November 2012, he had received 47 transfusions of red blood cells (RBCs) and platelets (PLTs), all derived from FPS donors. Repository plasma samples from the corresponding donations, which are required by Brazilian guidelines to be retained for 6 months after donation, were retested individually by NAT (Bio-Manguinhos) with nonreactive results. All implicated donors were invited to return to our blood center and provide a follow-up sample. Among the 37 donors that accepted this invitation, one seroconversion to anti-HIV was verified in a sample drawn on December 17, 2012, with a concomitant viral load of 39,658 copies/mL (HIV real-time viral load assay, Abbott). This donor has a history of two previous donations at our service before the implicated (index) donation that occurred on September 28, 2012. The repository sample from this donation also showed undetectable viremia by the same viral load test (95% limit of detection [LOD], 40 copies/mL). The HIV-infected recipient received the RBCs from this donation after 11 days of storage at 4°C, while the PLT component was transfused into another patient who died 2 days later, precluding further investigation. The plasma component from this donation was not transfused and subsequently retrieved, allowing for further study, as detailed below.

The HIV *pol* gene (protease and RT) for the donor and recipient follow-up samples were polymerase chain reaction amplified, sequenced, and submitted to phylogenetic analysis together with *pol* sequences from several contemporary HIV isolates from São Paulo city blood donors and from distinct HIV Group M subtypes. After nucleotide substitution model estimation, a maximum likelihood tree was reconstructed with support values estimated from 1000 bootstrap replicates. The donor and recipient *pol* sequences showed 100% sequence identity, and according to the phylogenetic tree, belong to B subtype clade, the commonest among Brazilian patients (Fig. 1).

The plasma bag from the index donation was thawed and used to prepare three coded panels consisting of 20 replicates of the undiluted index plasma, five replicates of this plasma diluted 6× with negative plasma, mimicking minipools of 6, and one sample from the diluent plasma. These panels were submitted to testing using three different NAT platforms: cobas TaqScreen MPX (95% LOD, 49 IU/mL; Roche, Branchburg, NJ), Procleix Ultrio (95% LOD, 20.72 copies/mL; Gen-Probe, San Diego), and NAT hepatitis C virus and HIV (95% LOD, 30 IU/mL or 13-39.9 copies/mL; Bio-Manguinhos); all three HIV RNA 95% LOD values were obtained from the respective package inserts. Reactivity was verified in a fraction of the undiluted aliquots by the Roche (3/20) and Novartis (13/20) assays. Interpolation of the detection rates verified into the probit analysis from the package inserts allows the estimation of the viral load to be in between 7.5 and 3.7 IU/mL, respectively, for Ultrio and TaqScreen (conversion factor, 0.6 copies/IU) confirming very low viremia in the index donation, well below the 40 copies/mL detection limit of viral load and minipool NAT assays (Table 1). This result suggests that the donor was in the eclipse or very early ramp-up phase at the time of the implicated donation, probably a few days after the infection.

The implicated donor denied all risk factors in the interview performed at the time of the index donation and continued to deny high-risk behavior in three subsequent visits to the blood bank, after his HIV status was disclosed to him. He also missed the opportunity of self-deferral given by the confidential unit exclusion process preceding the implicated donation. It has been estimated that approximately 7% of our blood donors are test seekers[2] and that the prevalence of test seekers is actually higher among first-time (FT) community donors than FT replacement donors. However, this individual, a 22-year-old male, was a repeat community donor, with a history of three previous donations at our center.

Three relatives of the HIV-infected patient, who were the best candidates for marrow donation based on HLA compatibility typing, were tested for the CCR5Δ32 deletion[3] in consideration of possible transplant to cure both his leukemia and his HIV infection, but unfortunately all were wild-type homozygous, not bearing the mutation associated with resistance to HIV infection. Highly active antiretroviral therapy was introduced and, after 60 days, the viral load was reduced to 69 copies/mL, when marrow transplantation was carried out. Up to March 28, 2013, the patient was alive and did not show any clinical sign of progression to AIDS.

