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Independent Effects of Nevirapine Prophylaxis and HIV-1 RNA Suppression in Breast Milk on Early Perinatal HIV-1 Transmission

Michael H. Chung, MD, MPH*, James N. Kiarie, MBChB, MMed, MPH†, Barbra A. Richardson, PhD‡,§, Dara A. Lehman, MHS||,¶, Julie Overbaugh, PhD§,¶, Francis Njiri, BSc†, and Grace C. John-Stewart, MD, PhD*,#

*Department of Medicine, University of Washington, Seattle, WA

†Department of Obstetrics and Gynaecology, Kenyatta National Hospital, Nairobi, Kenya

‡Department of Biostatistics, University of Washington, Seattle, WA

§Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

||Department of Molecular and Cellular Biology, University of Washington, Seattle, WA

¶Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA

#Department of Epidemiology, University of Washington, Seattle, WA

Abstract

Background—The mechanism of action of single-dose nevirapine on reducing mother-to-child transmission of HIV-1 may involve reduction of maternal HIV-1 or prophylaxis of infants.

Methods—In a study that randomized pregnant mothers to HIVNET 012 nevirapine versus short-course antenatal zidovudine, we compared breast milk HIV-1 RNA viral shedding and administration of single-dose nevirapine between mothers who transmitted HIV-1 to their infants at 6 weeks postpartum and those who did not.

Results—In multivariate analyses, maximum breast milk HIV-1 RNA levels (hazard ratio [HR] = 2.50, 95% confidence interval [CI]: 1.25 to 4.99; $P = 0.01$) and nevirapine use (HR = 0.12, 95% CI: 0.02 to 0.97; $P = 0.05$) were each independently associated with perinatal transmission at 6 weeks postpartum. Mothers who transmitted HIV-1 to their infants had significantly higher HIV-1 RNA levels in their breast milk between the second day and sixth week postpartum. Among mothers with maximum breast milk virus levels less than a median of 3.5 log₁₀ copies/mL, the administration of nevirapine further decreased HIV-1 transmission risk from 22.2% to 0.0% ($P = 0.04$).

Conclusions—Peripartum administration of single-dose nevirapine to mother and infant decreases early perinatal HIV-1 transmission by means of breast milk HIV-1 RNA suppression and, independently, by providing the infant with exposure prophylaxis.

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Correspondence to: Michael H. Chung, MD, MPH, International AIDS Research and Training Program, Box 359909, University of Washington, Seattle, WA 98104 (mhchung@u.washington.edu).

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The trial was conducted and completed before clinical trials were required to be registered by this journal. Therefore, there are no URL and registration number associated with this study and article.

Keywords

Africa; breast milk; HIV-1; mother-to-child transmission; nevirapine; prevention of perinatal transmission

Single-dose nevirapine given to mother and infant has been widely used to prevent mother-to-child transmission of HIV-1 in resource-limited settings.¹ The finding of persistent nevirapine-resistant strains in mothers who have taken this perinatal regimen has raised concerns that future antiretroviral treatment of these mothers may be compromised.²⁻⁴ Some have advocated that single-dose nevirapine be removed or adapted to exclude maternal dosing.^{5,6} Thus, elucidating the mechanisms by which nevirapine works to limit mother-to-child transmission of HIV-1 is important to optimize the effective use of single-dose nevirapine in multidrug regimens or in the rational design of alternate strategies.⁷

Antiretroviral medications to prevent mother-to-child transmission of HIV-1 exert their effect by decreasing maternal systemic/mucosal HIV-1 levels, by pre- or postexposure prophylaxis (drug effects within the host after or before viral exposure) of infants, or by a combination of these effects. Perinatal HIV-1 transmission is strongly associated with maternal HIV-1 RNA levels in plasma, genital secretions, and breast milk.⁸⁻¹⁰ Prolonged suppression of breast milk HIV-1 RNA may be one of the mechanisms through which nevirapine decreases transmission in a breast-feeding population.^{11,12} Nevirapine also decreases perinatal HIV-1 transmission without maternal dosing, however, demonstrating the role of exposure prophylaxis in the infant.^{13,14}

To assess the mechanism of effect of single-dose nevirapine given to mother and infant on early perinatal transmission, we examined breast milk HIV-1 RNA suppression associated with nevirapine and compared breast milk viral levels and administration of nevirapine between women who transmitted HIV-1 to their infants and those who did not at 6 weeks postpartum.

