# ADHERENCE-RELATED MECHANISMS OF BREASTMILK HIV-1 TRANSMISSION

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ABSTRACT

Nicole Lane Davis: Adherence-related mechanisms of breastmilk HIV-1 transmission
(Under the direction of William C. Miller)

Postpartum HIV transmission through breastfeeding accounts for up to 44% of infant HIV infections. Antiretroviral (ARV) drugs are administered to either the HIV-infected mother or HIV-exposed infant throughout breastfeeding to reduce HIV transmission risk. Suboptimal adherence to prescribed ARV regimens may partly explain breastmilk HIV transmission despite ARV use. The purpose of this dissertation was to 1) estimate adherence to postpartum ARVs, 2) quantify the effect of postpartum adherence on breastmilk HIV transmission, and 3) evaluate breastmilk HIV RNA concentration as a mediating factor between ARV adherence and breastmilk HIV transmission.

We included mother-infant pairs randomized to receive 28 weeks of either triple maternal ARV or daily infant nevirapine as part of the Breastfeeding, Antiretrovirals, and Nutrition study. Among these, a cohort of 1479 mother-infant pairs was used to estimate the association between postpartum ARV adherence and breastmilk HIV-1 transmission between 5-38 weeks of age. Additionally, a case-cohort study including 263 mother-infant pairs was used to assess breastmilk viral load as a mediating factor between postpartum adherence and breastmilk HIV transmission. Adherence was measured over four time intervals using pill count, suspension bottle weight, and maternal self-report. RNA concentration was measured in both plasma and breastmilk at 5 time points. Infant HIV-1 infection was determined by DNA PCR every 2-6 weeks.

Using pill count and bottle weight information, having ≥90% adherence was associated with a 52% (95% CI 3-76%) relative reduction in the adjusted rate of breastmilk HIV-1 transmission by 38 weeks of age, compared with having <90% adherence. Having partial adherence (81-98%) was associated with a 77% (95% CI 33-92%) relative reduction in the adjusted odds of having detectable (≥40 copies/ml) breastmilk HIV RNA, compared with having poor adherence (0-80%). Detectable breastmilk HIV RNA was associated with 4.5 (95% CI 2.0-9.9) times the adjusted relative rate of BMK HIV-1 transmission by 28 weeks of infant's age, compared to having undetectable (<40 copies/ml) breastmilk HIV RNA.

Maternal ARV adherence is associated with reduced breastmilk HIV transmission, at least partly mediated by reduced breastmilk HIV RNA. Effective adherence interventions may help to further prevent breastmilk HIV transmission.

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## PREFACE

I met Charlie Warde as an undergraduate. Dr. Warde was my general chemistry professor, and later became my academic advisor, life mentor, and a father figure. He introduced me to the field of public health, and helped me to find the vision and confidence to return for advanced degrees. He beamed with pride when he and his wife attended my Master's degree graduation, and again when I began my PhD studies. He will no doubt be looking down with joy and an Irish smirk when I give my final defense and walk across one last graduation stage. I can see him celebrating with a beer in hand and telling whoever will listen that I am one of the bravest people he knows, a title that took many years for me to be able to wear comfortably. He saw so much potential in me, and somehow knew that I could achieve more than I had ever dreamed for myself. He helped me to dream bigger, and to keep learning. There is no greater example of a mentor or teacher. I will forever be grateful for his early belief in me, and the profound impact he has had on my life.

I belong to a family of strong and resilient women, and I am so thankful to have a Mom and sister that have stood firmly by my side through all of life's valleys and peaks. There are no words to fully express my gratitude for the amount of sacrifices that my Mom graciously endured in order to provide a home, education, and opportunities for my sister and me. In addition, there is no one who gets more excited about my successes, or who is more comforting during times of turmoil, than my Stepdad. He has brought so much laughter, joy, inspiration, comfort, and meaning into my life. I hope that he will somehow always remember how incredibly much I love him.

Last but not least, I thank all of the people in my life that have made the last five years more fun. You have at times renewed my hope in humanity, consoled me, celebrated with me, and helped me put things into perspective. For all of the laughs, the joy, the encouragement, and the comfort, I genuinely thank you.

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| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| ANC | Antenatal Care |
| ARV | Antiretroviral |
| ART | Antiretroviral Therapy |
| BAN | Breastfeeding, Antiretrovirals, and Nutrition |
| BSU | Biostatistical Support Unit |
| CD4 | Cluster of Differentiation 4 |
| CDC | Centers for Disease Control and Prevention |
| CI | Confidence Interval |
| DAG | Directed Acyclic Graph |
| DBS | Dry Blood Spot |
| DNA | Deoxyribonucleic acid |
| HIV | Human Immunodeficiency Virus |
| HPDP | Health Promotion and Disease Prevention |
| HR | Hazard Ratio |
| IQR | Interquartile Range |
| MTCT | Mother-to-Child Transmission of HIV |
| NVP | Nevirapine |
| OR | Odds Ratio |
| PCR | Polymerase Chain Reaction |
| PMTCT | Prevention of Mother-to-Child Transmission of HIV |
| RNA | Ribonucleic acid |
| SD | Standard Deviation |

| | |
|---|---|
| UN | United Nations |
| UNC | University of North Carolina |
| UNAIDS | Joint United Nations Programme on HIV/AIDS |
| UNICEF | United Nations Children's Fund |
| VL | Viral load |
| WHO | World Health Organization |

## CHAPTER ONE: SPECIFIC AIMS

Prevention of mother-to-child HIV transmission (PMTCT) is one of the HIV research arena's biggest success stories.(1-12) We know from controlled studies that intrauterine and intrapartum transmission can be reduced by more than 80%; and postpartum transmission through breastfeeding can be reduced more than 70% by extended postpartum antiretroviral prophylaxis.(7, 8, 12) Despite these extraordinary scientific achievements and wide implementation of basic PMTCT interventions, implementation of more complex and efficacious interventions has been challenging in resource-limited settings.(13-15) Postpartum prophylaxis during breastfeeding is promising, but could be improved by understanding the extent to which adherence needs to be maintained during this extended period, and how suboptimal adherence affects transmission.(14)

The Breastfeeding, Antiretrovirals, and Nutrition (BAN) clinical trial was conducted in Malawi between 2004 and 2010 using a factorial design.(7) Mothers were randomized to receive a lipid-based nutrient supplement. All mothers and infants received one dose of nevirapine (NVP) and seven days of zidovudine and lamivudine. Mother-infant pairs were randomized to also receive one of three postpartum ARV regimens: 1) maternal triple ARV throughout breastfeeding; 2) infant NVP throughout breastfeeding; or 3) no further drugs postpartum ("enhanced" control). Infants randomized to maternal ARV and infant NVP experienced a 2.9% and 1.7% risk of HIV infection between 2 and 28 weeks of age, respectively.(7) Despite well-controlled implementation of an efficacious prophylaxis regimen,

breastmilk HIV transmission still occurred. One possible explanation for transmission could be suboptimal adherence to the prescribed antiretrovirals, which in turn can affect plasma drug concentrations and the duration and rate antiretrovirals suppress viral replication (pharmacokinetics). Continued viral replication results in increased HIV viral load, and higher viral loads have been associated with greater risk of HIV transmission.(16) Understanding the association between adherence, viral load, and resulting HIV transmission is critical.

Using clinical data along with stored plasma and breastmilk specimens from BAN, we estimated the association between postpartum antiretroviral adherence and the following: plasma HIV RNA concentration (viral load or VL), breastmilk HIV VL, and subsequent breastmilk HIV infection. In addition, we considered the following contrasts: plasma HIV RNA and breastmilk HIV RNA, and breastmilk HIV RNA and breastmilk HIV transmission. BAN provided a unique opportunity to conduct this ancillary study in a timely and cost-efficient manner. We used more frequent measures of the time-varying exposure (adherence) and outcomes (plasma and breastmilk viral load, transmission) than previously reported studies. In addition, we provided the first estimates of: adherence to an extended infant NVP regimen using the objective measure of suspension bottle weights, the association between postpartum adherence and breastmilk transmission, and the association between postpartum adherence and breastmilk viral load.

Quantifying the association between postpartum ARV adherence and breastmilk HIV transmission has significant practical implications for roll-out of World Health Organization (WHO) PMTCT guidelines for resource-limited settings, which promote extended antiretroviral prophylaxis until complete cessation of breastfeeding and beyond.

**Aim 1. Estimate the association between postnatal maternal and infant antiretroviral adherence and breastmilk HIV transmission between 5 and 38 weeks of life.**

*Hypothesis: Suboptimal adherence (<90%) results in increased breastmilk transmission of HIV.*

*Overview:* This prospective cohort study consisted of mother-infant pairs enrolled in BAN and randomly assigned to the two treatment arms. Adherence was measured by maternal ARV pill count, infant NVP suspension bottle weight, and maternal self-report.

**Aim 2. Estimate the association between postpartum maternal antiretroviral adherence, plasma HIV viral load, breastmilk HIV viral load, and breastmilk HIV transmission between 2 and 28 weeks of life.**

*Hypothesis: Suboptimal adherence (<80%) results in increased plasma and breastmilk HIV RNA, and increased HIV transmission via breastmilk.*

*Overview:* A case-cohort study design was employed. All HIV-infected infants were considered cases; a 15% subsample of HIV-uninfected infants served as non-cases. Plasma and breastmilk specimens taken at 2, 6, 12, 18, and 24 weeks were used for viral load analyses.

## CHAPTER TWO: BACKGROUND AND SIGNIFICANCE

### Prevention of mother-to-child HIV transmission

Incidence of perinatal HIV infection has declined in recent years, but still remains a significant public health problem.(17) In 2009, 370,000 children were infected with HIV through mother-to-child transmission; down from 500,000 estimated vertical transmissions in 2001.(18) While this 25% reduction in transmissions shows progress, greater reductions are achievable given current knowledge. This "impact gap" is greatest in sub-Saharan Africa, which bears the brunt of the pediatric HIV epidemic with 90% of vertical transmissions occurring in the region.(18)

Without any intervention, 25-45% of HIV-exposed infants will become HIV infected either in-utero, during labor and delivery, or through breastfeeding.(19, 20) Transmission can occur through fetal contact with HIV viral particles or HIV-infected immune cells found in one or more of the following: maternal blood, cervical or vaginal secretions, and breastmilk.(21) In-utero transmission is thought to occur in less than 10% of HIV-infected pregnant women, and may occur via transplacental crossing of blood, though this has not been proven.(22) The greatest proportion of infections occur during the intrapartum period, when the fetus may experience prolonged contact with virus found in maternal blood and/or cervical and vaginal secretions.(22) However, postpartum transmission through breastfeeding has been shown to account for up to 44% of infant infections, with the majority of

infections occurring in the first few months of life.(23) Breastmilk transmission is thought to occur when infant ingestion of cell-free or cell-associated virus in breastmilk is absorbed in mucosal gut surfaces, tonsils, or adenoids.(20)

Advanced maternal immune deficiency, prolonged rupture of the amniotic membranes, and extended breastfeeding are some of the factors associated with an increased risk of vertical transmission during various stages of the peripartum period (Table 2.1).(21, 24-27) However, high maternal viral load, possibly due to acute infection or advanced disease, is among the most important risk factors for transmission during pregnancy and breastfeeding.(16, 24, 28, 29) Transmission potential is thought to be virtually 0% at plasma HIV RNA concentration less than 1,000 copies/ml, and increase to over 40% at a plasma HIV RNA concentration of more than 100,000 copies/ml.(16) Most successful PMTCT interventions have focused on reducing fetal exposure to the virus, either by avoiding labor and delivery via elective caesarian section, avoiding breastfeeding via use of infant formula, or by reducing viral replication through use of antiretroviral medications.

