Late Postnatal Transmission of HIV-1 and Associated Factors

Taha E. Taha,1 Donald R. Hoover,2 Newton I. Kumwenda,3
Susan A. Fiscus,3 George Kabaluula,4 Chiwawa Nkhoma,3 Shu Chen,1
Estelle Piwowar,2 Robin L. Broadhead,5 J. Brooks Jackson,1
and Paolo G. Miotti3

1Department of Epidemiology, Bloomberg School of Public Health, and 2Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, and 3Office of AIDS Research, National Institutes of Health, Bethesda, Maryland; 4Department of Statistics and Institute for Health, Health Care Policy and Aging Research, Rutgers University, Piscataway, New Jersey; 5Department of Microbiology and Immunology, University of North Carolina, Chapel Hill; 6Departments of Obstetrics and Gynecology and of Pediatrics, College of Medicine, University of Malawi, and 7Johns Hopkins University–College of Medicine–Ministry of Health Research Project, Blantyre, Malawi

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Background. The present study was undertaken to determine the risk and timing of late postnatal transmission (LPT) of human immunodeficiency virus type 1 (HIV-1).

Methods. Breast-fed infants previously enrolled in 2 trials of antiretroviral prophylaxis were monitored in Malawi. Kaplan-Meier and proportional hazard models assessed cumulative incidence and association of factors with LPT.

Results. Overall, 98 infants were HIV infected, and 1158 were uninfected. The cumulative risk of LPT at age 24 months was 9.68% (95% confidence interval, 7.80%–11.56%). The interval hazards at 1.5–6, 6–12, 12–18, and 18–24 months were 1.22%, 4.05%, 3.48%, and 1.27%, respectively.

Conclusions. The risk of LPT beyond 6 months is substantial. Waning at 6 months could prevent >85% of LPT.

In Africa, HIV-infected women continue breast-feeding their babies for various reasons [1]. We previously reported that short postexposure prophylaxis with nevirapine (NVP) and zidovudine (ZDV) was associated with reductions in mother-to-child transmission (MTCT) of HIV at 6–8 weeks of age [2, 3]. NVP has a long half-life and might affect breast milk HIV load, which has been shown to be associated with risk of HIV postnatal transmission [4]. Whether these simple regimens can influence the magnitude and timing of late postnatal transmission (LPT) associated with breast-feeding is unknown. In this study in Malawi, we assessed the risk of LPT of HIV from 6–8 weeks to 24 months and examined factors associated with LPT.

Subjects, materials, and methods. Two concurrent randomized clinical trials (NVP–ZDV [NVAZ] studies) were conducted in Blantyre, Malawi [2, 3]. Infants were randomized to receive orally a single dose of NVP (2 mg/kg weight) or NVP (same single dose) plus ZDV (4 mg/kg weight) orally twice daily for 1 week. Mothers of these infants received intrapartum NVP if they presented early for delivery (early presenters) or did not receive NVP if they presented late for delivery with unknown HIV status (late presenters). All women were counseled and consented for HIV testing, and those who were HIV infected were enrolled after signing an informed consent form. None of the women received antiretroviral treatment while breast-feeding. Mother-infant pairs returned for follow-up visits at infant age 1 and 6–8 weeks, and 3, 6, 9, 12, 15, 18, and 24 months. At each visit, maternal-infant information was obtained, including breast-feeding status and type of feeding (exclusive or mixed).

The NVAZ studies were approved by the University of Malawi College of Medicine Research and Ethics Committee and the Johns Hopkins Bloomberg School of Public Health Committee on Human Research. Clinical care and referral were available to all participants at the study clinics.

In the present study, LPT was defined as HIV transmission occurring between 6–8 weeks and 24 months in breast-fed infants (infant HIV RNA negative at 6–8 weeks and HIV infected at a subsequent visit). All HIV-uninfected infants at 6–8 weeks in the NVAZ studies were eligible. Infants were classified as HIV infected if at least 1 HIV RNA test was positive or 2 ELISA tests and Western blot test were positive at or after 18 months, and infants were classified as HIV uninfected if HIV RNA tests were negative after 6–8 weeks or if HIV serologic tests were negative at or after 18 months. Infants with unclassifiable HIV status were excluded. The estimated timing of infant HIV infection was the midpoint between last negative and first positive HIV test. Infant follow-up was censored at date of weaning; extending this date to the next visit did not change the results.

