

Early Initiation of Antiretroviral Therapy Following In Utero HIV Infection Is Associated With Low Viral Reservoirs but Other Factors Determine Viral Rebound

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Background. Early HIV diagnosis allows combination antiretroviral therapy (cART) initiation in the first days of life following in utero (IU) infection. The impact of early cART initiation on infant viral reservoir size in the setting of high-frequency cART nonadherence is unknown.

Methods. Peripheral blood total HIV DNA from 164 early treated (day 0–21 of life) IU HIV-infected South African infants was measured using droplet digital PCR at birth and following suppressive cART. We evaluated the impact of cART initiation timing on HIV reservoir size and decay, and on the risk of subsequent plasma viremia in cART-suppressed infants.

Results. Baseline HIV DNA (median 2.8 log₁₀ copies/million peripheral blood mononuclear cells, range 0.7–4.8) did not correlate with age at cART initiation (0–21 days) but instead with maternal antenatal cART use. In 98 infants with plasma viral suppression on cART, HIV DNA half-life was 28 days. However, the probability of maintenance of plasma aviremia was low (0.46 at 12 months) and not influenced by HIV DNA load. Unexpectedly, longer time to viral suppression was associated with protection against subsequent viral rebound.

Conclusions. With effective prophylaxis against mother-to-child transmission, cART initiation timing in the first 3 weeks of life is not critical to reservoir size.

Keywords. early infant diagnosis; in utero HIV; HIV reservoir; reservoir decay; pediatric HIV; early treatment; digital droplet PCR; viral rebound.

For children living with human immunodeficiency virus (HIV), although lifelong viral suppression with combination antiretroviral therapy (cART) is possible, rates of treatment failure and mortality on cART are higher compared to adults [1, 2], particularly for infants [3–5]. HIV DNA in resting long-lived CD4⁺ T cells, or the latent HIV reservoir, is established in the first days of infection [6–8] and in children and adults reactivates in the days to weeks following cART cessation [9–11], thus constitutes

the ultimate barrier to HIV remission or cure. The only intervention that has been shown to reduce the latent HIV reservoir is early initiation of cART, whereby lower reservoir levels are achieved by not only restricting the initial size [12, 13] and sequence diversity [14, 15] of the viral reservoir, but also its cellular localization, skewing the reservoir to exist in shorter-lived, transitional CD4⁺ T-cell memory cells which decay faster than longer-lived central memory cells [16]. Because most in utero (IU) HIV infections occur in the final weeks of pregnancy [17, 18], it is possible routinely to treat HIV-infected newborns early in infection when diagnosed at birth. Early treatment along with a favorable tolerogenic neonatal immune milieu [19–21] has led to great scientific interest in the early treatment of IU HIV-infected infants as a model for HIV cure [22–24]. However, the precise age, whether it is hours or days of life, that defines early treatment to realize these benefits is unclear. In a cohort of IU HIV-infected infants in South Africa for whom cART was initiated within the first days of life, we quantified peripheral HIV DNA to determine the size and decay of the latent HIV

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reservoir, and the relationship with subsequent plasma virological control.

METHODS

Study Participants

Ucwangingo Lwabantwana is an observational study based in KwaZulu-Natal, South Africa, exploring early (aged <48 hours) cART for HIV-infected infants [25]. HIV-infected mothers and infants aged <21 days with confirmed positive total nucleic acid HIV polymerase chain reaction (PCR) testing at birth either via point-of-care (PoC) testing (whole blood Gene Xpert; Cepheid) or laboratory-based test result from dried blood spots (DBS; COBAS AmpliPrep/COBAS TaqMan version 2; Roche Molecular Diagnostics). Routine HIV care was provided by local Department of Health clinic staff according to South African guidelines [26]. First-line maternal cART was fixed-dose combination emtricitabine, tenofovir disoproxil fumarate, and efavirenz. Neonatal prophylaxis was commenced for all HIV-exposed infants: nevirapine (NVP) only, or NVP and zidovudine (AZT) where maternal viremia >1000 HIV RNA copies/mL was noted. Infant cART consisted of AZT/lamivudine (3TC)/NVP until 28 days for term infants or 42 weeks corrected gestational age for preterm infants, after which the regimen was switched to abacavir/3TC/ritonavir-boosted lopinavir. The study was approved by the KwaZulu-Natal and Oxfordshire Biomedical Ethics Review Committees. Written informed consent was signed by the infant's mother or guardian. The study presented here is an analysis of all data available prior to 31 December 2019. Latent HIV reservoir measurements were undertaken at baseline then longitudinally where there was optimal cART adherence, as demonstrated by declining plasma HIV RNA.

