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Dynamics of breast milk HIV-1 RNA with unilateral mastitis or abscess

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Abstract

Background—Mastitis and abscess in HIV-infected women increase risk of breastfeeding transmission of HIV. Guidelines encourage women to stop breastfeeding on the affected breast and feed on the contralateral breast. However, impact of breast pathology on breast milk HIV dynamics is unknown.

Methods—HIV RNA was quantified in 211 breast milk samples collected before, during and after a clinical mastitis or abscess diagnosis from 38 HIV-infected women participating in a Zambian breastfeeding study. HIV RNA quantity was compared between affected and unaffected breasts over time using generalized estimating equation models. A sample of 115 women without breast pathology was selected as a control group.

Results—In the affected breast, breast milk HIV RNA quantity increased from the pre- to during-pathology period by log₁₀ 0.45 copies/mL (95% CI: 0.16, 0.74) and after symptom resolution, HIV RNA levels were no different from pre-pathology levels (log₁₀ -0.04 copies/mL 95% CI: -0.33, 0.25). In the contralateral unaffected breast, HIV RNA quantity did not significantly increase (log₁₀ 0.15 copies/mL, 95% CI: -0.41, 0.10). Increase was more marked in women with abscess or with a greater number of mastitis symptoms. HIV RNA was not significantly different between affected and unaffected women, except at the time of diagnosis.

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Conclusions—Breast milk HIV RNA increased modestly in the affected breast with unilateral mastitis or abscess and returned to pre-pathology levels with symptom resolution. Contralateral HIV RNA was not affected. Results support guidelines encouraging feeding from the contralateral breast to minimize risk of HIV transmission associated with unilateral breast pathology.

Keywords

Infant feeding; HIV; Exclusive breastfeeding; Breast problem; Mastitis; Abscess

Introduction

In the absence of antiretrovirals, approximately 10–15% of infants breastfed by their HIV-infected mothers will acquire infection via this route.¹ Risk factors for breast milk transmission include low maternal CD4 count, high plasma or breast milk viral load, and presence of breast pathology, including sub-clinical and clinical mastitis as well as abscess.^{2–5} Sub-clinical mastitis has been associated with increased levels of breast milk HIV-1 RNA as well as increase in milk inflammatory cytokines that could facilitate transmission in the infant by cellular activation.^{6–9} Due to increased risk of transmission during mastitis and since most mastitis is unilateral (i.e. confined to only one breast), the World Health Organization (WHO) recommends that HIV-infected women with mastitis or other unilateral breast pathology stop breastfeeding on the affected breast and feed on the contralateral (or unaffected) breast; once symptoms resolve, women should again feed from both breasts.¹⁰ Although the quantity of HIV in breast milk and sub-clinical mastitis has been correlated,^{11, 12} no longitudinal data on viral dynamics in breast milk before, during and after clinically-detectable breast pathology are available. Thus, WHO guidelines are based on two unsubstantiated assumptions. First, the recommendation assumes that breast milk viral quantity declines to pre-mastitis levels after clinical symptom resolution. Second, that breast milk viral quantity in the contralateral breast does not increase. Theoretically, fever, inflammation, and other constitutional signs of clinical mastitis could lead to high HIV RNA in the clinically-unaffected breast.¹³ Empirical data on the temporal and lateral dynamics of breast milk HIV RNA before and after mastitis are needed in order to develop evidence-based counseling for HIV-infected breastfeeding women with mastitis or abscess.

Mastitis can be classified as either clinical or sub-clinical. Clinical mastitis is characterized by observable signs and symptoms, e.g. engorgement, redness, induration, pain and fever. Clinical mastitis and other overt breast pathology, including abscess, have been associated with increased risk of mother-to-child HIV transmission (MTCT), but studies have not directly measured changes in breast milk HIV RNA with clinical mastitis or abscess.^{4, 8} Sub-clinical mastitis is generally defined by markers of breast epithelium permeability—sodium or ratio of sodium:potassium concentrations. Sub-clinical mastitis tends to correlate with breast milk HIV concentrations^{6, 14} and with inflammation,¹⁵ possibly explaining associations between sub-clinical mastitis and increased HIV transmission. Interpretation of studies on sub-clinical mastitis are complicated by normal developmental changes over time in these biochemical markers as breastfeeding is established.¹⁶ Moreover, these biochemical changes may not necessarily be directly relevant to the clinical circumstance of overt breast morbidity.

To address the clinical problem of how to counsel HIV-infected breastfeeding women with mastitis or other generally unilateral breast pathology, this study examined longitudinal patterns of breast milk viral shedding in affected and unaffected contralateral breasts before, during, and after clinically-detectable breast pathology.