Initially, PMTCT programs in the developing world only used antiretrovirals to reduce in-utero and intrapartum HIV transmission. To limit postpartum transmission, mothers were encouraged to replacement feed if acceptable, feasible, appropriate, sustainable and safe (AFASS), or to exclusively breastfeed and wean early.(30) While antiretrovirals substantially reduced peripartum transmissions, postpartum transmissions still occurred at a relatively high rate.(13) HIV-free survival (proportions of infants that were alive and free of HIV) between formula-fed and breast-fed HIV-exposed infants proved to be similar by feeding choice.(31) Gains made in reducing vertical HIV transmission by avoiding breastfeeding were met with increases in death due to mixing formula with unsafe water, improper

hygiene, and a lack of breastmilk immunologic benefit.(27, 31) Reducing breastmilk HIV transmission while encouraging mothers to breastfeed gained focus and research attention, resulting in several randomized trials that assigned mother-infant pairs to postpartum antiretroviral prophylaxis or treatment until complete cessation of breastfeeding.(4, 6-8) Extending antiretrovirals through the breastfeeding period proved to be safe and effective at dramatically reducing postpartum HIV transmission via breastmilk, and is now recommended by WHO.(32)

**ARV adherence and virological suppression**

Achieving and maintaining virological suppression is dependent on adherence to prescribed regimens, as demonstrated in HIV-infected adults.(33) The class of ARV drug is thought to determine the level of adherence needed to maintain virological suppression, but it is unclear what level of adherence is needed to become and remain virologically suppressed in the late ARV era of boosted protease inhibitors and ultrasensitive measures of viral load.(34)

Greater than 95% adherence is thought to be needed to maintain virological suppression among a minimum of 80% of patients when unboosted protease inhibitors are included in the ARV regimen.(35) Pharmacokinetic boosting of protease inhibitors (PI) with drugs such as ritonavir has been used to extend the plasma half-life of the active PI (i.e. lopinavir) by inhibiting drug metabolism during absorption, and also in the liver.(36, 37) With boosted PIs, 80% adherence may be sufficient for a minimum of 80% of patients to achieve virological suppression.(38-40) In contrast, the adherence needed for non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimens is less understood.(36) NNRTIs typically have a longer half-life than PIs, resulting in potential mono-therapy

and development of drug resistance if patients are non-adherent for extended periods of time.(36) In that PIs have a high genetic barrier to resistance, they are often thought to be more 'forgiving' than NNRTIs.(36)

While much is known about adherence in HIV-infected adults, less is known about adherence to PMTCT regimens, including extended regimens to prevent breastmilk transmission. Among pregnant women, non-adherence was associated with higher HIV viral loads in two United States studies, and compliance to short-course zidovudine was associated with decreased vertical transmission in a Kenyan study.(41-43) However, information on postpartum adherence and adherence-related outcomes in resource-limited settings is scant. To address this knowledge gap, we assessed the effects of adherence to both NNRTI and PI-based postpartum ARV regimens on virological suppression and HIV transmission in a resource-limited setting.

**Maternal and infant adherence to antiretroviral drug regimens.**

Adherence to short-course and extended antiretroviral regimens may be measured using a variety of approaches ranging in sophistication, including the following: self-report, pill count, electronic pill-boxes, visual analog scale, pharmacy dispense records, patient attendance at scheduled medical visits, and measurement of blood drug levels.(44) None of the adherence measurement approaches are completely accurate, and most are not practical for routine use in clinical settings.(45) Self-reported adherence has the most potential for bias (usually in the form of overestimating adherence) due to recall and social desirability bias, but is the measurement tool most often used because it is easy and inexpensive to administer.(46) Adherence measurements based on pill count and suspension bottle

weight are thought to have greater validity and take into account adherence over the entire monitoring period, rather than just the three days prior to the self-report assessment visit.(47) More expensive and sophisticated methods of adherence measurement, such as electronic pill-boxes, may have even greater validity.(47) However, they do not account for the possibility of removing medication doses in advance, and are not available for routine use in Malawi. Assays of drug levels to determine ARV adherence have been sparingly used, and only in clinical trial settings. Expense and the need for highly trained laboratory personnel and specialized equipment contribute to their scant use. Plasma drug levels are also limited to the period of time right before the clinic visit, and when used alone provide limited data.(47) We used pill count and suspension bottle weight data taken at multiple visits as our main adherence measure. In sensitivity analyses, we compared pill count and bottle weight adherence with maternal self-reported adherence. Both pill count and self-report are thought to be feasible for implementation in resource-limited settings.

Adherence to combination ARV may be lower than adherence to simpler but less efficacious PMTCT regimens, according to pooled data from multiple PMTCT studies.(48) Several reasons for non-adherence to ARV and PMTCT regimens have been cited, including: time and financial costs of accessing care, stigma, forgetfulness or changes in routine, and side effects of medications.(43, 49-54) Continued exposure over time to structural and economic barriers, stigma, and routine barriers such as medication side effects or changes in routine may contribute to adherence decay with prolonged ARV, compared to short-course regimens. Ensuring gains in efficacy are not countered by deteriorating adherence-affected outcomes is paramount.

Adherence to extended infant nevirapine has only been sparingly estimated, with no known studies to date assessing infant adherence using suspension bottle weight. The same barriers that contribute to declines in postpartum adherence to maternal ARV may also lead to reductions in adherence to infant NVP during the breastfeeding period. If mothers are not receiving postpartum antiretrovirals, then adherence to extended infant NVP regimens until complete cessation of breastfeeding is critically important. Understanding the levels of infant adherence maintained during breastfeeding, and how they affect adherence-related outcomes is needed.

Postpartum adherence to PMTCT regimens was shown to be lower than antepartum adherence in the United States, but in resource limited settings, postpartum adherence is largely unknown.(41, 43, 55) A postpartum decline in adherence may be caused by several different factors, including, increased stress and changes in routine related to caring for a newborn, and concern over the newborn's HIV status.(56) Decreased adherence among HIV-positive adults has been associated with time since HIV diagnosis and duration of time on ARVs, which may also contribute to postpartum adherence decline in the era of extended prophylaxis through breastfeeding.(56) In that declines in adherence during the postpartum period have been seen in some settings, it is imperative to know the implications for sub-optimal postpartum adherence on HIV transmission during the critical breastfeeding period.

Only approximately 53% of women have adherence levels $\geq$ 80% during the postpartum period, according to a recent meta-analysis.(48) Therefore, nearly half of postpartum women had adherence levels below the current recommended level for virological suppression even when boosted protease inhibitors are used. Nonadherence has been associated with increased HIV viral load among pregnant women in the United States.(41, 43) However, the effect of nonadherence on MTCT has only been

evaluated in one resource-limited setting, which found that women who were compliant to a short-course zidovudine regimen tended to have a lower transmission rate compared to those who were non-compliant.(42) This was the first study to assess the effects of sub-optimal postpartum adherence on both plasma and breastmilk HIV viral load and HIV transmission in a resource-limited setting.

### Adherence and antiretroviral pharmacokinetics

Adequate therapeutic levels of antiretrovirals are needed to suppress viral replication and prevent development of resistance.(33) The degree of adherence to ARV regimens is directly related to the maintenance of adequate therapeutic antiretroviral levels.(33) However, even with adequate adherence, inter-patient drug exposure may vary due to differences in absorption, distribution, metabolism and elimination. While there are some data on ARV adherence and virological suppression, the level of adherence needed to maintain therapeutic drug levels, an important intermediary step, is not well known.(57)

### Adherence and breastmilk HIV transmission

To date, the level of adherence needed for postpartum maternal ARV regimens or extended infant NVP to prevent breastmilk HIV transmission has not been studied. While both maternal ARV and extended infant NVP have been efficacious in drastically reducing transmission rates, transmissions have still occurred even in closely monitored clinical trials.(4, 6-8, 58) Adherence has remained a well-documented challenge for HIV treatment in general, and specifically in PMTCT programs. The effect of adherence on breastmilk HIV transmission is important for prevention programming, and currently not

well understood. We quantified the effect of adherence to both maternal ARV and extended infant NVP on breastmilk transmission of HIV.

Several factors affect an infant's exposure to antiretrovirals through breastfeeding, and include the following: concentration of drug in the mother's plasma, amount of drug excreted into the breastmilk, and the amount of milk the infant ingests.(57) Drug disposition during the postpartum period can be unpredictable due to the physiologic changes that take place during pregnancy which affect drug disposition, and the intra-subject variability in the time it takes for these changes to revert back to pre-pregnancy levels.(57) In addition, two potential mechanisms are thought to be at play regarding virus populations in breastmilk. The first, which is thought to constitute the largest proportion, is by continual trafficking from blood into breastmilk of cell-associated virus which resides inside the cell (measured as HIV-DNA), or cell-free virions (measured as HIV-RNA).(59) The second is transient local production of virus in the breastmilk.(59, 60) To further add to the complexity, there are differential levels of antiretroviral exposure into breastmilk and infant plasma both within and between classes of drugs.(57) Only ZDV and 3TC have concentrated to any real extent in breastmilk, among the ARVs that have been evaluated to date.(60) In addition, ARV has been shown to suppress HIV RNA in plasma and breastmilk, while HIV DNA in breastmilk persisted.(60) These challenges have resulted in incomplete knowledge regarding the antiretroviral therapeutic levels that are needed to prevent drug resistance to the infant, and to ultimately prevent breastmilk HIV transmission. However, adherence to ARV regimens is undoubtedly required if therapeutic levels of antiretrovirals are going to be achieved and maintained.

**Summary**

Mother-to-child transmission of HIV has been greatly reduced, but challenges still remain.(15) WHO recently revised their PMTCT guidance for resource-limited settings, and now recommend all HIV-infected pregnant and breastfeeding women receive maternal antiretroviral treatment through the breastfeeding period and beyond.(32) Effective implementation of the revised WHO guidance relies on adherence to prescribed antiretroviral regimens.(32) Maintaining adherence to even short-course antiretroviral prophylaxis has been challenging for PMTCT programs in resource-limited settings.(54, 61-64) Adherence to more efficacious and complex maternal ARV regimens has been even lower than adherence to short-course PMTCT regimens, with almost half of HIV-positive mothers not maintaining an adherence level thought to be needed for virological suppression.(48) Being virologically suppressed has been widely shown to reduce the risk of HIV transmission.(16) Adherence is needed to achieve and maintain therapeutic levels of antiretrovirals in plasma, inhibit viral replication, and achieve viral suppression.(33)

We used a comprehensive approach for assessing the role of adherence on HIV transmission by assessing the causal pathway that includes both plasma and breastmilk HIV viral load. Greater understanding of the interconnectedness between adherence, viral load, and HIV transmission is a major step in eradicating breastmilk HIV transmission, and making breastfeeding safe for all infants. In addition, the data are of great importance to national PMTCT programs as they implement the revised WHO guidance and evaluate their adherence counseling messages.

**Table 2.1** Selected important risk factors for vertical HIV transmission by peripartum period.

| Stage | Risk Factors |
|---|---|
| Pregnancy | High HIV viral load (acute infection, advanced disease) |
| | Low CD4 count |
| | Other infections (e.g..: Hepatitis C, CMV, STIs) |
| Labor & Delivery | High maternal HIV viral load |
| | Prolonged rupture of membranes |
| | Preterm delivery (especially <32 weeks) |
| | Vaginal delivery |
| | Chorioamnionitis (inflammation of fetal membranes due to bacterial infection) |
| Breastfeeding | High maternal HIV viral load |
| | Low maternal CD4 count |
| | Maternal illness |
| | Non-exclusive breastfeeding |
| | Prolonged breastfeeding |
| | Cracked nipples, mastitis |
| | Infant co-infections |

## CHAPTER THREE: RESEARCH DESIGN AND METHODS

### PARENT STUDY

The Breastfeeding, Antiretroviral and Nutrition (BAN) randomized control trial was conducted in Malawi between 2004 and 2010 to assess in part the benefit and safety of antiretroviral medications given either to infants or to their mothers to prevent HIV transmission during breastfeeding.(7) Mothers were recruited at antenatal clinics in Lilongwe, Malawi where HIV testing was conducted. Eligibility criteria for mother-baby pairs included the following: maternal HIV infection, maternal CD4 $\geq$ 250 cells/μL ($\geq$ 200 cells/ μL before July 24, 2006), have used no antiretroviral drugs previously (including SD NVP), infant birth weight of at least 2000 grams, no infant or maternal condition that would preclude the use of a study drug, and able to be enrolled within 36 hours of delivery.(7)

All mothers and infants enrolled in BAN received a single dose of NVP along with seven days of zidovudine and lamivudine postpartum. In addition, mother-infant pairs were randomized to receive one of the following PMTCT prophylaxis regimens postpartum: 1) 28 weeks of a triple antiretroviral regimen given to the mother only (maternal ARV); 2) 28 weeks of daily nevirapine given to the infant only (infant NVP); or 3) no further drugs postpartum (enhanced control).(7)

Infant dried blood spots (DBS) were collected at labor and delivery, 1, 2, 4, 6, 8, 12, 18, 24, 28, 32, 36, 42 and 48 weeks of age, and were used to narrowly define the time of infant HIV infection by

polymerase chain reaction (PCR). In addition, maternal plasma and breastmilk specimens were obtained at labor/delivery, 2, 4, 6, 8, 12, 18, and 24 weeks postpartum. Maternal plasma continued to be collected at 28, 36, and 48 weeks postpartum. Infant plasma was collected at birth, 2, 6, 12, 18, 24, 28, 36, and 48 weeks. Self-reported adherence data was collected at 1, 4, 8, 21, and 28 weeks postpartum, using three-day recall; pill counts and bottle weights were calculated at 2, 4, 8, 12, 18, 24, and 28 weeks.(65)

**STUDY SETTING**

Malawi is a landlocked country in southeast Africa that has one of the lowest gross domestic products per capita ($860 based on purchasing power parity), and one of the highest maternal and infant mortality rates (460 deaths per 100,000 live births and 79 deaths per 1,000 live births, respectively).(66, 67) Approximately 10.8% of adults aged 15-49 in Malawi are HIV-infected. More than 90% of pregnant women attend at least one antenatal visit in Malawi, and HIV testing coverage within antenatal clinics in Lilongwe is greater than 90 percent.(68) Fifty-four percent of pregnant women have an institutional delivery with a skilled attendant and three percent receive a caesarean section.(68) The vast majority of women (72%) exclusively breastfeed their infants in the first months of life and 77% of women continue breastfeeding for at least two years.(68)

Lilongwe is the capital of Malawi, with a population of 670,000 people.(67) While Lilongwe is the most populous city in the country, it is still deemed peri-urban. The study population should allow the results of this research to be generalized to other parts of Malawi, and potentially other countries with generalized HIV epidemics in sub-Saharan Africa.