Definitions

- Acute maternal infection was defined as a mother with a negative HIV rapid antibody test at antenatal booking, followed by a positive test later in pregnancy or at delivery.
- All infants received a DBS test at birth but were subgrouped into PoC and DBS according to whether or not they were first diagnosed using a PoC test.
- Viral suppression was defined as 1 viral load (VL) measurement lower than the limit of detection. Where an HIV DNA measurement at suppression was not available, the measurement was made at a time point within 3 months of suppression.
- Two definitions of viral rebound were used: (1) low-level rebound, as 1 VL measurement >100 HIV RNA copies/mL, and (2) high-level rebound, as 1 VL measurement >1000 HIV RNA copies/mL.
- A "blip" was defined as either (1) 1 VL >100 HIV RNA copies/mL preceded and followed by an undetectable VL, or (2) a VL that increased <1 log₁₀ HIV RNA copies/mL

between consecutive monthly measurements in the context of initial VL decline.

- A viral suppression (VS) subgroup (n = 98) was defined by those infants who were followed for ≥6 months postenrolment, had reached VL suppression by 6 months of age, had ≥1 HIV DNA measurement, had a VL that was less than, the same, or <1 log₁₀ higher in consecutive monthly measurements.
- Adherence was documented at each clinic visit using self-reports and by measuring returned medication (if available). Nonadherence was defined as evidence or reports of missing ≥2 doses.

Laboratory Methods

VL was measured by Nuclisens EasyQ version 2.0 HIV-1 RNA PCR (bioMérieux) with a limit of detection of 20 HIV RNA copies/mL but <100 for low-volume samples. Lysed extracts from thawed peripheral blood mononuclear cells (PBMC) were used to measure total HIV DNA by droplet digital PCR (ddPCR; BioRad) with 5' long terminal repeat or *gag* primers and probes, depending on the efficiency of detection in each patient [27]. The HIV DNA count was normalized using RPP30 housekeeping gene quantification to give a value per million PBMC with a 95% Poisson confidence interval, as estimated across replicates by the QuantaSoft (BioRad) software. A limit of detection for each sample was estimated according to input cell number and where HIV DNA was undetectable, the result was recorded as the limit of detection. Results were excluded if HIV DNA was undetectable and the limit of detection was >25 copies/million PBMC.

Statistics

Statistical analyses were performed using R Software version 3.6.1 [28] and GraphPad Prism version 8. Comparisons were performed using the χ^2 and Fisher exact test for categorical variables. Two-group continuous variables were compared using the *t* test (parametric) or the Mann-Whitney *U* test (nonparametric). Univariate and multivariate linear regression were calculated using the *lm* R function. In the baseline HIV DNA analysis, where a large number of covariates were analyzed, a penalized multivariate linear regression model was used whereby the set of relevant predicting covariates was selected using the LASSO penalty approach [29] and the optimal penalty (λ) was determined via 10-fold cross-validation using the *glmnet* R package [30]. The LASSO-selected covariates were then used in a (post-LASSO) multivariate linear regression to give unpenalized regression coefficient estimates. The resulting *P* values in a post-LASSO analysis should be interpreted with caution because variable selection via a penalized approach is a separate concept to significance. A potentially nonlinear ln-HIV DNA decay over time in the VS infants was estimated and visualized via a generalized additive mixed model (GAMM) with a random effect (intercept only) on the participant, then again in a

multivariate analysis to assess for covariates influencing HIV DNA level over time, both using the *mgcv* R package [31]. The HIV DNA half-life ($t_{1/2}$) was calculated by using the time at which the HIV DNA level was half of its value at baseline, via the *predict()*, *gam* function. Pearson correlation was used to determine age of cART initiation correlation with age of VL suppression. Kaplan-Meier curves were used for time to viral suppression and rebound analyses, and Cox proportional hazard models using the *coxph* R function for multivariate analyses where incorporated covariates were those either significant in the baseline HIV DNA analysis or those of potential clinical relevance. All *P* values were 2-sided with an α of .05.

RESULTS

Baseline HIV DNA

Of 191 IU HIV-infected infants enrolled, a baseline HIV DNA measurement was available for 164 infants, whose characteristics at baseline are displayed in Table 1. cART was initiated at a median age of 6.5 days (interquartile range [IQR], 1–11 days,

maximum 21 days). The median infant baseline HIV DNA level was 2.8 \log_{10} copies/million PBMC (IQR, 2.3–3.4), but levels were variable, ranging from 0.7 to 4.8 \log_{10} copies/million PBMC. The baseline HIV DNA levels in the 77/164 (47%) PoC-tested infants did not differ from the infants diagnosed via DBS testing ($P = .7$, not shown). Furthermore, there was no significant relationship between baseline HIV DNA level and infant age at the time of measurement (β coefficient = $-.01$, $P = .3$; Figure 1A), even when adjusted for infant CD4 percentage and VL ($P = .14$, not shown) or analyzed by PoC/DBS group.

