

Abbreviations: ART = antiretroviral therapy, AZT = zidovudine, DNA = deoxyribonucleic acid, HIV = human immunodeficiency virus, NVP = nevirapine, PCR = polymerase chain reaction, PMTCT = prevention of mother-to-child transmission, RNA = ribonucleic acid.

Keywords: adolescent, Botswana, HAART, HIV, viral rebound

1. Introduction

There is currently no cure or an effective vaccine against human immunodeficiency virus (HIV). Antiretroviral therapy (ART) can result in full suppression of HIV replication but does not eliminate the virus due to existence of proviral reservoir. The proviral reservoir, comprised of a pool of latently infected cells, is a major obstacle to achieving a cure. During the early phase of HIV infection, proviral deoxyribonucleic acid (DNA) is harbored in multiple cells such as long-lived CD4+ T cells in the periphery and sanctuaries, establishing latency.^[1–4] Viral replication and rebound can occur following reactivation of these latently infected cells, particularly in the absence of ART.^[5–9] Hence, HIV-infected individuals should remain on treatment for life. With the current “Test and Treat all” recommendations^[10] caution must be exercised in clinical management of cases with long-term ART and viral suppression.

Currently, early ART initiation among infants perinatally infected with HIV is common, resulting in many HIV-positive children and adolescents receiving long-term ART. Botswana and other countries demonstrated in clinical trials that ART can prevent most (>98%) mother-to-child HIV transmissions,^[11–17] and that early initiation of ART in the breakthrough cases is feasible.^[18–20] Early infant diagnosis and initiation of ART has also been shown to be of critical importance in reducing infant morbidity and mortality.^[21,22]

Recent studies on proviral HIV reservoirs suggest that early initiation of ART reduces the size of the reservoir and prolongs time to virus rebound, while ART interruption leads to virus rebound.^[4,23] What remains unknown is the impact of combining early ART initiation and long-term ART on the size of the proviral reservoir and detection of HIV antibodies and viral nucleic acid. In virologically suppressed HIV-infected individuals, rapid tests for HIV-1 antibody and/or nucleic acid tests could produce false-negative results, which may mislead health care providers and patients. Here we report a case from a routine clinical practice in Botswana in which a perinatally HIV-infected adolescent tested negative for HIV antibodies after 14 years of ART, stopped their ART and experienced viral rebound 9 months later.

2. Case history

We present a case study of an adolescent male (“the participant”) with confirmed perinatal HIV infection within 1 month of life. Accompanying records (Table 1) indicated that the participant’s mother was enrolled in 2002 in a prevention of mother-to-child transmission (PMTCT) clinical trial known as “The Mashi study” (ClinicalTrials.gov Identifier: NCT00197587; 2002–2005) with no prior ART history and was given standard prophylaxis of nevirapine (NVP) and zidovudine (AZT) from 34 weeks’ gestation through delivery.^[18,19] The mother was randomized to the breastfeeding arm of the study. The mother had a viral load of 2,090 HIV-1 ribonucleic acid (RNA) copies/mL at the time of delivery.

HIV DNA polymerase chain reaction (PCR) for the baby at birth (negative), 1 month (positive), and 2 months (positive) after birth was performed using the Roche Amplicor, version 1.5 (Roche Molecular Systems, Inc, Branchburg, NJ). The participant

initiated ART (AZT + lamivudine + NVP) at 9 months of age, and remained on this regimen for 14 years (Fig. 1). The participant had high median follow-up CD4+ T cell count of 2068 cells/mm³ (interquartile range 1117–2938) and consistent viral suppression (<400 copies/mL) over the 14 years of routine follow-up in a local clinic.

At the age of 14 years 4 months, the participant decided to visit a local voluntary HIV testing center, where a rapid HIV test was negative and the viral load was undetectable (<400 HIV-1 RNA copies/mL). To confirm these results, additional HIV rapid tests – determine HIV-1/2 (Abbott Laboratories, North Chicago, IL) and UniGold HIV (Trinity Biotech, Ireland) – and a DNA PCR test – COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 Qualitative Test (Roche Molecular Systems, Inc), were performed, all with negative results. The test results were discussed extensively among the participant, his parent and health care provider. The decision was made to terminate ART when the participant was aged 14 years 11 months. The authors of this report did not play any role in the decision to terminate ART.

Using the Mashi study records, the participant at the age of 15 years 8 months was approached and recruited to the new HIV reservoir study in 2018. The study aimed at assessing the serological, virological outcomes, and proviral reservoir in adolescents during long-term ART. The study included adolescents (15–17 years of age) perinatally infected with HIV who received ART for >10 years.

