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Increasing Diagnostic Uncertainties in Children With In Utero HIV Infection

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Abstract: We present a case of an in utero HIV-infected child, who on day 1 of life had a positive whole blood total nucleic acid test but viral load <20 RNA copies/mL. Dried blood spot total nucleic acid testing was negative on day 1, 10 and at 3 months, while on ART prophylaxis then positive at 5 months after prophylaxis ended. Retrospective peripheral blood mononuclear cells HIV DNA testing from day 1 of life was positive, confirming in utero infection.

Key Words: point-of-care test, in utero HIV infection, prevention of mother-to-child transmission

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CASE REPORT

In 2016 in KwaZulu-Natal, South Africa, a female baby was born via vaginal delivery to an HIV-positive mother. In her first pregnancy in 2011, the mother had initiated combination antiretroviral therapy (cART) and delivered an HIV-uninfected female. During this second pregnancy, the mother did not attend antenatal clinic, but reported taking her cART haphazardly. The mother presented in labor to a secondary level hospital. At delivery, she had an absolute CD4 count of 452 cells/mm^3 (24%), and a plasma viral load of 12,000 HIV RNA copies/mL (c/mL) (Nuclisens EasyQ v2.0 HIV-1 RNA PCR, bioMérieux, Marcy l'Etoile, France). The baby received nevirapine prophylaxis at 2 hours of age. At 19 hours of age, the baby tested HIV positive using a total nucleic acid (TNA) polymerase chain reaction (PCR) point-of-care test on whole blood [GeneXpert (GXP) Qualitative HIV-1 PCR, Cepheid, Sunnyvale, CA, USA]. The cycle threshold measured 41.3, just below the positive cutoff value 42.0. Blood for plasma viral load testing (NucliSens) and a dried blood sample for laboratory-based HIV TNA PCR [COBAS AmpliPrep/COBAS TaqMan HIV-1 Qualitative PCR Version 2, Roche Molecular Diagnostics, Basel, Switzerland] was drawn before infant cART, comprising zidovudine, lamivudine and nevirapine, was initiated at 20 hours of age. The mother exclusively breast-fed.

The infant's absolute CD4+ T cell count was 1226 cells/mm^3 (36%), below the 10th centile for an HIV-uninfected newborn. However, the plasma viral load was lower than the detectable limit (<20 HIV RNA c/mL), and HIV TNA PCR on dried blood was negative. On day 10, these tests were repeated, and the results were unchanged.

In the absence of a confirmatory HIV test, cART was switched to zidovudine and nevirapine as prophylaxis against postpartum transmission. At 3 months, the child returned to clinic and both the child's plasma viral load (NucliSens) and dried blood HIV TNA PCR (CAP/CTM) remained undetectable. The absolute CD4+ T cell count and percentage remained low. Prophylaxis was stopped after this. When the child next attended clinic 5 months of age, HIV TNA on dried blood was now detectable by PCR (CAP/CTM) and the subsequent plasma viral load was >10^7 HIV RNA c/mL (COBAS 6800/8800 HIV RNA PCR, Roche Molecular Diagnostics, Basel, Switzerland) (Fig. 1A). At this time, the mother's plasma viral load had risen to 212,000 c/mL (COBAS) and she was still breast-feeding. cART was reinitiated for the infant and by 18 months of age, her plasma viral load was almost suppressed (120 HIV RNA c/mL), although the absolute CD4+ T cell count and percentage remained low at 1446 cells/mm^3 (19%).

To distinguish between in utero or post-partum infection, DNA was extracted from the child's peripheral blood mononuclear cells (PBMCs) stored from separate blood samples from day 1 and day 10 of life. Using droplet digital PCR (ddPCR; BioRad, Hercules, California, USA), the HIV cell-associated DNA viral load was measured at 40.7 (95% CI: 13.6–88.1) and 48.5 (95% CI: 17–106.7) copies per million PBMC, respectively (Fig. 1B). HIV *gag* was amplified and sequenced[2] from plasma stored from the mother and from proviral DNA from the child from day 1 of life, showing phylogenetic clustering of the sequences (Fig. 1C), confirming in utero infection of the child.

Mass spectrometry detected emtricitabine, tenofovir and efavirenz in the mother's plasma from day 1 postpartum.

