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

Roger L. Shapiro,1,2 Laura Smeaton,1 Shahin Lockman,2,4 Ihou Thior,5 Raabya Rossenkhant,4 Carolyn Wester,5 Lisa Stevens,3 Claire Moffatt,5 Peter Arimi,3 Patrick Ndase,5 Aida Asmelash,5 Jean Leidner,3 Vladimir Novitsky,2 Joseph Makhema,5 and Max Essex2

1Beth Israel Deaconess Medical Center, Division of Infectious Diseases, 2Department of Immunology and Infectious Diseases and 3Center for Biostatistics in AIDS Research, Harvard School of Public Health, and 4Birmingham and Women’s Hospital, Infectious Disease Unit, Boston, Massachusetts; 5Botswana–Harvard School of Public Health AIDS Initiative Partnership for HIV Research and Education, Gaborone, Botswana

Risk factors for mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) via breast-feeding were evaluated in a randomized trial. HIV-infected women and their infants received zidovudine as well as single-dose nevirapine or placebo. Infants were randomized to formula-feed (FF) or breast-feed (BF) in combination with zidovudine prophylaxis. Of 1116 at-risk infants, 6 (1.3%) in the FF group and 7 (1.3%) in the BF group were infected between birth and 1 month (P = .99). Maternal receipt of nevirapine did not predict early MTCT in the BF group (P = .45). Of 547 infants in the BF group at risk for late MTCT, 24 (4.4%) were infected. Maternal HIV-1 RNA levels in plasma (P < .001) and breast milk (P < .001) predicted late MTCT. These findings support the safety of 1 month of breast-feeding in combination with maternal and infant antiretroviral prophylaxis.

Trial registration. ClinicalTrials.gov identifiers: NCT-00197691 and NCT00197652.

The optimal infant feeding strategy for HIV-infected women in Botswana remains unknown. Breast-feeding has been associated with 4%–12% absolute risk of mother-to-child transmission of HIV (MTCT) through 6 months [1–4], yet replacement feeding with infant formula carries an excess risk of infant mortality [2, 5]. We performed a randomized clinical trial (the Mashi Study) comparing 6 months of breast-feeding in combination with zidovudine (ZDV) prophylaxis with formula feeding among infants born to HIV-infected women in Botswana. At 18 months, there was no difference in the rate of HIV-free infant survival [2].

A closer analysis of these data can define the specific risks for early and late MTCT via breast-feeding and help determine groups of women for whom breast-feeding may be recommended. We therefore studied MTCT via breast-feeding in the Mashi Study to determine the following: (1) the contribution of early breast-feeding to MTCT identified between birth and 1 month, (2) the impact of maternal receipt of single-dose nevirapine (NVP) on early and late MTCT via breast-feeding, and (3) the risk factors for late MTCT.

Methods. The Mashi Study enrolled 1200 HIV-infected pregnant women at 4 sites in Botswana. The study employed a randomized, factorial design to determine the following: (1) whether single-dose NVP given to mothers and infants provided additional prevention of MTCT if mothers and infants were also receiving ZDV [6] and (2) whether prophylactic ZDV given to breast-fed infants for 6 months prevented MTCT via breast-feeding [2]. Mothers received antenatal ZDV from 34 weeks’ gestation and intrapartum ZDV plus either single-dose NVP or placebo. In October 2002, highly active antiretroviral therapy (HAART) became available through the Botswana Government Antiretroviral Treatment Program and was offered to women with CD4 cell counts <200 cells/mm3 or AIDS, either during the antenatal or postnatal period. Infants initially received single-dose NVP or placebo at birth and ZDV prophylaxis for 1 month (formula-fed [FF] arm) or 6 months (breast-fed [BF] arm) (era 1). After 17 months of enrollment, the study was modified and all infants received single-dose NVP (era 2). Informed consent was obtained from all participants, approval for the study was granted by human subjects committees in Botswana and at the Harvard School of Public Health, and the human experimentation guidelines of the US Department of Health and Human Services were followed in this trial.

We considered infants who had a negative DNA polymerase chain reaction (PCR) result at birth (defined as a test result ob-
tained \( \leq 15 \) days after birth) and their first positive DNA PCR result at the 1 month visit (defined as a test result obtained \( \leq 45 \) days after birth) as potentially infected intrapartum or during early breast-feeding. Late MTCT was considered to have occurred in Infants who had a negative DNA PCR result at the 1 month visit (defined as above) and a first positive test result at any point thereafter. Infant DNA PCR testing was performed at birth, at 1, 4, 7, 9, and 12 months, and by ELISA at 18 months. Maternal demographic characteristics, baseline CD4 cell count, and baseline plasma HIV-1 RNA level were assessed at 34 weeks gestation. Expressed breast milk samples were collected at 2 weeks, 2 months, and 5 months postpartum. The HIV-1 RNA level in breast milk was determined for available samples.