Maternal baseline (enrollment) plasma viral load was mea-
sured using Roche HIV-1 RNA Amplicor Monitor (version 1.5). Infant blood, collected on filter paper cards at birth and at every follow-up visit, was tested for HIV RNA using NucliSens QL (BioMerieux) [2, 3]. The HIV RNA tests were performed at the University of North Carolina, Chapel Hill. Breast milk viral load (HIV-1 RNA in whole breast milk) from all transmitters (mothers of infants who became HIV infected) and a matched sample of nontransmitters (mothers of HIV-uninfected infants) was measured using the BioMerieux HIV RNA QT assay in accordance with the manufacturer’s instructions [5] at a reference laboratory (University of North Carolina, Chapel Hill). The samples from nontransmitters and transmitters were matched on date of delivery and presentation of the mother at delivery. Serial samples were tested starting from the time of infant HIV infection and proceeding backward to the time when the infant was not infected. NVP concentration in breast milk and maternal plasma and infant plasma was measured using a validated high-performance liquid chromatography method [6] at the HIV Prevention Trials Network Central Laboratory at the Johns Hopkins University, Baltimore, Maryland. Maternal plasma NVP measurements were on samples collected at birth and 6–8 weeks from early presenters. Infants’ plasma samples were also collected at the same time points.

Breast milk HIV RNA levels were log_{10} transformed to compare transmitters and nontransmitters at each visit using exact tests. The probability of detecting differences in breast milk viral load between transmitters and nontransmitters and between women who had received or had not received NVP were statistically tested using robust covariance logistic regression from the same individuals and multiple visits. Median values and ranges were calculated for NVP concentrations. Cumulative risk of LPT and interval hazards were obtained for infants not already HIV infected who became infected at 1.5–6, 6–12, 12–18, and 18–24 months using Kaplan-Meier (K-M) survival analysis. HIV-free survival (infant alive and not HIV infected) was estimated from the K-M analysis. Cox proportional hazard models assessed the association of potential risk factors with LPT; hazard ratios (HRs) and 95% confidence intervals (CIs) were obtained. SAS software (version 9.1; SAS Institute) was used for all statistical analyses.

**Results.** Of 2000 infants enrolled in the NVAZ studies, 554 were excluded because they were HIV infected at birth or at age 6–8 weeks (n = 322), died between birth and 6–8 weeks (~1.5 months) (n = 30), lost to follow-up between birth and 1.5 months (n = 189), or had no breast-feeding data (n = 13). Of 1446 HIV-uninfected infants who presented at 1.5 months, 190 were excluded because HIV results were not available after 1.5 months. Therefore, 1256 infants were included in the longitudinal analysis: 1158 HIV uninfected and 98 HIV infected.

Figure 1 shows interval risks (hazard) of postnatal transmission of HIV based on K-M analysis. Of the 2000 infants enrolled in the NVAZ study, 190 (9.50% [95% CI, 8.21%–10.79%]) were infected at birth. Of the remaining 1810 infants who were not infected, 132 infants were HIV infected at or before 1.5 months, corresponding to 8.43% (95% CI, 7.05%–9.80%) from the K-M analysis. Among the 1256 infants included in the longitudinal study and not previously infected, the interval risk (hazard) of HIV infection was 1.22% (95% CI, 0.61%–1.83%) during the period 1.5–6 months of age and increased to 4.50% (95% CI, 2.89%–5.19%) during the period 6–12 months. After 12 months, there was a gradual decline in hazard of HIV infection: 3.48% (95% CI, 2.24%–4.70%) during

**Figure 1.** Interval hazard of infant HIV infection. Estimates are based on Kaplan-Meier survival analyses.
12–18 months and 1.27% (95% CI, 0.33%–2.19%) during 18–24 months. At 24 months, the cumulative risk of LPT of HIV was 9.68% (95% CI, 7.80%–11.56%). Overall, 87.4% (9.68%–1.22%/9.68%) of LPT infections occurred after 6 months. The probability of HIV-free survival among infants not infected at 1.5 months of age was 98.7% at 6 months (95% CI, 98.1%–99.3%), 94.6% at 12 months (95% CI, 93.2%–95.9%), 90.2% at 18 months (95% CI, 88.4%–92.0%), and 87.4% at 24 months (95% CI, 85.3%–89.6%).

Baseline maternal plasma viral load was significantly associated with LPT (adjusted HR [AHR], 3.67 [95% CI, 2.55–5.27] per log10 unit). Additionally, primiparity (AHR, 4.82 [95% CI, 1.46–15.91]) and clinical mastitis (AHR, 4.94 [95% CI, 1.53–16.02]) were significantly associated with LPT. Other factors controlled for and not statistically significant were maternal age, hemoglobin level, body mass index, early versus late presentation, and sex of infant.

