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Maternal and Breast Milk Viral Load: Impacts of Adherence on Peri-Partum HIV Infections Averted - the BAN Study

Nicole L. Davis, MPH, PhD[1,2,*], **William C. Miller, MD, PhD, MPH**[2], **Michael G. Hudgens, PhD**[3], **Charles S. Chasela, PhD**[4], **Dorothy Sichali, BSc**[5], **Dumbani Kayira, MBBS**[5], **Julie A. E. Nelson, PhD**[6], **Susan A. Fiscus, PhD**[6], **Gerald Tegha, BSc**[5], **Deborah D. Kamwendo, MSc**[5], **Joseph Rigdon, PhD**[3], **Jeffrey S. A. Stringer, MD**[7], **Jonathan J Juliano, MD, MSPH**[2], **Sascha R. Ellington, MSPH**[8], **Athena P. Kourtis, MD, PhD, MPH**[8], **Denise J Jamieson, MD**[8], **Charles Van Der Horst, MD**[2], and **for the Ban study team**

[1]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA

[2]Division of Infectious Diseases, Department of Medicine, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

[3]Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA

[4]Division of Epidemiology and Biostatistics, School of Public Health, University of Witwatersrand, Parktown, South Africa

[5]University of North Carolina, UNC Project, Lilongwe, Malawi

[6]Department of Microbiology and Immunology, School of Medicine; Center for AIDS Research, University of North Carolina, Chapel Hill, NC, USA

[7]Department of Obstetrics & Gynecology, Global Women's Health Division; Institute for Global Health and Infectious Diseases, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

[8]Division of Reproductive Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Abstract

Background—Antiretroviral interventions are used to reduce HIV viral replication and prevent mother-to-child transmission. Viral suppression relies on adherence to antiretrovirals.

Corresponding Author: Nicole L. Davis, Centers for Disease Control and Prevention, 4770 Buford Hwy MS F-74, Atlanta, GA 30341-3717, (fax) 770-488-6291, (phone) 770-488-6385, dwg4@cdc.gov.

*Current affiliation: Division of Reproductive Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Contributions: ND, WM, and MH designed the study. CC, DS, DK, GT, DK, JN, SF, SE, AK, DJ, and CvdH participated in data collection and laboratory testing. ND and JR analyzed the data. ND, WM, MH, JN, SF, JR, JS, JJ, and CvdH interpreted the data. ND wrote the manuscript. All authors reviewed, edited, and approved the final manuscript.

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Methods—A two-phase study was conducted using data from the Breastfeeding, Antiretrovirals and Nutrition study. We included mothers randomized to 28 weeks of postpartum antiretrovirals with ≥1 plasma or breastmilk specimen. All mothers who transmitted HIV to their infants from 2-28 weeks (n=31) and 15% of mothers who did not (n=232) were included. Adherence was measured by pill count [categorized as poor (0-80%), partial (81-98%) and near perfect (>98%)]. Associations between adherence and breastmilk RNA were assessed using mixed effects models. Cox models were used to estimate associations between breastmilk RNA and HIV transmission. Using Monte Carlo simulation, we estimated the number of transmissions that would occur had everyone randomized to maternal ARVs been 90% and 100% adherent.

Results—Partial or near perfect antiretroviral adherence significantly reduced the odds of having detectable (≥40 copies/ml) breastmilk RNA, compared to poor adherence (OR 0.23, 95% CI 0.08-0.67; OR 0.36, 95% CI 0.16-0.81, respectively). Detectable breastmilk RNA was associated with increased breastmilk transmission, compared to undetectable breastmilk RNA (HR 3.8, 95% CI 1.2-12.1). All transmitting mothers had ≥1 plasma viral load specimen >100 copies/ml. An estimated similar number of transmissions would occur with 90% adherence compared with 100%.

Conclusions—Helping patients adhere to antiretrovirals throughout breastfeeding is important for realizing the full potential of recommended antiretroviral interventions to prevent mother-to-child HIV transmission. Maintaining plasma viral load <100 copies/ml may prevent breastmilk transmission.

