Correlates of Mother-to-Child Human Immunodeficiency Virus Type 1 (HIV-1) Transmission: Association with Maternal Plasma HIV-1 RNA Load, Genital HIV-1 DNA Shedding, and Breast Infections

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To determine the effects of plasma, genital, and breast milk human immunodeficiency virus type 1 (HIV-1) and breast infections on perinatal HIV-1 transmission, a nested case-control study was conducted within a randomized clinical trial of breast-feeding and formula feeding among HIV-1–seropositive mothers in Nairobi, Kenya. In analyses comparing 92 infected infants with 187 infants who were uninfected at 2 years, maternal viral RNA levels >43,000 copies/mL (cohort median) were associated with a 4-fold increase in risk of transmission (95% confidence interval [CI], 2.2–7.2). Maternal cervical HIV-1 DNA (odds ratio [OR], 2.4; 95% CI, 1.3–4.4), vaginal HIV-1 DNA (OR, 2.3; 95% CI, 1.1–4.7), and cervical or vaginal ulcers (OR, 2.7; 95% CI, 1.2–5.8) were significantly associated with infant infection, independent of plasma virus load. Breast-feeding (OR, 1.7; 95% CI, 1.0–2.9) and mastitis (relative risk [RR], 3.9; 95% CI, 1.2–12.7) were associated with increased transmission overall, and mastitis (RR, 21.8; 95% CI, 2.3–211.0) and breast abscess (RR, 51.6; 95% CI, 4.7–571.0) were associated with late transmission (occurring >2 months postpartum). Use of methods that decrease infant exposure to HIV-1 in maternal genital secretions or breast milk may enhance currently recommended perinatal HIV-1 interventions.

The majority of infant human immunodeficiency virus type 1 (HIV-1) infection can be prevented by antiretroviral therapy, cesarean section, and formula feeding, but these interventions are difficult to implement in regions of the world with the highest prevalence of maternal HIV-1 infection [1–3]. All 3 interventions require an established infrastructure, to identify HIV-1–infected pregnant women. In addition, the cost of these interventions exceeds the public health capacity of many resource-poor countries. While efforts are under way to improve access to these interventions, it remains important to develop more globally feasible intervention strategies for prevention of infant HIV-1 infection.

To develop new intervention strategies, it is important to understand the pathogenesis of mother-to-child transmission of HIV-1. Studies in the United States and Thailand have shown that maternal plasma HIV-1 RNA levels are highly correlated with the risk of infant HIV-1 infection [4–7]. Maternal genital HIV-1 RNA burden was also found to be associated with increased perinatal HIV-1 transmission in a study conducted in Thailand [7]. The effects of maternal systemic and genital HIV-1 burden on mother-to-child HIV-1 transmission are less well-defined for African populations and may differ from those seen in other settings, because of differences in viral subtype, prevalence of sexually transmitted diseases (STDs), maternal nutritional and immune status, and breast-feeding practices.

We conducted a prospective randomized clinical trial of breast-feeding and formula feeding in Kenya. In this study, the risk of HIV-1 transmission was 36.7% among women randomized to breast-feed and 21% among those randomized to formula feed, and the majority of breast milk HIV-1 transmission was observed in the first 6 months of life [8]. Within this study, we studied correlates of mother-to-child HIV-1 transmission, specifically evaluating the effect of plasma, genital, and breast milk HIV-1, antenatal STDs, breast-feeding, and breast infections on perinatal HIV-1 transmission.

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Informed consent was obtained from all study participants, and human experimentation guidelines of the US Department of Health and Human Services were followed. The study was approved by the institutional review boards of the University of Washington and the University of Nairobi.