To explore the potential influence of a large number of baseline maternal and infant characteristics on baseline HIV DNA, a penalized linear regression model (LASSO) was used to select influencing covariates from a possible set of 26 variables, as listed in Table 1. In the model representing all 164 infants, relatively, the baseline covariates that were the most influential on baseline infant HIV DNA were infant VL and CD4⁺ T-cell percentage, followed by absence of antenatal maternal cART (Figure 1B and Supplementary Figure 1A). Infant age at blood

Table 1. Baseline Characteristics of All Infants Versus Those Virally Suppressed

Characteristic	Baseline Infants (n = 164)	Virally Suppressed Infants (n = 98)	<i>P</i> Value
Infant			
Sex male, No. (%)	60 (36.6)	36 (36.7)	.1
Median birth weight, kg, median (IQR)	2.8 (2.4–3.1)	2.9 (2.5–3.2)	.2
Median gestational age at birth, wk, median (IQR) ^a	38 (36–39)	37.5 (37–39)	.4
Dual prophylactic ART, No. (%)	111 (67.7)	72 (73.5)	.2
PoC tested, No. (%)	77 (47)	41 (41.8)	.1
DBS PCR result indeterminate or negative, No. (%)	15 (9)	11 (11.2)	.4
Age of cART initiation, d, median (IQR) ^b	6.5 (1–11)	7 (1–11)	.6
Age of blood draw, d, median (IQR)	7 (1–11)	7 (1–11)	.6
Neonatal admission, No. (%)	54 (32.9)	29 (29.6)	.4
Breast feeding, No. (%)	130 (79.3)	77 (78.6)	.7
CD4 count, cells/ μ L, median (IQR)	2120 (1291–2702)	2222 (1371–2777)	.6
CD4 percentage, median (IQR)	44.5 (35.3–52)	45.5 (38–53)	.11
CD8 count, cells/ μ L, median (IQR)	1088 (685–1587)	983 (616–1433)	.3
CD8 percentage, median (IQR)	23 (18–32)	22 (18–28)	.3
CD4:CD8, median (IQR)	1.84 (1.17–2.77)	2.08 (1.45–2.84)	.2
Plasma viral load, copies/mL, median (IQR)	9700 (1200–56500)	3700 (310–30500)	.04
\log_{10} HIV DNA copies/million PBMC, median (IQR)	2.8 (2.3–3.4)	2.6 (2.2–3.3)	.1
Mother			
Age, y, median (IQR)	25.2 (21.9–30.3)	24.5 (21.2–29.7)	.3
Acute maternal infection, No. (%)	43 (26.2)	29 (29.6)	.6
cART initiation postnatal, No. (%)	15 (9.1)	8 (8.2)	.1
Duration cART in pregnancy, d, median (IQR)	104 (13–217)	95 (8–178)	.3
Self-reported maternal nonadherence, No. (%) ^c	70 (54)	36 (48)	.5
CD4 count, cells/ μ L, median (IQR)	460 (317–646)	516 (350–650)	.4
CD4 percentage, median (IQR)	24 (19–31)	25 (19–32)	.4
CD8 count, cells/ μ L, median (IQR)	952 (706–1272)	910 (660–1332)	.6
CD8 percentage, median (IQR)	52.5 (43.3–58.8)	49 (41–58)	.4
CD4:CD8, median (IQR)	0.46 (0.32–0.7)	0.5 (0.33–0.74)	.3
Plasma viral load, HIV RNA copies/mL, median (IQR)	3750 (488–85957)	2250 (253–20750)	.2

Abbreviations: cART, combination antiretroviral therapy; DBS, dried blood spot; HIV, human immunodeficiency virus; IQR, interquartile range; PCR, polymerase chain reaction; PoC, point-of-care.

^aExcluded from multivariate analysis because of missing data.

^bReplaced by cART prior to blood draw in multivariate analysis.

^cOf all mothers who were initiated on cART prior to labor.