Keywords

adherence; antiretroviral; breastfeeding; HIV transmission; PMTCT

Introduction

Maternal HIV RNA concentration (viral load or VL) in plasma and breastmilk is among the most important risk factors for HIV transmission during breastfeeding.[1-3] Transmission through breastmilk is thought to be due to two potential mechanisms. First, cell-associated virus or cell-free virions may continually traffic from blood into breastmilk.[4] Second, virus may be transiently produced locally.[4, 5] Both cell-associated and cell-free virus in breastmilk appear to increase HIV transmission. To date, no breastmilk viral threshold has been identified below which breastmilk HIV transmission has not occurred.[6, 7]

Breastmilk transmission is thought to occur when infant ingestion of cell-free or cell-associated virus is absorbed in mucosal gut surfaces, tonsils, or adenoids.[8] To prevent mother-to-child HIV transmission (PMTCT), antiretroviral (ARV) interventions have been used to reduce HIV viral replication in breastmilk and maternal blood. Adherence to ARV regimens is necessary to achieve and maintain therapeutic plasma drug levels, and in turn plasma viral suppression.[9, 10] However, ARV concentrations in breastmilk differ within and between classes of drugs.[11] Nevirapine passes into breastmilk, with breastmilk concentrations 67-90% those of mother's plasma. Zidovudine and lamivudine have higher exposures in breastmilk than maternal plasma.[5, 12]

In this study, we use a comprehensive approach to assess the role of adherence on HIV transmission by assessing the causal pathway that includes both maternal plasma and breastmilk HIV VL. We then compare the estimated number of transmissions that would have occurred from 2-28 weeks postpartum if all mothers randomized to maternal ARVs had been 0%, 90%, and 100% adherent.

Methods

The Breastfeeding, Antiretrovirals and Nutrition (BAN) study, conducted in Lilongwe, Malawi to assess the benefit and safety of maternal or infant ARVs to prevent HIV transmission during breastfeeding, recruited and followed HIV-infected pregnant women from antenatal clinics in 2004-2010. Eligibility criteria for mother-infant pairs included: maternal CD4+ ≥250 cells/μL (≥200 cells/μL before July 24, 2006), no previous ARV drug use (including single dose nevirapine [NVP]), infant birth weight ≥2000 grams, no condition that would preclude use of study drug, and enrollment <36 hours after delivery.[13]

Details of the BAN Study have been reported previously.[14, 15] Briefly, mothers were randomized after birth via factorial design to receive or not receive a lipid-based nutrient supplement throughout breastfeeding and to receive one of the following postpartum PMTCT regimens: 1) 28 weeks of maternal triple ARVs (maternal ARV); 2) 28 weeks of infant NVP; or 3) no further drugs postpartum.[13] All mothers and infants received one dose of NVP at delivery or birth and seven days of postpartum zidovudine and lamivudine.[13] Mothers were counseled to exclusively breastfeed for 24 weeks and wean from 24-28 weeks postpartum.[13] Ethical approval was obtained from the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention.

Study design

Using a cohort study design with two-phase sampling [16], we included all mothers in the maternal ARV or infant NVP arms with ≥1 available plasma or breastmilk sample between 2-24 weeks postpartum and an HIV transmission event between 2-28 weeks postpartum (n=31, 94% of transmission events). We also included 15% of mothers randomized to the two treatment arms of BAN who did not transmit HIV to their infant by 28 weeks postpartum (n=232); sampling was primarily based on stored specimen availability. When multiple births occurred only first-born multiples were included (n=5). Our analysis included a total of 263 mothers at risk of transmitting HIV via breastmilk from 2-28 weeks postpartum.

Adherence measures

Maternal adherence was calculated using pill counts taken by trained pharmacy staff on contiguous visits for the following postpartum time intervals: 2-4, 8-12, and 13-18 weeks. Specifically, adherence was calculated by dividing the difference in the number of pills distributed and returned by the number of pills expected to be consumed under perfect compliance.[17] The observed distribution of maternal pill count adherence was skewed, with most women randomized to maternal ARVs having adherence values greater than 90%. The

observed adherence distribution was anticipated due to mothers' high motivation to adhere to study drug in order to prevent HIV transmission to their infant. Mothers randomized to the infant NVP arm were assigned a maternal adherence value of zero for all intervals. In our main analyses, maternal adherence was categorized as poor (0-80%), partial (81-98%) and near perfect (>98%) using disjoint indicator variables. Categories were chosen based on previous studies and the observed adherence distribution, allowing for a relatively balanced number of observations across three adherence categories (poor, partial, and near perfect adherence). In sensitivity analyses, we treated adherence as a dichotomous variable (>90% versus ≤90%, and >80% versus ≤80%).