METHODS

Study Population

Subjects in this study participated in a randomized clinical trial conducted in Nairobi, Kenya comparing the effect of single-dose nevirapine (HIV Network for Prevention Trials [HIVNET] 012 regimen) and short-course zidovudine (Thai Centers for Disease Control and Prevention [CDC] regimen) on the quantity of HIV-1 RNA in breast milk over the first 6 weeks postpartum.^{15,16} The methods for enrollment, randomization, postpartum follow-up, and breast milk collection have been described elsewhere.¹¹ Briefly, pregnant women were randomized at 34 weeks of gestation to receive a single 200-mg oral dose of nevirapine at the onset of labor and a single 2-mg/kg (6 mg if birthweight >2.5 kg) oral dose of nevirapine suspension given to the neonate within 72 hours of delivery, or oral administration of 300 mg of zidovudine twice daily from 34 weeks of gestation until the onset of labor and 300 mg orally every 3 hours from the onset of labor until delivery.

During the first 6 weeks after delivery, 2 to 5 mL of breast milk was collected at home from each woman at 14 time points separated by at least 1 day. During each home visit, peer counselors collected information and supported mothers to engage in exclusive breast-feeding practices. At or within 3 days of delivery, maternal blood was obtained for HIV-1 RNA levels and neonatal blood was collected on filter paper for HIV-1 DNA. For mothers and newborns in whom early collection was not achieved, specimen blood collection was

performed within 2 weeks of delivery. At 6 weeks postpartum, maternal blood was drawn for HIV-1 RNA levels and infant blood was collected on filter paper for HIV-1 DNA.

Informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Boards at the Kenyatta National Hospital, Kenya and the University of Washington, Washington. The sample size of 50 women in each arm was calculated to detect a 0.75 log₁₀ HIV-1 RNA difference in breast milk with >90% power.

Laboratory Methods

Laboratory methods for HIV-1 RNA and DNA viral analysis in this study have been described elsewhere in detail.¹¹ After centrifugation, which was performed immediately after milk collection, breast milk supernatant samples were frozen at -70°C and shipped from Nairobi, Kenya on liquid nitrogen to Seattle, Washington, where they were stored at -70°C. Maternal plasma and breast milk HIV-1 RNA levels were measured using the Gen-Probe HIV-1 viral load assay (Gen-Probe Incorporated, San Diego, CA). Based on parallel testing of HIV-1-negative samples and samples spiked with a low number of copies of HIV-1 RNA, the lower limit of detection of HIV-1 RNA in breast milk was defined to be 100 copies/mL.¹¹ Infant HIV-1 infection status was determined by polymerase chain reaction (PCR) for HIV-1 DNA.¹⁷

Statistical Analysis

HIV-1 RNA viral load data in breast milk were log₁₀ transformed, and the mean of observations for each woman was taken within each of 7 separate time periods (0 to 2 days; 3 to 7 days; and 2, 3, 4, 5, and 6 weeks postpartum). Viral levels were compared between transmitters and nontransmitters using the Mann-Whitney *U* test. Transmission was defined as those infants testing positive for HIV-1 at any time point between 48 hours after delivery and 6 weeks postpartum. Breast milk HIV-1 RNA levels that measured less than the lower limit of detection (100 copies/mL) were assigned a value at the midpoint between the lower limit of detection and 0 (50 copies/mL). Time to HIV-1 infection in the infant was defined as the midpoint between the last negative and first positive HIV-1 PCR result. Cox regression analysis was used to determine the association between nevirapine use, plasma and breast milk viral loads, and time to HIV-1 transmission. Collinearity of nevirapine use, plasma viral load, and breast milk viral load was determined by assessing the standard errors of the coefficients; it was determined that plasma and breast milk viral loads were highly collinear, and only breast milk viral load was retained in the final model. Time to HIV-1 infection for 4 risk categories based on maternal breast milk viral load and nevirapine use was compared using Kaplan-Meier analysis and the log-rank test. Analyses were performed using SPSS version 14.0 (SPSS, Chicago, IL) and S-Plus 2000 (MathSoft, Seattle, WA).

