Cumulative Exposure to Cell-Free HIV in Breast Milk, Rather Than Feeding Pattern per se, Identifies Postnatally Infected Infants

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Background. We quantified the relationship between human immunodeficiency virus (HIV) RNA shedding in breast milk, cumulative RNA exposure, and postnatal transmission, relating timing of infection in the infant to estimated total volume of milk exposure.

Methods. Nested case-control study of 36 infants of HIV-infected mothers. Case patients were infants who acquired HIV infection through breastfeeding from age 6 through 28 weeks, and control subjects were uninfected infants matched on age at obtainment of a breast milk sample. Mothers and infants received peripartum single-dose nevirapine prophylaxis. Feeding data were collected daily; breast milk samples were collected and infant anthropometry was performed at 6 weeks and monthly thereafter. Volume of milk ingested was estimated using infant weight and feeding pattern.

Results. Before HIV acquisition in case patients, feeding pattern (exclusive breastfeeding; median duration, 65 vs 70 days; $P = .6$) and daily milk intake (mean volume, 638 vs 637 mL; $P = .97$) did not differ significantly between case patients and control subjects. Case mothers were more likely to shed virus (64% vs 9% always, 22% vs 20.5% intermittently, 14% vs 70.5% never shed; overall, $P < .001$). Case patients ingested ~15 times more HIV-1 RNA particles than did control subjects (196.5 vs 13×10^6 copies; $P < .001$). Allowing for maternal antenatal CD4 cell count and plasma HIV-1 load, child sex and duration of mixed breastfeeding, the association between HIV RNA exposure and infection remained statistically significant ($P < .001$).

Conclusions. Postnatal acquisition of HIV-1 is more strongly associated with cumulative exposure to cell-free particles in breast milk than with feeding mode. Reducing breast milk viral load through antiretroviral therapy to mother or child can further decrease postnatal transmission in exclusively breastfed infants.

The 2009 World Health Organization infant feeding recommendations for human immunodeficiency virus (HIV)–infected mothers in settings where replacement feeding is neither safe nor affordable are to breastfeed the infant for the first year, with antiretroviral treatment and/or prophylaxis for mothers or their infants [1]. This advice aims to reduce the risk of mother-to-child transmission of HIV through breastfeeding, which is estimated to be 4% during the first 6 months of exclusive breastfeeding and 1% per additional month of breastfeeding thereafter [2–5]. It has previously been estimated that the risk of acquisition of infection

through breast milk is .00064 per liter of breast milk ingested [6]. However, this estimate does not account for breastmilk viral load, the intermittent nature of virus RNA shedding in milk, and the intensity of breastfeeding [7]. Three major HIV reservoirs coexist in breast milk: RNA, which represents cell-free viral particles; proviral DNA as cell-associated virus integrated in latent T cells; and intracellular RNA representing cell-associated virus in activated producing T cells [8–13]. Their respective role in breast milk transmission of HIV-1 is poorly understood.

Because of the low estimated probability of transmission through breast milk per liter of milk ingested and the intermittent pattern of cell-free virus shedding in milk, postnatal HIV transmission through breastfeeding likely depends on the cumulative HIV exposure (ie, the overall amount of cell-free and cell-associated viral particles ingested by the infant during breastfeeding) and pattern or intensity of feeding [exclusive vs mixed or partial] and possibly by factors other than HIV. We aimed to quantify the relationship between cell-free HIV shedding in breast milk, cumulative cell-free HIV exposure, and postnatal acquisition of infection at age 6–28 weeks.