**SPECIFIC AIM 1**

***Estimate the association between postnatal maternal and infant antiretroviral adherence and breastmilk HIV transmission between 5 and 38 weeks of life.***

*Rationale:* Vertical HIV transmissions have occurred even in the setting of highly suppressive prophylaxis regimens. One possible explanation for transmissions is poor antiretroviral adherence. As extended PMTCT regimens that continue until complete cessation of breastfeeding and beyond are now promoted by WHO, it is imperative to know the relationship between adherence and HIV transmission during breastfeeding.

*Design:* We used an observational prospective cohort study to look at the relationship between adherence to the assigned prophylaxis regimen and breastmilk HIV transmission among all mothers and infants in the two treatment arms (maternal ARV and infant NVP).

*Study Population:* Our study population will consist of mother-infant pairs randomized to the two treatment arms of BAN, maternal ARV (n=849) and infant NVP (n=852). Mother-infant pairs that were randomized to the enhanced control arm of BAN (n=668) will be excluded because they were not exposed to the extended postnatal regimen. Infants in the two treatment arms that were diagnosed as HIV-positive between birth and 4 weeks of age (n=86) were excluded, as we had no adherence measure prior to their becoming HIV-infected. Similarly, infants in the two treatment arms that were lost to follow-up (n=84) or died (n=6) before by 4 weeks of age were excluded. In addition mother-infant pairs

with unknown breastfeeding status were excluded (n=46), as we could not ensure the infants were at risk of breastmilk HIV transmission. Our analysis thus included a total of 1479 mother-infant pairs.

*Exposure and Outcome Assessment*: Our main exposure of interest was adherence to the prescribed postpartum antiretroviral prophylaxis regimen. Adherence was measured at the following intervals using maternal ARV pill count and NVP suspension bottle weight from contiguous visits: 2-4 weeks, 8-12 weeks, 13-18 weeks, and 24-28 weeks. Adherence measures were not available for weeks 5-7, and 19-23. For analysis purposes, the unobserved adherence during weeks 5-7 and weeks 19-23 were assumed to be equal to the observed adherence during weeks 8-12 and weeks 24-28, respectively. Percent adherence was treated as a time-varying exposure, and calculated using the BAN dosing regimens and the following two formulae:

*Pill count*: $\frac{\text{(\# of pills distributed at previous visit - \# of pills returned at current visit)}}{\text{(days between visits * pills prescribed per day)}}$

*Bottle weight*: $\frac{\text{(\# of grams distributed at previous visit - \# of grams returned at current visit)}}{\text{(days between visits * grams prescribed per day)}}$

Adherence to each drug included in the maternal ARV regimen was calculated separately. In that all three drugs contained in the ARV regimen need to be taken for the regimen to be complete, we used the percent adherence for the least adherent drug as the percent adherence for the ARV regimen at each visit. Only NVP adherence was used for the infant NVP arm. Percent adherence was coded in

the following ways: continuous using any necessary transformations (e.g..: splines); dichotomous (≥ 90% vs. < 90%, based on previous studies and expected distribution of our adherence data); and as data-driven categories. We assessed the choice of cutoff for our dichotomous adherence measure in sensitivity analyses described below.

Pill count and bottle weight adherence measures were compared to self-reported adherence. Self-reported adherence was measured at 4, 8, 21, and 28 weeks postpartum based on the mother's answers to the following question: "During the past three days excluding today, have you/your baby missed any doses of (name of each individual antiretroviral prescribed)?" Self-reported adherence was considered a time-varying dichotomous variable (no reported missed doses of *any* drugs vs. at least one reported missed dose of *any* drug). Dichotomous self-reported and dichotomous pill count or bottle weight adherence measures were compared. However, the two measures assess adherence over different time periods (pill count and bottle weight: an interval of 2-6 weeks, self-report: an interval of 3 days) and were therefore expected to differ.

Our main outcome was breastmilk HIV transmission, determined by Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA) at 2, 12, 28, and 48 weeks to indicate infant HIV status. PCR positive results were confirmed by testing an additional blood specimen, and the window of infection was narrowed with tests of infant dried blood-spot specimens taken at 4, 6, 8, 18, 24, 32, and 36 weeks. Breastmilk HIV transmission was treated as a dichotomous variable. PCR detected HIV in infant DBS specimens between 5 and 38 weeks of age were considered as having the outcome. The date of the first PCR positive test was considered as the time transmission occurred. The date of the last PCR negative test was used for infants that remained HIV-uninfected during the study period. The frequency

of DBS collection and a narrow window for time of infection provided us with more accurate allocation of person-time by HIV-status than previously reported studies.

*Covariates:* Covariates consisted of randomization assignment, demographic characteristics, and health status information (Table 3.1). All covariates were measured at baseline as part of the BAN trial, with the exception of breastfeeding status that was assessed using standardized questionnaires administered regularly during BAN. Data for each covariate was obtained from the BAN database.

*Data Management:* Data management for BAN was supervised by the Biostatistical Support Unit (BSU) of the UNC Center for Health Promotion and Disease Prevention (HPDP Center). The BSU facilitated data management activities such as creating datasets, establishing database storage procedures, and archiving file backups. Statisticians at UNC and CDC, study investigators, and the BSU worked in collaboration on all data management activities.

Data collection forms (DCFs) were provided for each subject. Study documents did not include any names of study participants. Instructions for DCF use, data collection, and DCF scanning into the computerized database were provided by the BSU. Data collection form components were formatted for use through Teleform®, were optically scanned in Lilongwe via Teleform, and were uploaded to a database in Chapel Hill. Range checks were built into all choice option fields, and data verification was done on-screen. Teleform software has been used for two other large studies conducted by UNC, and is currently the method of data management used in studies run through the Department of Health Promotion and Disease Prevention.(65)

Laboratory specimens contained only a coded number in order to maintain subject confidentiality. Unique patient identification numbers were created by the BSU, and were used to link patient specific laboratory and demographic data. All electronic files were password-protected; access to files was only granted to authorized study personnel.

Demographic information, adherence assessments, infant HIV status, etc. were collected on various BAN study forms and stored in SAS datasets. Viral load data were stored in an Excel spreadsheet and imported into a SAS file. All relevant SAS datasets for this study were merged and checked for data integrity.

**Specific Aim 1: Statistical Analysis**

*Descriptive Analysis*: Distributions of all exposure, outcome and covariate variables were assessed to identify any outliers or unexpected values. Frequencies, means, and medians were also calculated for all variables.

*Modeling Approach*: Binomial regression models using generalized estimating equations (GEE) and an exchangeable correlation matrix were used to test whether or not adherence changed over time. A Cox model with time-varying covariates was used to estimate the hazard ratio for infant HIV infection by adherence status. Use of the Cox model allowed us to take into account lost-to-follow-up, death, time-varying adherence, time-dependent breastfeeding status, and time-fixed confounders. The Cox model follows the below formula, where the log of the hazard at time t is equal to the log of the baseline hazard function at time t ($h_0(t)$) plus the beta coefficients for both the time-varying exposure

($\beta_1 x_{i1}(t)$) and time-fixed confounders ($\beta_2 x_{i2}$). The hazard ratio is obtained by exponentiating the beta coefficient for the exposure at time t ($e^{(\beta 1 x i1(t))}$) holding all other variables constant.

$$\log h_i(t) = h_0(t) + \beta_1 x_{i1}(t) + \beta_2 x_{i2}$$

*Effect Measure Modification*: Effect measure modification was assessed by comparing the hazard ratio estimates and 95% confidence intervals from models with and without an interaction term between adherence and the covariate of interest. Covariates that produced adjusted hazard ratio estimates that were different enough to be clinically or programmatically relevant were considered effect measure modifiers. Potential effect measure modifiers are listed in Table 3.1.

*Confounding*: A directed acyclic graph (DAG) was used to identify potential confounders and a minimally sufficient adjustment set. Given the limited number of outcomes, a more parsimonious model was generated by removal of potential confounders that had minimal effect on the hazard ratio estimate or precision. Potential confounders are listed in Table 3.1.

*Multivariable Associations*: A backward elimination model strategy was implemented in order to evaluate each covariate in the presence of other covariates, and to consider joint effects. Covariates were sequentially removed from the full model individually and then replaced to allow for assessment of joint effects. The change-in-estimate was calculated for each model with a single covariate removed (compared to the full model). The covariate producing the smallest change-in-estimate was then

removed permanently and the process repeated. The effect of removing a covariate on the precision of the hazard ratio estimate for the main exposure-outcome relationship was assessed by the confidence limit ratio (upper limit/lower limit).

*Sensitivity Analyses*: Three main sensitivity analyses were conducted. First, sensitivity analyses were conducted around a range of values for ARV adherence during the study period. Specifically we conducted sensitivity analyses using the following: 1) a cutoff of 85% pill count and bottle weight adherence for our dichotomous main exposure variable; 2) a cutoff of 95% pill count and bottle weight adherence for our dichotomous main exposure variable; and 3) our self-reported adherence measure as our main exposure, instead of pill count/bottle weight.

Second, we assessed the impact of adjustment for baseline maternal viral load, which was not included in the main regression analyses. Baseline maternal viral load information was not available for 3 mother-infant pairs, including one mother-infant pair who experienced a HIV-1 transmission event. However, due to the known association between viral load and HIV transmission, we included log-transformed baseline maternal viral load in both the imputation and analysis models as a sensitivity analysis.

Differential detection of HIV using nucleic acid testing may occur in a setting of infant NVP versus maternal ARV exposure. Under daily NVP drug pressure an infant may have very low levels of virus, resulting in a limited number of cells that are infected with HIV, and a delayed positive DNA assay result. In order to account for this possibility, we conducted a sensitivity analysis and assumed transmission among infants in the infant NVP arm occurred at the time of the last HIV-negative test

before receiving an HIV-positive test, rather than assuming transmission occurred at the time of the first HIV-positive test.

*Missing Data*: Multiple imputation was used in order to account for missing adherence measures.(69) Adherence was assumed to be missing at random. The probability of being adherent for each missing adherence measure was predicted from a fitted logistic regression model comprised of variables believed a priori to be predictive of adherence and for which there was minimal missing data. Covariates used to predict adherence included ARV study arm, maternal age (continuous), parity (0, ≥1), marital status, (married, not married), education status (≤primary, >primary), baseline maternal CD4+ category (200-350, 351-500, >500), baseline maternal hemoglobin (<11mg, ≥11mg), previous visit(s) adherence, time-dependent breastfeeding status, infant's outcome, and log survival time. Five hundred complete data sets were imputed based on the logistic regression predicted probabilities. Extended unadjusted and adjusted Cox models were then fit to obtain parameter and variance estimates for each of the 500 datasets, and these estimates were combined to obtain a final mean hazard ratio estimate and 95% confidence interval.(70)

*Tied data:* The number of transmissions was small, therefore tied event times (transmissions occurring at the same time) were not thought to be problematic. However, tied data were handled using the exact method in SAS which assumes time is continuous and that ties arise from grouping continuous, untied data.

All data analyses and power calculations were conducted using SAS version 9.2 and 9.3 (SAS Institute, Cary, North Carolina, USA) and Stata version 11 (StataCorp LP, College Station, Texas, USA).

*Power Calculations:* There were 12 (1.7%) and 21 (2.9%) infections that occurred between 2 and 28 weeks in the NVP and ARV arms, respectively. Low transmission rates lead us to assume that most women and infants were adherent to their prescribed regimen. In addition, the desire to protect their infant from acquiring HIV is thought to provide high motivation for mothers to be adherent to their medication, and the administration of medication to their infant. High adherence motivation coupled with the close follow-up from a clinical trial is also thought to result in high rates of adherence. We further assumed that the majority of transmissions that occurred were due to poor adherence to medication, and that a smaller proportion of transmissions occurred despite perfect adherence, potentially due to drug resistance or other biological mechanisms. Specifically, we assumed adherence rates would be between 85-95% and that between 10-40% of transmissions would occur in the adherent group. Based on these assumptions, we had over 80% power to detect hazard ratios between 3.7 (85% adherence; 40% transmissions in adherent group) and 15.0 (95% adherence; 10% transmissions in adherent group) for combined maternal and infant adherence (Figure 3.1).

*Limitations:* We used pill count and bottle weight to categorize exposure status, which may lead to some misclassification of exposure. We were also reliant on the frequency with which mothers returned for visits and study staff collected complete adherence information. Missing pharmacy forms resulted in extended and inconsistent intervals between adherence measures, and an incomplete overall adherence estimation. We used multiple imputation to account for missing adherence measures.

