

Case Report

Detection of HIV-1 infections in blood donors during the pre-seroconversion window period in São Paulo, Brazil

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Abstract

By decreasing the pre-seroconversion window period, nucleic acid testing (NAT) has improved the safety of blood products and reduced the risk of transfusion-transmitted infections. Between 2011 and 2017, NAT determinations for approximately 898,202 donations were performed at *Fundação Pró-Sangue/Hemocentro de São Paulo* (FPS-HSP). Three seronegative HIV-viremic donations were detected. The NAT yield rate per million donations was 3.34 for HIV, and the acute HIV-1 infections detected are described, followed by a brief review of the situation in Brazil.

Keywords: HIV. NAT-HIV. Window period.

INTRODUCTION

According to current legislation in Brazil, high-sensitivity antigen/antibody-based assays for HBV, HCV, HIV-1/2, Chagas disease, syphilis, and HTLV-1/2, as well as nucleic acid testing (NAT) for HBV, HCV, and HIV, are mandatory for blood donation screening¹. At *Fundação Pró-Sangue/Hemocentro de São Paulo* (FPS-HSP), one of the largest blood banks in Latin America, 120,000 units of blood are collected annually. However, due to a positive reaction for one or more infection markers, approximately 2% of these donations are discarded; of these, the HIV discard rate was 0.11% in 2016. Overall, the introduction of nucleic acid testing (NAT) has improved the safety of blood products and reduced the risk of transfusion-transmitted infections by decreasing the pre-seroconversion window period². Our study aims to determine the HIV NAT-yield and to describe the acute HIV-1 infections detected between 2011 and 2017.

CASE REPORT

Between 2011 and 2017, NAT determinations for approximately 898,202 donations were performed at FPS-HSP,

and 3 seronegative HIV-viremic donations were detected. The NAT yield rate per million donations was 3.34 for HIV and the cases are described below.

HIV-1/2 antibody and antigen screening was performed using the chemiluminescent immunoassay (CMIA) kit HIV Ag/Ab Combo Architect System (Abbott, Germany) and/or the enzyme immunoassay (EIA) kit Genscreen Ultra HIV Ag/Ab (Biorad, France). Additionally, NAT assays were performed in minipools (MP) of six samples using the Kit NAT HIV/HCV/HBV (Bio-Manguinhos, Brazil; sensitivity: 95%; LOD: 30 IU/mL or 13-39.9 copies/mL for HIV), and reactive minipools were resolved by individual donation (ID)-NAT screening. CMIA HIV-reactive samples were confirmed by HIV western blotting (MP Diagnostics, Singapore).

In the samples with sufficient serum, a quantitative HIV RNA real-time reverse transcription-polymerase chain reaction (RT-PCR), targeting a portion of the HIV genome LTR region (sensitivity: 100 copies/mL), was performed using 10 µL of extracted RNA (Magna Pure Compact, Roche, Germany), 300 nM of each primer, 100 nM probe³, and 1X TaqMan Fast Virus One Step Master Mix (Thermo Fisher Scientific, USA). The HIV subtypes and mutation analyses were determined by direct sequencing of the HIV reverse transcriptase (RT) and protease regions, as previously described⁴, and use of the online Stanford HIV-SEQ program (<http://hivdb.stanford.edu>).

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Donor 1

A sporadic male donor, 36 years old, resident of São Paulo (Carapicuíba), donated whole blood on April 16, 2013, his eleventh donation in FPS-HSP. Sporadic donors are those whom repeat donation after more than 12 months since the previous donation. The last negative donation for all screening tests was on August 1, 2011. He was married and had finished high school. No common risk factor for HIV was identified by the donor questionnaire. The donation was EIA and CMIA-HIV Ag/Ab-negative, but HIV RNA-positive in ID-NAT (ct 32). The unit was discarded, and the donor was recalled to the blood bank. The laboratory result is presented in **Table 1**. Seventeen days after the index donation, on May 3, 2013, the new sample was HIV Ag/Ab-reactive, HIV RNA-positive in ID-NAT (ct 17), and the HIV western blot was indeterminate. Twenty-eight days after index donation, on May 14, 2013, all markers were positive, including HIV western blot. A further interview was performed as part of the counseling procedure. On this occasion, the patient answered questions about the potential risk factors involved in exposure to HIV. The donor reported that he had been intimate with four partners (women) in the preceding 12 months and had never used condoms. He was referred to the infectious disease department for management.

Donor 2

A 36-year-old, sporadic male donor, living in São Paulo, donated whole blood on June 11, 2016 (sixth donation). The last negative donation for all screening tests was on July 27, 2013. He was single and graduated in pharmacy faculty. No common risk factors for HIV were identified by the donor questionnaire. The donation was HIV NAT-positive and non-reactive for EIA-HIV. ID-NAT confirmed the presence of HIV-1 RNA (ct 32). Seventeen days after the index donation, on June 28, 2016, the donor tested positive for HIV-1 RNA using ID-NAT screening (ct 24). The donation was EIA-HIV Ag/Ab-reactive and the HIV-1 western blot was positive. He reported no risk on the donor return questionnaire. He was referred for treatment.