METHODS

We nested a case-control study in a large infant feeding intervention cohort (Vertical Transmission Study) of women attending 9 clinics (8 rural and 1 urban) in KwaZulu-Natal, South Africa, which aimed to examine breastfeeding and HIV transmission in a community with a high prevalence of HIV infection [2, 14]. Single-dose nevirapine was provided to all HIV-1–infected women and their infants peripartum; women were counselled antenatally on infant feeding options in accordance with policy recommendations at the time: commercial formula feeds or exclusive breastfeeding for the first 6 months of life. Women were supported in their feeding choice by lay-workers who visited breastfeeding mothers at home. Maternal socioeconomic level was defined by education level and household water type [2]. Venous samples were taken from women at enrollment and at 6 months after delivery, for plasma RNA load assessment and CD4 cell count. Daily infant feeding data were collected at weekly home visits. Infant weight was collected at birth. Breast milk samples and dried blood spot samples from infants were collected and anthropometry was performed at 6 weeks and monthly thereafter; an additional dried blood spot sample was taken from infants after delivery [14, 15].

Postnatal transmission was defined as HIV infection acquired at age 6–28 weeks. The estimated age at HIV-1 infection was taken as the midpoint between the last negative RNA polymerase chain reaction (PCR) result and the first positive RNA PCR result [2]. Case patients were postnatally infected infants [2, 14]; 42 infants received a diagnosis of HIV infection at age 49.5–197 days. Control subjects were HIV-uninfected infants matched for infant age at the time of obtainment of breast milk samples that was closest to a case patient's age at first positive PCR result and last negative PCR result (in a 1:1 ratio).

Breast milk lactoserum, including the lipid fraction, was collected from stored (−80°C) whole breast milk samples. RNA was isolated from 500 μL of lactoserum with use of the magnetic particle-based ASPS method (Abbott), and HIV load was quantified using the HIV Charge Virale assay (Biocentric) on the MJ MiniOpticon quantitative PCR detection platform (Biorad), with a sensitivity of 375 copies per mL of lactoserum [16]. This method enabled accurate assessment of cell-free viral load that is preferentially entrapped by lipids [17]. Feeding categories followed World Health Organization definitions [2, 14, 15].

The Vertical Transmission Study and breast milk analyses were approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal.

Statistical Methods

Case-control pairs with information on feeding pattern, infant weight, and breast milk viral load were included in the present analysis. Duration of breastfeeding was estimated using the Kaplan-Meier method and was compared between case patients and control subjects with use of the log-rank test. Half the value of the threshold (375/2 copies/mL of lactoserum; 50/2 copies/mL of plasma) was assigned to samples with undetectable HIV RNA load for the purpose of logarithmic transformation. Viral shedding in breast milk was categorized as never, intermittent, and permanent shedders [18].

The volume of milk ingested per day was estimated according to Arcus-Arth [19] as (−.312*age +157.7*weight) in exclusively breastfed infants, with a multiplicative correction factor when, in addition to breast milk, the infant was given water (1), formula (.7), solids (.9), or at least 2 other foods (.7) [20]. Monthly weight measurements were linearly interpolated to obtain daily weight. In 3 control subjects and 1 case patient, missing birth weights were replaced by the median birth weight observed in the overall cohort (3100 g) [2]. The probability of transmission per liter of breast milk ingested was computed using estimated milk volume ingested and estimated risk of postnatal transmission through breastfeeding in the Vertical Transmission Study cohort [2]. Daily HIV RNA exposure (ie, the amount of cell-free viral particles in the volume of milk ingested) was equal to the product of linearly interpolated milk HIV RNA load and daily milk intake. By assuming that there was no predominant breast, mean daily HIV RNA exposure between breasts could be estimated. Cumulative HIV RNA exposure was estimated as the sum of daily RNA exposure between the first breast milk sample at ∼6 weeks and HIV acquisition, with left and right truncating to elicit summation over the same period in each case-control pair. For each woman, the slope of HIV RNA load between the

last negative HIV PCR result and the first positive test result was computed as the ratio of HIV RNA load variation over the time between the 2 PCR tests.

Associations between parameters with non-Gaussian distributions were assessed using the Spearman correlation coefficient. The Wilcoxon signed-rank test for paired data was used to compare HIV RNA exposure and RNA load between case patients and their matched control subjects and between right and left breasts. To estimate the risk of postnatal transmission associated with cumulative HIV exposure in breast milk, we built a conditional logistic regression model with use of the PHREG procedure in SAS, version 9.1 (SAS Institute). Variables shown to be associated with postnatal transmission on the basis of a P value <.2 in univariate analysis were included in the multivariable model, after verifying the absence of multicollinearity.