Methods

Study Population

Breast milk samples were selected from HIV-infected women with mastitis or abscess enrolled in the Zambia Exclusive Breastfeeding Study (ZEBS) in Lusaka, Zambia. ZEBS was approved by all participating institutions' ethical review boards, and is described in detail elsewhere.^{17, 18} In brief, ZEBS was a randomized trial to assess the impact of exclusive breastfeeding with abrupt weaning at four months on postnatal HIV transmission and child survival. All women were counseled to exclusively breastfeed until 4 months; half were randomized to stop all breastfeeding at 4 months and the other half to continue breastfeeding for a duration of their own choice. This analysis is restricted to 943 randomized women who initiated breastfeeding and did not receive antiretroviral therapy (ART), other than single-dose Nevirapine at onset of labor. ART was not available in Zambia for most of the study period. Bilateral maternal breast milk samples were collected at 1 week, 1, 4, 4.5, 6, 9, 12, 15, 18, 21, and 24 months post-partum, as long as breastfeeding continued. A protocol change in September 2004 restricted milk collection to 1, 4, 4.5, and 12 months post-partum. Single dose nevirapine is known to affect HIV RNA quantity in breast milk, thus samples from both breasts were examined as the effect of nevirapine would be bilateral, yet the effect of mastitis is unilateral.^{19,20}

Trained study nurses undertook a clinical breast examination and structured symptom history at clinic visits scheduled at 1 week, 1, 2, 3, 4, 4.5, 5, 6, 9, 12, 15, 18, 21, and 24 months postpartum. A diagnosis of unilateral clinical mastitis or abscess was made in 86/943 (9.1%) women and at 110/10,987 (1.0%) visits. Clinical mastitis was defined as recent or current fever ($>37^{\circ}\text{C}$) and at least one of the following signs and symptoms: engorgement, red/shiny breasts, blocked duct(s), and/or recent or current painful breasts. At each visit, nurses determined presence or absence of breast abscess, a clinically-evident pathology characterized by a pus-filled, hard mass in the breast tissue. Women were also asked whether they had visited a clinic or hospital due to illness since the prior visit.

Of the 86 women diagnosed with unilateral clinical mastitis/abscess diagnoses at 110 visits, 38/86 (44.2%) women had breast milk samples taken from both breasts prior to, at the time of and after diagnosis. The remaining 72 cases of mastitis or abscess in 48 women had missing samples due to the standardized milk collection protocol, missed visits, or cessation of breastfeeding (SDC: Figure 1). Pre-pathology samples were taken from the clinic visit completed just prior to the visit when the pathology was first diagnosed. Mastitis and abscess have rapid onset^{21, 22} and are typically caused by non-infectious factors, namely milk stasis.¹⁰ Therefore, a visit completed at least two weeks prior should be free of pathology. Samples were defined as being during the condition if collected at the same visit when mastitis or abscess was diagnosed as described above. Data on the duration of symptoms were not collected. Resolution of mastitis varies according to the severity of symptoms; in the absence of antibiotics, symptoms typically resolve within 24 hours to 8 days.^{10, 22–25} Post-pathology samples were taken from the next available milk sample after diagnosis when breast problems were not reported and a minimum of 9 days after the diagnosis. Of 228 samples selected for testing (left and right breast milk samples at 3 time points), 211 (92.5%) had valid results obtained. Of the 38 women, 37 (97.3%) had results in the affected breast at the pre-, 31 (81.6%) at the during, and 34 (89.5%) at the post-diagnosis visit. Of the 38 women, 36 (94.7%) had results in the unaffected contralateral breast pre-, 36 (94.7%) during, and 37 (97.4%) post-diagnosis.

The 211 breast milk samples in 38 women with breast pathology were compared to 115 milk samples in 115 women who did not experience breast pathology. These 115 women were selected as those without breast pathology from the 138 HIV-infected women with breast

milk samples collected at 1 week, 1 month and 4 months post-partum analyzed in a previous report describing breast milk HIV RNA across breasts.¹⁶

Infant HIV infection was determined by HIV DNA PCR testing on dried blood samples collected at birth, 1 week and 1, 2, 3, 4, 4.5, 5, 6, 9, 12, 15, 18, 21, and 24 months postpartum. All positive results were confirmed on a second sample if possible. All negative results were confirmed negative by quantification of betaglobulin to ensure sample adequacy.

Laboratory methods

Copies of HIV-1 RNA per milliliter of breast milk were measured with the Roche Amplicor Ultrasensitive HIV-1 1.5 kit (Roche Molecular Systems, Inc., Branchburg, NJ) with a lower detection limit of 50 copies/mL. The assay has been validated for quantitation of HIV-1 RNA in breast milk.²⁶ As RNA quantity was non-normally distributed, log-transformed data were used for analyses. For samples below the detection limit of the assay, a value of 49 copies/mL was imputed.