RESULTS

Study Population

A detailed description of the participants and primary endpoints in this randomized clinical trial has been published.¹¹ Between March 5, 2003, and October 31, 2003, 1865 pregnant women received HIV-1 testing, of whom 319 (17%) were HIV-1-seropositive, and 76 were enrolled in the trial. Those who enrolled expressed interest in exclusive breast-feeding, had no previous exposure to antiretroviral drugs, and presented earlier than 32 weeks of gestation. Of the 76 participants at enrollment, 60 remained in the study, were randomized, and had breast milk collected after delivery: 30 subjects were randomized to short-course zidovudine and 30 to single-dose nevirapine.

HIV-1 Mother-to-Child Transmission

Sixty neonates, 30 in the zidovudine arm and 30 in the nevirapine arm, were born and had HIV-1 viral assays performed within 2 weeks of delivery. Among these newborns, 1 from the nevirapine arm tested positive for HIV-1 <48 hours after delivery and was considered to be infected in utero and excluded from analyses. Two newborns from the zidovudine arm, who tested positive for HIV-1 at 5 and 7 days postpartum without a negative test result <48 hours postpartum, were included in the analysis after calculations excluding them determined similar results but with slightly less power to test the hypotheses. Between 2 weeks postpartum and the 6-week postpartum endpoint, 3 infants were lost to follow-up and 2 died. Maternal CD4 cell counts and plasma viral levels during pregnancy and delivery were similar between those who were lost or whose babies died and those who were retained in the study ($P = 0.5$ and $P = 0.8$, respectively). At 6 weeks postpartum, 55 infants (92%) had blood samples collected for HIV-1 DNA. Excluding the single in utero transmission, 9 infants, 1 from the nevirapine arm and 8 from the zidovudine arm, were infected with HIV-1 at 6 weeks. The mothers of these 9 infected infants were defined as postnatal transmitters.

At enrollment and delivery, sociodemographic characteristics were comparable between mothers who transmitted HIV-1 and those who did not transmit HIV-1 to their infants at 6 weeks postpartum (Table 1).

Breast Milk HIV-1 RNA Levels and HIV-1 Transmission

During the first 6 weeks postpartum, 745 breast milk samples were collected from 54 women. Eighty percent of the women had all breast milk samples collected from the same breast. Of those who had breast milk collected from both breasts, 86% of the samples were collected from the same breast. A total of 8 breast milk samples (1%) were drawn from breasts that exhibited signs of mastitis, bleeding, or abscess at the time of collection. All mothers were reported to have exclusively breast-fed their infants. One hundred twenty-six samples were collected from 9 women who transmitted HIV-1 to their infants, and 619 breast milk samples were collected from 45 women who did not transmit HIV-1 by 6 weeks postpartum. Three hundred eighty-one breast milk samples were collected from 28 women who took single-dose nevirapine at delivery.