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Methods

Clinical Methods

The enrollment of women, counseling and randomization methods, follow-up of infants, and specimen collection schedule in this clinical trial have been described elsewhere [8]. In brief, after giving informed consent, HIV-1–seropositive women were enrolled in the study during pregnancy and were randomized to breast-feeding or formula feeding at ~32 weeks' gestational age. At 32 weeks' gestation, blood specimens were obtained for plasma virus load and T cell subsets. Pelvic speculum examination was conducted for collection of cervical and vaginal specimens for HIV-1 DNA polymerase chain reaction (PCR) assays and screening for genital infections. Cervical and vaginal specimens for HIV-1 DNA were collected by using sterile Dacron swabs, as described elsewhere [9]. STDs (gonorrhea, chlamydiosis, or trichomoniasis) or genital infections (candidiasis or bacterial vaginosis) diagnosed at this screening visit were treated according to standard Kenyan Ministry of Health STD treatment guidelines for pregnant women. None of the women participating in the study received antiretrovirals. Women and infants in the cohort were followed up after delivery for 2 years. Infant follow-up visits were conducted monthly in the first year of life and every 3 months in the second year. Infant visits included an interim history and physical examination. At each visit, all women were asked about breast-feeding during the past month. Infants of women reporting any breast-feeding at any time during follow-up were considered to have breast milk exposure. Blood specimens were obtained from infants at birth, 6 weeks, 14 weeks, and every 3 months thereafter. Breast disease (cracked nipples, bleeding nipples, mastitis, or breast abscess) was assessed by history and physical examination at every maternal postpartum visit, and breast milk samples were obtained from breast-feeding mothers at 3-month intervals. Blood was collected for HIV-1 serology from spouses who requested testing.

Laboratory Methods

Microbiology. *Neisseria gonorrhoeae* was detected by culture, *Chlamydia trachomatis* by antigen testing (Clearview; Unipath or Microtrak; Syva), bacterial vaginosis by Gram stain score [10], and candida and *Trichomonas vaginalis* infection by potassium hydroxide and wet preparation, respectively. Serologic testing for syphilis was conducted by using the rapid plasma reagin test (Becton Dickinson).

HIV-1 detection and quantitation. HIV-1 seropositivity was diagnosed on the basis of 2 separate ELISA tests (Behring and Cambridge Biotech). The PCR techniques used for HIV-1 DNA detection in breast milk and genital specimens, with a sensitivity for detection of HIV-1 *gag* DNA of 99.2% and a specificity of 99.7%, have been described elsewhere [9, 11, 12]. Maternal quantitative plasma viral HIV-1 RNA levels were determined using a quantitative HIV-1 assay that is sensitive for all subtypes in Kenya (Gen-Probe) [13]. HIV-1 DNA *gag* sequences in infant peripheral blood monocytes or filter paper were detected by using PCR assays [8, 14].

Infant HIV-1 infection status. Infant HIV-1 infection status was defined by using PCR and ELISA testing [8]. In this study, the sensitivity of PCR testing for a single infant specimen was 95% and the specificity 98% [8]. Infants were determined to be infected (1) if PCR testing of blood samples obtained on 2 consecutive dates yielded positive results for HIV-1 DNA; (2) if a single PCR test result was positive, if the sample was obtained at the last visit the child was seen; or (3) if an ELISA test result at $\geq$15 months of age was positive and PCR results were unavailable for that date. Infants were determined to be uninfected at 24 months if none of the criteria for infection were met and PCR testing of a blood specimen collected at $\geq$24 months of age was negative for HIV-1 DNA or if an HIV-1 ELISA at $\geq$24 months of age was negative (and there was no PCR assay result for that date).

Vitamin A assays. Vitamin A levels were measured using high-performance liquid chromatography.

Statistical Methods

Classification of timing of infection. The sensitivity of HIV-1 PCR assays for detection of HIV-1 infection in non–breast-feeding infants is estimated to be 93% by 2 weeks of age [15]. Thus, infants in our study who had a negative PCR assay at $\geq$2 months of age with a subsequent positive PCR analysis almost certainly had acquired HIV-1 infection via breast-feeding rather than through in utero or intrapartum infection. Hence, infants in the study whose first positive PCR test occurred before 2 months of age were considered to have been infected early (either in utero, intrapartum, or through early breast milk exposure). Infants testing PCR negative at $\geq$2 months of age with a subsequent positive PCR or a positive ELISA at $\geq$15 months of age were considered to have acquired infection late postnatally (through breast-feeding). Infants who first tested positive for HIV-1 after 2 months of age and who had no negative tests at or after 2 months were considered indeterminate for timing of infection and were excluded from the analyses of early and late transmission.

