HIV-1 NAT minipool during the pre-seroconversion window period: detection of a repeat blood donor

P. Palla,1 M. L. Vatteroni,2 L. Vacri,1 F. Maggi2 & U. Baicchi1

1Blood Center and NAT Section, Azienda Ospedaliera-Universitaria, Pisa, Italy
2Virology Section, Department of Experimental Pathology, University of Pisa, Pisa, Italy

Background The introduction of nucleic acid amplification technology (NAT) for screening pooled or individual donations remarkably improved the safety of blood products. The size of mini-pooled NAT is considered critical for identification of HIV-1 infected donors during pre-seroconversion phase of infection. We describe a case of HIV-1 infection in a serologically negative repeat blood donor identified by 16 minipool (MP) NAT.

Materials and Methods The donation was tested by Roche Cobas AmpliScreen HIV-1 Test with manual extraction (MultiPrep Specimen Processing Procedure). The sensitivity of different MP sizes was observed. Serial samples of infected donor were examined with different third and fourth generation HIV-1 serological assays.

Results In the index donation viral load was 515 copies/ml corresponding to about 50 IU when diluted in 16 MP. Abbott third and fourth generation EIA tests detected the seroconversion four days later the index donation.

Conclusion The report emphasizes the relevance of a very small size of MP to really reduce the window serologic phase of current EIA test by HIV-1 NAT test.

Key words: HIV EIA sensitivity, HIV-1 NAT, individual NAT, minipool NAT, pre-seroconversion period.

Introduction

The implementation of nucleic acid amplification technology (NAT) to screen blood donors has remarkably reduced the risk of transfusion-transmitted HIV-1 and hepatitis C virus (HCV) infections by reducing the diagnostic window period in recent infections [1]. In Italy, blood screening for HCV RNA was made mandatory in July 2002, whereas screening for HIV RNA and HBV (hepatitis B virus) DNA was decided at regional government level, and introduced in Tuscany in July 2002 for HIV and January 2005 for HBV.

Prior to implementation of NAT testing in Italy, the residual risk for transfusion-transmitted infections by antibody-screened donations was estimated as 1 in 435 000 and 1 in 127 000 for HIV-1 and HCV, respectively [2]. After NAT implementation, the estimated risk for HCV and HIV blood-transmitted infections was reduced to 0·5 and 1·1 per million units, respectively [3]. This residual post-transfusional risk was mainly sustained by minipool NAT (MP NAT) rather than by single-unit (individual NAT – ID NAT) screened donations [4].

Different reports have described HIV-1 transmission following transfusions with MP-NAT screened blood donations from donors in the pre-seroconversion phase of infection. It has been suggested that the dilution factor associated to minipool size (16–24 samples) was implicated in the failure of NAT assays to detect low levels of HIV RNA [5].

In this case report, we describe an acute HIV-1 infection in a repeat blood donor, detected by HIV-1 16 MP NAT 4 days before the seroconversion.

Case report

The donor, a 43-year-old male, donated blood 49 times, nine times in 2004, the last of which was on 15 December 2005. At the index donation, on 8 January 2005, no common risk
factor for HIV was identified by the donor questionnaire or the medical interview. The donor was in good health. Physical examination did not reveal any abnormality. The donation was HIV-1 antibody-negative. The donation was included in a 16 minipool that tested positive for HIV-1 RNA. It was confirmed in a single donation NAT test. The stored sample from the previous donation on 15 December 2004 was tested in a single donation NAT with negative results.

A new blood sample, taken 4 days later, confirmed the HIV-1 RNA positivity. Anti-HIV-1 antibodies were detected in the serum. A further interview was undertaken as part of the counselling procedure. On questioning the donor, who was married with two sons, revealed an incident of unprotected heterosexual sex on 24 December 2004. He subsequently developed an influenza-like syndrome. He was referred to the Infectious Disease Department for management. Serological and NAT tests performed on the wife were negative 1 month after the husband’s donation.

Materials and methods

HIV-1 serological test

HIV-1 antibodies were evaluated by third-generation assays AxSYM HIV 1/2 gO (Abbott Diagnostics, Abbott Park, IL) and Enzygnost Anti-HIV1/2 Plus (Dade Behring, Marburg, Germany). Three fourth-generation assays were also utilized, comprising AxSYM HIV Ag/Ab Combo (Abbott Diagnostics), COBAS CORE HIV Combi (Roche, Branchburg, NJ) and Enzygnost HIV Integral (Dade Behring). Western blot was performed by HIV Blot version 2-2 (Genelabs Diagnostic, Singapore). HIV-1 p24 antigen was detected by HIV Ag EIA (Roche) without an immune-complex dissociation step.

Assays for HIV-1 RNA

NAT tests were performed on MP by Roche’s COBAS AmpliScreen HIV-1 Test version 1-5 (Branchburg, NJ) with MultiPrep Specimen Processing Procedure (manual extraction) according to manufacturer’s instruction. This manual procedure includes a preliminary centrifugation step to concentrate viral particles followed by isopropanol precipitation.