After the first 2 days and up to 1 week postpartum, HIV-1 RNA levels in breast milk among transmitters were significantly higher than viral levels in breast milk among nontransmitters (median $\log_{10}$ HIV-1 RNA: 2.96 vs. 1.94; $P = 0.04$; Table 2). Breast milk HIV-1 RNA viral elevation associated with transmitters compared with nontransmitters persisted significantly throughout the second (median $\log_{10}$ HIV-1 RNA: 2.97 vs. 1.85; $P < 0.001$), third (median $\log_{10}$ HIV-1 RNA: 3.99 vs. 2.17; $P = 0.002$), fourth (median $\log_{10}$ HIV-1 RNA: 3.85 vs. 2.30; $P = 0.008$), fifth (median $\log_{10}$ HIV-1 RNA: 4.30 vs. 1.92; $P = 0.006$), and sixth (median $\log_{10}$ HIV-1 RNA: 3.65 vs. 2.04; $P = 0.03$) weeks postpartum (Fig. 1). In univariate analyses, higher maximum breast milk HIV-1 RNA levels during 6 weeks of follow-up was associated with an increased risk of HIV-1 transmission (hazard ratio [HR] = 2.39, 95% confidence interval [CI]: 1.32 to 4.30; $P = 0.004$) and was independently predictive of transmission after adjusting for plasma HIV-1 levels around the time of delivery (HR = 2.04, 95% CI: 1.03 to 4.04; $P = 0.04$).

Nevirapine, Breast Milk and Plasma HIV-1 RNA Levels, and HIV-1 Transmission

In a univariate analysis, perinatal administration of single-dose nevirapine to mother and infant (HR = 0.10, 95% CI: 0.01 to 0.79; $P = 0.03$), maximum breast milk HIV-1 RNA levels (HR = 2.39, 95% CI: 1.32 to 4.30; $P = 0.004$), and plasma HIV-1 RNA levels around the time of delivery (HR = 3.64, 95% CI: 1.29 to 10.26; $P = 0.02$) were each associated with a decreased risk of HIV-1 transmission at 6 weeks postpartum. In a multivariate model that

included all 3 variables, maximum breast milk HIV-1 RNA levels (HR = 2.02, 95% CI: 0.87 to 4.68; $P = 0.1$) and nevirapine administration (HR = 0.14, 95% CI: 0.02 to 1.16; $P = 0.07$) had a trend for independent effects on HIV-1 transmission, whereas plasma HIV-1 RNA levels around the time of delivery did not (HR = 1.57, 95% CI: 0.54 to 4.57; $P = 0.4$). Because maximum breast milk viral level was a more significant factor in this model and tests for collinearity and correlation between breast milk viral levels and plasma viral levels near delivery were highly significant ($r = 0.58$, $P < 0.001$), we excluded plasma viral levels in a subsequent model and found that maximum breast milk HIV-1 RNA levels were associated with increased transmission (HR = 2.50, 95% CI: 1.25 to 4.99; $P = 0.01$) independent of nevirapine and that administration of nevirapine had a protective effect even after controlling for breast milk viral levels (HR = 0.12, 95% CI: 0.02 to 0.97; $P = 0.05$). Restricting to women with a CD4 count ≥ 200 cells/mm³, breast milk HIV-1 RNA suppression remained significantly associated with decreased transmission ($P = 0.008$), whereas a trend for the protective effects of nevirapine was seen ($P = 0.1$). Analyses were repeated with random exclusion of up to 2 of the 9 infected babies to model misclassification of timing of transmission attributable to potential incomplete sensitivity of infant filter paper HIV-1 detection. Results remained essentially the same in these analyses (remained significant or trends with $P < 0.1$).

Including prenatal CD4 cell count or CD4 cell count around the time of delivery in the model did not significantly alter the relation between HIV-1 perinatal transmission risk and nevirapine or maximum breast milk viral levels. In multivariate analyses that included CD4 count < 500 cells/mm³ at 32 weeks of gestation, maximum breast milk HIV-1 RNA levels, and nevirapine, maximum breast milk HIV-1 RNA levels (HR = 3.67, 95% CI: 1.48 to 9.07; $P = 0.005$) and nevirapine (HR = 0.11, 95% CI: 0.01 to 0.92; $P = 0.04$) were significantly associated with decreased mother-to-child HIV-1 transmission risk, whereas CD4 count was not (HR = 0.26, 95% CI: 0.05 to 1.38; $P = 0.1$). In similar multivariate analyses that included CD4 count < 500 cells/mm³ around the time of delivery, CD4 cell count continued not to be significantly associated with HIV-1 transmission risk (HR = 0.83, 95% CI: 0.20 to 3.38; $P = 0.8$), whereas maximum breast milk HIV-1 RNA levels (HR = 2.58, 95% CI: 1.24 to 5.38; $P = 0.01$) was and nevirapine (HR = 0.13, 95% CI: 0.02 to 1.04; $P = 0.055$) showed a strong trend.