Statistical Analysis

First, the analysis included a within-person, within-breast comparison of HIV RNA in breast milk across the three time-points. Second, the contralateral breast at the same time point was compared. Third, comparisons to women who did not experience breast pathology were conducted. Crude analyses compared the distributions of HIV RNA in breast milk over time, across breasts and between the groups. Relationships between breast pathology, breast milk HIV RNA and infant feeding patterns, acute maternal illness, and postnatal age of the child were examined. Generalized estimating equations (GEE) were used to compare breast milk HIV RNA quantity between groups adjusting for repeat measures per subject. Change in HIV RNA quantity between visits was compared with generalized linear models (PROC GLM). All analyses were completed with SAS, version 9.1.3 (Cary, NC).

Results

Demographic/Social Characteristics

Of the 86 women with unilateral mastitis or abscess, 38 (42.2%) had breast milk samples collected at a visit prior to, during, and after diagnosis. The 38 women with samples available were similar to the 48 who had breast pathology but lacked milk samples (Table 1). Women included in the analysis who had breast pathology had a mean CD4 count of 322 cells/uL (23.6% CD4 count <200 cells/uL) and mean plasma viral load of log₁₀ 4.62 copies/mL at enrollment during pregnancy. Four (11.8%) women reported a history of breast mastitis or abscess during previous experiences of breastfeeding.

Timing and Symptoms of Mastitis and Abscess

The timing and symptoms associated with mastitis or abscess are presented in Table 2. The majority of events occurred early in the post-partum period; most women were exclusively breastfeeding at this time. Women had a mixture of symptoms, defined by number and severity, associated with clinical mastitis and abscess. Women excluded from this analysis had mastitis diagnosed earlier and more often had pathology on the left side; otherwise, women were similar (Table 2).

Breast Milk HIV RNA in the Affected Breast

Breast milk HIV RNA during mastitis or abscess (log₁₀ 2.93 copies/mL) was significantly higher than before diagnosis (log₁₀ 2.56 copies/mL, p=0.03). After resolution of symptoms,

mean HIV RNA quantity decreased to levels comparable to those observed pre-pathology ($\log_{10}$ 2.46 copies/mL, $p=0.52$) (Table 3, SDC: Figure 2). Differences were slightly stronger when the analysis was restricted to women who had HIV RNA >50 copies/ml (Table 3). However, if considered simply as above or below the threshold for detection (50 copies/ml), there were no significant differences.

Mean change in HIV RNA concentrations from the pre- to the during-pathology visit was $\log_{10}$ 0.45 copies/mL (95% CI: 0.16, 0.74) in the affected breast (Table 4). With resolution of mastitis/abscess, breast milk HIV RNA decreased on average $\log_{10}$ -0.54 (95% CI: -0.19, -0.89) copies/mL in the affected breast. Breast milk HIV RNA concentrations were similar in the samples collected prior to and after resolution of symptoms (change of $\log_{10}$ -0.04 (95% CI: -0.33, 0.25) copies/mL in the affected breast).

Breast Milk HIV RNA in the Contralateral Unaffected Breast

In the contralateral breast, HIV RNA did not significantly change between the pre- ($\log_{10}$ 2.52 copies/ml), during ($\log_{10}$ 2.60 copies/mL, $p=0.56$) or post-diagnosis periods ($\log_{10}$ 2.36 copies/mL, $p=0.20$) (Table 3, SDC Figure 2). There were no significant increases or decreases in viremia before and after diagnosis of pathology in the unaffected breast (Table 4). Nor were there changes in the contralateral unaffected breast in the proportion with detectable HIV RNA before, during or after pathology.

At the visit when mastitis or abscess was diagnosed, the affected breast ($\log_{10}$ 2.93 copies/mL) had significantly higher breast milk HIV RNA concentrations than the contralateral unaffected breast ($\log_{10}$ 2.60 copies/mL, $p=0.05$). At the visits before and after the diagnosis, breast milk HIV RNA was not different across breasts ($p=0.55$).

Signs, symptoms, and breast milk HIV RNA

Each of the signs and symptoms associated with mastitis and abscess were examined separately for impact on HIV RNA breast milk concentrations. Mean breast milk HIV RNA quantity from the sample taken during pathology in the affected breast was significantly higher in those women who had a red/shiny breast ($\log_{10}$ 3.47 vs. $\log_{10}$ 2.65 copies/mL, $p=0.05$) or abscess ($\log_{10}$ 3.48 vs. $\log_{10}$ 2.62 copies/mL, $p=0.03$). Women who reported painful breasts also tended to have higher viral concentrations at the time of pathology ($\log_{10}$ 3.05 vs. $\log_{10}$ 2.17 copies/mL, $p=0.07$). Mean change in HIV RNA quantity from the pre- to the during-pathology visit varied by specific sign or symptom (SDC: Figure 3). Women with red/shiny breasts had a $\log_{10}$ 0.82 increase in HIV RNA concentration compared to women without this sign ($\log_{10}$ 0.26 copies/mL, $p=0.08$). Women with abscess had the largest increase in breast milk HIV RNA ($\log_{10}$ 0.89 vs. $\log_{10}$ 0.18 copies/mL, $p=0.03$).