Breast-feeding women were counseled at each visit to breast-feed exclusively, and the actual feeding method as well as adverse events were evaluated each month for 6 months and at least every 3 months thereafter. Women were encouraged to wean infants from breast milk between months 5 and 6 and were provided formula and weaning foods. Women with HIV-infected infants were encouraged to continue breast-feeding.

Laboratory testing was performed at the Botswana-Harvard Partnership HIV Reference Laboratory in Botswana. Infant HIV testing was performed by PCR DNA assay (AmpliCord HIV-1 Test; Roche Diagnostic Systems). ELISA for HIV antibodies was used at 18 months (Ortho AB-Capture, Ortho Clinical Diagnostics; Murex HIV2.0, Abbott-Murex). The HIV-1 RNA copy number in plasma was quantified by using the automated standard protocol, with a lower limit of detection of 400 copies/mL, employing the COBAS Amplicor/Ampli Prep HIV-1 Monitor Test V1.5 (Roche Molecular Systems). The HIV-1 RNA level in breast milk supernatant was quantified by using the automated ultrasensitive protocol, with a lower limit of detection of 40 copies/mL, employing the COBAS Amplicor/Ampli Prep HIV-1 Monitor Test.

Statistical analyses were performed by using SAS (version 9.1, SAS Institute). Infant HIV-1 infection rates between birth and 1 month were compared by use of Fisher's exact test. After 1 month of age, infection rates were estimated by the Kaplan-Meier method, and risk factors were evaluated by using Cox proportional hazards modeling. Univariable models were used to estimate associations, with some covariates (diarrhea, pneumonia, and anemia) formulated as time varying to use the most recent status (presence vs. absence) of these comorbidities at each risk-set time in the model. To reduce reverse causality, we excluded time-varying events that occurred during the month before the at-risk time estimation. A stepwise, backward elimination procedure was used to fit multivariable Cox models, retain effect modifiers, and pick the covariate with the fewest missing values and most clinical relevance among collinear covariates (namely, the HIV-1 RNA level in plasma vs. that in breast milk).

**Results.** Baseline characteristics were well-matched between feeding arms (data not shown). Infant PCR testing occurred within 4 days of birth for 96% of infants, and later PCR testing occurred within 4 days of the scheduled 1-, 4-, and 7-month visits for 92% of infants. Of 1116 infants alive and HIV-free at birth, 6 (1.1%) in the FF arm and 7 (1.3%) in the BF arm were identified as infected between birth and 1 month (\( P = .99 \)), representing transmission either intrapartum or during early breast-feeding. In the FF arm, no breast-feeding was reported to have occurred among the 6 infants who were infected during the first month.

Maternal receipt of single-dose NVP was not a significant predictor for MTCT between birth and 1 month in the BF arm (\( P = .45 \)). There was a nonsignificant trend for early protection from maternal receipt of NVP in the FF arm (\( P = .12 \)); this trend was only apparent during the first study era, before all infants received prophylactic NVP and before HAART became available for women with advanced disease. Table 1 displays the breakdown of transmission between birth and 1 month by maternal and infant receipt of NVP or placebo in each feeding arm. Pharmacologic testing supported maternal self-report of NVP receipt for 95 of 96 randomly selected women [6]. Because 937 (80%) of 1179 live-born infants in the study were randomized to receive single-dose NVP, our ability to assess the importance of the infant dose for preventing transmission of HIV during early breast-feeding was diminished. In the latter half of the study, 71 women who were receiving HAART at the time of delivery (i.e., women with a CD4 cell count <200 cells/mm3 or AIDS) did not receive single-dose NVP or placebo, which limited our ability to evaluate its effect at lower CD4 cell counts.

Of 547 infants in the BF arm who were alive and HIV-uninfected at 1 month of age, late MTCT occurred in 24 (4.4%); it occurred in 15 infants before 4 months of age, in 6 during the period from month 4 to 6, and in 3 during the period from month 7 to 24 (table 1). An additional 4 transmissions occurred during the first 2 months of life, but the timing could not be determined to be either early or late because of missing PCR results at birth (for 1 infant) or at 1 month (for 3 infants). In the FF arm, 2 infants became infected after the 1 month visit, and 2 became infected at an undetermined time point.