We were also limited by the relatively small number of transmissions that occurred in the two BAN treatment arms between 5-38 weeks postpartum (n=45), which prevented us from making comparisons of the association between adherence and transmission by study arm. In addition, unmeasured factors may have affected the relationship between adherence and transmission, biasing our observed associations.

*Alternative approaches:* A randomized control trial that randomizes mothers or infants to sub-optimal adherence would not be ethical. This research built upon an existing cohort of mother-infant pairs that were and were not adherent to their randomized prophylaxis regimen, and allowed us to answer the specific aim by means of an observational cohort study.

**SPECIFIC AIM 2**

***Estimate the association between postpartum maternal antiretroviral adherence, plasma HIV viral load, breastmilk HIV viral load, and breastmilk HIV transmission between 2 and 28 weeks of life.***

*Rationale*: Suboptimal adherence to prescribed antiretroviral prophylaxis regimens may affect the rate at which the antiretrovirals begin to suppress viral replication, and/or the duration of the effect on viral replication (pharmacokinetics), resulting in increased HIV viral load. Higher viral loads have been associated with higher risk of HIV transmission. This study allowed us to estimate the association between antiretroviral adherence, resulting plasma and breastmilk HIV viral load, and subsequent HIV infection. The effect of adherence on viral load and HIV transmission have important implications for

adherence counseling messages and determining the cost-effectiveness of routine viral load screening among HIV-positive breastfeeding mothers.

*Design*: We used a cost efficient case-cohort design to take into account plasma and breastmilk HIV viral load when assessing the association between adherence and breastmilk transmission of HIV.

*Study Population*: We included all mother-infant pairs in the 28 weeks of triple maternal ARV or daily infant NVP arms with available plasma or breastmilk samples and an HIV transmission event between 2 and 28 weeks of the infant's life (n=31). We also included a 15% sample of mother-infant pairs randomized to the two treatment arms of BAN where HIV transmission did not occur within 28 weeks of life (n=232); sampling was primarily based on stored breastmilk and plasma availability. Mother-infant pairs who experienced transmission between birth and two weeks of age (n=86) were excluded and information on first-born multiples was used when multiple births occurred (n=5). Our analysis thus included a total of 263 mother-infant pairs at risk of breastmilk HIV transmission between 2 and 28 weeks of age.

*Exposure and Outcome Assessment*: Exposure and outcome varied by contrast estimated and included the following: 1) adherence (exposure) and plasma HIV VL (outcome), 2) adherence (exposure) and breastmilk HIV VL (outcome); 3) plasma HIV VL (exposure) and breastmilk HIV VL (outcome), and 4) breastmilk HIV VL (exposure) and breastmilk HIV transmission (outcome) (Table 3.2).

Maternal adherence was calculated for the following postpartum time intervals: 2-4 weeks, 8-12 weeks, and 13-18 weeks. Adherence to triple maternal ARVs was calculated using pill counts taken by trained pharmacy staff on contiguous visits, and has been described in detail in Aim 1. Briefly, maternal ARV adherence was calculated from the difference in the number of pills distributed at the previous visit and the number returned at the current visit, compared with the number of pills that should have been taken between visits if the mother was perfectly compliant. Mothers randomized to the infant NVP arm were assigned a maternal adherence value of zero for all intervals. Maternal adherence was categorized into the following using disjoint indicator variables, 0-80% ("poor adherence", referent), 81-98% ("partial adherence"), and >98% ("near perfect adherence"). Categories were chosen based on previous studies and the observed adherence distribution.(34)

HIV RNA was quantified from blood plasma and whole breastmilk at enrollment, 2, 6, 12, 18, and 24 weeks postpartum. In addition, breastmilk HIV RNA was measured at 4 and 8 weeks post-delivery. Plasma VL was quantified using the Abbott RealTime HIV assay (Abbott Molecular, Des Plaines, IL) and the 0.6ml protocol according to the package insert (lower limit of quantitation 40 copies/ml). Breastmilk VL was quantified from 0.6 ml whole breastmilk pre-treated with 209ul Abbott RNA sample prep lysis buffer and 60ul Abbott Proteinase K (53°C incubation for 20 min) using the Abbott RealTime HIV assay (Abbott Molecular, Des Plaines, IL; lower limit of quantitation 56 copies/ml). HIV RNA concentrations that were detected but below the limit of quantitation were assigned a value of 39 for plasma and 55 for breastmilk (lower limit of quantitation minus one), and RNA concentrations that were undetectable were assigned a value equal to 50% of the lower limit of quantitation (plasma: 20, breastmilk: 28). VL was then categorized as detectable (plasma: $\geq$40 copies/ml, breastmilk: $\geq$56 copies/ml) or undetectable

(plasma: <40 copies/ml, breastmilk: <56 copies/ml) when treated as a binary variable, and log10-transformed when treated as a continuous variable.

Infant HIV status was determined at 2, 12, 28, and 48 weeks by Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA). PCR positive results were confirmed by testing an additional blood specimen, and the window of infection was narrowed with tests of infant dried blood-spot specimens taken at 4, 6, 8, 18, 24, and 32 weeks. Breastmilk transmission was defined as first detection of HIV infection by PCR in infant blood between 2 and 28 weeks of age, and treated as a dichotomous variable. Covariates included randomization assignment, demographic characteristics, and health status as described in Aim 1 (Table 3.1).

*Covariates:* Covariates consisted of randomization assignment, demographic characteristics, and health status information (Table 3.1). All covariates were measured at baseline as part of the BAN trial, with the exception of breastfeeding status that was assessed using standardized questionnaires administered regularly during BAN. Data for each covariate was obtained from the BAN database.

**Specific Aim 2: Statistical Analysis**

Descriptive Analysis and Weighting Structure: Descriptive analyses were conducted as described in Aim 1. Sampling weights were used to take into account the sampling of cases and non-cases, realizing that the sampled cases and non-cases represent a larger population. Specifically, the reciprocal of the study inclusion probability was used as a sampling weight in all regression analyses.

General Modeling Approach: We used generalized linear mixed models with identity link and Gaussian distribution to estimate the difference in log10 plasma VL by adherence category, and logit link with binomial distribution to estimate odds ratios for detectable breastmilk VL by adherence and plasma VL category. Single and multivariable Cox models were used to estimate the hazard ratio for the rate of breastmilk HIV transmission by 28 weeks of age. Mother-infant pairs were censored at reported cessation of breastfeeding unless HIV transmission occurred within 30 days of reported cessation, and mother-infant pairs that were lost to follow-up were censored at the time of their last PCR negative HIV test.(71)

Effect Measure Modification: Effect measure modification was assessed by comparing model-specific effect estimates (i.e. beta coefficients, odds ratios, and hazard ratios) and 95% confidence intervals from models with and without an interaction term between the exposure and covariate of interest. Covariates that produced adjusted estimates that were different enough to be clinically or programmatically relevant were considered effect measure modifiers. Potential effect measure modifiers are listed in Table 3.1.

Confounding: Confounding was assessed by using a DAG, identifying a minimally sufficient adjustment set, and then using a backward elimination approach as described in Aim 1. However, the effect estimates for Aim 2 included beta coefficients, odds ratios and hazard ratios. Potential confounders are listed in Table 3.1.

Multivariable Associations: A backward elimination model strategy was implemented as described in Aim 1. However, the effect estimates for Aim 2 included beta coefficients, odds ratios and hazard ratios.

Missing Data: Missing adherence, plasma VL, and breastmilk VL measures were multiply imputed using proc mi in SAS. Dichotomous variables were imputed using logistic regression, and continuous variables were imputed using predictive mean matching.

Power Calculations**:** Our sample size was fixed, as it utilized laboratory results from an existing protocol (PI: Susan Fiscus). Power for all sub-aims was based on 33 cases (all HIV-infected infants in ARV intervention arms) and 158 non-cases (10% of the HIV-uninfected infants), and is described in detail below. Power calculations used exact methods due to the small sample size. An alpha level of 0.05 was used for all power calculations. Power may slightly decrease after adjusting for necessary covariates, but this study had sufficient power to detect meaningful comparisons in the differences in viral load and risk of HIV transmission by adherence category.

As in Aim 1 we assumed that most mothers and infants would adhere to their prescribed drug regimen, and therefore only a minority would have a detectable HIV RNA viral load in plasma and breastmilk. We also assumed that the majority of those with a detectable viral load would be in the non-adherent group, while realizing that a small proportion of those that are adherent may still have a detectable viral load due to factors such as drug resistance. We used a conservative estimate of power

and present the minimum power that would be expected, as it assumed only one sample would be used per subject. However, power was expected to increase due to the use of repeated measures.

We had 84% power to detect an odds ratio of 0.15 assuming 85% adherence and that 70% of those in the non-adherent group would have a detectable viral load (Table 3.3). We thought it reasonable to assume that there is a large association between adherence and viral load, and therefore anticipated the odds ratios outlined in Table 3.3.

Similarly, we assumed that the vast majority of transmissions would occur in subjects with a detectable viral load. We had over 80% power to detect a hazard ratio of 2.4 assuming 30% would have a detectable viral load and 20% of those with an undetectable viral load would result in a transmission event (Table 3.4). We thought it reasonable to assume that there is a large association between viral load and resulting transmission, and therefore anticipated fairly large hazard ratios.

All data analyses and power calculations were conducted using SAS version 9.2 and 9.3 (SAS Institute, Cary, North Carolina, USA), and Stata version 11 (StataCorp LP, College Station, Texas, USA).

*Limitations*: Potential limitations regarding our adherence measure and unmeasured factors are as described in Aim 1. Another limitation was a lack of plasma or breastmilk samples at the selected time points, which introduced large gaps in timelines. We were dependent on the frequency with which mothers returned for visits and study staff collected blood and breastmilk specimens at each visit.

Specimens were collected and stored more frequently than in other PMTCT studies, making this less of a problem than in studies where specimens were collected much less often.

Due to the small number of transmissions that occurred in the BAN trial we combined data from the maternal ARV and infant NVP arm. While it would be ideal to look at the effects of adherence to maternal ARV and infant NVP separately, we were not powered to study such differences.

We did not have HIV DNA viral loads for breastmilk specimens. Maternal ARV adherers may have suppressed breastmilk HIV RNA but had persistent breastmilk HIV DNA, potentially explaining transmissions that occurred despite perfect adherence. In addition, if compartmentalization or local viral evolution was occurring within the breast, then transmission may have resulted despite perfect adherence due to viral replication or evolution taking place where only 3TC and ZDV have been shown to concentrate.(72) Inflammation in the breast and/or drug resistance may have also contributed to transmission, but was not included in this study. Future work is being planned by Sabrina Zadrozny and Julie Nelson to examine the role that mastitis and resistance play in transmission, respectively. We took all of these limitations into account when generating conclusions about the relationships examined in this study.

*Alternative approaches*: A cohort design would have been optimal to look at the effects of plasma and breastmilk HIV viral load on the association between adherence and transmission; however, such a design would be extremely costly. Use of a case-cohort design allowed us to achieve Aim 2 in a

cost-efficient and timely manner, while maintaining enough power to detect meaningful and significant differences.

**Table 3.1.** Potential effect measure modifiers or confounders for all aims.*

| Covariate | Definition and coding |
|---|---|
| ARV randomization | Categorical: maternal ARVs not including lopinavir/ritonavir, maternal ARVs including lopinavir/ritonavir, infant NVP |
| Nutritional randomization | Dichotomous: nutritional supplement (=1) vs. no supplement (=0) |
| Mothers: | |
| Age | Age in years, continuous† |
| Parity | Continuous† |
| Marital status | Categorical: married/cohabitating, divorced/separated/never married, widowed |
| Education | Dichotomous: ≤ primary school (=1) vs. > primary school (=0) |
| Baseline CD4 count | Count per $mm^3$, continuous† |
| Serious adverse events | Dichotomous: ≥ 1 (=1) vs. none (=0) |
| ARV regimen | Categorical: ZDV/3TC/NVP, ZDV/3TC/NFV, ZDV/3TC/LPV/r |
| Infants: | |
| Sex | Dichotomous: male (=1) vs. female (=0) |
| Birth weight | Weight in kilograms, continuous† |
| Age | Age in months, continuous† |
| Serious Adverse events | Dichotomous: ≥ 1 (=1) vs. none (=0) |

*Parameters were decided based on expert knowledge and use of a causal diagram

†Continuous variables may be re-coded as categorical variables for analyses.