Comparison of Women with Mastitis to Women without Mastitis

The 38 women with mastitis or abscess were compared to 115 women without breast pathology. The 38 women with mastitis or abscess were older (27.4 ± 6.1 vs. 25.0 ± 4.7 years, $p=0.01$) and had lower CD4 count at enrollment (322 ± 149 cells/uL vs. 403 ± 207 cells/uL, $p=0.03$). Women with mastitis or abscess were more likely at enrollment to report swollen glands in more than one place (10.5%) compared to non-mastitis women (1.7%) ($p=0.03$). However, HIV-1 plasma viral load at enrollment was similar ($\log_{10}$ 4.62 ± 0.80 copies/mL vs. $\log_{10}$ 4.46 ± 0.83 copies/mL, $p=0.44$) between the groups (Table 1).

Of the breast milk samples from women with mastitis or abscess, 164/211 (77.7%) had detectable HIV RNA ≥ 50 copies/mL compared to 61/115 (54.9%) breast milk samples from women without breast pathology ($p<0.01$, Odds Ratio [OR]=2.86 95% CI: 1.74, 4.69). The

mean breast milk HIV RNA concentration in the affected breast milk sample at the time of breast pathology diagnosis ($\log_{10}$ 2.93 $\pm$ 0.19 copies/mL) was significantly higher than among women without breast pathology ($\log_{10}$ 2.41 $\pm$ 0.09 copies/mL, $p=0.01$). Breast milk HIV RNA in samples collected from women without breast pathology was similar to breast milk HIV RNA concentrations in samples collected from the unaffected breasts at all time points and from the affected breast at pre- and post-diagnosis time points (Table 5).

Mother to Child Transmission of HIV

Of the 38 pairs, 8 (21.1%) of the children were HIV-infected by 24 months of age. The overall rate of MTCT is similar to that of the larger cohort. Among the 8 infected children, two were infected *in utero*, 3 intrapartum or in the early postnatal period (first positive at 1 month) and 3 during breastfeeding after 1 month of age. Of the 6 non-intrauterine infections, 4 (66.7%) were found to be infected after mastitis or abscess occurred in the mother. The 4 post-breast pathology transmitters were compared to the 30 non-transmitting women. Transmitters had higher plasma viral load than non-transmitters ($\log_{10}$ 4.78 vs. $\log_{10}$ 4.20 copies/mL, $p=0.05$), non-significantly lower CD4 counts (282 vs. 328, $p=0.55$), and higher breast milk HIV RNA quantity before ($\log_{10}$ 3.50 vs. $\log_{10}$ 2.32 copies/mL, $p=0.03$) and after mastitis/abscess ($\log_{10}$ 3.53 vs. $\log_{10}$ 2.08 copies/mL, $p=0.02$). Additionally, mean breast milk HIV RNA quantity at the time of mastitis/abscess tended to be higher in the transmitters ($\log_{10}$ 3.60 vs. $\log_{10}$ 2.73 copies/mL, $p=0.24$), although it was not statistically significant.

Discussion

Breastfeeding complications, including mastitis and abscess are common, occurring among an estimated 10–33% of all nursing women.^{10, 27} Breast pathology can be painful and many women stop breastfeeding after experiencing mastitis.²⁸ Mastitis and abscess have the added risk among HIV-infected women of increasing the risk of postnatal MTCT.^{3, 4} WHO recommendations for breastfeeding in the presence of mastitis/abscess in HIV-infected women encourage women to continue to breastfeed **only** from the contralateral, unaffected breast until symptoms resolve. Our study provides the first empiric data to support this recommendation. In this cohort of 38 women with mastitis or abscess, significant, albeit modest, increases in breast milk HIV RNA were observed in the affected breast. It is important to note that even small changes in viral load seem to impact HIV heterosexual transmission; similar trends are likely to be true in breast milk MTCT.²⁹ In the majority of women, breast milk HIV RNA concentrations in the affected breast decreased from the elevated level at the time of symptoms to its baseline level or lower with symptom resolution. In the contralateral breast, breast milk HIV RNA did not change over the period of mastitis.

Our results suggest that there is no long-lasting impact of unilateral mastitis on breast milk viral shedding in either breast. This is consistent with the results from studies of HIV dynamics during acute illness and vaccination. In both circumstances, acute increases in HIV RNA are transient with a return to baseline levels.^{30, 31} Our results also demonstrate that breast milk HIV RNA concentrations are not elevated in the affected breast prior to the mastitis event. Furthermore, it appears that the regulation of HIV entry into breast milk is independent between breasts, since local pathology in one breast does not negatively impact the contralateral breast. Thus the influence of mastitis and abscess on the risk of transmission to the child is temporary and confined to the affected breast.