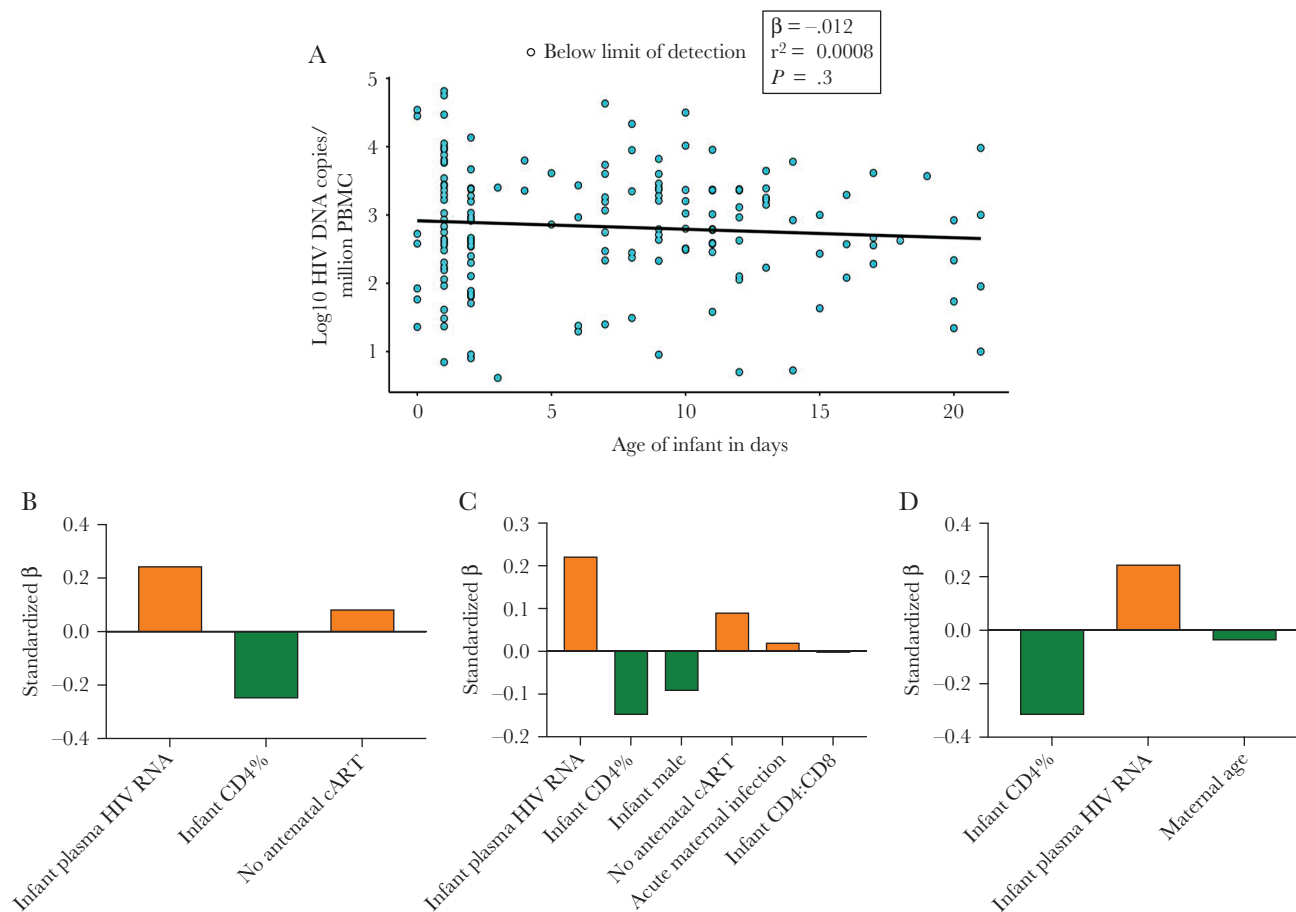


Figure 1. Baseline infant HIV DNA levels. *A*, Baseline HIV DNA levels as determined using droplet digital PCR on PBMC from 164 infants at a median age 7 days. Undetectable HIV DNA is represented by an open circle at the limit of detection for that measurement. Measurements are displayed according to the age of the infant at the time of measurement. Univariate linear regression model shown by the black line. *B–D*, Covariates selected using a penalized linear regression model (LASSO) are shown left to right along the x-axis in order of standardized β coefficient magnitude, which represents their relative influence on HIV DNA. Positive, orange bars indicate covariates associated with higher HIV DNA, while negative, green bars indicate covariates associated with lower HIV DNA. These selected covariates were used in the post-LASSO linear regression to calculate unpenalized, unstandardized β coefficients (Table 2). *C* and *D*, LASSO model for all 164 infants. *C* and *D*, Point-of-care tested infant and dried blood spot tested infant subgroups, respectively. Abbreviations: cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction.

draw did not influence HIV DNA level. The selected influential covariates were then input in a (post-LASSO) multivariate linear regression to determine their absolute influence on infant HIV DNA (Table 2). The absence of antenatal maternal cART increased infant HIV DNA by 0.6 \log_{10} copies/million PBMC.