Overall, 794 breast milk samples were tested for HIV RNA from 313 women. The proportion of samples with detectable breast milk viral load was consistently higher in transmitters than nontransmitters at all visits (figure 2); these differences were significant at each visit (P < .02) except visits at 3 (P = .07), 18 (P = .07), and 24 (P = .41) months. The probability of detecting HIV in breast milk was lower during the period 1.5–9 months, compared with 12–24 months (nontransmitters: odds ratio [OR], 0.38 [95% CI, 0.16–0.91]; transmitters: OR, 0.57 [95% CI, 0.38–0.99]). Among nontransmitters, the proportion of women with detectable breast milk HIV RNA during 1.5–3 months was significantly lower (P = .03, exact test) in women who received NVP (early presenters) than in women who did not receive NVP (late presenters); the numbers were too small to compare differences at only 1.5 months.

NVP was detectable in all birth breast milk samples tested for 33 women who received NVP intrapartum (early presenters). In these samples, NVP concentrations were very high (median, 1470 ng/mL; in 32/33 women NVP concentration ranged from 679 to 4432 ng/mL). At 1 week, NVP was detectable in breast milk in 45 (83.3%) of 54 samples tested (median, 140 ng/mL; range, 29–460 ng/mL). At 6–8 weeks, only 1 (1.3%) of 75 women tested had detectable NVP (41 ng/mL) in breast milk. NVP in maternal plasma at 1.5 months was undetectable in all 67 samples tested. At birth, plasma samples from 31 infants of early presenting women were tested for NVP concentration, and 25 (80.6%) had detectable NVP.
(median, 674 ng/mL; range, 52–1609 ng/mL). At 1.5 months, 10 (4.3%) of 231 infants tested (born to early or late presenters) had detectable plasma NVP (median, 74 ng/mL; range, 35–811 ng/mL).

Discussion. This study shows that the risk of LPT associated with breast-feeding remains substantial. By age 24 months, the cumulative risk of HIV infection among infants who received short antiretroviral prophylactic regimens and were uninfected at 1.5 months was 9.7% (95% CI, 7.8%–11.6%). This estimate is comparable to a LPT probability of 9.3% (3.8%–14.8%) during 1–18 months reported from a meta-analysis involving multiple African sites [7]. The interval hazard of HIV infection was very low (1.22%) during the period 1.5–6 months, and LPT after 6 months accounted for >85% of infant HIV infections (figure 1).

We speculate that several factors may explain the relatively low risk between 1.5 and 6 months and the increase thereafter. First, NVP being highly lipophylic and widely distributed throughout the body [8] may have resulted in a more sustained lowering of viral load in breast milk. Analyses of the breast milk viral load and NVP concentration data in the present study provide some clues but are inconclusive. For example, among nontransmitters, the detection of breast milk HIV RNA during the period 1.5–3 months was lower among women who received NVP (early presenters) than in those who did not (late presenters). However, consistent with results of other studies [9], NVP levels were rarely detected after 1.5 months. Additional analyses (data not shown) did not show statistically significant differences in association of early presenters (NVP received intrapartum), compared with late presenters with LPT after adjusting for infant prophylaxis and maternal plasma viral load.

Second, we speculate that the appearance and then fading of NVP resistance mutations may have influenced LPT in this study. In the NVAZ studies, 64% of infants had detectable NVP resistance mutations at 1.5 months [10], and other studies showed that NVP resistance mutations fade and were no longer detected by 12 months [11]. NVP resistant virus might not be easily transmitted or fit to adequately replicate in the infant during the period 1.5–6 months, thus contributing to the low risk of infant infection at this age. The rise in LPT after 6 months could be associated with disappearance of NVP resistant virus and emergence of wild-type virus.

Third, exclusive breast-feeding has been shown to be associated with lower postnatal transmission of HIV-1 in African infants [12]. In the present study, the frequency of exclusive breast-feeding was high early postnatally and gradually declined (99% at week 1, 90% at 1.5 months, 56% at 3 months, and 3% at 6 months). Although these data are unlikely to explain the low risk before 6 months, this factor remains potentially important because of misclassification and reporting errors of breast-feeding. In the NVAZ studies, the median durations of exclusive and mixed breast-feeding were 2 and 12 months, respectively [13].

The major risk factors associated with LPT were higher maternal plasma viral load, clinical mastitis, and primiparity. These are consistent with findings of other studies, including earlier studies from Malawi [14, 15]. A difficulty encountered when evaluating interventions to curtail MTCT of HIV is how to balance benefits of breast-feeding with risk of infant HIV infection. Our findings suggest that if breast feeding were stopped by 6 months >85% of LPT could be prevented. HIV-infected women should consistently be counseled about the risks of HIV transmission through breast-feeding and adequately counseled on safer feeding methods after weaning.

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