Donor 3

On March 27, 2017, a 25-year-old, first-time male donor living in São Paulo donated whole blood at FPS-HSP. No common risk factor for HIV was identified through clinical screening. NAT-HIV in pooled samples was positive and CMIA HIV Ag/Ab screening was negative. ID-NAT confirmed the presence of RNA-HIV (ct 28) and viral load was 3,500 copies/mL. On June 2, 2017, 36 days after the index donation, ID-NAT was positive (ct 32) and viral load was 200 copies/mL. HIV Ag/Ab was detected in the serum, and the HIV-1 western blot complement test was positive. Sequence analysis indicated HIV-1 subtype B, and no resistance to reverse transcriptase and protease inhibitors was found. The donor, who was single, reported that he had been intimate with two partners in the last 12 months, had irregularly used condoms, and had received a piercing more than a year previously. He was referred to the infectious disease department for treatment.

DISCUSSION

Between 2011 and 2017, NAT determinations were performed in the FPS-HSP for approximately 898,202 donations. Three seronegative HIV-viremic donations were detected. The NAT yield rate per million donations was 3.34 for HIV, which is slightly lower than the rates observed in Brazil, varying from 4.38 to 4.62 per 1,000,000 donations⁵. In the USA and Europe, these ratios are less than 0.5 and 0.66 per 1,000,000 donations, respectively, and the rates in Africa are highest (close to 36.78 per 1,000,000 donations)⁶.

The main risk for transfusion safety is a donation during the window period, and both prevalence and incidence are important in estimating the risk of transfusion-transmitted infections. In Brazil, although the prevalence of HIV among donors has been declining over the years, it remains high, at 92.2/100,000 donations⁷, when compared to other countries, such as the USA, which has a rate of 8.3/100,000 donations⁸. The incidence of new HIV infections in blood donors has been steady for decades, with rates ranging from 22.6 to 25.9/100,000^{4,7}; nonetheless, it is still almost higher than that in the USA, with a rate of 3.5/100,000 persons/year⁸. Thus, the estimated residual risk in Brazil, from 4.2 to 11.3/1,000,000 donations⁷, is higher than that in the USA, estimated to be at 1/1,100,000 donations⁸. Overall, transfusion safety depends greatly on measures or strategies for reducing the incidence of new infections.

Transmission of HIV through transfusion of contaminated blood components was documented despite the implementation of NAT screening. A patient with acute myeloid leukemia received a unit of red blood cells that contained HIV at a concentration too low to be detected in MP-NAT. Perhaps the use of an individual NAT, or more sensitive methodologies, might have prevented this transmission⁹. Indirect strategies, such as methods for raising awareness of the immunological window period among donors, methods for understanding the motives of the donor, and the identification of test takers, may also aid in increasing transfusion safety¹⁰.

In the present study, the donor 3 sample was classified as HIV-1 subtype B, which is the most prevalent subtype in our population. The distribution of HIV subtypes in Brazil is reported to be 75–81% for subtype B, 7–15% for subtype F, 3.8–5% for subtype C, and 4–7.6% for recombinant subtypes among blood donors^{4, 11}. In the USA and Europe, the most prevalent, subtype B, ranges from 81 to 95%¹². No antiretroviral drug resistance variants were detected in the studied case. Other studies have verified an increase in primary drug resistance over the years, with a frequency of 6% between 1998 and 2002 and 19% between 2007 and 2011 among blood donors^{4,11}. In the USA and some regions of Europe, resistance rates range from 11 to 14%¹².

In conclusion, the NAT yield rate per million donations was 3.34 for HIV, and detection of these cases during the pre-seroconversion window period proves that NAT has improved the testing of transfused blood. Blood donors are considered a sentinel population and studies of prevalence, incidence, and molecular characterization of the circulating strains in this group are important to assist in the measures of prevention

TABLE 1: HIV serologic and molecular test results of seronegative HIV-viremic donations.

CASE	DATE	CMIA HIV (cutoff value of 1.0)	EIA HIV (cutoff value of 0,271)	Minipool-NAT-HIV	Individual NAT-HIV	RT-PCR HIV (copies / mL)	WBlot HIV (Positive bands)
donor 1	April 16, 2013	non-reactive	non-reactive	positive ct 34	positive ct 32	ND	ND
	May 03, 2013	reactive 571,990	reactive 3,000	ND	positive ct 17	ND	undetermined gp120
	May 14, 2013	reactive 7,850	reactive 2,777	ND	positive ct 23	ND	positive p17, p24, gp120, and gp160
donor 2	Jun 11, 2016	ND	non-reactive	positive ct 34	positive ct 32	ND	non-reactive
	Jun 28, 2016	ND	reactive 3,000	ND	positive ct 24	ND	positive p24, gp160
donor 3	March 27, 2017	non-reactive	ND	positive ct 30	positive ct 28	positive 3,500	ND
	June 2, 2017	reactive 107.73	ND	ND	positive ct 32	positive 200	positive p17, p24, p39, gp41, p66, gp120, and gp160

CMIA: chemiluminescent immunoassay, **EIA:** enzyme immunoassay, **Ct:** cycle threshold, **ND:** not done.

and dissemination of infections. Continuous research and surveillance about HIV prevalence among blood donors are needed to maintain and evenly increase blood safety in Brazil.

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Conflict of Interest

The authors declare that they have no competing interests.

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