Correlates of transmission. Analyses were conducted using SPSS Version 8.0 (SPSS). Exposures of potential interest were compared between infected and uninfected infants, using Pearson's χ^2 and Fisher's exact tests for dichotomous variables and Mann-Whitney *U* tests for continuous variables. To define correlates of early transmission, infants infected before 2 months of age were compared with infants uninfected at 2 years of age. A similar analysis was conducted for infants infected at $\geq$2 months of age, to define correlates of late postnatal transmission. Breast milk HIV-1 DNA analyses were based on 1 sample per woman, collected in the first 3 months postpartum. Cox proportional hazards regression with time dependent covariates was used to evaluate the effects of maternal breast pathologies (cracked nipples, bleeding nipples, mastitis, and breast abscess) and infant thrush on late infant HIV-1 infection among the subset of women reporting breast-feeding. Median duration of breast-feeding among breast-fed infants was assessed by using Kaplan-Meier analysis, and differences in age at cessation of breast-feeding between infected and uninfected infants was compared, using the log rank test.

Effect on transmission was measured as relative risk (RR) for time-dependent covariates and as an odds ratio (OR) for fixed covariates. Multivariate logistic regression models or proportional hazard models were used to determine whether associations persisted after adjustment for plasma virus load. Because of the colinearity of several variables significant in univariate analyses (vaginal HIV-1 DNA, cervical HIV-1 DNA, CD4 count, and plasma HIV-1), we did not create models adjusting for all cofactors si-

multaneously. Rather, we adjusted only for maternal plasma virus load, which has been shown in other studies to be the most important independent predictor of mother-to-child transmission of HIV-1 [4–7].

Results

Of 401 liveborn infants, 384 had follow-up, of whom 92 were determined to be HIV-1 infected and 187 uninfected at 2 years of age. The remaining 105 were HIV-1 uninfected at the time of last determination but did not have an HIV-1 status end point at 2 years. Analyses for correlates of transmission were restricted to the 279 mother/infant pairs with a defined HIV-1 infection status end point. All the measured variables of the 279 mother/infant pairs included in the correlates analysis were similar to those of the mother/infant pairs excluded from the analysis. Among the 92 infected infants, 53 were determined to have acquired infection early (<2 months of age) and 27 to have acquired infection late (≥2 months). For the remaining 12 infants, timing of infection could not be ascertained, because their first positive PCR assay occurred after 2 months of age without a previous negative PCR.

Characteristics of the cohort. Among women included in this analysis, the mean age was 23 years (table 1). Most women (80%) had no history of HIV-1–related symptoms (fever, cough, or diarrhea >1 month's duration, itchy rash, or weight loss >5 kg). The median CD4 count was 416 cells/mm^3, with 29 (11%) women having absolute CD4 counts <200 cells/mm^3. Among 251 women with plasma HIV-1 RNA load quantified at 32 weeks' gestation, the median virus load was 43,185 copies/mL (range, 112–1,228,475).

At ~32 weeks' gestational age, 13% of women had cervical or vaginal ulcers, 6% gonorrhea, 11% chlamydiosis, 24% trichomoniasis, and 7% syphilis. Bacterial vaginosis was diagnosed in 50% and vaginal candidiasis in 31% of women. Cervical HIV-1 DNA was detected in 98 (39%) and vaginal HIV-1 DNA in 44 (17%) of 253 women.

Ninety-two percent of deliveries were vaginal. Cesarean deliveries were conducted for emergency indications rather than on an elective basis. Of 189 infants on whom Dubowitz maturity scoring was conducted, 7 (4%) were premature (<37 weeks) [16].

Breast-feeding was reported by 175 women (63%). The median duration of breast-feeding was 17 months (range, 3 days to 31 months). Thirty-one (18%) of 170 breast-feeding women had cracked nipples, 18 (11%) had bleeding nipples, 18 (11%) had mastitis, and 20 (12%) had a breast abscess, by history or examination, at least once during the course of follow-up. Breast milk specimens collected within 3 months postpartum from 141 breast-feeding mothers were evaluated for HIV-1 DNA. One hundred nineteen women (81%) had detectable HIV-1–infected cells in their first specimen. The median concentration of HIV-1–infected cells was 1 per 10^4 breast milk cells (range, 0–367). Fifty-two (41%) women had a high concentration of HIV-1–infected cells (≥10 infected cells/10^4 breast milk cells).