All analyses were performed using SAS version 9.1 (SAS Institute).

RESULTS

Of 42 case-control pairs, 6 were excluded from further analyses because only 1 breast milk sample was available (5 pairs) or feeding data were collected after the estimated age of HIV acquisition (1 pair). In the remaining 36 pairs, the median estimated infant age at HIV acquisition was 89.5 days (interquartile range [IQR], 66–128 days; range, 49.5–186.5 days), with a last negative PCR test result at a minimum of 39 days; the median duration between the last negative and the first positive PCR test results was 28 days (IQR, 28–43 days). Case patients were mostly male, with a nonsignificantly higher birth weight, significantly higher maternal socioeconomic status, significantly lower maternal antepartum and postpartum CD4 cell count, and higher antepartum plasma RNA load (Table 1). Breast health problems, particularly serious breast pathologies, were rare (2 mothers of case patients) [21].

Table 1. Maternal and Infant Characteristics of HIV-1–Infected Infants and HIV-1–Uninfected Infants[a]

	HIV-1–infected infants		HIV-1–uninfected infants		
	No.		No.		P value
Maternal characteristic					
Age at delivery, years	36	25.1 (22–28.2)	36	26.4 (20.0–30.8)	.88
Enrolment clinic, no. (%)					.77
Urban		9 (25)		8 (22.2)	
Semi-urban		13 (36.1)		11 (30.6)	
Rural		14 (38.9)		17 (47.2)	
Highest level of education, no. (%)					.59
No education		2 (5.6)		3 (8.3)	
Some primary		9 (25)		13 (36.1)	
Secondary and tertiary		24 (66.7)		20 (55.6)	
Unknown		1 (2.8)			
Water type, no. (%)					.06
Borehole, tank, well		1 (2.8)		3 (8.3)	
River, stream		8 (22.2)		14 (38.9)	
Piped water		27 (75)		17 (47.2)	
Other				2 (5.6)	
Previous liveborns, no. (range)	35	0 (0–2)	36	1 (0–2)	.07
Mode of delivery, no. (%)					.71
Vaginal		31 (86)		33 (92)	
Caesarean		5 (14)		3 (8)	
Duration of rupture of membranes, h	24	0.25 (.1–9.5)	31	0.5 (.1–5)	.83
Antenatal CD4 cell count, cells/μL	34	369 (223–558)	35	519 (443–600)	.037
Antenatal plasma HIV-1 RNA load, log10 copies/mL	32	4.38 (4.02–4.90)	34	4.00 (2.99–4.82)	.02
CD4 cell count at 6 months postpartum, cells/μL	28	376 (224–666)	36	623 (417–703)	.01
Plasma HIV-1 RNA load at 6 months post-partum, log10 copies/mL	27	4.45 (3.88–4.92)	30	4.06 (3.08–4.65)	.095
Infant characteristics					
Sex male, no. (%)		22 (61)		15 (42)	.098
Birth weight, g	35	3200 (2800–3500)	33	3000 (2650–3300)	.37
Age at last HIV negative test result (day)	36	68 (44–116)	36	458 (410–548)	
Age at first HIV positive test result (day)	36	115 (88–154)		Not applicable	

[a] median (inter-quartile) are reported for quantitative variables

Breastfeeding Pattern

Overall, during the first 28 weeks of life, data on the breastfeeding pattern was collected up to 200 days for control subjects and for a slightly shorter period for case patients (median, 200 days; IQR, 198–200 days). Control subjects were exclusively breastfed for longer than case patients (median duration, 183 vs. 157 days; $P = .003$), although the overall duration of any breastfeeding was not significantly different between the 2 groups ($P = .17$). Before the age at HIV acquisition in the case patients (and matched age in the control subjects), cumulative feeding patterns did not differ significantly: 20 case patients (69%) and 25 control subjects (76%) were exclusively breastfed from birth ($P = .55$). The median duration of exclusive breastfeeding before HIV acquisition was 65 days (IQR, 51–95 days) for case patients and 70 days (IQR, 53–107) for control subjects ($P = .6$), with again nonsignificant difference in median duration of any breastfeeding for both case patients and control subjects (90 days; IQR, 67–128 days).