HIV RNA and DNA testing

HIV RNA was quantified from blood plasma and whole breastmilk at enrollment, 2, 6, 12, 18, and 24 weeks postpartum. In addition, breastmilk HIV RNA was measured at 4 and 8 weeks postpartum. Plasma VL was quantified using the Abbott RealTime HIV assay (Abbott Molecular, Des Plaines, IL) by the 0.6ml protocol according to the package insert (lower limit of quantitation 40 copies/ml). Breastmilk VL was quantified from 0.6 ml whole breastmilk pre-treated with 209μl Abbott RNA sample prep lysis buffer and 60μl Abbott Proteinase K (53°C incubation for 20 min) using the Abbott RealTime HIV assay (lower limit of quantitation 56 copies/ml). HIV RNA concentrations that were detected but below the limit of quantitation were assigned a value of 39 for plasma and 55 for breastmilk, and RNA concentrations that were undetectable were assigned a value equal to 50% of the lower limit of quantitation (plasma: 20, breastmilk: 28).

Infant HIV status was determined at 2, 12, 28, and 48 weeks by Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA). PCR positive results were confirmed by testing an additional blood specimen. The window of infection was narrowed by testing infant dried blood-spots collected at 4, 6, 8, 18, 24, and 32 weeks. Breastmilk transmission was defined as first detection of HIV infection by PCR in infant blood between 2-28 weeks of age. Infants who tested HIV-1 DNA PCR positive at either birth or by two weeks of age were considered to be infected *in utero* or intrapartum and were excluded from all BAN analyses of breastmilk transmission. Sensitivity of HIV-1 DNA PCR for diagnosing intrapartum HIV transmission has been shown to reach 93% by 14 days of age, thus most perinatal infections will be detected within two weeks of birth.[18]

Data Analyses

We used generalized linear mixed models with a random effect (i.e., random intercept) for each mother-infant pair. Identity link and Gaussian distribution were used to estimate differences in $\log_{10}$ plasma VL by adherence category, and logit link with binomial distribution were used to estimate odds ratios for detectable breastmilk VL by adherence and plasma VL category. Adherence was paired with VL measured at the end of the adherence time interval. Therefore, only VL information from 6, 12, and 18 weeks postpartum was used when adherence was the exposure. The reciprocal of the study inclusion probability was used as a sampling weight in all regression analyses to account for the two-phase sampling.[19]

Single and multivariable Cox models were used to estimate the relative hazard of breastmilk HIV transmission by 28 weeks. Breastmilk VL was treated as a time-varying covariate in Cox models, lagged by one interval. Time until infant HIV infection was right censored at the first occurrence of the following: time of infant death, time of infant's last PCR negative test if lost to follow-up or at 28 weeks if the infant remained HIV-uninfected, or at reported cessation of breastfeeding unless HIV transmission occurred within 30 days of reported cessation.[14]

Effect measure modification was assessed by comparing unadjusted and adjusted estimates and 95% confidence intervals using an interaction term between the exposure and variable of interest. Study arm was identified as an effect measure modifier when assessing the association between breastmilk viral load and HIV transmission. No other effect measure modification was identified. A directed acyclic graph was used to identify potential confounders and a minimally sufficient adjustment set.[20] Potential confounding variables consisted of randomization assignment, demographic characteristics, and health status information collected at enrollment (hereafter referred to as baseline) (Table 1). Missing adherence and VL measures were accounted for using multiple imputation[21] assuming data were missing at random.

Monte Carlo simulation was used to estimate the number of transmissions that would have occurred if all mothers randomized to maternal ARVs (n=848) had been 0%, 90%, and 100% adherent. Data sets with the same sample size as the maternal ARV arm of BAN were simulated under four adherence scenarios. Under the first scenario, longitudinal adherence values were generated by randomly sampling from the observed adherence values for all mothers in the maternal ARV arm. Under the remaining scenarios, all mothers were simulated to be 0%, 90%, and 100% adherent. Simulation results from the first scenario were compared with the actual observed data from BAN to ensure the simulation model was correctly calibrated. For all scenarios, time-varying breastmilk VLs were then randomly generated using the fitted mixed effects logistic regression model from the observed data conditional on simulated adherence values. A transmission time of infant HIV infection was then simulated based on a Cox model with constant baseline hazard and simulated time-varying breastmilk VL. Censoring times were simulated in accordance with the 12% observed dropout in BAN. Finally, the number of infant infections estimated to have occurred with 0%, 90%, and 100% adherence were compared.