DISCUSSION

In South Africa, HIV diagnosis following in utero mother-to-child transmission (MTCT) is becoming increasingly problematic.[3] Initial HIV plasma viral loads at birth for in utero infected

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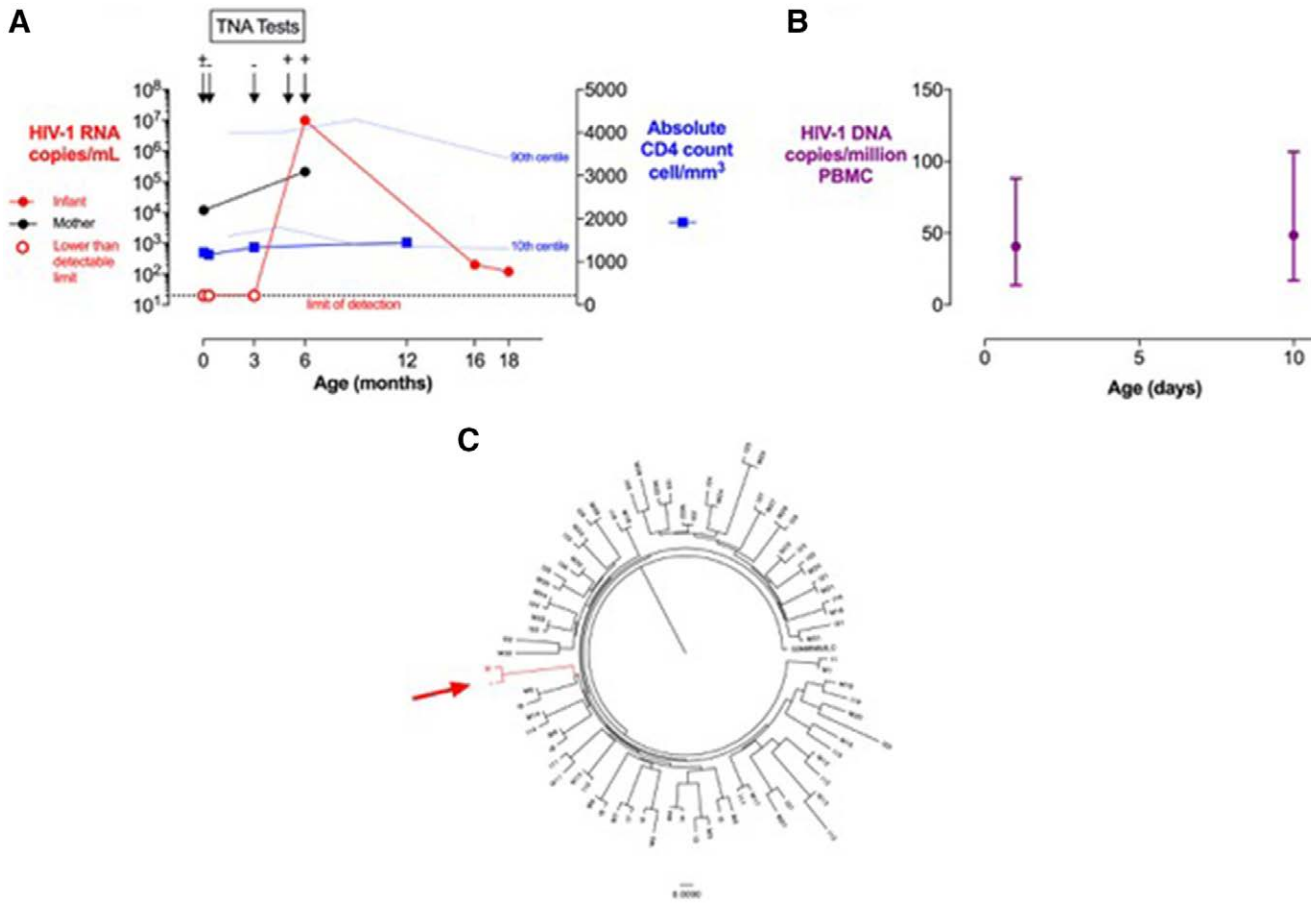

FIGURE 1. Infant test results. A: Summary of the infant's and mother's plasma HIV-1 RNA viral load, the infant's absolute CD4 count and the TNA PCR tests (+ indicates a positive test and – indicates a negative test). The dotted lines represent the absolute CD4 count 90th and 10th centiles for healthy HIV unexposed infants.[1] B: Detectable HIV-1 DNA as measured using ddPCR, 95% CIs is shown. C: Phylogenetic clustering of mother–infant pairs. A maximum likelihood phylogenetic tree was constructed using full-length gag sequences. Population sequences were generated by Sanger sequencing from 40 mother–infant pairs from the study cohort in KwaZulu-Natal, South Africa and rooted on a subtype C consensus sequence. The mother–infant pair that is the subject of this report is shown by the red arrow. The legend indicates phylogenetic distance between mother–infant pairs and is expressed as nucleotide substitutions per site.

infants are approximately 15-fold lower than in the pre-ART era[4,5] and after birth infants born to HIV-infected mothers receive prophylaxis against post-partum MTCT, further lowering plasma viral load in infected infants. The impact of nevirapine, even single dose at birth, is to reduce plasma viral load by a median of 1.3 $\log_{10}$ HIV RNA c/mL in the first days of life.[6] Thus, an initial HIV TNA PCR test positive shortly after birth may become indeterminate or negative when repeated for confirmation of diagnosis after a week of prophylaxis.