Correlates of HIV-1 infection among infants on univariate analysis. Mothers of infected infants had significantly lower absolute CD4 cell counts (384 vs. 454 cells/mm^3; $P < .0001$) and higher plasma HIV RNA levels (88,965 vs. 29,512 copies/mL; $P < .0001$) than mothers of uninfected infants (table 1). The transmission risk among women who had virus loads in the lowest quartile (<10,000 copies/mL) was 9%, compared with 45% among women with virus loads above the median (>43,000 copies/mL). Women with virus loads above the median had a 4-fold higher risk of transmission (95% confidence interval [CI], 2.2–7.2). Although maternal virus load strongly predicted transmission, the relationship was not absolute. One woman with a plasma virus load >1 million copies/mL did not transmit infection, whereas 3 with <5000 copies/mL transmitted infection.

Infant HIV-1 infection was significantly associated with the presence of HIV-1 DNA in maternal cervical (OR, 2.7; 95% CI, 1.6–4.7) and vaginal (OR, 2.3; 95% CI, 1.2–4.4) secretions during pregnancy. Vaginal or cervical ulcers during pregnancy were also associated with increased transmission risk (OR, 2.3; 95% CI, 1.2–4.8), and there was a trend for an association with gonorrhea ($P = .1$).

Infant HIV-1 infection was significantly associated with prematurity, as determined by Dubowitz scoring (OR, 16.0; 95% CI, 1.9–134.9).

Randomization to breast-feeding (OR, 1.8; 95% CI, 1.3–2.6) and any reported exposure to breast milk were associated with infant HIV-1 infection (OR, 1.7; 95% CI, 1.0–2.9). Among breast-feeding mothers, mastitis was associated with a significantly increased transmission risk (RR, 3.9; 95% CI, 1.2–12.7). There was a trend for a higher median concentration of HIV-1–infected cells in breast milk among mothers of infected infants than among mothers of uninfected children (9.3 vs. 1.1 infected cells/10^4 cells; $P = .09$).

Multivariate analyses of correlates of infant HIV-1 infection. In multivariate models, cervical shedding of HIV-1 DNA, vaginal shedding of HIV-1 DNA, cervical or vaginal ulcers, prematurity, breast-feeding, and mastitis remained significantly associated with increased risk of infant HIV-1 infection, after adjusting for plasma virus load (table 2).

Correlates of early infant infection. Infants who were infected by 2 months of age were compared with uninfected infants in a second analysis (table 3). Seven correlates of overall transmission (cervical HIV-1 DNA, vaginal HIV-1 DNA, cervical or vaginal ulcers, prematurity, breast-feeding, plasma virus load, and immunosuppression) were significantly associated with early transmission. In multivariate analyses, cervical HIV-1 shedding, vaginal HIV-1 shedding, cervical or vaginal ulcers, prematurity, and breast milk exposure remained significantly associated with early infant infection, after adjusting for plasma

virus load (data not shown). Bleeding nipples were also associated with early infant HIV-1 infection.

Correlates of late infant infection. Although detection of virus before 2 months of age could result from infection occurring in utero, at delivery, or through breast-feeding, infants who were infected at ⩾2 months of life were most likely infected through breast-feeding. Late infant infection was associated with increased maternal plasma viral RNA levels, mastitis (RR, 21.8; 95% CI, 2.3–211.0), and breast abscess (RR, 51.6; 95% CI, 4.7–571.0) (table 3). Mastitis (RR, 47.3; 95% CI, 4.2–530.2) and breast abscess (RR, 42.7; 95% CI, 3.7–488.9) remained associated with late transmission after adjustment for plasma virus load in multivariate analyses. Women with a high concentration of HIV-1–infected breast milk cells tended to have higher risk of postnatal transmission than women without detectable breast milk HIV-1 (20% vs. 10%; $P = .09$).

Discussion

We have identified several factors associated with perinatal HIV-1 transmission in this Kenyan cohort that may provide insight into the mechanism of transmission. We observed a highly significant relationship between cervical or vaginal shedding of HIV-1 provirus and infant infection, and this relationship was independent of maternal plasma virus load. Although there is evidence that the majority of HIV-1 transmission among non–breast-feeding infants occurs close to the time of delivery, it is unknown whether this transmission is parenteral (maternal blood contact with infant blood secondary to breaches in the maternal-infant blood barrier) or mucosal (infant conjunctival, oral, or nasopharyngeal exposure to infected maternal genital secretions) [17]. Previous studies, demonstrating that risk of perinatal HIV-1 transmission is increased in vaginal as com-

Table 1. Correlates of infant HIV-1 infection.