All data analyses were conducted using SAS version 9.3 (SAS Institute, Cary, North Carolina, USA) and R version 3.0.2 (R Core Team, Vienna, Austria).

Results

Baseline characteristics of mother-infant pairs included in this two-phase study were comparable to those in the full BAN cohort (Table 1).[13] At baseline, mothers had a median CD4+ count of 408/μL (IQR: 301-544), $\log_{10}$ plasma VL of 4.4 (IQR: 3.8-4.9), and hemoglobin of 10.6 g/dl (IQR: 9.7-11.5).

Overall median $\log_{10}$ plasma VL did not meaningfully differ between transmitting and non-transmitting mothers randomized to infant NVP (4.1 and 4.2 copies/ml, respectively), nor between transmitting mothers randomized to infant NVP and transmitting mothers randomized to maternal ARVs (4.1 and 3.9 copies/ml, respectively) (Table 2). However, overall median $\log_{10}$ plasma VL was two logs less among non-transmitting mothers randomized to maternal ARVs (2.2 copies.ml) compared to the aforementioned.

A greater percentage of transmitting mothers had detectable breastmilk VL compared with non-transmitters at nearly all time points, regardless of study arm (Table 2). However, 85% (540/632) of breastmilk specimens from the maternal ARV arm had undetectable virus, compared to 56% (385/691) for women enrolled in the infant NVP arm. Median $\log_{10}$ breastmilk VL was similar between breastmilk specimens from transmitting and non-transmitting mothers and between breastmilk specimens from mothers randomized to the maternal ARV and infant NVP arms, due in part to the large number of specimens with undetectable virus.

Adherence and HIV viral load

At least one paired maternal adherence and plasma HIV VL was available for 245 (93%) mothers meeting our study inclusion criteria. Among mothers in the maternal ARV arm, overall mean adherence was 88% [median 0.96, IQR 0.86-1.00] at 2-18 weeks postpartum and most received a boosted protease inhibitor regimen of zidovudine, lamivudine, and lopinavir/ritonavir (70%). Based on the unadjusted model, having partial adherence was associated with a 0.82 (95% confidence interval (CI) 0.55-1.09) $\log_{10}$ reduction in plasma VL compared to having poor adherence (Table 3). Having near perfect adherence was associated with a 0.69 (95% CI 0.45-0.93) $\log_{10}$ reduction in plasma VL compared to poor adherence. Adjusting for nutritional randomization and baseline maternal characteristics yielded similar results. Multiple imputation produced similar though attenuated associations between increased adherence and lower plasma VL.

Maternal adherence had an analogous association with suppression of virus in breastmilk. Among 211 (80%) mothers with paired adherence and breastmilk HIV VL data, partial adherence and near perfect adherence were associated with a 76% (95% CI 28-92%) and a 62% (95% CI 14-83%) relative reduction in the odds of having detectable breastmilk VL, respectively, compared to poor adherence (Table 4). Adjustment for potential confounding variables did not appreciably change the estimates or precision. The associations remained similar but less precise when using multiple imputation. Results are not presented for the strata of mothers randomized to infant NVP, as they did not contribute to the >80-98% or >98% adherence categories.

In sensitivity analyses, adherence was treated as a dichotomous variable (>90% versus ≤90%, and >80% versus ≤80%). Choice of adherence categorization did not change our overall conclusions, as higher adherence remained associated with reduced log10 plasma viral load and reduced odds of detectable breastmilk viral load, compared with lower adherence (data not shown).

Plasma and breastmilk HIV viral load

Among the 221 (84%) mothers with ≥1 paired plasma and breastmilk HIV VL, mothers with detectable breastmilk VL typically had detectable plasma VL. Two (<1%) mothers had detectable breastmilk VL (56 and 77 copies/ml) and undetectable plasma VL at 6 weeks postpartum. Mothers with a detectable plasma VL had 59 (95% CI 21-169) times the odds of having a detectable breastmilk VL, compared with mothers with an undetectable plasma VL (Table 4). Adjusting for study arm, baseline maternal CD4+ count, and baseline maternal plasma VL resulted in a slightly attenuated association between detectable plasma and breastmilk VL (OR 40, 95% CI 15-107). Using multiple imputation produced similar though attenuated findings.