Our data further indicated that the degree of clinical symptomatology correlated with HIV concentrations in breast milk. Women with abscess or red/shiny breast had greater change in HIV RNA concentrations from baseline to the symptomatic visit in the affected breast.

Potentially, the severity of the symptoms is an indicator of increased breast permeability or inflammation, allowing entry into or increased replication of virus in the breast compartment. Given the data presented and the important correlation between increased RNA quantity and HIV transmission, lactation counselors and nurses counseling HIV-infected women should support appropriate breastfeeding techniques and encourage mastitis-affected women to feed from the contralateral breast only. Improvements in breastfeeding counseling have been shown to correlate with reduced breast problems; therefore, enhanced counseling could both prevent problems developing as well as provide appropriate advice for those with mastitis.²⁸

A strength of this study is the utilization of women as their own control, which essentially eliminates confounding on fixed and time-dependent variables. The study is further strengthened by the consistency of the results from the within-person analysis to the results from conventional two exposure group comparisons. Our study has the added advantage of milk samples from both breasts over time allowing within-person comparisons at single time points.

The study is limited by small sample size; although this is one of the only cohorts with longitudinal milk samples collected from both breasts. Second, due to the length of time between the mastitis/abscess visit and post-pathology visit (average of 92 days), it was not possible to determine how quickly breast milk HIV RNA returned to baseline levels. Third, measures of breast milk cell-associated HIV, a predictor of breast milk transmission of HIV, were not available.^{11, 32} Fourth, we had only limited data on some time-varying variables that may be potential confounders, such as other acute illnesses in the mother. We were able to examine if clinic attendance between visits modified the study results, and they did not. Finally, handedness may have been helpful to determine if mastitis was more likely to occur in one breast or the other in our population, as has been demonstrated in other studies.³³

With respect to breast complications, such as mastitis and abscess, in HIV-infected women, the current WHO recommendations appear to be appropriate. Breast milk HIV RNA concentrations increase during a period of mastitis or abscess in the affected breast, thus placing the child at increased risk of MTCT. HIV-infected women should refrain from breastfeeding on the affected breast but can continue to breastfeed from the contralateral breast. Breast milk HIV RNA concentrations in the contralateral breast did not significantly increase despite breast pathology in the other breast. Further studies of the physiology of mastitis and abscess and their relationships with breast milk virologic dynamics are required to enhance the current guidelines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. De Cock KM, Fowler MG, Mercier E, et al. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. *Journal of the American Medical Association*. 2000; 283:1175–1182. [PubMed: 10703780]

2. Fowler MG, Kourtis AP, Aizire J, Onyango-Makumbi C, Bulterys M. Breastfeeding and transmission of HIV-1: epidemiology and global magnitude. *Adv Exp Med Biol.* 2012; 743:3–25. [PubMed: 22454338]
3. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. *Journal of Infectious Diseases.* 1999; 180:93–98. [PubMed: 10353866]
4. Embree JE, Njenga S, Datta P, et al. Risk factors for postnatal mother-child transmission of HIV-1. *AIDS.* 2000; 14:2535–2541. [PubMed: 11101065]
5. Shapiro RL, Smeaton L, Lockman S, et al. Risk factors for early and late transmission of HIV via breast-feeding among infants born to HIV-infected women in a randomized clinical trial in Botswana. *J Infect Dis.* 2009; 199:414–418. [PubMed: 19090775]
6. Willumsen JF, Filteau SM, Coutoudis A, et al. Breastmilk RNA viral load in HIV-infected South African women: effects of subclinical mastitis and infant feeding. *AIDS.* 2003; 17:407–414. [PubMed: 12556695]
7. Willumsen JF, Filteau SM, Coutoudis A, Uebel KE, Newell ML, Tomkins AM. Subclinical mastitis as a risk factor for mother-infant HIV transmission. *Advances in Experimental Medicine & Biology.* 2000; 478:211–223. [PubMed: 11065074]
8. Semba RD. Mastitis and transmission of human immunodeficiency virus through breast milk. *Annals New York Academy of Sciences.* 2000; 918:156–162.
9. Gantt S, Shetty AK, Seidel KD, et al. Laboratory indicators of mastitis are not associated with elevated HIV-1 DNA loads or predictive of HIV-1 RNA loads in breast milk. *J Infect Dis.* 2007; 196:570–576. [PubMed: 17624843]
10. WHO. Mastitis: Causes and Management. Geneva, Switzerland: World Health Organization; 2000. Report No.: WHO/FCH/CAH/00.13.
11. Kantarci S, Koulinska IN, Aboud S, Fawzi WW, Villamor E. Subclinical mastitis, cell-associated HIV-1 shedding in breast milk, and breast-feeding transmission of HIV-1. *J Acquir Immune Defic Syndr.* 2007; 46:651–654. [PubMed: 18043320]
12. 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; 50:762–769. [PubMed: 20121424]
13. Georgeson JC, Filteau SM. Physiology, immunology, and disease transmission in human breast milk. *AIDS Patient Care STDS.* 2000; 14:533–539. [PubMed: 11054937]
14. Semba RD, Kumwenda N, Taha TE, et al. Mastitis and immunological factors in breast milk of lactating women in Malawi. *Clin Diagn Lab Immunol.* 1999; 6:671–674. [PubMed: 10473515]
15. Gantt S, Carlsson J, Heath L, et al. Genetic analyses of HIV-1 env sequences demonstrate limited compartmentalization in breast milk and suggest viral replication within the breast that increases with mastitis. *J Virol.* 2010; 84:10812–10819. [PubMed: 20660189]
16. 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; 47:320–328. [PubMed: 18398972]
17. Thea DM, Vwalika C, Kasonde P, et al. Issues in the design of a clinical trial with a behavioral intervention--the Zambia exclusive breast-feeding study. *Control Clin Trials.* 2004; 25:353–365. [PubMed: 15296810]
18. Kuhn L, Aldrovandi GM, Sinkala M, et al. Effects of early, abrupt weaning for HIV-free survival of children in Zambia. *N Engl J Med.* 2008; 359:130–141. [PubMed: 18525036]
19. Rossenkan R, Ndung'u T, Sebunya TK, et al. Temporal reduction of HIV type 1 viral load in breast milk by single-dose nevirapine during prevention of MTCT. *AIDS Res Hum Retroviruses.* 2009; 25:1261–1264. [PubMed: 20001515]
20. Aizire J, McConnell MS, Mudiope P, et al. Kinetics of nevirapine and its impact on HIV-1 RNA levels in maternal plasma and breast milk over time after perinatal single-dose nevirapine. *J Acquir Immune Defic Syndr.* 2012; 60:483–488. [PubMed: 22217678]
21. Amir LH, Lumley J. Women's experience of lactational mastitis--I have never felt worse. *Aust Fam Physician.* 2006; 35:745–747. [PubMed: 16969450]