**Figure 3.1.** Power curve for Aim 1; maternal ARV and infant NVP arms combined

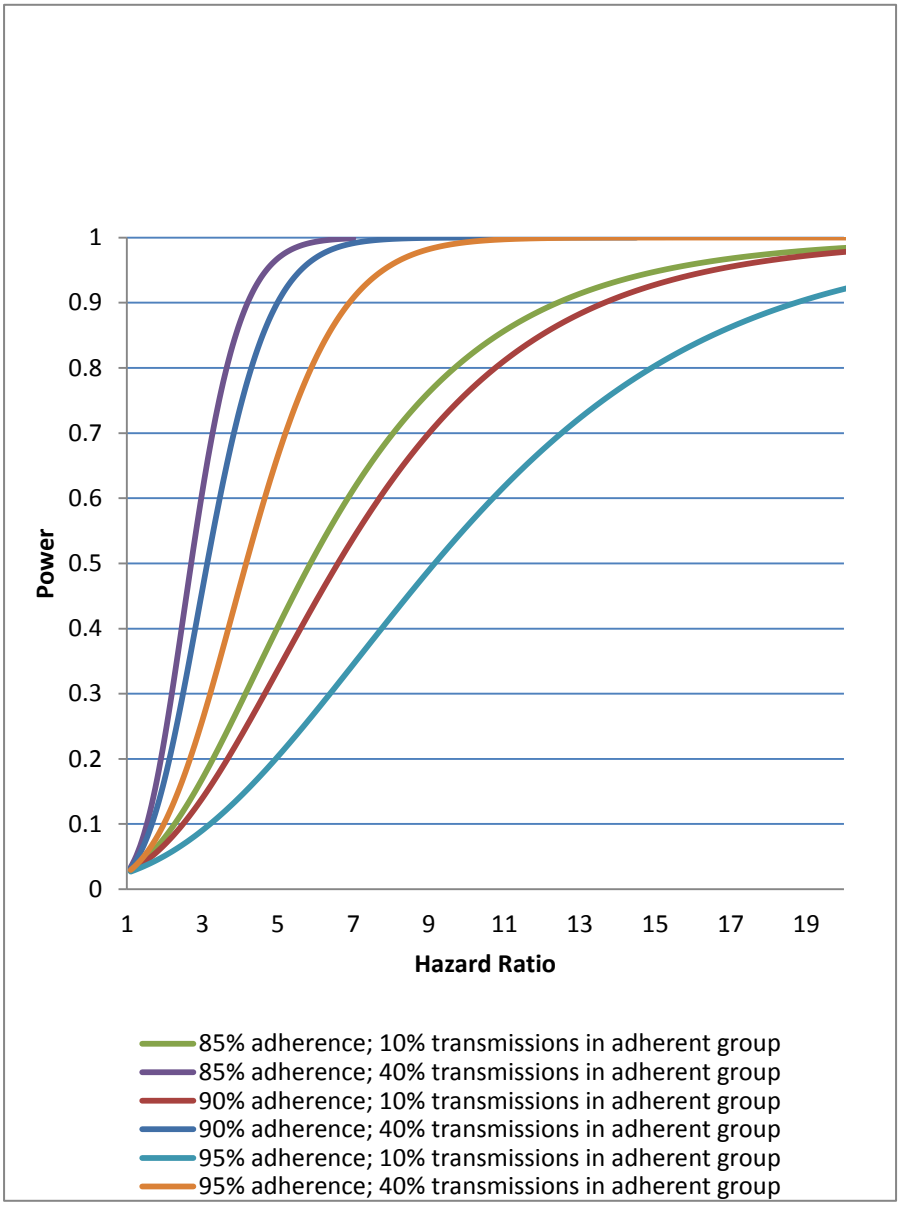

**Table 3.2.** Summary of contrasts evaluated in Aims 1 and 2

| Aim | Exposure | Exposure Structure | Outcome | Outcome Structure | Proposed Model |
|---|---|---|---|---|---|
| 1 | Adherence | Continuous, Categorical, Dichotomous | HIV Transmission | Dichotomous | Cox model |
| 2.1 | Adherence | Continuous, Categorical, Dichotomous | Viral Load | Continuous, Dichotomous | Mixed effects models |
| 2.2 | Viral Load | Continuous, Categorical, Dichotomous | HIV Transmission | Continuous, Dichotomous | Cox model |

**Table 3.3.** Aim 2 assumptions and power calculations for the association between adherence and viral load

| % adherence | % detectable VL among non-adherent group | % Power | Odds Ratio |
|---|---|---|---|
| 85 | 50 | 74 | 0.15 |
| 85 | 70 | 84 | 0.15 |
| 85 | 90 | 89 | 0.10 |
| 90 | 50 | 66 | 0.10 |
| 90 | 70 | 81 | 0.10 |
| 90 | 90 | 95 | 0.05 |

**Table 3.4**. Aim 2 assumptions and power calculations for the association between viral load and HIV transmission

| % detectable viral load | % transmissions in undetectable VL group | % Power | Hazard Ratio |
|---|---|---|---|
| 20 | 15 | 81 | 2.8 |
| 20 | 20 | 84 | 2.6 |
| 30 | 15 | 87 | 2.8 |
| 30 | 20 | 83 | 2.4 |
| 40 | 15 | 82 | 2.6 |
| 40 | 20 | 81 | 2.3 |

## CHAPTER FOUR: SPECIFIC AIM 1 RESULTS
## ADHERENCE TO EXTENDED POSTPARTUM ANTIRETROVIRALS IS ASSOCIATED WITH DECREASED BREASTMILK HIV-1 TRANSMISSION

### INTRODUCTION

The World Health Organization (WHO) now recommends that all pregnant and breastfeeding women with HIV infection receive lifelong antiretroviral therapy (ART) in an effort to simplify and standardize prevention of mother-to-child HIV transmission (PMTCT) prophylaxis regimens, and achieve global goals of eliminating new infant HIV infections and keeping their mothers alive.(32) Effective implementation of this WHO guidance relies on adherence to the prescribed antiretroviral regimen.(14) Maintaining adherence to antiretroviral (ARV) prophylaxis regimens has been challenging for patients, and predicting who will be non-adherent and intervening in an effective way has been challenging for PMTCT programs.(54, 61-64, 73) During the postpartum period, almost one half of patients included in a recent meta-analysis did not maintain an adherence level thought to be needed for virological suppression (>80%).(48)

Non-adherence to ART and PMTCT regimens may occur for several reasons, including: time and financial costs of accessing care, stigma, forgetfulness or changes in routine, and side effects of medications.(43, 49-54) Among adults who are not pregnant or breastfeeding, greater than 95% adherence is thought to be needed to maintain virological suppression when unboosted protease inhibitors (PI) are included in the ART regimen.(35) Pharmacokinetic boosting of PIs with drugs such as ritonavir has been used to extend the plasma half-life of the active PI by inhibiting drug metabolism.(36,

37) With boosted PIs, 80% adherence may be sufficient for adults who are not pregnant or breastfeeding to achieve virological suppression.(38-40) To our knowledge this is the first study to assess the level of adherence needed for postpartum maternal ARV regimens or extended infant nevirapine (NVP) to prevent breastmilk HIV transmission.

Despite the efficacy of ART in PMTCT, transmission does occur even in the context of closely monitored clinical trials for poorly understood reasons.(4, 5, 7, 8, 74) In this paper, we use data from a recent randomized PMTCT trial in Malawi to: 1) estimate adherence to a postpartum maternal triple antiretroviral regimen and daily infant NVP using pill counts, bottle weights, and maternal self-report; 2) compare the characteristics of mother-infant pairs by adherence category; and 3) quantify the effect of adherence to both a maternal triple antiretroviral regimen and extended daily infant NVP on breastmilk HIV transmission.

## METHODS

The Breastfeeding, Antiretrovirals and Nutrition (BAN) trial was conducted in Malawi between 2004 and 2010 using a factorial design to assess the benefit and safety of antiretroviral medications given either to infants or to their mothers to prevent HIV transmission during breastfeeding.(7) Mothers were recruited at antenatal clinics in Lilongwe, where HIV testing was conducted. Eligibility criteria for mother-infant pairs included the following: antibody-confirmed maternal HIV infection, maternal CD4 $\geq$250 cells/μL ($\geq$200 cells/μL before July 24, 2006), no previous antiretroviral drug use (including single dose NVP), infant birth weight of at least 2000 grams, no infant or maternal condition that would preclude the use of a study drug, and able to be enrolled within 36 hours of delivery.(7)

All mothers and infants enrolled in BAN received one dose of NVP and seven days of zidovudine and lamivudine postpartum.(7) In addition, mother-infant pairs were randomized to receive one of the following postpartum PMTCT prophylaxis regimens: 1) 28 weeks of a maternal triple antiretroviral regimen (maternal ARV); 2) 28 weeks of infant NVP; or 3) no further drugs postpartum (enhanced control).(7) Mothers were also randomized to either receive or not receive a nutritional intervention consisting of a lipid-based nutrient supplement throughout breastfeeding.(7) All mothers were advised to breastfeed exclusively for the first 24 weeks postpartum with weaning between 24 and 28 weeks.(7) Details of the drug regimens and nutrition supplement have been reported previously.(71, 75, 76)

**Study design**

The study population consisted of mother-infant pairs randomized to the two treatment arms of BAN, maternal ARV (n=849) and infant NVP (n=852). We excluded mother-infant pairs that were not randomized to an extended postnatal antiretroviral regimen (n=668), and infants who had one of the following outcomes between birth and four weeks of age: HIV infection (n=86), death (n=6), lost to follow-up (n=84), or unknown breastfeeding status (n=46) (rationale described in more detail below). Information on first-born multiples was used when multiple births occurred (n=31). Our analysis thus included a total of 1479 mother-infant pairs at risk of breastmilk HIV transmission between 5 and 38 weeks of age.

**Data analyses**

Breastmilk HIV transmission was determined by Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA) at 2, 12, 28, and 48 weeks to indicate infant HIV status. PCR positive

results were confirmed by testing an additional blood specimen, and the window of infection was narrowed with tests of infant dried blood-spot specimens taken at 4, 6, 8, 18, 24, 32, and 36 weeks. The primary outcome was breastmilk transmission, defined as first detection of HIV infection by PCR in infant blood between 5 and 38 weeks of age, and treated as a dichotomous variable. The secondary outcome was time until either first detection of infant HIV infection or infant death by 38 weeks. The process for death ascertainment has been described previously.(71)

The main exposure of interest was adherence to the prescribed postpartum ARV regimen. We measured adherence two ways: 1) maternal ARV pill counts or infant NVP suspension bottle weights, taken by trained pharmacy staff (hereafter referred to simply as "adherence"); and 2) maternal self-report, using a standardized questionnaire (used in sensitivity analyses, hereafter referred to as "self-reported adherence"). Only pill counts and bottle weights measured on contiguous visits were used, and included visits at the following weeks of infant age: 2, 4, 8, 12, 18, 24, and 28. Adherence was then calculated for the following time intervals: 2-4 weeks, 8-12 weeks, 13-18 weeks, and 24-28 weeks of age. Adherence measures were not available for weeks 5-7, and 19-23. For analysis purposes, the unobserved adherence during weeks 5-7 and weeks 19-23 were assumed to be equal to the observed adherence during weeks 8-12 and weeks 24-28, respectively. Adherence was calculated using BAN dosing regimens (71) and the following two formulae.

$$\textbf{\textit{Pill count}}: \frac{\text{(\# of pills distributed at previous visit - \# of pills returned at current visit)}}{\text{(days between visits * pills prescribed per day)}}$$

$$\textbf{\textit{Bottle weight}}: \frac{\text{(\# of grams distributed at previous visit - \# of grams returned at current visit)}}{\text{(days between visits * grams prescribed per day)}}$$

If adherence was outside of the 0 or 1 bounds, we truncated it to 0 or 1, respectively. Because all three prescribed ARV drugs are necessary for full regimen activity, we made separate calculations for each drug and used the lowest percentage of the three to define a patient's ARV adherence at a given interval. A cutoff of 90% was then used to dichotomize adherence (<90% adherent=non-adherent; ≥90% adherent=adherent). The choice of adherence cutoff was based on previous studies and the expected adherence distribution.(34)

Self-reported adherence was measured at 4, 8, 21, and 28 weeks postpartum and based on the mother's answer to the following question: "During the past three days excluding today, have you/your baby missed any doses of (name of each individual antiretroviral prescribed)?" Self-reported adherence was considered a time-varying dichotomous variable (≥1 reported missed dose of *any* drug; no reported missed doses of *any* drugs).

Potential confounding variables consisted of randomization assignment, demographic characteristics, and health status information (Table 4.1). All covariates were measured at baseline as part of the BAN trial. Frequencies, means, and medians were also calculated, as appropriate, to compare characteristics of mother-infant pairs by exposure (adherence) and outcome (HIV status) category. Binomial regression models using generalized estimating equations (GEE) and an exchangeable correlation matrix were used to test whether or not adherence changed over time.

A Cox model was used to assess the association between adherence and the rate of breastmilk HIV transmission and breastmilk HIV transmission or infant death by 38 weeks of age, adjusting for

potential prognostic factors. Adherence was included in the Cox model as a time varying covariate, lagged by one interval to ensure the exposure occurred prior to the outcome. Mother-infant pairs lost to follow-up were censored at the time of their last PCR negative HIV test.