The index donation was quantified by Roche’s COBAS AmpliPrep/AmpliCor HIV-1 Monitor test version 1-5. In this system, extraction was performed by DNA probe-capture technique.

Subtype assignment was carried out by comparison to representative HIV-1 group M, N, O sequences stored either in the Stanford and in the Los Alamos databases (HIV-BLAST).

External run controls

In order to confirm the proficiency and correct performance of all the steps of the assay in our centre, working reagents for HIV and HCV supplied by Istituto Superiore di Sanità (ISS) and calibrated against the respective WHO International Standards were used in addition to the positive and negative controls provided with the kits. HIV-1 RNA ISS0103 (genotype B) [6] diluted at 100 IU/ml and HCV RNA ISS0102 (genotype 1) [7] diluted at 50 IU/ml were used as external run controls (RC) to validate routine assay run. Starting from January 2005, HBV DNA ISS0501 (genotype A, serotype ayw) reagent diluted at 100 UI/ml was also introduced in every run and considered as internal criteria of run validation.

Results

The results of HIV testing, both serological and NAT, for the donor identified as HIV RNA-positive are shown in Table 1.

The viral load on the index donation was 515 copies/ml, corresponding to 815 IU/ml (Roche conversion factor 0-64). HIV subtyping indicated the isolate in clade F1.

Four days after index donation, the donor tested HIV-positive by Abbott third-generation test (HIV 1/2 g0) and by fourth-generation Roche and Abbott immunoassays. Fourth-generation Enzygnost HIV Integral gave positive results only on 28 January, and Enzygnost HIV1/2 Plus tested negative until 7 February blood collection. HIV1/2 Western blot was p24-reactive [++++] on 28 January. HIV-1 p24 antigen was always negative.

The results of replicates testing of the index donation in pool sizes are shown in Table 2.

Discussion

Between June 2002 and December 2004, our Transfusion Service performed 197 912 NAT determinations on samples from about 88 000 donors/year living in west Tuscany. The donations were screened for HIV-1 and HCV by 20 MP NAT with the Roche system. A manual extraction procedure was utilized. Since January 2005, blood donations were screened also for HBV DNA. Based on previous evaluations of HBV analytical sensitivity (data not shown), the minipool size was reduced to 16 donations in order to enable detection of the low viraemia levels associated with occult HBV infections (< 500 IU/ml) [8].

Our case describes an acute HIV-1 infection of a repeat blood donor with a secondary-school diploma and high frequency of donation (15 in the last 2 years). When informed about his health status, he declared one heterosexual risk on 24 December 2004. The interval between donations in the previous 2 years was over 45 days, whereas the last break was of 23 days. He admitted that the index donation in January 2005 was performed to exclude infectious risks. This suggests that recently infected persons may be more likely to donate in early stage of infection.
The finding of an HIV-1 NAT-positive, antibody-negative, repeat donor shortly following a reduction in pool size led us to investigate the possibility that pool size might influence test sensitivity. In the runs carried out with 1:20 dilution (20 MP) and 1:16 dilution (16 MP) 3 out of 10 and 1 out of 10 replicates resulted respectively negative, confirming the need for smaller pool sizes to obtain a 100% of positive results. This was in accordance with the analytical sensitivity claimed by manufacturer at 50 IU, (positivity frequence of 85-8%), because the index donation viraemia was 805 IU/ml and corresponded to 40 IU/ml and 50 IU/ml when diluted in 20 and 16 MP, respectively.

Currently used EIA tests generally comprise p24 antigen and IgM/IgG detection (Ag/Ab combined fourth-generation assays) and reduce the diagnostic window from 3 to about 2 weeks in comparison to third-generation antibody tests [9]. In our setting, third-generation HIV 1/2 g0 EIA had the same sensitivity as two fourth-generation assays in the sample collected 4 days after the index donation. Though daily samples of donor were not available, the weak positive results (D.O. < two times the cut-off) indicate a serological window of only 18 days in both EIA tests.

Moreover, p24 EIA antigen test was negative, suggesting the presence of immunocomplex to interfere with the capture of antigenic protein by EIA tests. We suppose that negative results obtained with fourth-generation Enzygnost kits HIV Integral (both Plus and Integral) were not so due to a lower analytical sensitivity in detecting HIV antigen (100–250 pg/ml), if compared to others (< 20 pg/ml), as to a lower sensitivity in the detection of anti-HIV IgM.

According to the division of early period of HIV-1 infection in four major stages, as described by Busch and co-workers, our case is located in stage III a (between MP NAT detectability and serological detection) [10].

In conclusion our HIV-1 NAT 16-donation minipool system is able to detect below 1000 IU/ml HIV-1 RNA on individual donations, revealing infection in a donor only 4 days before serological evidence of infection by the current available HIV antibody tests.

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References