22. Wambach KA. Lactation mastitis: a descriptive study of the experience. *J Hum Lact.* 2003; 19:24–34. [PubMed: 12587642]
23. Barbosa-Cesnik C, Schwartz K, Foxman B. Lactation mastitis. *Journal of the American Medical Association.* 2003; 289:1609–1612. [PubMed: 12672715]
24. Fetherston CM, Lai CT, Hartmann PE. Relationships between Symptoms and Changes in Breast Physiology during Lactation Mastitis. *Breastfeeding Medicine.* 2006; 1:136–145. [PubMed: 17661590]
25. Fetherston C. Characteristics of lactation mastitis in a Western Australian cohort. *Breastfeed Rev.* 1997; 5:5–11. [PubMed: 9699467]
26. Ghosh MK, Kuhn L, West J, et al. Quantitation of human immunodeficiency virus type 1 in breast milk. *J Clin Microbiol.* 2003; 41:2465–2470. [PubMed: 12791866]
27. ABM clinical protocol #4: mastitis. Revision, May 2008. *Breastfeed Med.* 2008; 3:177–180. [PubMed: 18778213]
28. Michie C, Lockie F, Lynn W. The challenge of mastitis. *Arch Dis Child.* 2003; 88:818–821. [PubMed: 12937109]
29. Modjarrad K, Chamot E, Vermund SH. Impact of small reductions in plasma HIV RNA levels on the risk of heterosexual transmission and disease progression. *AIDS.* 2008; 22:2179–2185. [PubMed: 18832881]
30. Sulkowski MS, Chaisson RE, Karp CL, Moore RD, Margolick JB, Quinn TC. The effect of acute infectious illnesses on plasma human immunodeficiency virus (HIV) type 1 load and the expression of serologic markers of immune activation among HIV-infected adults. *Journal of Infectious Diseases.* 1998; 178:1642–1648. [PubMed: 9815216]
31. Spach DH. Immunizations for HIV-infected adults: indications, timing and response. *Topics in HIV Medicine.* 2006; 14:154–158. [PubMed: 17237556]
32. 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. *Journal of Infectious Diseases.* 2004; 190:1880–1888. [PubMed: 15499546]
33. Stables D, Hewitt G. The effect of lateral asymmetries on breast feeding skills: can midwives' holding interventions overcome unilateral breast feeding problems. *Midwifery.* 1995; 11:28–36. [PubMed: 7731373]

Characteristics of 86 HIV-Infected, breastfeeding women diagnosed with unilateral mastitis or abscess who had or did not have breast milk samples available for analysis and characteristics of 115 unaffected women.