The standard first-line Early Infant Diagnosis (EID) HIV test in South Africa is the HIV TNA PCR (CTM/CAP) on dried blood spot that has a similar sensitivity and limit of detection to the whole blood point-of-care TNA PCR (GXP) used in this case, with 95% probability limits of detection 278 (95% CI: 253–304) and 221.8 (95% CI: 195.6–260.5) HIV RNA c/mL, respectively.[7,8] However, as with this case, and as is becoming increasingly common, when the copy number of HIV TNA is lower than the limit of detection, false negative results ensue.

In addition to the increase in false negatives, with the decline in the MTCT rate thus the lower HIV prevalence in infants, the positive predictive value of EID testing is reduced. Consequently, despite the high specificity of EID tests used, it has been estimated that, without confirmatory testing (repeat TNA PCR on dried blood spot) that is standard in South Africa, more than 10% of infants initiated on cART would be diagnosed falsely positive.[9] Therefore, omitting confirmatory testing would result in a significant number of infants unnecessarily committed to the potential toxicity, cost and burden of lifelong cART. For this reason, World Health Organization (WHO) does not recommend reliance on a single initial positive EID HIV test.[10]

The optimal confirmatory test should be more sensitive and specific than the original screening test, to truly confirm infection. The sensitivity of our ddPCR assay has a variable limit of detection, depending on the concentration of cells used, but mostly ranges from 3 to 12 HIV DNA copies per million PBMCs, therefore is much more sensitive than the standard TNA PCR tests, enabling it in this case to confirm in utero infection.

More sensitive tests such as ddPCR and viral sequencing are more expensive and require a higher level of expertise. However, in South Africa, confirmatory testing remains cost-effective unless the confirmatory test exceed US$ 400.[9] However, it may be reasonable to limit the more sensitive confirmatory testing to those initial test

results that fall within an indeterminate range. The GXP test did not have an indeterminate range as recommended by WHO,[10] but it is likely a cycle threshold value of 41.3 would have been indeterminate and could have been an indication to use a more sensitive confirmatory test.

If more sensitive testing had been performed on this infant in the first days of life instead of retrospectively, a definitive diagnosis could have been made while the plasma viral load and cell-associated HIV DNA were still low. This would have negated the need for a "treatment interruption" as occurred after stopping infant prophylaxis thereby preserving the low viral burden, increasing the possibility of an HIV remission, such as in the case of the Mississippi baby.[11]

What was not initially clear from this case is why the infant had an undetectable plasma viral load when it was presumed that the mother was not taking cART as prescribed. Given that the mother's plasma viral load was a log lower at delivery than 6 months later, it suggests that the mother was taking enough cART for partial treatment, further demonstrated by the ART detected in her plasma the day after delivery. Presumably, it was this ART that lowered her own and therefore her infant's viral load, which was then further reduced by infant prophylaxis.

This child was confirmed positive for HIV infection 5 months of age after stopping prophylaxis. The phenomenon of repeated negative plasma RNA PCR testing on prophylaxis followed by a positive result following discontinuation has been described before,[12] but here, uniquely, we have captured the initial low-level infection using the sensitive PBMC ddPCR assay and have proven infection with viral sequencing.

This case demonstrates that prevention of MTCT strategies involving ART in pregnancy and during the breast-feeding period, while highly successful in reducing transmission, also can complicate diagnosis of in utero infection in infants. Although this particular instance is a rare and extreme case, the difficulties with EID in the ART era are an increasingly common one. On a public health level, the important messages are first to recognize that now with a lower prevalence of in utero HIV, the positive predictive values of standard HIV PCR tests are reduced. Therefore, confirmatory EID tests following a positive or indeterminate result are imperative. Second, given the higher proportion of infants with an initial plasma viral load levels lower than the limit of the detection of the standard TNA PCR tests, if feasible, the confirmatory test should be a more sensitive test such as a cell-associated HIV DNA test, particularly if the initial test result is in the indeterminate range. In the absence of more sensitive tests, standard tests should be repeated frequently and most crucially, after infant prophylaxis cessation.

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