Correlate	Infected ($n = 92$)[a]	Uninfected ($n = 187$)[a]	Odds ratio (95% CI)	P
Sociodemographic and clinical variables				
Age, years	23 (17–40)	23 (16–38)	—	.7
Spouse HIV-1 infected	24/42 (57)	44/65 (68)	0.6 (0.3–1.4)	.3
HIV-related symptoms[b]	15/89 (17)	39/185 (21)	0.8 (0.4–1.5)	.4
Physical examination findings				
Genital warts	14/90 (16)	23/184 (13)	1.3 (0.6–2.7)	.4
Cervical or vaginal ulcers	18/90 (20)	18/184 (10)	2.3 (1.2–4.8)	.02
Vulvar ulcers	3/89 (3)	6/184 (3)	1.0 (0.3–4.2)	1.0
Vaginal discharge	45/89 (51)	95/184 (52)	1.0 (0.6–1.6)	.9
Cervical mucopus	31/88 (35)	50/183 (27)	1.4 (0.8–2.5)	.2
Genital infections				
Gonorrhea	8/89 (9)	8/178 (5)	2.1 (0.8–5.8)	.1
Chlamydiosis	9/86 (11)	19/178 (11)	1.0 (0.4–2.3)	1.0
Trichomoniasis	13/45 (29)	18/83 (22)	1.5 (0.6–3.4)	.4
Bacterial vaginosis	37/74 (50)	86/171 (50)	1.0 (0.6–1.7)	1.0
Candida	27/86 (31)	52/173 (30)	1.1 (0.6–1.9)	.8
Syphilis	8/91 (9)	11/183 (6)	1.5 (0.6–3.9)	.4
Laboratory findings				
Hemoglobin, g/dL	10.8 (6.5–18)	10.9 (6.4–15.5)	—	.9
CD4 count, cells/mm^3	384 (15–881)	454 (46–1207)	—	<.0001
Vitamin A, g/dL	26 (6–59)	27 (8–44)	—	.4
Severe vitamin A deficiency (<20 g/dL)	21/68 (31)	29/132 (22)	1.6 (0.8–3.1)	.2
Viral markers				
Plasma viral RNA levels, copies/mL	88,965 (850–1,228,475)	29,512 (112–1,116,725)	—	<.0001
Plasma virus load greater than median	58/79 (73)	70/172 (41)	4.0 (2.2–7.2)	<.0001
Cervical HIV-1 DNA	45/82 (55)	53/171 (31)	2.7 (1.6–4.7)	<.0001
Vaginal HIV-1 DNA	21/82 (26)	23/174 (13)	2.3 (1.2–4.4)	.01
Breast milk HIV-1 DNA[c]	42/53 (79)	72/88 (82)	0.9 (0.4–2.0)	.7
Labor and delivery				
Prolonged ruptured membranes (⩾4 h)	37/91 (41)	60/182 (33)	1.4 (0.8–2.3)	.2
Emergency cesarean section	10/92 (11)	11/187 (6)	1.9 (0.8–4.8)	.1
Low birth weight (<2.5 kg)	8/86 (9)	9/173 (5)	1.9 (0.7–5.6)	.2
Maturity by Dubowitz scoring, weeks	40 (32–43)	40 (32–43)	—	.007
Prematurity (<37 weeks)	6/56 (11)	1/133 (1)	16.0 (19–134.9)	.003
Breast-feeding				
Randomized to breast-feed	61/92 (66)	84/187 (45)	1.8 (1.3–2.6)	.001
Breast-fed ever (reported)	65/92 (71)	110/187 (59)	1.7 (1.0–2.9)	.06
Duration, months[c]	18 (0.2 to >24)	16 (0.1 to >24)	—	.08

NOTE. CI, confidence interval; HIV-1, human immunodeficiency virus type 1.

[a] Values are median (range) or proportion (%).

[b] HIV-related symptoms include fever, cough, or diarrhea of >1 month's duration, itchy rash, or weight loss >5 kg.

[c] Among breast-feeders.

Table 2. Multivariate analyses of risk factors for infant HIV-1 infection (adjusting for maternal plasma viral RNA levels).

Variable	Adjusted odds ratio (95% CI)	*P*
Cervical HIV-1 DNA	2.4 (1.3–4.4)	.004
Vaginal HIV-1 DNA	2.3 (1.1–4.7)	.03
Cervical or vaginal ulcers	2.7 (1.2–5.8)	.01
Prematurity	15.6 (1.8–139.0)	.01
Reported exposure to breast milk	1.8 (1.0–3.3)	.04
Mastitis	3.4 (1.0–11.4)[a]	.05
CD4 count <200 cells/mm^3	1.9 (0.8–4.9)	.15

NOTE. CI, confidence interval; HIV-1, human immunodeficiency virus type 1.