Milk Intake and Risk of Postnatal Transmission through Breast Milk

The estimated milk volume ingested at age 6–28 weeks (Figure 1A) or before HIV acquisition (Figure 1B) did not differ significantly betw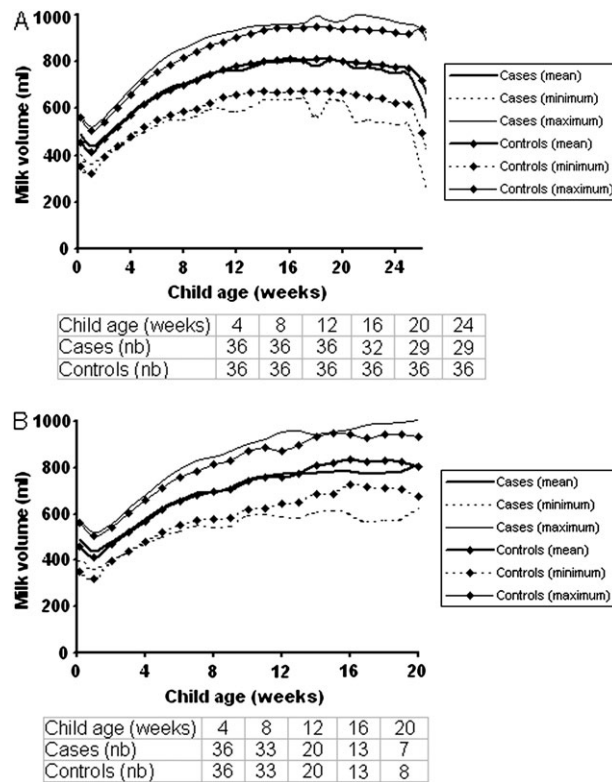een case patients and control subjects, with a mean daily milk intake of 638 mL in case patients and 637 mL in control subjects ($P = .97$).

The estimated risk of postnatal transmission through breastfeeding in this study was previously estimated at .032 per 100 child-days (95% confidence interval [CI], .0222–.0455 per 100 child-days)[2], which translates to an estimated probability of .0005 (95% CI, .00035–.00071) per liter of breast milk ingested.

HIV Shedding in Breast Milk in the First 6 Months of Life

From 34 days through 28 weeks postpartum, there were a total of 318 samples from both breasts taken at the same visit; median number of breastmilk samples per woman was 5 in case mothers (range, 2–6) and 4 in control mothers (range, 3–5). The mean breast milk HIVRNA load over the first 28 weeks per mother was inversely correlated with maternal antepartum CD4 cell count ($\rho = -.47$; 95% CI, $-.63$ to $-.26$; $n = 69$) and positively with maternal plasma HIV RNA level before ($\rho = .46$; 95% CI, .24–.63; $n = 66$) or 6 months after delivery (Appendix Tables A1 and A2, Figures A1 A and B).

Undetectable HIV RNA in milk was quasi-uniformly distributed over time (Appendix Figures A2 A and B), and there was no statistically significant variation in viral load slope (mean difference, $-.0001$; $n = 33$ in right breast; $-.0000$; $n = 34$ in left

Child age (weeks)	4	8	12	16	20	24
Cases (nb)	36	36	36	32	29	29
Controls (nb)	36	36	36	36	36	36

Child age (weeks)	4	8	12	16	20
Cases (nb)	36	33	20	13	7
Controls (nb)	36	33	20	13	8

Figure 1. *A,* Daily milk volume before 28 weeks of age in HIV-infected infants (case patients) and in uninfected infants (control subjects). *B,* Daily milk volume before HIV acquisition in HIV-1–infected infants (case patients) and in uninfected infants (control subjects).