The infants who were diagnosed by PoC testing were analyzed separately from the infants who were diagnosed by DBS testing to account for any selection bias between the 2 groups [25]. The median infant age at baseline blood draw in these groups was 1 day (IQR, 1–2) and 11 days (IQR, 8–14), respectively. The separate baseline HIV DNA multivariate analyses are shown in Figure 1C and 1D and Supplementary Figure 1B and 1C. In both groups, infant VL and CD4⁺ T-cell percentage still had the strongest relationship with HIV DNA level. Male sex was additionally associated with lower DNA load and acute maternal infection during the pregnancy with higher DNA load in the PoC group (Table 2 and Figure 1C and 1D). We observed

that, after adjusting for other influencing variables, for those infants tested at birth, males had HIV DNA levels 0.5 \log_{10} HIV copies/million PBMC lower than female infants (Table 2).

HIV DNA Decay

Despite early treatment, in general, virological response to cART in this cohort was suboptimal [25]. However, a subgroup of 98 infants with successful viral suppression (VS infants) were further analyzed via longitudinal measurements of HIV DNA load to examine HIV DNA decay in early treated infants (Supplementary Figure 2). To check for representativeness, the VS infants' baseline characteristics were compared to all the infants with measurements at baseline (Table 1). The VS infants had significantly lower baseline VL (median 3700 vs 9700 HIV RNA copies/mL, $P = .04$) but baseline characteristics were otherwise similar. In the VS group, median age at VL suppression was 60 days (IQR, 29–90 days) and this was not

Table 2. Variables Associated With Infant Baseline HIV DNA Level, a Post-LASSO Multivariate Analysis

Variable	All Infants (n = 164)			PoC Infants (n = 77)			DBS Infants (n = 87)		
	β	95% CI	P Value ^a	β	95% CI	P Value ^a	β	95% CI	P Value ^a
Infant plasma viral load, log ₁₀ HIV RNA copies/mL	.22	.14 to .3	<.0001	.22	.09 to .35	.001	.26	.17 to .35	<.0001
Infant CD4, %	-.03	-.04 to -.02	<.0001	-.01	-.04 to .01	.2	-.03	-.04 to -.02	<.0001
No antenatal cART	.61	.24 to .98	.002	.76	.13 to 1.39	.02
Male infant	-.49	-.88 to -.09	.02
Acute maternal infection29	-.08 to .66	.1
Infant CD4:CD8	-.08	-.28 to .11	.4
Maternal age, y	-.02	-.04 to 0	.03

Adjusted r^2 all infants = 0.37 ($P \leq .0001$); PoC infants = 0.39 ($P \leq .0001$); DBS infants = 0.5 ($P \leq .0001$).

Abbreviations: β , unstandardized regression coefficient estimate; cART, combination antiretroviral therapy; CI, confidence interval; DBS, dried blood spot tested; HIV, human immunodeficiency virus; PoC, point-of-care tested.

^aP values for post-LASSO linear regression models should be interpreted with caution as they ignore the selection by LASSO.

significantly correlated with age at cART initiation ($r^2 = 0.18$, $P = .08$, not shown), even when adjusted for baseline CD4⁺ T-cell percentage and VL ($\beta = .02$, $P = .1$, not shown). VS infant HIV DNA measurements were censored at VL rebound, resulting in 274 measurements in the first 3.5 years of life, of which 40/274 (14.5%) were below the limit of detection. The HIV DNA over time GAMM was nonlinear, well representative of the data and highly significant (adjusted R^2 0.84, $P < .0001$; Figure 2).

Rate of decay was estimated from the model, giving an initial (ie, from baseline) half-life of 28 days. At 90 days the half-life had increased to 194 days. A multivariate GAMM was used to determine the factors influencing the change in HIV DNA over time (Table 3). VL over time and age at viremia suppression significantly influenced HIV DNA decay while age of cART

initiation, blips, documented nonadherence, infant sex, and CD4⁺ T-cell percentage over time did not (Table 3).