Plasma viral load and HIV transmission

At least one plasma VL between 2-24 weeks postpartum was available for 27 of 33 (82%) mothers of HIV-infected infants. Among these, one transmission event occurred from a mother with undetectable baseline plasma VL. However, transmission occurred at 24 weeks postpartum and after three VL measures ranging from 8,000-108,000 copies/ml. The remaining 26 transmitting mothers had baseline plasma VL>3500 copies/ml.

Among 116 mothers with complete plasma VL information, five had undetectable plasma VL at all measured time points between 2-24 weeks. All five were in the maternal ARV arm, also had undetectable breastmilk VL at all measured time points, and none transmitted HIV to their infant. Additionally, plasma VL from the time point immediately preceding transmission was available for 21 mothers, with VL ranging from 102 to >636,000 copies/ml. No mother who consistently maintained a plasma viral load <100 copies/ml transmitted HIV to her infant during breastfeeding.

Breastmilk viral load and HIV transmission

Complete time-dependent breastmilk VL and infant HIV status were available for 134 (51%) mothers. Of these, 15 experienced a HIV transmission event between 2-28 weeks postpartum (maternal ARV arm: 11, infant NVP arm: 4). Among these 15 mothers, 11 (73%) had at least one detectable breastmilk VL before transmission occurred, and 8 (53%) had a detectable breastmilk VL at the last measured time point before transmission.

Using multiply imputed data (n=31 transmission events), detectable breastmilk VL was associated with 3.8 (95% CI 1.2-12.1) times the rate of breastmilk HIV transmission, compared with undetectable breastmilk VL after adjusting for study arm and baseline maternal characteristics (Table 5). Breastmilk VL had a somewhat diminished association with HIV transmission in unadjusted analyses (HR 2.9, 95% CI 1.0-8.4). In complete case analyses, detectable breastmilk VL had a similar unadjusted and increased adjusted association with HIV transmission (unadjusted HR 2.7, 95% CI 1.4-5.4; adjusted HR 7.4, 95% CI 3.2-17.1). However, the adjusted complete case analysis results should be interpreted with caution due to the number of adjustment factors and limited number of transmission events.

Simulating perfect adherence

Using Monte Carlo simulation, the calibration model predicted the same number of HIV transmissions between 2-28 weeks postpartum as observed in the maternal ARV arm of BAN (n=21). If all 848 mothers randomized to maternal ARVs had been 100% adherent, the model predicted 20.4 transmissions (95% prediction interval (PI) 12, 30). In contrast, if all of these mothers had been 0% adherent, the model predicted 42.3 transmissions (95% PI 30, 56), indicating an average of approximately 22 infections averted due to perfect maternal ARV adherence. Simulations where all 848 women were 90% adherent yielded similar results (20.5 transmission, 95% PI 12, 30) to the 100% adherence scenario.

Discussion

We have shown that better adherence is associated with lower breastmilk HIV RNA and lower risk of transmission of HIV to the infant. We also estimated that a similar number of infant infections would have occurred with 90% maternal ARV adherence as estimated to have occurred with 100% maternal ARV adherence. While we were able to detect HIV RNA at lower concentrations than several previous studies, we were unable to identify a breastmilk HIV RNA threshold below which transmission did not occur. However, no transmissions occurred when maternal plasma VL was consistently <100 copies/ml.

Non-adherence to ARVs has been associated with higher plasma HIV VL in pregnant women.[22] Similarly, we found non-adherence to postpartum maternal ARVs to be associated with substantially higher plasma and breastmilk HIV RNA concentration during the breastfeeding period, and we provide the first estimates of the association between maternal ARV adherence and breastmilk HIV VL. In addition, plasma VL has been correlated with breastmilk VL.[1, 22, 23] Using more frequently measured paired plasma and breastmilk specimens, we also found a strong positive association between plasma and breastmilk HIV RNA concentration for mothers randomized to maternal ARVs and mothers randomized to infant NVP.