Table 2. Median HIV RNA Load in Breast Milk in Episodes with Detectable HIV RNA

	Case patients		Control subjects		
	No of samples	HIV RNA load, $\log_{10}$ copies/mL (IQR)	No of samples	HIV RNA load, $\log_{10}$ copies/mL (IQR)	P
Before HIV-1 acquisition					
Right breast	45	3.37 (3.13–3.82)	6	2.93 (2.72–3.08)	0.005
Left breast	43	3.45 (3.07–4.02)	9	3.06 (2.83–3.28)	.045
Before 28 weeks					
Right breast	98	3.32 (3.05–3.75)	12	2.99 (2.74–3.15)	.001
Left breast	103	3.43 (2.98–4.01)	16	3.06 (2.91–3.51)	.09

Abbreviations: IQR, interquartile range.

breast). Comparing left and right breast, breast milk HIV-1 RNA load was always at least .3 $\log_{10}$ copies/mL higher in the left breast in 2 case mothers (3%), intermittently higher in 1 breast in 44 (61%) mothers (33 case patient and 11 control subjects), and always similar in both breasts (difference, < .3 $\log_{10}$ copies/mL) in 26 mothers (36%; 1 case patient and 25 control subjects). Breast milk viral load did not vary statistically significantly between breasts (mean difference, −.04 $\log_{10}$ copies/mL; $P = .29$; $n = 318$), and loads in breasts per woman were strongly correlated ($\rho = .61$; 95% CI, .54–.68). Further analysis assumed that there was no predominant breast.

By 28 weeks postpartum, mothers of case patients were more likely to shed virus from either breast than were mothers of control subjects (44% vs 3% always, 53% vs 35% intermittently, and 3% vs 62% never shed; overall $P < .001$).

Accounting for episodes with detectable breast milk viral load, either for all or only for those before HIV acquisition in case patients, the mean HIV RNA load was significantly higher in breast milk of case mothers (Table 2).

Cumulative Cell-Free HIV Exposure Through Breast Milk Before HIV Acquisition

Cumulative HIV RNA exposure was estimated from a median age of 44.5 days (range, 38–68 days) for a median duration of 41 days IQR, 22–72 days). Infants ingested a median estimated amount of 231,325 free HIV particles daily IQR, 138,439–1,416,627 particles; case patients: median, 1,349,530; IQR, 341,400–4,328,963; control subjects: median, 142,118; IQR, 125,116–179,289). We estimated that case patients ingested ∼15 times more cell-free HIV RNA particles than did control subjects (196.5 × 10^6 vs 13.0 × 10^6; $P < .001$). To investigate whether the association remained after allowing for maternal disease progression, we analyzed the 12 case-control pairs in which both members had a maternal postpartum CD4 cell count >350 cells/μL. In this comparison, infected infants were still estimated to have been exposed to significantly more cell-free HIV particles than control subjects (22.4 × 10^6 vs. 8.05 × 10^6; $P < .001$) before HIV acquisition;

maternal antepartum CD4 cell count (median, 518 vs 510 cells/μL; $P = .66$), and maternal antepartum plasma HIV-1 RNA load (median, 3.98 vs 4.04 $\log_{10}$ copies/mL; $P = .56$) did not differ significantly between case patients and control subjects in these pairs. After adjustment for infant sex, maternal antepartum CD4 cell count, maternal antepartum plasma viral load, and duration of mixed breastfeeding, a 1 × 10^7 increase in HIV-1 RNA ingested particles was associated with a 2-fold increased risk of postnatal infection in the infant (adjusted odds ratio, 2.06; 95%CI, 1.02–4.16) (Table 3).