HIV DNA and Plasma Viremia Control

The relationship between HIV DNA levels and plasma viremia control was explored, first by examining the time to virological suppression in all 164 infants. Baseline infant VL, CD4⁺ T-cell percentage and HIV DNA levels independently influenced time to suppression of plasma viremia, based on univariate Cox regression analyses and a multivariate analysis (Table 4). The addition of infant sex, age of cART initiation, and PoC testing did not individually influence time to suppression nor improve the fit of the multivariate model. In the final multivariate model, baseline log₁₀ VL had the strongest influence on time to suppression (hazard ratio [HR], 0.55; 95% confidence interval [CI],

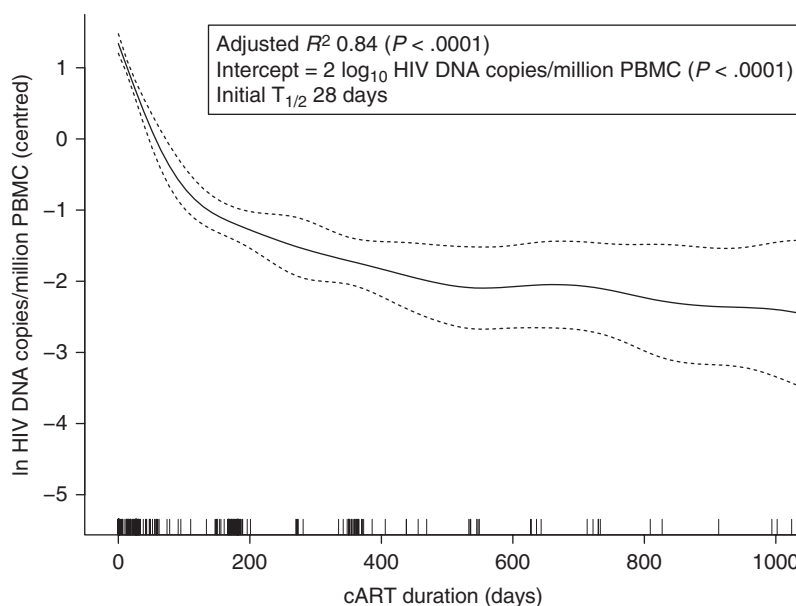


Figure 2. HIV DNA decay in the viral suppression infants. Generalized additive mixed model of log₁₀ HIV DNA over time on cART with individual infant as a random effect (intercept only). Dashed lines, 95% confidence intervals. Vertical spikes on the x-axis denote timing of observations. Abbreviations: cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell.

Table 3. Variables Influencing HIV Decay

Variable	Estimate (95% CI)	P Value
Parametric coefficients		
Intercept	2.12 (1.8 to 2.43)	<.0001
Infant sex	0.004 (-.28 to .29)	.98
Blips ^a	0.12 (-.31 to .55)	.6
Documented cART nonadherence ^b	-0.08 (-.22 to .06)	.3
PoC tested	0.16 (-.34 to .66)	.5
Approximate significance of smooth terms		
	EDF	P Value
cART duration, d	3.19	<.0001
Infant age at cART initiation, d	1.00	.80
Infant age suppressed, wk	1.00	.0003
Plasma viral load, log ₁₀ HIV RNA copies/mL over time	1.00	<.0001
CD4 percentage over time	2.02	.3

Generalized additive mixed model adjusted $R^2 = 0.86$.

Abbreviations: cART, combination antiretroviral therapy; CI, confidence interval; EDF, effective degrees of freedom; HIV, human immunodeficiency virus; PoC, point-of-care tested.

^aA blip was defined as one plasma viral load >100 HIV RNA copies/mL preceded and followed by an undetectable plasma viral load, or a plasma viral load that increased <1 log₁₀ HIV RNA copies/mL between consecutive monthly measurements during initial viremia decline.

^bEvidence or reports of missing ≥ 2 doses.

.45-.67; $P < .0001$; Table 4). After this adjustment, baseline HIV DNA level still had a significant effect, where an increase of 1 log₁₀ HIV DNA copies/million PBMC decreased the rate to suppression by 26% ($P = .01$; Table 4).

Next, maintenance of plasma aviremia on cART was evaluated in the VS infants with regard to (1) HIV DNA level at baseline and (2) HIV DNA at the time point VL suppression was achieved. HIV DNA load was assessed both as a continuous variable, and a categorical variable (above and below the median value). Median time to viral rebound >100 HIV RNA copies/mL was 423 days (95% CI, 320-666) and 571 days (95% CI, 280-∞) for >1000 HIV RNA copies/mL. The probability of viral rebound >1000 HIV RNA copies/mL at 12 months was 0.46 (95% CI, .34-.56). The univariate analyses did not find any significant relationship between HIV DNA load at baseline or at the time of suppression and time to viral rebound for either definition (Table 5). The best fit multivariate model for time to viral rebound >1000 HIV RNA copies/mL was statistically significant, but older infant age at viral suppression was associated with a slower rebound rate (HR,

0.93; 95% CI, .88-.98; $P = .009$) and not with HIV DNA load at baseline or HIV DNA load at the time point VL suppression was achieved (Table 5). The best fit multivariate model for time to low-level viral rebound was not statistically significant (Table 5).