Effect measure modification was assessed by comparing unadjusted and adjusted hazard ratio estimates and 95% confidence intervals using an interaction term between adherence and the variable of interest. Covariates that produced adjusted hazard ratio estimates that were different enough to be clinically or programmatically relevant were considered effect measure modifiers. A directed acyclic graph (DAG) was used to identify potential confounders and a minimally sufficient adjustment set.(77) Given the limited number of outcomes, a more parsimonious model was generated by removal of potential confounders that had minimal effect on the hazard ratio estimate or precision.

Multiple imputation was used in order to account for missing adherence measures.(69). Adherence was assumed to be missing at random. The probability of being adherent for each missing adherence measure was predicted from a fitted logistic regression model comprised of variables believed a priori to be predictive of adherence and for which there was minimal missing data. Covariates used to predict adherence included ARV study arm, maternal age (continuous), parity (0, $\geq$1), marital status, (married, not married), education status ($\leq$primary, >primary), baseline maternal CD4+ category (200-350, 351-500, >500), baseline maternal hemoglobin (<11mg, $\geq$11mg), previous visit(s) adherence, time-dependent breastfeeding status, infant's outcome, and log survival time. Five hundred complete data sets were imputed based on the logistic regression predicted probabilities. Extended unadjusted and adjusted Cox models were then fit to obtain parameter and variance estimates for each of the 500

datasets, and these estimates were combined to obtain a final mean hazard ratio estimate and 95% confidence interval.(70)

Three sensitivity analyses were conducted to address self-reported adherence, baseline maternal viral load, and timing of infant HIV infection. First, we compared self-reported adherence with pill count and bottle weight adherence, and compared the association between adherence and breastmilk HIV transmission using each adherence measurement method. To do this, self-reported adherence was considered as the main exposure in sensitivity analyses, instead of adherence measured by pill count or bottle weight. Second, we assessed the impact of adjustment for baseline maternal viral load, which was not included in the main regression analyses. Baseline maternal viral load information was not available for 3 mother-infant pairs, including one mother-infant pair who experienced a HIV transmission event. However, due to the known association between viral load and HIV transmission, we included log-transformed baseline maternal viral load in both the imputation and analysis models as a sensitivity analysis. Third, we assessed potential measurement error associated with assignment of transmission timing due to the use of daily infant NVP. In a setting of infant NVP versus maternal ARV exposure, differential detection of HIV using nucleic acid testing may occur. Under daily NVP drug pressure an infant may have very low levels of virus, resulting in a limited number of cells that are infected with HIV, and a delayed positive DNA assay result. In a sensitivity analysis, transmission was assumed to occur at the time of the last HIV-negative test rather than the time of the first HIV-positive test for infants in the infant NVP arm. All data analyses were conducted using SAS version 9.3 (SAS Institute, Cary, North Carolina, USA).

## RESULTS

A total of 1479 mother-infant pairs were included in analyses (Figure 4.1). The median maternal age was 26 years [interquartile range (IQR): 23 to 29]. Most mothers were married (93%) and reported at least one previous live birth (87%) (Table 4.1). Among those randomized to the maternal ARV arm, most received a boosted protease inhibitor regimen of zidovudine, lamivudine, and lopinavir-ritonavir (79%) (Table 4.1). Mothers had a median baseline CD4+ count of 477 per uL (IQR: 330 to 582), a median baseline log10 viral load of 4.1 copies per milliliter of blood (IQR: 3.6 to 4.7), and a median hemoglobin of 10.8 grams per dL (IQR: 10.0 to 11.7). The median infant birth weight was 3.0 kg (IQR: 2.7 to 3.3).

Overall, mean pill count or bottle weight adherence was 87% [median 0.98; IQR: 0.82 to 1.00], and did not meaningfully differ by study arm (maternal arm 87%, infant arm 86%). Adherence changed over time in both the maternal and infant arm ($p<0.001$), but no consistent pattern was identified (Table 4.2).

Among all mother-infant pairs with at least one pill count or bottle weight adherence measure, 22-40% were <90% adherent at any given interval (maternal ARV arm 24-42%, infant NVP arm 17-51%) (Table 4.2). A larger proportion of mother-infant pairs with a HIV transmission event were <90% adherent (41%) during any given interval compared with mother-infant pairs whose infants remained HIV-uninfected (33%) (Table 4.2), but the difference was not statistically significant ($p=0.1$).

**Complete case**

A total of 501 mother-infant pairs had complete pill count or bottle weight adherence information (complete cases). Of these, 146 (29%) were always ≥90% adherent and 35 (7%) were never ≥90% adherent (Table 4.3).

Among complete cases, 23 experienced a breastmilk HIV transmission event by 38 weeks of age (maternal ARV arm: 18, infant NVP arm: 5), and 28 experienced the composite outcome of breastmilk HIV transmission or infant death by 38 weeks (maternal ARV arm: 21, infant NVP arm: 7). Having ≥90% adherence was associated with a 60% relative reduction in the rate of breastmilk HIV transmission by 38 weeks of age among complete cases, compared with having <90% adherence (Hazard Ratio (HR) 0.40; 95% confidence interval (CI) 0.17-0.91) (Table 4.4). Adjustment for maternal age, baseline maternal CD4+ count, baseline maternal hemoglobin level, time-dependent breastfeeding status, and study arm had little impact (HR 0.41, 95% CI 0.18-0.94) (Table 4.4).

**Multiple imputation**

Adherence measured by pill count and bottle weight was imputed for 29% (431/1479) of mother-infant pairs at 4 weeks, 27% (387/1411) at 12 weeks, 29% (401/1375) at 18 weeks, and 43% (560/1310) at 28 weeks. No variables were significant predictors of adherence at all time points.

Having ≥90% pill count or bottle weight adherence was associated with a 50% relative reduction in the rate of breastmilk HIV transmission by 38 weeks of age, compared with having <90% adherence [unadjusted HR 0.50, 95% CI 0.25-0.99] when using multiply imputed data (Table 4.4). The estimated

hazard ratio was similar when unadjusted Cox models were fit separately for each study arm [maternal ARV HR 0.51, 95% CI 0.21-1.21; infant NVP HR 0.48, 95% CI 0.16-1.44) (Table 4.4). Adjustment for potential confounding did not meaningfully change the hazard ratio estimate (HR 0.48; 95% CI 0.24-0.97) (Table 4.4).

**Composite outcome: breastmilk HIV transmission or infant death**

Associations between adherence and the composite outcome of breastmilk HIV transmission or death did not appreciably differ from those using only HIV-transmission as the outcome (unadjusted HR 0.58, 95% CI 0.32-1.04; adjusted HR 0.59, 95% CI 0.33-1.06).

**Sensitivity analyses**

A total of 541 mother-infant pairs had complete self-reported adherence information. Of these, 22 experienced a breastmilk HIV transmission event (maternal ARV arm: 16, infant NVP arm: 6). Overall, 92% of mothers self-reported missing no pills in the last three days. Self-reported adherence was similar by study arm (infant NVP: 0.93, maternal ARV: 0.90). Among complete cases, reporting missing no pills in the prior 3 days (100% self-reported adherence) was associated with a 74% relative reduction in the rate of breastmilk HIV transmission, compared with those self-reporting <100% adherence during the last three days (HR 0.26, 95% CI: 0.09, 0.71) and after adjusting for study arm, time-dependent breastfeeding status, and baseline maternal age, CD4+ count, and hemoglobin. The association remained similar when using multiply imputed self-reported adherence (n=1479, adjusted HR 0.33, 95% CI: 0.14, 0.78).

In the remaining two sensitivity analyses, minimal changes were observed. Including baseline maternal viral load in the imputation and analysis model resulted in a nearly identical association between pill count or bottle weight adherence and the rate of breastmilk HIV transmission (complete case HR 0.40, 95% CI 0.18-0.92; imputed HR 0.49, 95% CI 0.25-0.97), compared to models that did not adjust for baseline maternal viral load. Similarly, allowing for potential differential detection of HIV in an environment of maternal ARV versus daily infant NVP did not change the association between adherence and breastmilk HIV transmission (adjusted HR 0.43, 95% CI 0.19-1.01).

## DISCUSSION

We have shown that adherence to antiretroviral regimens needs to be maintained throughout the breastfeeding period to maximize efforts to prevent breastmilk HIV transmission and improve infant HIV-free survival. To our knowledge, this study was the first in a resource-limited setting that assessed adherence over time to an extended postpartum prophylaxis regimen using pill count, bottle weight, and maternal self-report. The extent of missing adherence data in our study underscores the difficulty in consistently assessing adherence to postpartum antiretroviral medications, even in a well-monitored clinical trial with intensive follow-up.

Non-adherence to ART has been associated with higher HIV viral loads in pregnant women, and compliance to short-course maternal zidovudine has been associated with decreased HIV vertical transmission.(41-43) Maternal ARV adherence rates had a negative but not statistically significant correlation with MTCT rates in a recent meta-analysis.(48) However, most studies in the meta-analysis used short-course ARV regimens and reported only antepartum adherence.(48) In contrast, we measured postpartum adherence over four time intervals, and allowed adherence to vary over time. In

addition, we used methods that account for missing adherence measures, infants lost to follow-up, and infant death.

The number of transmission events was relatively small, despite the large sample size. The limited number of transmission events reduced the precision of our estimates, and prevented comparisons by study arm. We still observed a substantial effect of adherence on breastmilk HIV transmission, despite the small number of transmission events.

Adherence levels found in BAN are similar to reported adherence from other randomized controlled trials of either extended infant or maternal antiretroviral prophylaxis during breastfeeding, and to a pooled postpartum adherence estimate.(4, 8, 9, 48, 74) However, direct adherence comparisons are difficult due to differences in the definition and measurement of adherence across trials. Adherence may be measured using a variety of approaches ranging in sophistication, but all are imperfect.(46) Self-reported adherence has the most potential for bias (usually in the form of overestimating adherence) due to recall and social desirability bias, but is the measurement tool most often used because it is easy and inexpensive to administer.(46) Adherence measurements based on pill count and suspension bottle weight are subject to manipulation, but are thought to have greater validity and take into account adherence over the entire monitoring period, rather than just the three days prior to the self-report assessment visit.(47) In our study, self-reported adherence was higher than adherence measured by pill count and bottle weight. However, both adherence measures resulted in a consistent association with breastmilk HIV transmission, increasing the confidence in our findings.

We attempted to determine an adherence threshold for transmission prevention. We assessed multiple adherence cutoffs (e.g.: 80% and 95%) and imputed adherence as a continuous measure. However, we were unable to identify an adherence threshold. Our findings suggest that the association between adherence and transmission is a continuum, with higher adherence leading to higher protection against transmission (results not shown).

When adherence data are skewed, presenting only one measure of central tendency (e.g., mean or median) may fail to convey the characteristics of the adherence distribution, and be misleading. For example, median adherence in our study was 98%, suggesting that no further action is needed to maintain high levels of postpartum adherence. Mean adherence was 87%, suggesting that some work is needed to improve adherence, but adherence remained high. In contrast, 22-40% of mother-infant pairs in our study were <90% adherent during each interval, suggesting that increased focus is needed to improve postpartum adherence. Variation in measures implies that some mother-infant pairs had very low adherence, and that some had intermittent non-adherence. Given that transmission is associated with non-adherence, there is a need to consistently emphasize adherence throughout the postpartum period.

Non-adherence, however, did not account for all transmission events. Transmission may have occurred despite perfect adherence due to imperfection in our adherence measure, the length of time between maternal initiation of antiretrovirals and virological suppression, antiretroviral drug resistance, or other unidentified processes. Antiretroviral resistance testing could reveal whether resistance played a role in transmission events that occurred among adherent mother-infant pairs, and could also indicate non-compliance in the maternal ARV arm. The number of visits that could be used to assess adherence

resulted in inconsistent and extended time intervals between adherence measures. Adherence was held constant during the interval, and lagged to ensure the exposure occurred before the outcome. Therefore, measured adherence may not be the true adherence in the period immediately before transmission.

Our results reinforce the need to focus on maintaining adherence to meet PMTCT goals. Furthermore, the extent of non-adherence in this heavily monitored clinical trial setting has important implications for adherence counseling messages, antiretroviral resistance concerns, local PMTCT program planning efforts, and global modeling exercises predicting MTCT elimination. We measured adherence through 28 weeks postpartum. However, WHO guidelines now recommend lifelong maternal ART for all HIV-positive pregnant and breastfeeding women.(32) Assessment of adherence and adherence-related outcomes throughout one to two years of breastfeeding is urgently needed.