The viral load at enrollment (9 months after ART termination) was 186,762 HIV RNA copies/mL. Two HIV enzyme-linked immunosorbent assay tests – Murex HIV-1.2.O (Murex Biotech, Dartford, UK) and Biorad Genetic Systems HIV-1/HIV-2 Plus O (Redmond, WA) – and the HIV DNA PCR test – CAP/CTM HIV-1 qualitative test – were also positive at enrollment in the HIV reservoir study. The test results demonstrate that despite negative

Table 1
Diagnostic and clinical events of the case.

Characteristic	
Mother’s ART during pregnancy?	No
Type of feeding	Breastfeeding
Sex of child	Male
Child received prophylaxis at birth	Yes, AZT, NVP
DNA PCR result at birth	Negative
DNA PCR result at 1 mo	Positive
DNA PCR result at 2 mo	Positive
Viral load (HIV RNA copies/mL) at 8 mo	1210
CD4 T cell count (cells/mm ³) at 8 mo	3768
Age at HAART initiation	9 mo
HAART	AZT + 3TC + NVP
Age at ART termination	14 yr 11 mo
DNA PCR result at 15 yr 8 mo	Positive
HIV ELISA result at 15 yr 8 mo	Positive
Viral load (HIV RNA copies/mL) at 15 yr 8 mo	186,762
CD4 T cell count (cells/mm ³) at 14 yr 11 mo	961

3TC=lamivudine, ART=antiretroviral therapy, AZT=zidovudine, ELISA=enzyme-linked immunosorbent assay, HAART=highly active antiretroviral therapy, NVP=nevirapine.

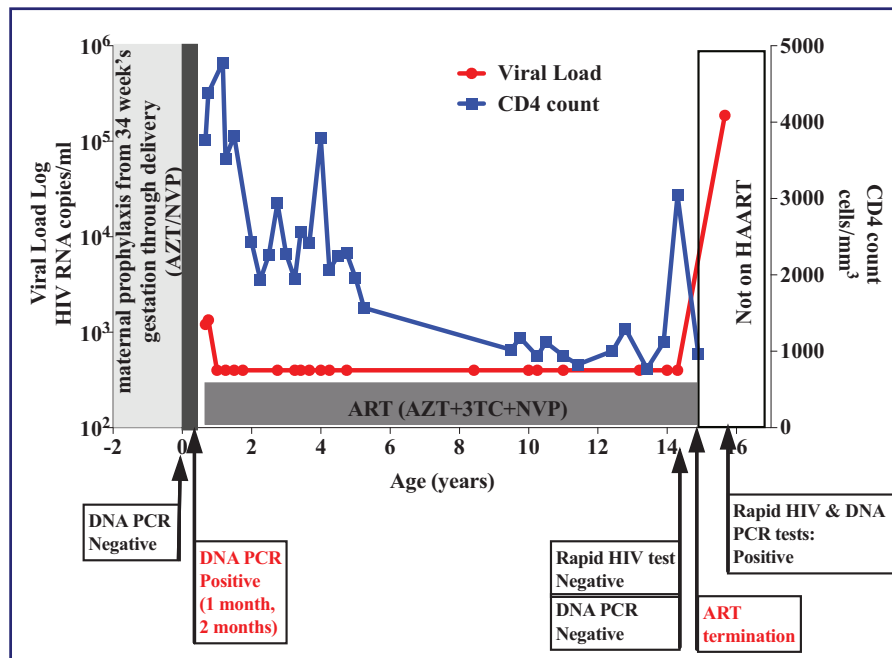


Figure 1. Longitudinal clinical and laboratory data for case 097-533.

HIV rapid and DNA PCR test results at age 14, the participant had latent infection, and termination of ART led to viral rebound.

3. Discussion

The case report describes an adolescent male who received HIV prophylaxis (AZT, NVP) and tested negative by HIV-1 DNA PCR at birth. Clinical notes confirm that his mother was randomized to the breastfeeding arm in the PMTCT Mashi study^[18] in the early 2000s. The HIV-1 DNA PCR became positive at 1 month and 2 months after birth suggesting that the transmission of HIV to the child occurred either through intrapartum or early breastfeeding. While on ART and virally suppressed for 14 years, the participant was tested for HIV at a local testing center on his own suspicion that the initial HIV tests were incorrect. However, in the vast majority of cases where virologic confirmation of HIV is made in an infant or child, viral rebound does occur when ART is terminated, even if ART was administered early during HIV infection and participants display very small viral reservoirs.^[4,23,24] With most perinatally-infected children now receiving early and long-term treatment, the numbers of cases similar to the one described in this report will likely increase, and caution should be exercised when interpreting “negative” diagnostic results.