This report illustrates the limitation of laboratory testing for prevention of transmission of infectious agents,

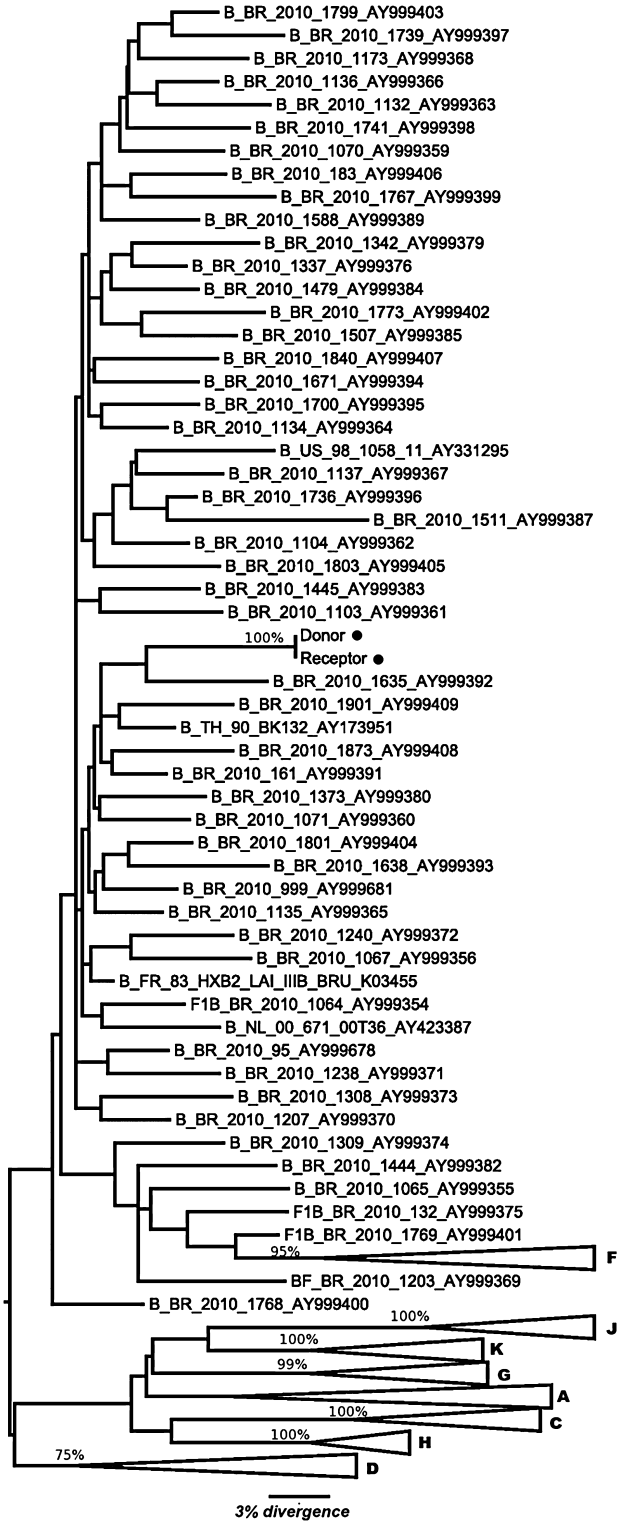

Fig. 1. HIV-1 protease and reverse transcriptase phylogeny. Maximum likelihood tree for 85 HIV-1 protease and reverse transcriptase sequences inferred with TIM1+I+G model for Group M subtypes. Donor and recipient samples sequenced in this work (●) clustered with 100% bootstrap within B subtype clade, supporting transmission linkage. Clades comprising non-B subtype viruses were collapsed for better visualization.

TABLE 1. Results of NAT of aliquots of plasma derived from the index donation (undiluted, n = 20; diluted 6×, n = 5) and the diluent plasma (n = 1) on three NAT platforms available in Brazil

	Number of replicates	cobas MPX N+	Procleix N+	Bio-Manguinhos N+
Neat	20	3	13	0
Diluted 1:6	5	0	1	0
Negative plasma	1	0	0	0

specifically HIV, by transfusion. In the literature there are several reports of HIV transfusion transmission by NAT-negative window period donations (compiled in Kleinman et al.[4]) although to our knowledge this case had the lowest documented viral load. In this situation, even individual NAT by the most sensitive methods available would have a reasonable chance of failing to detect HIV RNA in the donation sample. Consequently, it is important to continue to invest in understanding motivations for blood donation and raising consciousness of the risk posed by donations given shortly after risky behaviors. Efforts in this direction may prove as effective in protecting the blood supply as ultrasensitive NAT.

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CONFLICT OF INTEREST

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

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