Among the at-risk infants in the BF arm, univariable associations with late MTCT included higher baseline maternal plasma HIV-1 RNA level (\( P < .001 \)), higher breast milk HIV-1 RNA level (\( P < .001 \)), lower baseline maternal CD4 cell count (\( P = .004 \)), and maternal death (\( P = .006 \)). In multivariable analysis (which excluded breast milk HIV-1 RNA level, because it was colinear with plasma HIV-1 RNA level), maternal HIV-1 RNA level (\( P = .005 \)), maternal CD4 cell count (\( P = .06 \)), and lack of electricity in the home (\( P = .05 \)) predicted late MTCT (table 2). The duration of infant ZDV prophylaxis did not predict late MTCT (\( P = .98 \)). Four (16.7%) of the 24 late transmissions occurred among infants whose prophylactic ZDV had been stopped prior to their first positive HIV test result. Maternal receipt of single-dose NVP did not predict late MTCT. Al-
Table 1. Timing of mother-to-child transmission of HIV (MTCT) in the Mashi Study, by randomized feeding strategy.

<table>
<thead>
<tr>
<th>Timing of transmission</th>
<th>Breast-feeding + ZDV for 6 months (n = 588 live births)</th>
<th>Formula-feeding + ZDV for 1 month (n = 591 live births)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth: in utero MTCT</td>
<td>19/580 (3.3%)</td>
<td>22/576 (3.7%)</td>
</tr>
<tr>
<td>Birth to 1 month: peripartum and early breast-feeding MTCT</td>
<td>7/561 (1.3%)</td>
<td>6/555 (1.1%)</td>
</tr>
<tr>
<td>&gt;1 month: late breast-feeding MTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months 1 to 3</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Months 4 to 6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Months 7 to 24</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>24/547 (4.4%)</td>
<td>2/537 (0.4%)</td>
</tr>
</tbody>
</table>

NOTE. The timing of transmission could not be determined for 4 infants in the breast-feeding arm and for 2 infants in the formula arm. Denominators are for live infants at risk at the start of each interval. ZDV, zidovudine.

a All women received ZDV from 34 weeks’ gestation and were randomized to receive either single-dose nevirapine (NVP) or placebo during labor. Infants received ZDV for either 1 month (formula-fed arm) or 6 months (breast-fed arm). During the initial study era, infants were also randomized to receive single-dose NVP or placebo on the basis of their mothers’ assignment. The study design was modified after 17 months, and all infants subsequently received single-dose NVP (era 2).

b First positive polymerase chain reaction (PCR) result at 0–15 days.

c First positive PCR result at 16–45 days.

d For era 1, 1/110 (0.9%) maternal NVP/infant NVP and 2/117 (1.7%) maternal placebo/infant placebo; for era 2, 1/164 (0.6%) maternal NVP/infant NVP and 3/170 (1.8%) maternal placebo/infant NVP.

e For era 1, 0/119 (0%) maternal NVP/infant NVP and 4/108 (3.7%) maternal placebo/infant placebo; for era 2, 1/165 (0.6%) maternal NVP/infant NVP and 1/163 (0.6%) maternal placebo/infant NVP.

f First positive PCR result at 45 days.

g For era 1, 8/107 (7.5%) maternal NVP/infant NVP and 7/113 (6.2%) maternal placebo/infant placebo; for era 2, 6/162 (3.7%) maternal NVP/infant NVP and 3/168 (1.8%) maternal placebo/infant NVP.

Discussion. In the Mashi Study, in which mothers and infants received antiretroviral prophylaxis and where HAART became available during the study period, MTCT during early breast-feeding was rare, and maternal receipt of single-dose NVP did not impact the rate of MTCT during early or late breast-feeding. Higher HIV-1 RNA levels in plasma and breast milk were predictors for late MTCT.

The randomized feeding design allowed isolation of risk of MTCT via breast-feeding during the first month of life, when early transmission via breast-feeding is otherwise difficult to separate from intrapartum transmission. Only 1 previous study, performed in Nairobi, randomized women to either breast-feed or formula-feed their infants [7]. That study enrolled 425 women at a time when no antiretroviral interventions were available; HIV transmission was detected in 28 breast-fed infants and 13 formula-fed infants between birth and 6 weeks of age. In contrast, our study—performed in a setting where extensive prophylaxis was possible—detected few early transmissions in either arm. Although we had low power to detect a true difference in the rate of MTCT according to feeding arm because the study was not designed to evaluate transmission during early breast-feeding, it appears that prophylaxis and the availability of HAART blunted the risk of early transmission overall. The similarity between feeding arms suggests that most new infections that developed during this early period may have represented transmission that occurred intrapartum (rather than during breast-feeding).