The adherence levels and breastmilk HIV RNA concentration found in BAN are similar to levels found in previous studies,[7, 23-27] although direct comparisons are difficult due to differences in adherence measurement and viral assays, and the variety of thresholds used to define adherence and detectable VL. Nonetheless, we previously found consistent associations between adherence and breastmilk HIV transmission in BAN using pill count and self-reported adherence.[17]

Detectable breastmilk HIV VL and breastmilk HIV transmission occurred despite apparently perfect (100%) maternal ARV pill count adherence. This observation may be due to imperfection in our adherence measure, length of time between maternal initiation of antiretrovirals and viral suppression, mastitis, antiretroviral drug resistance, low levels of antiretrovirals in breastmilk, or other unidentified processes. In addition, adherence was held constant during the interval, and therefore may not reflect true adherence at the time immediately preceding the VL measure. Breastmilk HIV transmission also occurred despite undetectable breastmilk HIV VL, possibly due to periods of detectable breastmilk VL at

unmeasured time points, or persistent cell-associated HIV viral reservoirs in breastmilk not eliminated with triple ARVs. [28-31]

Detectable breastmilk HIV VL was associated with HIV transmission among mothers randomized to maternal antiretrovirals. Among mothers randomized to infant NVP, detectable breastmilk HIV VL also appeared to be associated with breastmilk HIV transmission, though the small number of transmission events limited our ability to make conclusions by study arm.

Slightly stronger associations between adherence and both plasma and breastmilk VL were estimated in the partial adherence group, compared to the near perfect adherence group. This paradoxical result may be due to adherence measurement error, including the possibility that partial adherers report more accurate pill returns whereas perfect adherers may return only the number of pills expected. However, both adherence categories were associated with reduced plasma and breastmilk HIV RNA concentration.

The limited number of available paired adherence and VL measures among mothers in the maternal ARV arm affected the precision of our estimates, and limited our ability to make conclusions by ARV study arm. Missing breastmilk viral load data were predominantly due to BAN Study protocol amendments which reduced the frequency of breastmilk collection in order to decrease staff workload and save costs. Complete description of BAN Study protocol amendments and their rationale have been previously published.[32] Missing adherence and VL measures were multiply imputed. Similar, though often attenuated, associations were seen for all contrasts when multiply imputed data were used. No substantial gains in precision were seen when using multiply imputed data, potentially due to the number of outcomes that had to be imputed and the lack of strong auxiliary variables.[33] However, mixed effects models are consistent under the missing at random assumption, increasing the confidence in our analogous findings using both complete case and multiply imputed data.

As we have previously shown, antiretroviral adherence needs to be maintained to maximize PMTCT efforts and increase infant HIV-free survival.[17] However, we now more conclusively show that maintaining antiretroviral adherence alone is unlikely to prevent all breastmilk transmission in the first 28 weeks postpartum if triple ARVs are initiated after delivery. The sufficient set of mechanisms that cause breastmilk HIV transmission remain incompletely determined. Provision of lifelong maternal ARV therapy to all pregnant and breastfeeding women, with currently available ARVs, will likely have to be implemented in concert with other prevention interventions to eliminate breastmilk HIV transmission.[34]

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Table 1
Baseline characteristics of 263 mother-infant pairs

	Total*	
	N	(%)
Antiretroviral randomization		
Maternal antiretroviral	129	(49)
Infant nevirapine	134	(51)
Nutritional randomization		
No supplement	125	(48)
Received supplement	138	(52)
Mothers:		
Age (years)		
15-25	144	(55)
26-35	105	(40)
36-45	13	(5)
Education (primary school only)	173	(66)
Married	242	(92)
Parity ≥1	227	(86)
CD4+ count per mm3		
200-350	95	(36)
351-500	81	(31)
>500	87	(33)
Plasma viral load copies/ml		
≤ 1,000	20	(8)
1,001-10,000	60	(23)
> 10,000	181	(69)
Hemoglobin <11 g/dl	160	(61)
Antiretroviral regimen**		
Nevirapine based	5	(4)
Nelfinavir based	29	(26)
Lopinavir/ritonavir based	78	(70)
Infants:		
Sex (female)	139	(53)
Birth weight <2.5 kg	27	(10)

*Maternal age is missing for 1 mother, baseline plasma viral load is missing for 2 mothers, and antiretroviral (ARV) regimen is missing for 17 mothers assigned to maternal ARVs