Table 1

	Affected women				Unaffected women			
	Included in the Analysis (n=38)		Excluded from the Analysis (n=48)		Control group (n=115)			
	N	%	N	%	N	%		
Categorical Characteristics								
	Married	33	86.8	39	81.3	92	80.0	
	Primary or No formal Education	24	63.2	32	66.7	68	59.1	
	Informal sector Employment**	14	36.8	8	16.7	30	26.1	
	Water Source Inside House/On Property	8	21.1	6	12.5	25	21.7	
	Cooking with Charcoal/Wood	23	60.5	36	75.0	83	72.2	
	Electricity in the home	17	44.7	15	31.3	40	34.8	
	Refrigerator in the home	5	13.2	4	8.3	12	10.4	
	Ran out of food 1 day in the last month	10	26.3	14	29.2	28	24.3	
Medical History								
	Malaria during this pregnancy	11	28.9	19	39.6	47	41.2	
	Weight loss in the last 6 months**	5	13.2	18	37.5	29	25.2	
	Cough for > 30 days	6	15.8	4	8.3	16	13.9	
	Swollen glands in more than one√ place	4	10.5	10	20.8	2	1.7	
	Severe rash over most of body	6	15.8	2	4.2	9	7.8	
	Ever been diagnosed with Tuberculosis	4	10.5	8	16.7	11	9.6	
	Ever been diagnosed with Thrush	4	10.5	8	16.7	7	6.1	
	Ever been diagnosed with Herpes Zoster	8	21.1	8	16.7	13	11.3	
	STD Treatment in last 8 months	7	18.4	9	18.8	26	22.6	
	Eligible for ARV+	28	73.7	37	77.1	75	65.2	
	Number of previous pregnancies	0	7.9	8	16.7	17	14.8	
	1–2	17	44.7	20	41.6	52	45.2	
	3	18	47.4	20	41.6	46	40.0	

Categorical Characteristics	Affected women				Unaffected women			
	Included in the Analysis (n=38)		Excluded from the Analysis (n=48)		Control group (n=115)			
	N	%	N	%	N	%	N	%
Breast-fed the previous baby ^{***}	32/34	94.1	38/38	100.0	94/98	95.9		
Report of prior episode of mastitis or abscess	4	10.8	6	12.5	7	6.4		
Took single dose NVP at delivery	34	89.5	44	91.7	110	95.6		
Continuous Characteristics	Mean (SD)		Median		Mean (SD)		Median	
Mother's Age (years) [∫]	27.5 (6.1)		27.5		25.7 (4.8)		25.0 (4.7)	
Plasma Viral Load during pregnancy (log ₁₀ copies/mL)	4.62 (0.80)		4.65		4.39 (0.84)		4.46 (0.83)	
CD4 Count during pregnancy (cells/uL) [∫]	322 (149)		294		350 (199)		403 (207)	
CD4 Count at 12 months post-partum (cells/uL)	374 (238)		367		382 (274)		474 (275)	
Hemoglobin during pregnancy (g/dL)	10.5 (1.7)		10.15		10.3 (1.3)		10.7 (1.8)	

^{**} p<0.05 comparing included to excluded affected women

[†] ARV Eligibility is defined as CD4 count is <350 cells/uL or WHO Stage III defined as defined as at enrollment: weight loss >6 months, thrush, fever, cough>6 months, tuberculosis, diarrhea>6 months

^{***} Missing data from 1 woman included and 2 women excluded on breastfeeding history.

[∫] p<0.05 comparing affected women to unaffected women

Table 2

Description of 110 episodes of unilateral mastitis/abscess diagnoses among 86 HIV-infected breastfeeding women by whether or not they could be included in the analysis

	Included n=38	Excluded n=72
Days between sample collection	Mean (std dev)	Range
Days pre- to during-pathology	44.1 (27.4)	14–106
Days during- to post-	92.2 (61.3)	9–374
Days pre- to post-pathology	135.9 (66.1)	56–415
	n (%)	n (%)
Post-partum months of diagnosis **		
1 week	0 (0%)	20 (27.8%)
1 month	25 (65.8%)	9 (12.5%)
2 months	1 (2.6%)	10 (13.9%)
3 months	0 (0%)	10 (13.9%)
4 months	6 (15.8%)	6 (8.3%)
4.5 months	3 (7.9%)	3 (4.2%)
5 months	0 (0%)	7 (9.7%)
6 months	1 (2.6%)	2 (2.8%)
9 months	2 (5.2%)	1 (1.4%)
15 months	0 (0%)	4 (5.6%)
Laterality **		
Pathology on left breast	23 (60.5%)	29 (40.3%)
Pathology on right breast	15 (39.5%)	43 (59.7%)
Infant feeding at time of diagnosis *		
Exclusive	35 (92.1%)	58 (80.6%)
Mixed	1 (2.6%)	11 (15.3%)
Complementary feeding	2 (5.3%)	3 (4.2%)
Symptoms		
Engorgement	13 (34.2%)	24 (33.3%)
Red/shiny breast	9 (23.7%)	12 (16.7%)
Blocked duct	3 (7.9%)	6 (8.3%)
Abscess	15 (39.5%)	27 (37.5%)
Painful breasts	30 (78.9%)	52 (73.6%)
Breast problem since last visit	26 (68.4%)	48 (66.6%)
Cracked/bleeding nipples	4 (10.5%)	7 (9.7%)
Swollen Nipples	5 (13.2%)	16 (22.2%)
Other	5 (13.2%)	15 (20.8%)
Number of concurrent symptoms		