DISCUSSION

This is the largest study of HIV DNA in early treated IU HIV-infected infants. Baseline infant HIV DNA levels were low, in some cases below the limit of detection, and this was associated with maternal antenatal cART use. However, there was no evidence to suggest that for infants receiving ART as prophylaxis, which is the standard of care for HIV-exposed infants, that there was expansion of the latent HIV reservoir in the first 3 weeks of life, nor that earlier initiation of cART resulted in faster HIV DNA decay. PoC diagnosis and the subsequent blood sampling within hours of birth provided insight into clinical factors potentially influencing HIV DNA in utero. Higher HIV DNA levels were demonstrated in female infants and those born to acutely infected mothers. Lower HIV DNA was found in infants

Table 4. Variables Influencing Time to Viral Suppression

Variable	Univariate		Multivariate	
	HR (95% CI)	P Value	HR (95% CI)	P Value
Baseline infant HIV DNA, log ₁₀ copies/million PBMC	0.59 (.49-.72)	<.0001	0.75 (.60-.94)	.01
Baseline infant plasma viral load, log ₁₀ HIV RNA copies/mL	0.52 (.43-.63)	<.0001	0.55 (.45-.66)	<.0001
Baseline infant CD4, %	1.03 (1.01-1.05)	<.0001	1.02 (1.01-1.04)	.008
Male infant	0.87 (.60-1.27)	.5
PoC tested	0.82 (.57-1.17)	.3
Infant age at cART initiation, d	1.01 (.98-1.04)	.7

Cox proportional hazard model. Wald test $P \leq .0001$.

Abbreviations: cART, combination antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio; PoC, point-of-care.

Table 5. Variables Influencing Time to Viral Rebound

Variable	Viral Load > 100 HIV RNA Copies/mL			Viral Load > 1000 HIV RNA Copies/mL		
	Univariate		Multivariate	Univariate		Multivariate
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Infant HIV DNA, log ₁₀ copies/million PBMC						
Baseline high vs low ^a	1.12 (.67–1.87)	.7	1.82 (.98–3.4)	.06	0.89 (.5–1.59)	.7
Baseline continuous	1.05 (.79–1.4)	.8	0.99 (.72–1.36)	.9
Suppression high vs low ^{a,b}	0.92 (.47–1.79)	.8	0.72 (.34–1.55)	.4
Suppression continuous ^b	1.05 (.67–1.64)	.8	0.89 (.56–1.42)	.6
Male infant	0.88 (.52–1.5)	.6	0.78 (.42–1.43)	.4
DBS tested	0.69 (.41–1.15)	.2	0.60 (.35–1.03)	.07	0.29 (.33–1.02)	.06
Infant age at viral suppression, wk	0.97 (.94–1.01)	.1	0.95 (.91–99)	.02	0.95 (.91–1)	.047
CD4 % at suppression	1.01 (.99–1.03)	.5	1.01 (.98–1.03)	.5

Cox proportional hazard model. Wald test viral load > 100 HIV RNA copies/mL $P = .06$; viral load > 1000 HIV RNA copies/mL $P = .02$.

Abbreviations: cART, combination antiretroviral therapy; CI, confidence interval; DBS, dried blood spot; HIV, human immunodeficiency virus; HR, hazard ratio.

^aAbove and below the median value.

^bHIV DNA measurement at the time point viral suppression is achieved or within 3 months.

with lower VL and higher CD4⁺ T-cell percentages, but it is unknown whether these correlations were causal or consequential. VL at baseline and age of suppression were significantly associated with HIV DNA decay rate, but this was not further influenced by blips or documented cART nonadherence. Although lower infant HIV DNA levels were associated with a faster time to VL suppression, they were not associated with suppression maintenance, suggesting that there are immunological, virological, or behavioral factors that override the potential biological advantages of low latent HIV reservoirs in early treated IU HIV-infected infants in the first years of life.

We found higher infant HIV DNA than the only 2 other studies of HIV DNA in IU HIV-infected prevention of mother-to-child transmission (PMTCT)-exposed infants in the first days of life (median 2.8 vs 2.1 and 2.2 log₁₀ HIV DNA copies/million PBMC) [12, 13], although this may be related to small sample sizes in the other studies (n = 9 and 7 vs n = 164 here). HIV DNA levels in the first days of life from all 3 studies were approximately 1 log₁₀ copies/million PBMC lower than in IU HIV-infected infants not exposed to PMTCT [32]. Maternal cART use in pregnancy in our study was common (approximately 75% [25]). The ratio of umbilical cord blood to maternal blood ART levels is close to 1 [33], and in South Africa, serum drug levels of NVP and AZT when administered at prophylaxis dosing are often indistinguishable from those observed following treatment dosing [26, 34, 35]. Thus, this substantial infant exposure to ART transplacentally and postnatally as prophylaxis prior to cART initiation is lowering or restricting infant latent HIV reservoir size.