**Among mothers randomized to maternal ARV arm

Table 2

Percentage of mothers with detectable maternal HIV RNA by study arm, week postpartum, and infant's 28 week HIV-infection status.*

	Maternal ARV arm						Infant NVP arm					
	Total mothers tested	Detectable plasma HIV RNA		Total mothers tested	Detectable breastmilk HIV RNA		Total mothers tested	Detectable plasma HIV RNA		Total mothers tested	Detectable breastmilk HIV RNA	
	N	n	(%)	N	n	(%)	N	n	(%)	N	n	(%)
HIV-transmitting mothers												
2 weeks	8	7	(88)	13	2	(15)	6	6	(100)	6	1	(17)
4 weeks	--	--	--	11	3	(27)	--	--	--	6	3	(50)
6 weeks	15	15	(100)	9	3	(33)	8	8	(100)	5	3	(60)
8 weeks	--	--	--	8	2	(25)	--	--	--	6	4	(67)
12 weeks	12	11	(92)	7	3	(43)	8	8	(100)	6	5	(83)
18 weeks	4	4	(100)	5	3	(60)	1	1	(100)	4	2	(50)
24 weeks	9	8	(89)	3	1	(33)	8	8	(100)	3	2	(67)
Overall median log_{10} copies/ml (IQR)	3.9 (2.3-4.7)			1.7 (1.7-2.1)			4.1 (3.4-4.8)			1.9 (1.7-2.7)		
Non-transmitting mothers												
2 weeks	74	63	(85)	90	14	(16)	82	78	(95)	101	15	(15)
4 weeks	--	--	--	87	15	(17)	--	--	--	106	38	(36)
6 weeks	97	71	(73)	89	9	(10)	111	108	(97)	96	50	(52)
8 weeks	--	--	--	91	7	(8)	--	--	--	93	53	(57)
12 weeks	93	51	(55)	85	12	(14)	105	105	(100)	92	45	(49)
18 weeks	55	32	(58)	72	9	(13)	70	70	(100)	89	45	(51)
24 weeks	71	39	(55)	62	9	(15)	92	92	(100)	78	40	(51)
Overall median log_{10} copies/ml (IQR)	2.2 (1.6-3.2)			1.7 (1.7-1.7)			4.2 (3.2-4.9)			1.7 (1.7-2.4)		

*Plasma specimens were not collected at week 4 or week 8. Plasma HIV RNA concentrations ≥40 copies/ml and breastmilk HIV RNA concentrations ≥56 copies/ml were considered detectable

Table 3

Mean difference in log_{10} maternal plasma HIV RNA by adherence category

	Complete case				Imputed Unadjusted			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	Mean diff**	(95% CI)	Mean diff**	(95% CI)	Mean diff**	(95% CI)	Mean diff**	(95% CI)
Maternal and Infant Arm								
81-98% adherent vs 0-80%	-0.82	(-1.09, -0.55)	-0.93	(-1.19, -0.68)	-0.45	(-0.76, -0.15)	-0.52	(-0.82, -0.22)
>98% adherent vs 0-80%	-0.69	(-0.93, -0.45)	-0.75	(-0.98, -0.52)	-0.37	(-0.65, -0.08)	-0.42	(-0.70, -0.15)
Maternal arm only†								
81-98% adherent vs 0-80%	-0.07	(-0.45, 0.30)	-0.08	(-0.45, 0.29)	0.01	(-0.31, 0.33)	0.01	(-0.31, 0.33)
>98% adherent vs 0-80%	0.09	(-0.25, 0.44)	0.10	(-0.24, 0.44)	0.11	(-0.19, 0.41)	0.12	(-0.18, 0.41)

*Adjusted for: baseline maternal age, baseline maternal CD4+ count, baseline log_{10} plasma viral load, and nutrition randomization

**Mean difference in log_{10} maternal plasma viral load by adherence category (referent: 0-80% adherence), based on mixed linear regression with log_{10} plasma viral load as outcome

†In complete case analyses, a total of 47, 89, and 117 pill count adherence measures were in the 0-80%, 81-98%, and >98% adherence categories, respectively

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Table 4

Odds ratios for adherence and detectable (≥40 copies/ml) plasma HIV RNA as a risk factor for detectable (≥56 copies/ml) breastmilk HIV RNA