		Included n=38	Excluded n=72
Days between sample collection		Mean (std dev)	Range
	One	11 (28.9%)	19 (26.4%)
	Two	14 (36.8%)	23 (31.9%)
	Three	5 (13.2%)	17 (23.6%)
	Four	5 (13.2%)	7 (9.7%)
	Five	3 (7.9%)	6 (8.3%)
Maternal clinic consultation reported			
	Pre-diagnosis	3 (7.9%)	n/a
	At diagnosis visit	16 (42.1%)	28 (36.1%)
	Post-diagnosis	8 (21.1%)	n/a

* Exclusive breastfeeding was defined as the provision of breast milk and prescribed medicines only. Mixed breastfeeding was defined as the provision of breast milk and any other food/liquid, including water at the child's age of 6 months or less. Complementary feeding was defined as provision of breast milk and other foods of a child older than 6 months.

** Chi-square test: $p < 0.05$

Table 3

Breast Milk HIV RNA quantity ($\log_{10}$ copies/mL) in 38 women with unilateral mastitis/abscess before, during and after diagnosis

		Pre- Diagnosis	During- Diagnosis	Post- Diagnosis	Pre- vs. During-	Pre- vs. Post-
P-value over time						
All women (n=211 samples)						
<i>Affected breast</i>	n	37	31	34		
	Mean	2.56	2.93	2.46	0.03	0.52
	Std. Error	0.15	0.19	0.19		
P-value across breasts						
Only if HIV RNA >50 copies/mL (N=164 samples)						
<i>Unaffected breast</i>	n	36	36	37		
	Mean	2.52	2.6	2.36	0.56	0.20
	Std. Error	0.13	0.15	0.11		
P-value across breasts						
P-value over time						
Only if HIV RNA >50 copies/mL (N=164 samples)						
<i>Affected breast</i>	n	29	25	22		
	Mean	2.77	3.22	2.82	0.02	0.80
	Std. Error	0.17	0.19	0.25		
P-value across breasts						
P-value over time						
<i>Unaffected breast</i>	n	29	29	30		
	Mean	2.72	2.82	2.54	0.53	0.22
	Std Error	0.14	0.16	0.12		
P-value across breasts						
P-value over time						
P-value across breasts						
P-value over time						

Table 4

Change in Breast Milk HIV RNA concentration (Log₁₀ copies/mL) in 38 HIV-infected women with unilateral mastitis/abscess

	Pre- to During-	During- to Post-	Pre- to Post-
<i>Affected breast</i>			
Mean	0.45	-0.54	-0.04
Std. Error	0.15	0.18	0.15
95% CI	+0.16, +0.74	-0.19, -0.89	-0.33, +0.25
<i>Unaffected breast</i>			
Mean	0.08	-0.21	-0.15
Std. Error	0.14	0.13	0.13
95% CI	-0.19, +0.35	-0.46, +0.04	-0.41, +0.10
<i>Difference in Change across breasts</i>	0.37	-0.33	0.11
(95% CI)	(+0.09, +0.63)	(-0.70, +0.05)	(-0.19, +0.42)
P-value of comparing differences in change across breasts	0.007	0.09	0.46

Table 5

Breast Milk HIV RNA quantity ($\log_{10}$ copies/mL) in 38 women with unilateral mastitis/abscess before, during and after diagnosis compared to 115 women without mastitis/abscess.

		Pre Diagnosis	During	Post Diagnosis
<i>Affected breast</i>	n	37	31	34
	Mean	2.56	2.93	2.46
	Std. Error	0.15	0.19	0.19
	% detectable (≥ 50 copies/mL)	78.40%	80.60%	67.50%
<i>Unaffected breast</i>	n	36	36	37
	Mean	2.52	2.60	2.36
	Std. Error	0.13	0.15	0.11
	% detectable (≥ 50 copies/mL)	80.60%	80.60%	78.40%
<i>Non-affected women</i>	n	115	115	115
	Mean	2.41	2.41	2.41
	Std. Error	0.09	0.09	0.09
	% detectable (≥ 50 copies/mL)	54.90%	54.90%	54.90%
P-value comparing means of mastitis-affected breast to non-affected women		0.28	0.01	0.69
P-value comparing means of non-affected breast to non-affected women		0.38	0.15	0.88