The negative HIV-1 rapid test and qualitative HIV-1 DNA PCR results and undetectable viral load should not have been surprising for this teenager. Similar cases of the loss of diagnostic markers have been reported elsewhere in perinatally HIV infected children who started early ART.^[25,26] Long-term viral suppression can lead to the absence of circulating HIV-1 RNA and disappearance of laboratory markers, but stable reservoirs of latent virus persist in CD4+ T cells^[24,25] and reactivation of these cells can lead to resurgence of the infection when ART is withdrawn. One case of post-ART remission (<20 HIV-1 RNA copies/mL) over 8 years was reported in 2017 in a South African

9-year-old, whose treatment was interrupted after 40 weeks in a clinical trial.^[27] Another case of post-treatment control was reported in France of an 18-year-old who started ART at birth and was stopped by her parents at 6 years.^[28] These case studies highlight the potential diagnostic complications of the loss of detectable laboratory markers after long-term ART.^[24,29] These cases are exceptions and may lead providers/patients to believe that they are cured, but similar to the “Mississippi baby,” we cannot predict if and when the latent virus will rebound. Importantly, for both the Mississippi baby and also for an Early Infant Treatment Cohort followed in Botswana, quantitative HIV DNA PCR positivity from peripheral blood mononuclear cells was detected at about 2 years of age, even when standard qualitative HIV DNA PCR assays were negative.^[20,24]

Studies have shown that very early ART initiation, such as during acute infection or immediately after birth leads to drastic reductions in DNA set-points, viral reservoirs and has led to longer duration of remission; however, ART interruption almost always lead to viral rebound.^[23,30–32] These findings have been similar across HIV subtypes. Our case started ART at 9 months of life, and was virally suppressed for more than 12 years. Although we did not have access to adherence data, the viral load kinetics suggested very good adherence. Other studies have demonstrated even longer duration to rebound.^[23] In the case of “Mississippi baby,” viral rebound was observed after 2 years of terminating ART^[33] and up to more than 12 years in the French ANRS EPF-CO10 pediatric cohort.^[28] Henrich et al observed HIV relapse in individuals initiating extremely early ART, during Fiebig stage 1 (detectable plasma RNA and antibody negative), despite loss of detectable HIV in blood and tissues using molecular and culture-based detection methods. Therefore, there is an urgent need for better HIV reservoir assays performed on standard specimens to distinguish potential or true remissions from latent infections that will ultimately rebound. In our case study, it possible that HIV could be detectable in other

compartments other than peripheral blood or with ultrasensitive assays. Undetectable DNA by standard commercial molecular assays; therefore, may not be enough rule out HIV infection. A major focus of the HIV cure studies is to identify potential markers predictive of viral rebound and the development of ultrasensitive viral reservoir assays such as single copy assay,^[34] total DNA quantification, viral reactivation, and growth.^[35–37]

The case described is the first report in Botswana in which termination of ART following negative HIV tests during long-term ART led to viral rebound. This case provides an important lesson for proper care and management of patients on ART. Improper ART termination in the reported case highlights the necessity for developing sensitive assays able to detect and assess the proviral reservoir. The reported case shows that negative diagnostic results from rapid and DNA PCR tests in individuals on long-term ART could be misleading. Health care providers dealing with individuals on long-term ART should interpret these results cautiously. In the event that a decision is made that it is in the patient's best interest to terminate ART, laboratory monitoring must be continued long-term, to assess possible viral rebound.

4. Conclusions

Although long-term ART is associated with reduced size of the proviral reservoir, it may also lead to the inability to detect HIV antibodies and DNA using standard methods. Evaluation for possible functional cure (or for false-positive initial testing) by termination of ART, especially without a highly sensitive quantification of proviral reservoir, may be misleading. It is advisable that standard HIV-1 DNA PCR negative tests in individuals on long-term ART are interpreted with caution, and treatment interruption should be avoided.

5. Ethical considerations

The study was approved by the Office of Human Research Administration (OHRA) of the Harvard T.H. Chan School of Public Health and by the Health Research and Development Committee of the Botswana Ministry of Health and Wellness. The parent and participant signed written informed consent and assent, respectively, which includes publication of results where de-identified and de-linked codes will be used.

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