**Figure 4.1.** Study population and outcomes

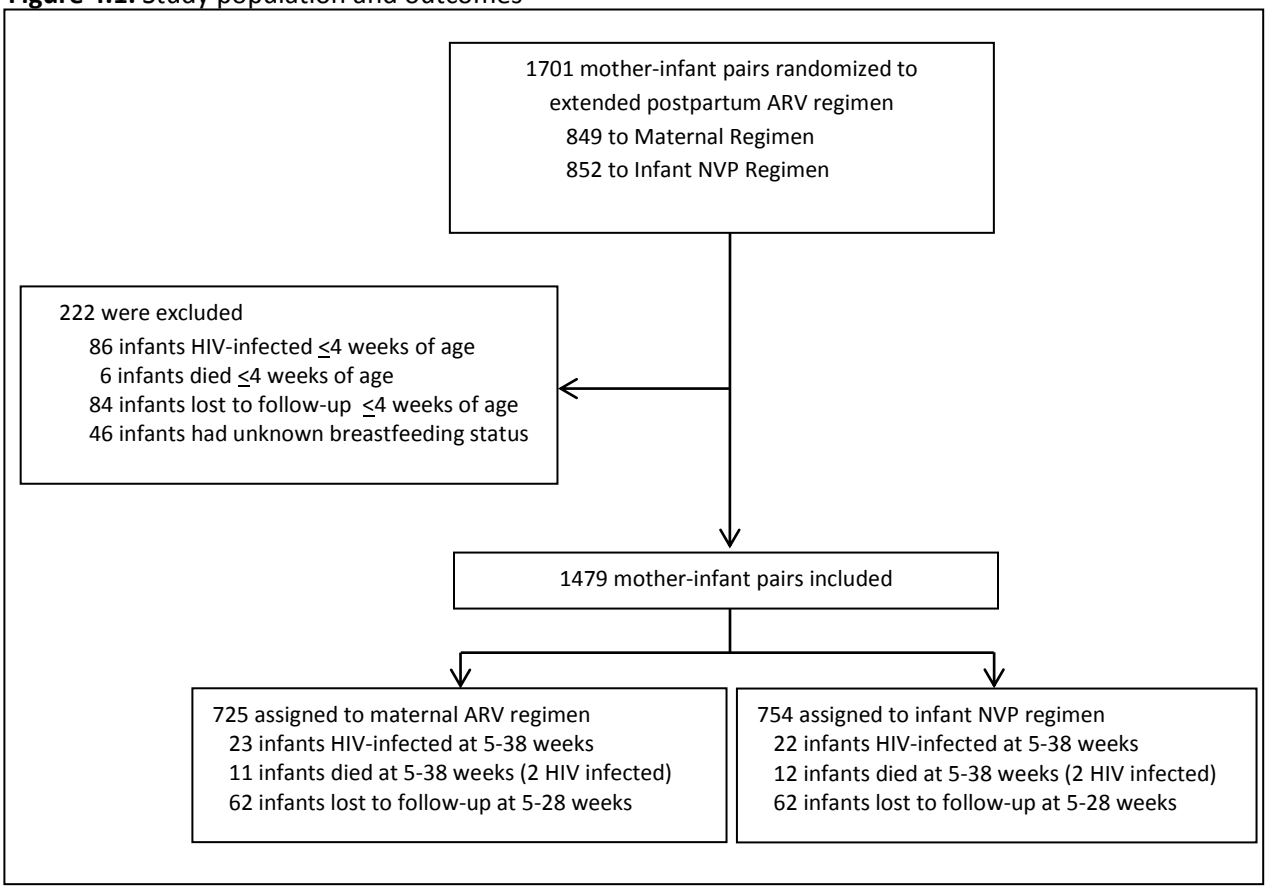

**Table 4.1**. Baseline characteristics of 1479 mother-infant pairs.

| | Total* (N=1479) | | HIV-1 infected infant** (N=45) | | HIV-1 uninfected infant† (N=1434) | |
|---|---|---|---|---|---|---|
| | **N** | **(%)** | **N** | **(%)** | **N** | **(%)** |
| Antiretroviral randomization | | | | | | |
| Maternal antiretroviral | 725 | (49) | 23 | (51) | 702 | (49) |
| Infant nevirapine | 754 | (51) | 22 | (49) | 732 | (51) |
| Nutritional randomization | | | | | | |
| No supplement | 733 | (50) | 17 | (38) | 716 | (50) |
| Received supplement | 746 | (50) | 28 | (62) | 718 | (50) |
| **Mothers:** | | | | | | |
| Age (years) | | | | | | |
| 15-25 | 720 | (49) | 30 | (67) | 690 | (48) |
| 26-35 | 680 | (46) | 14 | (31) | 666 | (47) |
| 36-45 | 76 | (5) | 1 | (2) | 75 | (5) |
| Education | | | | | | |
| Primary school only | 941 | (64) | 29 | (64) | 912 | (64) |
| > primary school | 536 | (36) | 16 | (36) | 520 | (36) |
| Married | | | | | | |
| No | 110 | (7) | 5 | (11) | 105 | (7) |
| Yes | 1369 | (93) | 40 | (89) | 1329 | (93) |
| Parity | | | | | | |
| 0 | 193 | (13) | 8 | (18) | 185 | (13) |
| $\geq$1 | 1280 | (87) | 37 | (82) | 1243 | (87) |

| | Total* | | HIV-1 infected infant** | | HIV-1 uninfected infant† | |
|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | N | (%) |
| CD4+ count per $mm^3$ | | | | | | |
| 200-350 | 450 | (30) | 20 | (44) | 430 | (30) |
| 351-500 | 478 | (32) | 13 | (29) | 465 | (32) |
| >500 | 551 | (37) | 12 | (27) | 539 | (38) |
| Plasma viral load copies/mL | | | | | | |
| ≤1,000 | 147 | (10) | 1 | (2) | 146 | (10) |
| 1,001-10,000 | 425 | (29) | 7 | (16) | 418 | (29) |
| >10,000 | 904 | (61) | 36 | (82) | 868 | (61) |
| Hemoglobin (g/dl) | | | | | | |
| <11 | 783 | (53) | 35 | (78) | 748 | (52) |
| ≥11 | 696 | (47) | 10 | (22) | 686 | (48) |
| ARV Regimen‡ | | | | | | |
| Nevirapine based | 20 | (3) | 0 | (0) | 20 | (3) |
| Nelfinavir based | 124 | (18) | 5 | (22) | 119 | (18) |
| Lopinavir/ritonavir based | 556 | (79) | 18 | (78) | 538 | (79) |
| **Infants** | | | | | | |
| Sex | | | | | | |
| Female | 752 | (51) | 23 | (51) | 729 | (51) |
| Male | 727 | (49 | 22 | (49) | 705 | (49) |
| Birth weight (kg) | | | | | | |
| <2.5 | 106 | (7) | 7 | (16) | 99 | (7) |
| ≥2.5 | 1370 | (93) | 38 | (84) | 1332 | (93) |

* Maternal plasma viral load not available for 3 mothers, ARV regimen not available for 25 mothers randomized to maternal ARV arm, and infant birth weight not available for 3 infants

** Infant tested PCR positive for HIV-1 between 5 and 38 weeks of age

† Includes 16 infants that tested PCR positive for HIV-1 after 38 weeks of age

‡ Among those randomized to maternal ARV arm.

**Table 4.2.** Pill count and bottle weight adherence for mother-infant pairs with ≥ 1 adherence measure.

| | | Percent adherence | | | | Dichotomous adherence |
|---|---|---|---|---|---|---|
| | n | Mean | SD* | Median | IQR** | % < 90% adherent |
| **Maternal + Infant regimen** | | | | | | |
| Overall | | 0.87 | 0.22 | 0.98 | 0.82-1.00 | 33 |
| Week 4 | 1048 | 0.86 | 0.23 | 0.98 | 0.82-1.00 | 34 |
| Week 12 | 1024 | 0.91 | 0.19 | 1.00 | 0.91-1.00 | 22 |
| Week 18 | 974 | 0.87 | 0.19 | 0.97 | 0.76-1.00 | 37 |
| Week 28 | 750 | 0.82 | 0.27 | 0.96 | 0.74-1.00 | 40 |
| **Maternal regimen** | | | | | | |
| Overall | | 0.87 | 0.21 | 0.97 | 0.85-1.00 | 32 |
| Week 4 | 571 | 0.86 | 0.22 | 0.96 | 0.82-1.00 | 37 |
| Week 12 | 552 | 0.89 | 0.19 | 0.98 | 0.89-1.00 | 26 |
| Week 18 | 522 | 0.91 | 0.17 | 0.99 | 0.90-1.00 | 24 |
| Week 28 | 401 | 0.81 | 0.27 | 0.94 | 0.73-1.00 | 42 |
| **Infant regimen** | | | | | | |
| Overall | | 0.86 | 0.23 | 1.00 | 0.78-1.00 | 34 |
| Week 4 | 477 | 0.86 | 0.24 | 1.00 | 0.81-1.00 | 31 |
| Week 12 | 472 | 0.92 | 0.19 | 1.00 | 0.95-1.00 | 17 |
| Week 18 | 452 | 0.82 | 0.20 | 0.87 | 0.67-1.00 | 51 |
| Week 28 | 349 | 0.82 | 0.27 | 0.98 | 0.74-1.00 | 39 |

| | | Percent adherence | | | | Dichotomous adherence |
|---|---|---|---|---|---|---|
| | n | Mean | SD* | Median | IQR** | % < 90% adherent |
| **HIV-infected infants** | | | | | | |
| Overall | | 0.84 | 0.26 | 0.96 | 0.83-1.00 | 41 |
| Week 4 | 30 | 0.85 | 0.22 | 0.93 | 0.84-1.00 | 47 |
| Week 12 | 24 | 0.89 | 0.22 | 0.99 | 0.89-1.00 | 29 |
| Week 18 | 18 | 0.82 | 0.30 | 0.98 | 0.75-1.00 | 39 |
| Week 28 | 11 | 0.71 | 0.35 | 0.83 | 0.45-1.00 | 55 |
| **HIV-uninfected infants** | | | | | | |
| Overall | | 0.87 | 0.22 | 0.98 | 0.82-1.00 | 33 |
| Week 4 | 1018 | 0.86 | 0.23 | 0.99 | 0.82-1.00 | 34 |
| Week 12 | 1000 | 0.91 | 0.19 | 1.00 | 0.91-1.00 | 22 |
| Week 18 | 956 | 0.87 | 0.19 | 0.97 | 0.76-1.00 | 37 |
| Week 28 | 739 | 0.82 | 0.27 | 0.96 | 0.74-1.00 | 40 |

*SD=standard deviation
**IQR=Interquartile range

**Table 4.3.** Characteristics of mother-infant pairs with complete adherence information (n=501)

| | Always ≥90% adherent (n=146) | | <90% adherent for ≥1 interval (n=355) | | Never ≥90% adherent (n=35)* | |
|---|---|---|---|---|---|---|
| | **N** | **%** | **N** | **%** | **N** | **%** |
| ARV randomization | | | | | | |
| Maternal antiretroviral | 99 | (68) | 229 | (65) | 23 | (66) |
| Infant nevirapine | 47 | (32) | 126 | (35) | 12 | (34) |
| Nutritional randomization | | | | | | |
| No supplement | 71 | (49) | 159 | (45) | 17 | (49) |
| Received supplement | 75 | (51) | 196 | (55) | 18 | (51) |
| **Mothers:** | | | | | | |
| Age (years) | | | | | | |
| 15-25 | 66 | (45) | 142 | (40) | 18 | (51) |
| 26-35 | 78 | (53) | 187 | (53) | 17 | (49) |
| 36-45 | 2 | (1) | 25 | (7) | 0 | (0) |
| Education | | | | | | |
| Primary school only | 90 | (62) | 226 | (64) | 19 | (54) |
| More than primary school | 55 | (38) | 129 | (36) | 16 | (46) |
| Married | | | | | | |
| No | 10 | (7) | 25 | (7) | 1 | (3) |
| Yes | 136 | (93) | 330 | (93) | 34 | (97) |
| Parity | | | | | | |
| 0 | 22 | (15) | 33 | (9) | 5 | (14) |
| ≥1 | 124 | (85) | 321 | (91) | 30 | (86) |

| | Always ≥90% adherent (n=146) | | <90% adherent for ≥1 interval (n=355) | | Never ≥90% adherent (n=35)* | |
|---|---|---|---|---|---|---|
| | **N** | **%** | **N** | **%** | **N** | **%** |
| CD4+ count per $mm^3$ | | | | | | |
| 200-350 | 46 | (32) | 116 | (33) | 11 | (31) |
| 351-500 | 41 | (28) | 110 | (31) | 11 | (31) |
| >500 | 59 | (40) | 129 | (36) | 13 | (37) |
| Plasma viral load copies/mL | | | | | | |
| ≤1,000 | 20 | (14) | 32 | (9) | 1 | (3) |
| 1,001-10,000 | 42 | (29) | 93 | (26) | 3 | (9) |
| >10,000 | 83 | (57) | 230 | (65) | 31 | (89) |
| Hemoglobin (g/dl) | | | | | | |
| <11 | 74 | (51) | 188 | (53) | 20 | (57) |
| ≥11 | 72 | (49) | 167 | (47) | 15 | (43) |
| ARV Regimen** | | | | | | |
| Nevirapine based | 4 | (4) | 5 | (2) | 1 | (4) |
| Nelfinavir based | 13 | (13) | 33 | (14) | 6 | (26) |
| Lopinavir/ritonavir based | 82 | (83) | 191 | (83) | 16 | (70) |
| **Infants** | | | | | | |
| Sex | | | | | | |
| Female | 67 | (46) | 195 | (55) | 20 | (57) |
| Male | 79 | (54) | 160 | (45) | 15 | (43) |
| Birth weight (kg) | | | | | | |
| <2.5 | 17 | (12) | 19 | (5) | 3 | (9) |
| ≥2.5 | 129 | (88) | 336 | (95) | 32 | (91) |

* Columns are not mutually exclusive. Those 'never ≥ 90% adherent' are also included in the '<90% adherent for ≥1 interval' column.