To clarify the relation between maximum breast milk HIV-1 RNA levels and nevirapine, 4 risk categories were created and the time to HIV-1 infection in these 4 groups was compared. The median level of HIV-1 RNA in all breast milk samples collected was 3.5 log₁₀ copies/mL. The 4 risk categories were based on maximum breast milk HIV-1 RNA levels greater than and at or less than the median and whether the mother and infant received nevirapine. Mother-infant pairs with maximum breast milk viral levels ≤ 3.5 log₁₀ copies/mL and who received nevirapine ($n = 16$) had a transmission rate of 0.0% at 6 weeks postpartum (Fig. 2). At the other end of the spectrum, mother-infant pairs with maximum breast milk viral levels > 3.5 log₁₀ copies/mL who received no nevirapine but only zidovudine ($n = 9$) had a transmission rate of 34.9% (95% CI: 12.3 to 57.5) at 6 weeks postpartum. Women with breast milk viral levels > 3.5 log₁₀ copies/mL who received nevirapine ($n = 12$) had a transmission rate of 8.3% (95% CI: 0.0 to 24.0) at 6 weeks postpartum, and those with breast milk viral levels ≤ 3.5 log₁₀ copies/mL who only received zidovudine ($n = 17$) had a transmission rate of 22.2% (95% CI: 0.0 to 49.4) at 6 weeks postpartum. There was a significant difference in the time to HIV-1 infection in the 4 categories ($P = 0.04$).

DISCUSSION

In this study, women who transmitted HIV-1 to their infants by 6 weeks postpartum had significantly higher levels of HIV-1 RNA in breast milk than those who did not transmit

HIV-1; there was a 2.5-fold increased risk in perinatal transmission for every log increase in breast milk HIV-1 RNA. In analyses that controlled for breast milk HIV-1 RNA levels, we also demonstrated that receiving nevirapine was an independent protective factor against HIV-1 transmission (HR = 0.12, 95% CI: 0.02 to 0.97; $P = 0.05$). Thus, our study suggests that nevirapine exerts its effects on mother-to-child HIV-1 transmission by means of suppression of breast milk HIV-1 and exposure prophylaxis. The duality of single-dose nevirapine's mechanism of action may explain its substantial additive benefit when combined with short-course zidovudine to prevent mother-to-child transmission of HIV-1 infection.¹⁸

Breast milk shedding of HIV-1 RNA is strongly associated with HIV-1 transmission from mother to infant.^{9,10,19} In our study, HIV-1 RNA levels in breast milk were highly associated with transmission risk and were persistently suppressed by nevirapine, suggesting that nevirapine's effect is attributable, in part, to breast milk HIV-1 viral suppression. There was a significant ~2-fold increased risk of HIV-1 transmission per log increase in breast milk HIV-1 RNA in analyses that adjusted for maternal plasma HIV-1 RNA near delivery ($P = 0.04$). Decreased breast milk HIV-1 RNA levels may reflect suppression of viral replication systemically or in the breast milk itself in which nevirapine levels have been measured for up to 21 days after single-dose therapy.²⁰

Exposure prophylaxis reduces mother-to-child HIV-1 transmission in newborn babies and is used to protect infants from untreated HIV-1 maternal infection.^{13,14} Among women with breast milk HIV-1 RNA levels $>3.5 \log_{10}$ copies/mL in this cohort, the administration of nevirapine decreased the transmission risk from 34.9% to 8.3%. Among women who had breast milk HIV-1 RNA levels $\leq 3.5 \log_{10}$ copies/mL but who did not receive nevirapine, the transmission risk was 22.2% in contrast to the 0.0% transmission risk among those who received nevirapine. Thus, with or without suppression of breast milk HIV-1 RNA, nevirapine given to mother and infant exerted a protective effect in this study. Part of this exposure prophylaxis may arise from ingestion of nevirapine through breast milk in addition to the infant dosing provided as part of the standard regimen.