DISCUSSION

We estimated the number of HIV cell-free particles in breast milk ingested by an infant before acquiring infection and showed that infants who became postnatally infected at 6–28 weeks of age ingested significantly more cell-free viral particles from breast milk than did uninfected infants, independently of maternal HIV CD4 cell count and plasma viral load. Because the estimated volume of breast milk consumed did not significantly differ between case patients and control subjects, the difference in exposure of the virus particles was driven by increased HIV shedding in breast milk from mothers of case patients. Our estimated probability of breast milk transmission (.0005 per liter ingested) was of the same order of magnitude as a previous estimate reported from a Kenyan study (.00064 per liter ingested) [6].

Our stusy was a case-control study nested in a well-designed prospective cohort, with intensive infant feeding support and follow-up and high-quality longitudinal data [2, 14, 22]. Mothers and infants were given single-dose nevirapine prophylaxis at or shortly after delivery only. Most importantly, the daily collected breastfeeding information, monthly collected maternal and child clinical data, infant HIV status, and breast milk samples from both breasts allowed the estimation of quantity of virus shedding in the breast milk, volume of milk intake, HIV RNA exposure, and assessment of the association of these factors with postnatal transmission. In addition,

Table 3. Risk of Postnatal HIV Infection Associated with Cumulative HIV RNA Exposure in Breast Milk Between 6 Weeks of Age and Estimated Age of HIV Infection

Variable	Adjusted OR	95% CI	P
Cumulative HIV-1 RNA exposure in milk (for each additional 10^7 copies)	2.06	1.02–4.16	.04
Maternal antepartum CD4 cell count (for each additional 100 cells/μL)	1.20	0.80–1.81	.37
Maternal antepartum plasma HIV load (for each additional log_{10} copies/mL)	1.05	0.45–2.46	.92
Duration of mixed breastfeeding (for each additional week)	1.04	0.94–1.15	.43
Male infants compared to female infants	3.40	0.44–26.40	.24

NOTE. The estimated age at mother-to-child HIV-1 transmission was taken as the midpoint between the last negative RNA PCR and the first positive RNA PCR tests. Estimated by conditional logistic regression with adjustment on the other factors reported in the table.
Abbreviations: CI, confidence interval; OR, odds ratio.

comparison between the 2 breasts and adjustment on confounding factors was also possible.

However, our study presents some limitations. We used the midpoint between the last negative and the first positive HIV PCR test results to estimate timing of acquisition of postnatal infection. This assumption may underestimate variance [23]; however, because the length of the interval (median, 28 days) was relatively short, compared with the 22-weeks duration of follow-up, the potential bias is unlikely to be substantial. Furthermore, we estimated daily milk intake using a formula based on infant weight and feeding pattern that was validated with healthy, full-term European or northern American infants [19]. We used correction factors based on a survey conducted in Brazil [20] to account for introduction of food other than breast milk. Although our cohort differs from these populations, our daily milk intake estimates are close to age-specific standards (658 mL vs standard 670 mL from day 8 through month 2, and 788 mL vs standard 750 mL from month 3 through month 5 [24]). The plateau in estimated volume of breast milk intake that we observed from 8 weeks postpartum is similar to that reported in previous studies in which investigators directly estimated milk volumes by weighing infants before and after each feed [25]. Finally, although it has been suggested that subclinical mastitis may be associated with higher breast milk viral load [9, 26], with the intensive breastfeeding counseling, episodes of clinical and subclinical mastitis were rare [21].

We confirm a strong correlation in breast milk HIVRNA load between breasts [27], and although we show differential shedding profiles between breasts, there seldom was a persistently predominant breast throughout lactation, suggesting that breast milk samples may be collected from either breast for studies investigating HIV shedding patterns in breast milk. We also confirm the correlation between breast milk and maternal plasma HIV RNA loads, with lower values in breast milk, and an inverse correlation with maternal antepartum CD4 cell count [10].

Intermittent HIV RNA shedding was common, supporting the need for frequent breast milk sampling to identify underlying mechanisms of shedding. Mothers of case patients were more likely to shed virus and at higher levels than were mothers of control subjects, which confirms breast milk HIV RNA load as a strong predictor of postnatal HIV-1 transmission [9]. There was no significant variation in HIV RNA load in breastmilk slope between the last negative and the first positive HIV PCR test result in case infants of mothers, which suggests that transmission is not explained by an abrupt increase of HIV RNA load and favors cumulative HIV exposure as important predictor of transmission.