From these low levels, HIV DNA decayed quickly with an initial HIV DNA half-life of 28 days. This was shorter than the 2.7 months observed by Veldsman et al in their analysis of infants with similar ages of cART initiation and VL suppression [13], although in their analysis the half-life was taken at 6 months, in comparison to the initial decline we have reported. IU HIV transmission is estimated to occur at a median of 14 days prior to delivery [17], and our infants were on average 1 week postdelivery, thus 3 weeks postinfection, but the HIV DNA decay rate in the infants was faster than the approximate half-life of decay of 12 weeks for adults treated in the first days of infection [7], demonstrating benefits of the neonatal immune system. It was intriguing to note that although HIV DNA decay was significantly associated with VL, it was not influenced by blips or documented nonadherence (Table 3). This raises the possibility that the initial degree and length of plasma viremia is more crucial in terms of reservoir size compared with short periods of viremia later, also shown in analytic treatment interruption studies in adults [36, 37]. However, it may have been that 3-monthly VL measurements and self-reports of adherence were simply insensitive markers for intermittent viremia.

Similar to our multivariate model of HIV DNA decay, most pediatric studies have found lower levels of HIV DNA associated

with younger infant age at suppression [10, 12, 13, 16, 38–45]. The key difference for our cohort was there was no association between age of cART initiation and age of suppression, probably because previous studies analyzed age of cART initiation in the order of months, compared to our narrow window of age of initiation (21 days). Interestingly, we found that time to rebound was faster in those infants who suppressed plasma viremia earlier. This may have been due to more effective HIV-specific immune responses in those infants who were exposed to plasma viremia for longer, delaying rebound [9]. Alternatively, this finding may instead be due to behavioral factors, where mothers of viremic infants in the first months receive the most attentive adherence support, addressing central issues such as disclosure, and developed robust cART administration skills.

Studies have shown correlations between lower peripheral total HIV DNA levels and superior maintenance of plasma aviremia in children [43, 46], but compared to these, our participants had a much higher probability of VL rebound (0.46 at 12 months compared with 0.01 in Kuhn et al [43]), but were also much younger, so less likely to be cART adherent ([3, 4]). It is possible that lower HIV DNA levels are useful in slowing viral rebound in the context of occasional missed doses, but not for the higher levels of nonadherence seen in younger children.

The findings of our study are relevant to settings like South Africa, with well implemented PMTCT programs, including antenatal cART, neonatal prophylaxis, and HIV testing at birth. Although we did not find any benefit in terms of latent HIV reservoir size for initiating cART in the first hours versus days of life, it is important to make the point that this should not be used as an argument against PoC testing for early infant diagnosis, which has been shown to decrease test turnaround time and increase rates of cART initiation [47, 48], crucial for KwaZulu-Natal, where only 25% of infants return for confirmatory HIV testing following an initial positive DBS test [49]. Furthermore, the innate immunological benefits of cART initiation in the first days of life are enabled by PoC testing [12].

We used total PBMC HIV DNA as a surrogate marker for latent HIV reservoir which may have overestimated HIV DNA able to contribute to plasma viremia. However, measurement of total HIV DNA still has clinical relevance, as defective and un-integrated HIV DNA triggers inflammation contributing to disease progression [50]. There was an inverse relationship between CD4⁺ T-cell percentage and HIV DNA, therefore in the case of low CD4⁺ T-cell count there would have been an even higher concentration of HIV DNA per CD4⁺ T cell than measured.

This HIV DNA decay analysis benefited from a large sample size, but its generalizability may be limited by excluding those infants with prolonged time to suppression and higher baseline plasma viral load who may have had intrinsically different viral or immune characteristics. However, inadequate plasma viral suppression for those infants was more likely secondary to cART nonadherence, a scenario for which HIV DNA decay is less relevant.

In conclusion, where PMTCT programs are implemented well, and the standard of care can achieve HIV diagnosis at birth and cART initiation within the first 3 weeks of life, the priority for IU HIV-infected infant cART initiation should be timed to maximize caregiver adherence to achieve and sustain viral suppression, rather than aiming for lower HIV DNA as a hallmark of HIV remission.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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