Several recent studies have suggested that nevirapine may primarily exert its effects through exposure prophylaxis and that eliminating maternal nevirapine administration in favor of infant ingestion alone may be equally effective and less associated with viral resistance.^{6,21} A study by Shapiro et al⁶ demonstrated no additional benefit from maternal nevirapine administration. The findings from that study may have differed from ours because of exclusion of some severely immunosuppressed women; inclusion of in utero perinatal infections, which made up 75% of all transmissions in that study; and the design, which incorporated administration of prophylactic zidovudine to all newborns for 1 month after delivery. Our study excluded known in utero infections, included women with a CD4 count <200 cells/mm³, and did not administer zidovudine to babies. Detailed breast milk HIV-1 viral shedding was assessed at multiple time points to enable analysis of the concurrent impact of maternal dose nevirapine on breast milk HIV-RNA and mother-to-child transmission in the early peripartum period.

This study has several limitations. It was a small sample size, the comparison group received short-course zidovudine, and HIV-1 transmission was assessed up to only 6 weeks postpartum. Some studies suggest that HIV-1-infected cells are a more predictive indicator of infectivity and that antiretroviral medications may not equivalently suppress RNA and DNA in breast milk.^{22,23} Thus, it is important to assess nevirapine's effect on HIV-1 DNA within breast milk to define drug effects on cell-associated and cell-free virus further. It is possible but less likely that decreased perinatal transmission ascribed to nevirapine administration may be attributable to increased risk secondary to rebound after stopping

zidovudine.⁹ Finally, discerning relative effects of plasma HIV-1, breast milk HIV-1 RNA, and breast milk HIV-1 DNA is challenging given the collinearity of the variables evaluated, each of which is strongly associated with the other and with transmission. In a model including plasma and breast milk HIV-1 RNA levels, however, breast milk HIV-1 RNA was more predictive of transmission, and adjusting for HIV-1 in maternal plasma and breast milk compartments enabled a distinct assessment of potential nevirapine exposure prophylaxis effects.

A strength of the study was its frequent sampling of breast milk. Fourteen breast milk vials were collected from each participant over 42 days after delivery and allowed a day-to-day analysis of breast milk HIV-RNA shedding. This sampling frame opened a unique window into the biologic mechanism of action of nevirapine.

Our findings suggest that reducing maternal breast milk HIV-1 RNA levels in breast milk and giving exposure prophylaxis to the infant reduces HIV-1 perinatal transmission risk and that single-dose administration of nevirapine to mother and baby around the time of delivery accomplishes both of these tasks. Suppressing breast milk viral shedding seems to decrease mother-to-child transmission, and nevirapine's role in reducing breast milk HIV-1 levels should be recognized, particularly among breast-feeding women. In a subset analysis of women with a CD4 count ≥ 200 cells/mm³, maternal breast milk HIV-1 RNA remained significantly associated with perinatal transmission in this study, suggesting a need for maternal suppression of breast milk HIV-1 RNA regardless of immune status. With additional perinatal anti-retroviral coverage recommended by the World Health Organization (WHO) to limit viral resistance, retaining maternal nevirapine as part of any regimen may enhance treatment efficacy.²⁴ At the same time, providing antiretroviral prophylaxis for the infant against HIV-1 exposure from the mother seems to be an important component of nevirapine's effectiveness, and alternate perinatal regimens seeking to match nevirapine's success should also consider the strength of this mechanism of action.