	Complete case				Imputed			
	Unadjusted		Adjusted		Unadjusted		Adjusted	
	Odds Ratio	(95% CI)	Odds Ratio	(95% CI)	Odds Ratio	(95% CI)	Odds Ratio	(95% CI)
Exposure: Adherence[†]								
Maternal and Infant Arm								
>80-98% adherent vs 0-80%	0.24	(0.08, 0.72)	0.23[*]	(0.08, 0.67)	0.30	(0.07, 1.30)	0.29[*]	(0.07, 1.19)
>98% adherent vs 0-80%	0.38	(0.17, 0.86)	0.36[*]	(0.16, 0.81)	0.42	(0.10, 1.83)	0.41[*]	(0.10, 1.69)
Maternal arm only								
>80-98% adherent vs 0-80%	0.47	(0.16, 1.41)	0.47[*]	(0.15, 1.40)	0.50	(0.11, 2.21)	0.50[*]	(0.11, 2.21)
>98% adherent vs 0-80%	0.64	(0.28, 1.43)	0.64[*]	(0.29, 1.43)	0.67	(0.15, 2.96)	0.68[*]	(0.15, 3.00)
Exposure: Plasma HIV RNA[‡]								
Maternal and Infant Arm								
Detectable vs. Undetectable	59	(21, 169)	40[**]	(15, 107)	43	(8, 236)	35[**]	(7, 169)
Maternal arm only								
Detectable vs. Undetectable	45	(16, 124)	40[**]	(15, 108)	41	(9, 185)	38[**]	(9, 168)

[*] Adjusted for: baseline maternal age, CD4+ count and $\log_{10}$ plasma viral load, as well as nutritional randomization.

[**] Adjusted for: baseline maternal CD4+ and baseline maternal plasma viral load. Study arm was included as a confounding variable in combined maternal and infant arm models, but was not included in maternal arm only models.

[†] In complete case analysis, a total of 516 specimens from both the maternal and infant arms were tested and 183 specimens had detectable breastmilk HIV RNA. Mothers randomized to infant NVP were assigned an adherence value of zero. For the maternal arm, a total of 224 specimens were tested; of these, 78 specimens were in the >80-98% adherent category, 102 specimens were in the >98% adherent category, and 33 specimens had detectable breastmilk HIV RNA (9 from the >80-98% adherent category and 19 from the >98% adherent category).

[‡] In complete case analysis, a total of 797 specimens from both the maternal and infant arms were tested; of these, 663 specimens had detectable plasma HIV RNA, 238 specimens had detectable breastmilk HIV RNA, and 236 specimens had both detectable plasma and detectable breastmilk HIV RNA. For the maternal arm only, a total of 382 specimens were tested; of these, 255 specimens had detectable plasma HIV RNA, 57 specimens had detectable breastmilk HIV RNA, and 55 specimens had both detectable plasma and detectable breastmilk HIV RNA.

Table 5

Hazard ratios for detectable (>56 copies/ml) breastmilk HIV RNA load as a risk factor for breastmilk HIV-1 transmission

	Complete Case				Imputed			
	Unadjusted*		Adjusted**		Unadjusted*		Adjusted**	
	Hazard Ratio	(95% CI)	Hazard Ratio	(95% CI)	Hazard Ratio	(95% CI)	Hazard Ratio	(95% CI)
Maternal and Infant Arm								
Detectable vs. Undetectable	2.7	(1.4, 5.4)	7.4	(3.2, 17.1)	2.9	(1.0, 8.4)	3.8	(1.2, 12.1)
Viral load by study arm								
Maternal ARV: Detectable vs. Undetectable	5.6	(2.5, 12.7)	7.8	(3.1, 19.2)	4.8	(1.5, 15.8)	4.4	(1.3, 15.2)
Infant NVP: Detectable vs. Undetectable	3.9	(0.8, 18.0)	6.0	(1.2, 31.4)	2.8	(0.5, 15.0)	2.9	(0.5, 17.7)

*Combined maternal and infant arm models contained no confounding variables. Viral load by study arm models contained study arm and an interaction term between breastmilk viral load and study arm.

**Adjusted for continuous baseline maternal age, baseline maternal CD4+, baseline maternal hemoglobin level, and baseline maternal plasma viral load. Study arm was included as a confounding variable in combined maternal and infant arm models. Viral load by study arm models included an interaction term between breastmilk viral load and study arm.