The World Health Organization recommends 1 week of infant ZDV prophylaxis when mothers receive a full antenatal ZDV course [8], but supporting data for this recommendation were derived from a study population that was not breast-feeding [9]. The low MTCT rate in our BF arm during the first month suggests that the full month of infant ZDV, and possibly the additional receipt of single-dose NVP for infant prophylaxis, may be beneficial. These data are consistent with recent studies suggesting benefit from extended NVP prophylaxis for breast-
feeding infants [10]. The availability of HAART for women with advanced HIV infection also may have reduced risk of MTCT during early breast-feeding.

Maternal receipt of single-dose NVP was not significantly associated with MTCT during early or late breast-feeding. The extended presence of NVP in breast milk has been demonstrated to reduce the level of HIV-1 RNA in breast milk [11, 12], but this may provide little additional protection against MTCT when full maternal and infant prophylaxis regimens are used. It should be noted that our study was not powered to detect a significant early difference for maternal receipt of single-dose NVP in the BF arm, but given the low transmission rate of 1.3% between birth and 1 month, a larger study to confirm these findings may not be feasible.

We identified a higher plasma HIV-1 RNA level as the most significant risk factor for MTCT during late breast-feeding in multivariable analyses. The level of HIV-1 RNA in breast milk was excluded from the multivariable analysis, but was a significant univariable predictor (and is a known predictor for late MTCT [13, 14]). Of the 24 late transmissions observed in our study, none occurred when baseline maternal HIV-1 RNA level was >3500 copies/mL. The range of CD4 counts at which transmission occurred was large, although the median baseline CD4 cell count among women who transmitted HIV was only 225 cells/mm³.

In summary, the main risk factors for MTCT via breast-feeding appear to be higher breast milk and plasma levels of HIV-1 RNA, and probably lower CD4 cell count. These factors may be modified by maternal use of HAART. In the setting of extensive maternal and infant prophylaxis, the rate of MTCT was low during the first month of life and neither breast-feeding nor maternal receipt of single-dose NVP predicted transmis-
sions. These findings may have policy implications in locations where early formula-feeding has been associated with high rates of infant mortality (and where a short period of breast-feeding in combination with infant prophylaxis might be a viable recommendation) and where NVP-based treatment and MTCT prevention programs coexist.

Acknowledgments

We are indebted to the patients who participated in this study, and thank the Mashi Study team, which included Chuka Anude, Jonas Chanda, Lillian Makori, Janet Banno Moorad, Taolo Agnes Modise, Tholakele Moyo, Dipotsu Arbi, Mateo Malamba, Kgomo Moto Koloi, Lerato Dube, Tumisang Mmolotsi, Setho Babitseng, and Dorcus Mere (Molepolole Site); Jenny Boyle, Jane Magetshe, Tumalano Sekoto, Venice Modikwa, Lebopo Garebatho, Margaret Tsuro, Merriam Sesinyi, and Kabo Kelebalekgosi (Mochudi Site); Zegabriel Tedla, Gloria Mayondi, Ntukunu Makubate, Keatile Sebinang, Lesedi Tsalaile, Boniny Tsule, Irene The-beetsile, Jessica Setswalo, Irene Leteane, and Oarabile Makgabana (Lobatse Site); Mpho Mogodi, Anchilla Owor, Innocent Hove, Aida Asmelash, Tebogo Kakhu, Phana Ramalepa, Joyce Lubinda, S’khatle Ndebele, Florence Modise, Chenesani Bohule, Kebong Motshabi, and Malegogo Nthihame (Gaborone Site). We also wish to thank Chris Rowley, Shabnam Za-vahir, Ria Madison, Tlhongbotho Masoloko, Mary Fran McLane, Edward Garmey, Sui Yuan Chang, Enoch Sepako, Gaseene Sebetso, Flor-ence Modise, Chenesani Bohule, Kebong Motshabi, and Malegogo Nthihame (Gaborone Site). We also wish to thank Chris Rowley, Shabnam Zavahir, Ria Madison, Tlhongbotho Masoloko, Mary Fran McLane, Edward Garmey, Sui Yuan Chang, Enoch Sepako, Gaseene Sebetso, Florence Modise, Chenesani Bohule, Kebong Motshabi, and Malegogo Nthihame (Gaborone Site). We also wish to thank Chris Rowley, Shabnam Zavahir, Ria Madison, Tlhongbotho Masoloko, Mary Fran McLane, Edward Garmey, Sui Yuan Chang, Enoch Sepako, Gaseene Sebetso, Florence Modise, Chenesani Bohule, Kebong Motshabi, and Malegogo Nthihame (Gaborone Site).

References