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FIGURE 1. Mean log₁₀ HIV-1 RNA in breast milk over days postpartum of HIV-1 mother-to-child transmitters (T) versus nontransmitters (NT) at 6 weeks postpartum (lines are Loess curves).

FIGURE 2.

Kaplan-Meier curves of maximum breast milk $\log_{10}$ HIV-1 RNA viral level (BMVL) greater than and at or less than the median ($3.5 \log_{10}$ copies/mL) and nevirapine (NVP) administration on cumulative HIV-1 perinatal infection. The P value for a difference in the 4 groups (log-rank test) is 0.04.

TABLE 1

Characteristics of Women Who Gave Birth by Transmission Status

	Transmitters		Nontransmitters		P
	n	Value	n	Value	
Total	9		45		
Median (IQR) age (y)	9	21.0 (20.5 to 27.5)	45	25.0 (21.5 to 29.5)	0.2
Median (IQR) schooling (y)	9	8 (8 to 12)	44	8 (7 to 12)	0.4
Unmarried (%)	9	1 (11%)	45	1 (2%)	0.2
Median (IQR) no. lifetime sexual partners	9	2 (1 to 4)	45	3 (2 to 4)	0.5
Median (IQR) age (y) at first intercourse	9	18 (16 to 19)	45	16 (15 to 18)	0.2
CD4 count (cells/ μ L) at 32 wk of gestation					
Median (IQR)	8	540 (326 to 839)	44	504 (296 to 701)	0.9
≤ 200	1	13%	5	11%	1.0
201 to 499	3	38%	17	39%	1.0
≥ 500	4	50%	22	50%	1.0
CD4 count (cells/ μ L) at delivery					
Median (IQR)	9	555 (364 to 773)	43	650 (429 to 981)	0.3
≤ 200	0	0%	3	7%	1.0
201 to 499	4	44%	12	28%	0.4
≥ 500	5	56%	28	65%	0.7
CD4 count (cells/ μ L) at 6 wk postpartum					
Median (IQR)	9	514 (178 to 634)	45	699 (490 to 928)	0.05
≤ 200	2	22%	3	7%	0.2
201 to 499	2	22%	9	20%	1.0
≥ 500	5	56%	33	75%	0.4
Median (IQR) plasma HIV-1 RNA ($\log_{10}$ copies/mL) at 32 wk of gestation	9	5.39 (4.66 to 5.58)	45	4.64 (4.27 to 5.20)	0.05

IQR indicates interquartile range.

TABLE 2

HIV-1 RNA Viral Levels in Breast Milk (Median of the Mean log₁₀ Copies/mL) and Plasma (Median log₁₀ Copies/mL) Over Collection Days Postpartum

Days Postpartum	Transmitters (Median [IQR])	n	Nontransmitters (Median [IQR])	n	P
Breast milk					
0 to 2	2.81 (1.85 to 3.91)	4	2.09 (1.70 to 3.52)	18	0.7
3 to 7	2.96 (1.91 to 4.20)	9	1.94 (1.70 to 2.84)	43	0.04
8 to 14	2.97 (2.62 to 4.53)	9	1.85 (1.70 to 2.33)	45	<0.001
15 to 21	3.99 (3.15 to 4.80)	9	2.17 (1.70 to 3.12)	45	0.002
22 to 28	3.85 (2.75 to 4.39)	9	2.30 (1.70 to 3.39)	44	0.008
29 to 35	4.30 (2.25 to 4.60)	9	1.92 (1.70 to 3.00)	45	0.006
36 to 42	3.65 (1.95 to 4.28)	9	2.04 (1.70 to 2.96)	44	0.03
Plasma					
0 to 14	5.44 (5.12 to 5.90)	9	4.62 (3.74 to 5.07)	45	0.003
~42	5.52 (4.51 to 5.75)	8	4.84 (4.15 to 5.20)	42	0.08

IQR indicates interquartile range.