[a] The value for mastitis indicates relative risk rather than odds ratio.

pared with elective cesarean deliveries, following prolonged exposure to ruptured membranes, and among first-born twins, suggest that exposure to infected secretions in the birth canal influences transmission [2, 18, 19]. In a randomized clinical trial conducted in Malawi, use of vaginal washes with chlorhexidine during labor and delivery resulted in decreased HIV-1 transmission to infants among women with prolonged ruptured membranes, which also suggests that exposure to maternal genital HIV-1 is an important determinant of infection [20]. Our observation suggests that infant exposure to HIV-1–infected cells in maternal genital secretions is a critical factor in intrapartum transmission and complements recent studies demonstrating that perinatal HIV-1 transmission is associated with genital HIV-1 RNA levels [7].

This finding has implications for the design of new perinatal HIV-1 intervention strategies. It will be important to determine the effect of short-course antiretroviral drugs on genital HIV-1 infection, specifically to determine whether a minimum treatment period is required for reduction of genital HIV-1 shedding. This information will be useful for women who present to antenatal care late in pregnancy. Topical antiseptic treatment during pregnancy or delivery is a safe, inexpensive, and easily implemented intervention that does not require antenatal HIV-1 screening and lacks the systemic side effects of antiretroviral therapy. Unfortunately, to date, intrapartum administration of topical antiseptics has not been effective in decreasing transmission of HIV-1 to infants. It is possible that alternative regimens of topically applied antiseptics or antivirals may have broader efficacy than that seen in the Malawi study [20]. Topical therapies may need to be initiated several days before delivery, to clear the genital passage of HIV-1. Ideally, new regimens should be evaluated for their effect on genital HIV-1 before the initiation of large-scale clinical trials.

Maternal genital ulcer disease, most likely due to herpes infection, was associated with significantly increased risk of infant HIV-1 infection. Despite antenatal treatment of STDs, maternal infection with gonorrhea at 32 weeks' gestation was also associated with a trend for increased infection. We did not observe an association between infant HIV-1 infection and other STDs or genital infections, including syphilis, chlamydiosis, bacterial vaginosis, candidiasis, or trichomoniasis. Our ability to detect an effect of these infections was limited, because all diagnosed infections were treated during pregnancy. In addition, trichomoniasis results were available only for a subset of women, and our study had insufficient power to definitively evaluate this STD. In several studies, STDs and genital ulcers have been associated with genital shedding and sexual transmission of HIV-1 [21–24]. We observed significantly increased perinatal HIV-1 transmission among women with genital ulcer disease. In many settings, screening for STDs and genital infections is not a routine part of antenatal care of HIV-1–infected women. Our study suggests that antenatal STD screening and treatment or empiric STD prophylaxis should be further explored as potential perinatal HIV-1 interventions.

Table 3. Correlates of infant HIV-1 overall, early, and late infection.

Correlate	Overall infant infection (n = 92)	Early infection (<2 months of age) (n = 53)	Late infection (≥2 months of age) (n = 27)
Cervical or vaginal ulcers	2.3 (1.2–4.8)	3.0 (1.4–6.8)	2.1 (0.7–6.5)
CD4 count <200 cells/mm^3	2.5 (1.1–5.4)	2.6 (1.1–6.5)	1.5 (0.4–5.7)
Prematurity	16.0 (1.9–134.9)	22.8 (2.6–202.2)	9.4 (0.6–159.1)
Low birth weight	1.9 (0.7–5.6)	2.5 (0.9–7.5)	1.5 (0.3–7.5)
Reported exposure to breast milk	1.7 (1.0–2.9)	2.0 (1.0–3.8)	1.7 (0.7–4.0)
Viral markers			
Virus load >43,000 copies/mL	4.0 (2.2–7.2)	4.0 (2.0–8.2)	2.6 (1.0–6.4)
Cervical HIV-1 DNA	2.7 (1.6–4.7)	2.9 (1.5–5.6)	1.9 (0.8–4.4)
Vaginal HIV-1 DNA	2.3 (1.2–4.4)	2.0 (1.0–4.5)	2.0 (0.7–5.4)
Breast milk HIV-1 DNA	2.4 (0.9–6.9)	3.0 (0.8–11.5)	1.7 (0.4–7.1)
Breast disease[a]			
Cracked nipples	2.2 (0.7–7.0)	2.5 (0.8–8.3)	0 ($-\infty$ to ∞)
Bleeding nipples	2.6 (0.4–19.2)	6.5 (1.6–26.3)	0 ($-\infty$ to ∞)
Mastitis	3.9 (1.2–12.7)	1.7 (0.4–7.3)	21.8 (2.3–211.0)
Abscess	1.1 (0.2–8.1)	0 ($-\infty$ to ∞)	51.6 (4.7–571.0)
Infant thrush[a]	1.8 (0.2–13.6)	0 ($-\infty$ to ∞)	3.0 (0.4–23.1)