Mechanisms of HIV breast milk transmission remain poorly understood. Because of the dynamic nature of the relationship between the source of HIV reservoirs (breast milk) and the potential target host (the maturing gastrointestinal tract of the young infant), multiple mechanisms are likely to be at stake. A remaining question relates to the nature of HIV reservoirs in milk involved in transmission, and the association of HIV RNA exposure per se with postnatal transmission does not necessarily prove causation between the 2 events. Breast milk cellular reservoirs are likely to play a major role in transmission [28]. Both B and T lymphocytes in breast milk harbor homing markers strongly suggesting migration from mucosal sites, particularly from the gut [11, 29]. Recent studies identified latently infected CD4 T cells [13] and spontaneously activated CD4 T cells [12] in breast milk as likely reservoirs involved in transmission. These 2 reservoirs are unaffected by maternal antiretroviral therapy [12, 13, 30] and are likely responsible for residual transmission from antiretroviral-treated lactating women. In the present study, one transmitting mother never shed HIV-1 in breast milk, despite multiple measurements, which confirms that at least some breast milk transmission is attributable to cell-associated HIV reservoirs [8, 10].

In conclusion, higher cumulative exposure to cell-free HIV RNA in breast milk is associated with higher rates of postnatal infection in the infant, independent of maternal CD4 cell count and plasma viral load; cumulative exposure is attributable to viral shedding in, rather than volume of, breast milk consumed. The contribution of exposure to cell-associated HIV remains to be determined, as do factors associated with compartmentalized shedding of HIV in breast milk.

Supplementary Material

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/).

Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

References

1. UNAIDS/UNICEF/WHO. Rapid advice: revised principles and recommendations on infant feeding in the context of HIV. Geneva, Switzerland: WHO/UNAIDS, 2009.
2. Coovadia HM, Rollins NC, Bland RM, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet **2007**; 369:1107–6.
3. The Breastfeeding and HIV International Transmission Study Group. Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. J Infect Dis **2004**; 189:2154–66.
4. Iliff PJ, Piwoz EG, Tavengwa NV, et al. Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. AIDS **2005**; 19:699–708.
5. Rollins NC, Becquet R, Bland RM, Coutsoudis A, Coovadia HM, Newell ML. Infant feeding, HIV transmission and mortality at 18 months: the need for appropriate choices by mothers and prioritization within programmes. AIDS **2008**; 22:2349–57.
6. Richardson BA, John-Stewart GC, Hughes JP, et al. Breast-milk infectivity in human immunodeficiency virus type 1-infected mothers. J Infect Dis **2003**; 187:736–40.
7. Willumsen JF, Newell ML, Filteau SM, et al. Variation in breastmilk HIV-1 viral load in left and right breasts during the first 3 months of lactation. AIDS **2001**; 15:1896–8.
8. Koulinska IN, Villamor E, Chaplin B, et al. Transmission of cell-free and cell-associated HIV-1 through breast-feeding. J Acquir Immune Defic Syndr **2006**; 41:93–9.
9. Lunney KM, Iliff P, Mutasa K, et al. Associations between breast milk viral load, mastitis, exclusive breast-feeding, and postnatal transmission of HIV. Clin Infect Dis **2010**; 50:762–9.
10. Rousseau CM, Nduati RW, Richardson BA, et al. Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission. J Infect Dis **2004**; 190:1880–8.
11. Petitjean G, Becquart P, Tuaillon E, et al. Isolation and characterization of HIV-1-infected resting CD4+ T lymphocytes in breast milk. J Clin Virol **2007**; 39:1–8.
12. Valea D, Tuaillon E, Al Tabaa Y, et al. Highly activated CD4+ T cells spontaneously producing human immunodeficiency virus type I in breast milk from women treated with antiretroviral Drugs. Vth IAS Conference on HIV Pathogenesis; 19-22 July 2009. Cape Town, South Africa, 2009.
13. Becquart P, Petitjean G, Tabaa YA, Valea D, Huguet MF, et al. Detection of a large T-cell reservoir able to replicate HIV-1 actively in breast milk. AIDS **2006**; 20:1453–5.
14. Bland R, Coovadia H, Coutsoudis A, Rollins N, Newell ML. Cohort profile: mamanengane or the Africa centre vertical transmission study. Int J Epidemiol **2009**; 39:351–60.
15. Patel D, Bland R, Coovadia H, Rollins N, Coutsoudis A, Newell ML. Breastfeeding, HIV status and weights in South African children: a comparison of HIV-exposed and unexposed children. AIDS **2010**; 24:437–45.
16. Rouet F, Chaix ML, Nerrienet E, Ngo-Giang-Huong N, Plantier JC, et al. Impact of HIV-1 genetic diversity on plasma HIV-1 RNA quantification: usefulness of the Agence Nationale de Recherches sur le SIDA second-generation long terminal repeat-based real-time reverse transcriptase polymerase chain reaction test. J Acquir Immune Defic Syndr **2007**; 45:380–8.
17. Ghosh MK, Kuhn L, West J, et al. Quantitation of human immunodeficiency virus type 1 in breast milk. J Clin Microbiol **2003**; 41:2465–70.
18. Rousseau CM, Nduati RW, Richardson BA, et al. Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease. J Infect Dis **2003**; 187:741–7.
19. Arcus-Arth A, Krowech G, Zeise L. Breast milk and lipid intake distributions for assessing cumulative exposure and risk. J Expo Anal Environ Epidemiol **2005**; 15:357–65.
20. Haisma H, Coward WA, Albernaz E, et al. Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. Eur J Clin Nutr **2003**; 57:1633–42.
21. Bland RM, Becquet R, Rollins NC, Coutsoudis A, Coovadia HM, Newell ML. Breast health problems are rare in both HIV-infected and HIV-uninfected women who receive counseling and support for breast-feeding in South Africa. Clin Infect Dis **2007**; 45:1502–10.
22. Gaillard P, Piwoz E, Farley TM. Collection of standardized information on infant feeding in the context of mother-to-child transmission of HIV. Stat Med **2001**; 20:3525–37.
23. Alioum A, Dabis F, Dequae-Merchadou L, et al. Estimating the efficacy of interventions to prevent mother-to-child transmission of HIV in breast-feeding populations: development of a consensus methodology. Stat Med **2001**; 20:3539–6.
24. Riordan J, Auerbach K. Breastfeeding and human lactation. Sudbury, MA: Jones & Bartlett Publishers, 1999.
25. Neville MC, Keller R, Seacat J, et al. Studies in human lactation: milk volumes in lactating women during the onset of lactation and full lactation. Am J Clin Nutr **1988**; 48:1375–86.
26. Willumsen JF, Filteau SM, Coutsoudis A, et al. Breastmilk RNA viral load in HIV-infected South African women: effects of subclinical mastitis and infant feeding. AIDS **2003**; 17:407–14.
27. Semrau K, Ghosh M, Kankasa C, et al. Temporal and lateral dynamics of HIV shedding and elevated sodium in breast milk among HIV-positive mothers during the first 4 months of breast-feeding. J Acquir Immune Defic Syndr **2008**; 47:320–8.
28. Dimitrov DS, Willey RL, Sato H, Chang LJ, Blumenthal R, Martin MA. Quantitation of human immunodeficiency virus type 1 infection kinetics. J Virol **1993**; 67:2182–90.
29. Tuaillon E, Valea D, Becquart P, et al. Human milk-derived B cells: a highly activated switched memory cell population primed to secrete antibodies. J Immunol **2009**; 182:7155–62.
30. Lehman DA, Chung MH, John-Stewart GC, et al. HIV-1 persists in breast milk cells despite antiretroviral treatment to prevent mother-to-child transmission. AIDS **2008**; 22:1475–85.