NOTE. Values are odds ratio (95% confidence interval [CI]) unless otherwise indicated. HIV-1, human immunodeficiency virus type 1.

[a] Time-dependent covariate; values are relative risk (95% CI).

Breast milk exposure was significantly associated with infant infection, with an ~2-fold increased risk of transmission. Our randomized clinical trial provided the most definitive currently available estimate of breast milk HIV-1 transmission, with a 2-year cumulative probability of HIV-1 infection of 37% among women randomized to breast-feed versus 21% among women randomized to formula feed [8]. Any reported breast-feeding is a crude measure of breast milk exposure, given that breast milk exposure ranged from 3 days to >2 years. However, in this case-control analysis, we found that any reported exposure to breast milk was a risk factor for transmission, with infected infants tending to have a longer duration of breast-feeding. Analyses incorporating data on volume and frequency of breast milk ingestion and the duration of the breast-feeding period will be necessary to determine the infectious risk per breast milk exposure and to determine whether there are periods of increased transmission risk during the postnatal course.

In many areas of the world where HIV-1 is highly prevalent, formula feeding may not be a feasible option for infected mothers, because of concerns regarding loss of confidentiality and stigmatization, risk of diarrheal mortality in areas with poor sanitation, or expense. When formula feeding is recommended for HIV-1–infected women, it remains important to develop interventions to reduce the risk of HIV-1 infection in infants whose mothers choose to breast-feed. Mastitis, breast abscess, and bleeding nipples were each associated with increased transmission risk in this cohort. The association between clinically diagnosed breast infections and HIV-1 transmission complements data from Semba and colleagues [25], who described an association of laboratory-diagnosed mastitis (as defined by sodium levels in breast milk) and HIV-1 transmission. Breast inflammation with recruitment of inflammatory cells may be hypothesized to up-regulate HIV-1 expression locally and to lead to higher levels of breast milk virus. The presence of HIV-1–infected cells or cell-free virus in blood-contaminated breast milk may have led to increased transmission in women with bleeding nipples. Our observations suggest that HIV-1–infected breast-feeding mothers who develop mastitis, bleeding nipples, or breast abscess should be discouraged from breast-feeding from the affected breast during the period of infection or bleeding.

Because our study had serial PCR data in a breast-feeding cohort followed up for 2 years postpartum, we were able to determine correlates of early infant infection and of late infection. We defined early infection as detection of HIV-1 within the first 2 months of life. Infants with early infection may have acquired infection in utero, intrapartum, or via early breast-feeding. This definition of early infection enabled a sufficient sample size to evaluate several potential cofactors for transmission. A limitation of our definition of early infection was that we could not discriminate cofactors specific for any one of the 3 transmission routes.

In conclusion, we have shown that maternal genital HIV-1–infected cells, plasma HIV-1 RNA levels, and breast-feeding independently predicted infant HIV-1 infection. In addition, local cofactors, such as maternal cervical or vaginal ulcer disease, mastitis, bleeding nipples, and breast abscess, were associated with enhanced transmission of HIV-1, perhaps because of erosion of normal maternal-infant barriers or recruitment of inflammatory cells. Currently, ~600,000 infants become HIV-1 infected annually, the majority in settings with limited access to effective interventions [26]. Although it is clear that current interventions have the capability to eradicate the majority of pediatric HIV-1 infections, the challenge remains to develop more widely practicable interventions for resource-poor countries. The identification of new correlates of perinatal HIV-1 transmission in this study provides information that is important for further development of interventions for these settings.

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