** Among those randomized to maternal ARV arm

**Table 4.4.** Estimates of adherence as a risk factor for breastmilk HIV-1 transmission and a composite outcome of breastmilk HIV-1 transmission or infant death by 38 weeks of age, among those HIV-1 uninfected at 5 weeks.*

| | Imputed adherence | | | | Complete case | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted | | Adjusted** | | Unadjusted | | Adjusted** | |
| | Hazard Ratio | (95% CI) | Hazard Ratio | (95% CI) | Hazard Ratio | (95% CI) | Hazard Ratio | (95% CI) |
| **Breastmilk HIV-1 transmission** | | | | | | | | |
| *Maternal and Infant Regimen* | | | | | | | | |
| *Adherent vs non-adherent* | 0.50 | (0.25, 0.99) | 0.48 | (0.24, 0.97) | 0.40 | (0.17, 0.91) | 0.41 | (0.18, 0.94) |
| *Adherence by study arm* | | | | | | | | |
| Maternal ARV adherent vs. non-adherent | 0.51 | (0.21, 1.21) | 0.49 | (0.20, 1.19) | 0.44 | (0.17, 1.11) | 0.44 | (0.17, 1.12) |
| Infant NVP adherent vs. non-adherent | 0.48 | (0.16, 1.44) | 0.47 | (0.16, 1.40) | 0.31 | (0.05, 1.87) | 0.32 | (0.05, 1.91) |
| **Breastmilk HIV-1 transmission or infant death** | | | | | | | | |
| *Maternal and Infant Regimen* | | | | | | | | |
| *Adherent vs non-adherent* | 0.58 | (0.32, 1.04) | 0.59 | (0.33, 1.06) | 0.42 | (0.20, 0.89) | 0.44 | (0.21, 0.94) |
| *Adherence by study arm* | | | | | | | | |
| Maternal ARV adherent vs. non-adherent | 0.51 | (0.23, 1.08) | 0.53 | (0.24, 1.17) | 0.38 | (0.16, 0.91) | 0.40 | (0.17, 0.95) |
| Infant NVP adherent vs. non-adherent | 0.66 | (0.28, 1.59) | 0.65 | (0.27, 1.55) | 0.58 | (0.13, 2.63) | 0.61 | (0.13, 2.75) |

*Having greater than or equal to 90% pill count or bottle weight adherence is considered adherent; less than 90% adherence is considered non-adherent.
**Adjusted for maternal age, CD4+, hemoglobin, and time-dependent reported breastfeeding status. Study arm was included as a confounding variable in combined maternal and infant regimen models. Adherence by study arm models included an interaction term between adherence and study arm.

# CHAPTER FIVE: SPECIFIC AIM 2 RESULTS
# POSTPARTUM ANTIRETROVIRAL ADHERENCE ASSOCIATED WITH REDUCED BREASTMILK HIV-1 RNA VIRAL LOAD AND BREASTMILK TRANSMISSION

## INTRODUCTION

Maternal HIV RNA concentration (viral load or VL) in plasma and breastmilk is among the most important risk factors for HIV transmission during breastfeeding.(24, 28, 29, 78) Risk of perinatal transmission is thought to be virtually 0% at plasma HIV VL less than 1,000 copies/ml, and increase to over 40% at plasma HIV VL of more than 100,000 copies/ml.(16) Two potential mechanisms are thought to be at play regarding virus populations in breastmilk. The first is by continual trafficking from blood into breastmilk of cell-associated virus (measured as HIV-DNA) or cell-free virions (measured as HIV-RNA).(59) The second is transient local production of virus in the breastmilk.(59, 60) Both cell-associated and cell-free virus in breastmilk have been associated with increased breastmilk HIV transmission, and no breastmilk HIV RNA threshold has been identified below which breastmilk HIV transmission has not occurred.(79, 80)

Breastmilk transmission is thought to occur when infant ingestion of cell-free or cell-associated virus in breastmilk is absorbed in mucosal gut surfaces, tonsils, or adenoids.(81) To prevent mother-to-child HIV transmission (PMTCT), antiretroviral (ARV) interventions have been used to reduce HIV viral replication in breastmilk and both maternal and infant blood. Adherence to ARV regimens is necessary to achieve and maintain therapeutic drug levels in plasma, and in turn achieve and maintain plasma viral

suppression.(33) However, there are differential levels of antiretroviral exposure into breastmilk and infant plasma both within and between classes of drugs.(57) Only zidovudine and lamivudine have concentrated to any real extent in breastmilk, among the ARVs that have been evaluated to date.(60, 72) To our knowledge, this study is the first to assess the association between maternal adherence to postpartum zidovudine- and lamivudine-containing regimens using ARV pill counts, and resulting breastmilk HIV VL.

Greater understanding of the interconnectedness between adherence, breastmilk HIV VL, and HIV transmission is a major step in eradicating breastmilk HIV transmission, and making breastfeeding safe for all infants. In this study, we will use a comprehensive approach to assess the role of adherence on HIV transmission by assessing the causal pathway that includes both maternal plasma and breastmilk HIV VL. We will estimate the following associations using clinical data along with stored plasma and breastmilk specimens from BAN: 1) postpartum maternal ARV adherence and both maternal plasma and breastmilk HIV VL; 2) maternal plasma and breastmilk HIV VL; and 3) breastmilk HIV VL and breastmilk HIV transmission.

## METHODS

The Breastfeeding, Antiretrovirals and Nutrition (BAN) randomized trial was conducted in Lilongwe, Malawi to assess the benefit and safety of maternal or infant ARV medications to prevent HIV transmission during breastfeeding.(7) Mothers were recruited between 2004 and 2010 at antenatal clinics. Eligibility criteria for mother-infant pairs included: antibody-confirmed maternal HIV infection, maternal CD4 ≥250 cells/μL (≥200 cells/ μL before July 24, 2006), no previous ARV drug use (including

single dose NVP), infant birth weight ≥2000 grams, no infant or maternal condition that would preclude use of a study drug, and enrollment <36 hours after delivery.(7)

A factorial design was used. Mothers were randomized to receive or not receive a nutritional intervention consisting of a lipid-based nutrient supplement throughout breastfeeding.(7) Mother-infant pairs were also randomized to receive one of the following postpartum PMTCT prophylaxis regimens: 1) 28 weeks of maternal triple ARVs (maternal ARV); 2) 28 weeks of infant NVP; or 3) no further drugs postpartum (enhanced control).(7) All mothers and infants received one dose of NVP and seven days of postpartum zidovudine and lamivudine.(7) Exclusive breastfeeding for the first 24 weeks was advised, with weaning between 24 and 28 weeks. (7) Details of the drug regimens and nutrition supplement have been reported previously.(71, 75, 76)

**Study design**

Using a case-cohort design, we included all mother-infant pairs in the 28 weeks of triple maternal ARV or daily infant NVP arms with available plasma or breastmilk samples and an HIV transmission event between 2 and 28 weeks of the infant's life (n=31, 98% of all transmission events). We also included a 15% sample of mother-infant pairs randomized to the two treatment arms of BAN where HIV transmission did not occur within 28 weeks of life (n=232); sampling was primarily based on stored breastmilk and plasma availability. Mother-infant pairs who experienced transmission between birth and two weeks of age (n=86) were excluded and information on first-born multiples was used when multiple births occurred (n=5). Our analysis thus included a total of 263 mother-infant pairs at risk of breastmilk HIV transmission between 2 and 28 weeks of age.

**Data Analyses**

We considered the following contrasts: 1) adherence and plasma HIV VL, 2) adherence and breastmilk HIV VL; 3) plasma and breastmilk HIV VL, and 4) breastmilk HIV VL and breastmilk HIV transmission.

Maternal adherence was calculated for the following postpartum time intervals: 2-4 weeks, 8-12 weeks, and 13-18 weeks. Adherence to triple maternal ARVs was calculated using pill counts taken by trained pharmacy staff on contiguous visits.(82) Briefly, maternal ARV adherence was calculated from the difference in the number of pills distributed at the previous visit and the number returned at the current visit, compared with the number of pills that should have been taken between visits if the mother was perfectly compliant. Mothers randomized to the infant NVP arm were assigned a maternal adherence value of zero for all intervals. Maternal adherence was categorized into the following using disjoint indicator variables, 0-80% ("poor adherence", referent), 81-98% ("partial adherence"), and >98% ("near perfect adherence"). Categories were chosen based on previous studies and the observed adherence distribution. (34)

HIV RNA was quantified from blood plasma and whole breastmilk at enrollment, 2, 6, 12, 18, and 24 weeks postpartum. In addition, breastmilk HIV RNA was measured at 4 and 8 weeks post-delivery. Plasma VL was quantified using the Abbott RealTime HIV assay (Abbott Molecular, Des Plaines, IL) and the 0.6ml protocol according to the package insert (lower limit of quantitation 40 copies/ml). Breastmilk VL was quantified from 0.6 ml whole breastmilk pre-treated with 209ul Abbott RNA sample prep lysis buffer and 60ul Abbott Proteinase K (53°C incubation for 20 min) using the Abbott RealTime HIV assay

(Abbott Molecular, Des Plaines, IL; lower limit of quantitation 56 copies/ml). HIV RNA concentrations that were detected but below the limit of quantitation were assigned a value of 39 for plasma and 55 for breastmilk (lower limit of quantitation minus one), and RNA concentrations that were undetectable were assigned a value equal to 50% of the lower limit of quantitation (plasma: 20, breastmilk: 28). VL was then categorized as detectable (plasma: >40 copies/ml, breastmilk: >56 copies/ml) or undetectable (plasma: <40 copies/ml, breastmilk: <56 copies/ml) when treated as a binary variable, and log10-transformed when treated as a continuous variable.

Infant HIV status was determined at 2, 12, 28, and 48 weeks by Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA). PCR positive results were confirmed by testing an additional blood specimen, and the window of infection was narrowed with tests of infant dried blood-spot specimens taken at 4, 6, 8, 18, 24, and 32 weeks. Breastmilk transmission was defined as first detection of HIV infection by PCR in infant blood between 2 and 28 weeks of age.

We used generalized linear mixed models with identity link and Gaussian distribution to estimate the difference in log10 plasma VL by adherence category, and logit link with binomial distribution to estimate odds ratios for detectable breastmilk VL by adherence and plasma VL category. Adherence was paired with VL measured at the end of the adherence time interval to ensure the exposure (adherence) occurred prior to the outcome (VL). Therefore, only VL information from 6, 12, and 18 weeks postpartum were used when adherence was the exposure. The reciprocal of the study inclusion probability was used as a sampling weight in all regression analyses to account for sampling of cases and non-cases.

Single and multivariable Cox models were used to estimate relative hazard of breastmilk HIV transmission by 28 weeks of age. Breastmilk VL was treated as a time-varying covariate in the Cox models, lagged by one interval to ensure the exposure occurred prior to the outcome. Mother-infant pairs were right censored at the time of infant death, the time of their last PCR negative test if the infant was lost to follow-up, or they were administratively censored at 28 weeks if the infant remained HIV-uninfected during the entire study period. Mother-infant pairs were also censored at reported cessation of breastfeeding unless HIV transmission occurred within 30 days of reported cessation.(71)

Effect measure modification was assessed by comparing unadjusted and adjusted hazard ratio estimates and 95% confidence intervals using an interaction term between the exposure and variable of interest. Covariates that produced adjusted hazard ratio estimates that were different enough to be clinically or programmatically relevant were evaluated further as effect measure modifiers. A directed acyclic graph (DAG) was used to identify potential confounders and a minimally sufficient adjustment set.(77) Potential confounding variables consisted of randomization assignment, demographic characteristics, and baseline health status information (Table 5.1). More parsimonious models were generated by removal of potential confounders that had minimal effect on the estimate or precision.

Missing adherence and VL measures were accounted for using multiple imputation (83) under the assumption the data were missing at random. For each analysis, 50 imputations were performed. The imputation model comprised variables believed a priori to be predictive of adherence and VL, and for which we had reasonably complete information. Covariates used to predict adherence and VL included ARV study arm, maternal age (continuous), parity (0, ≥1), marital status, (married, not married), education status (≤ primary, > primary), baseline maternal CD4+ category (200-350, 351-500, >500),

baseline maternal hemoglobin (<11 mg, ≥11 mg), baseline maternal log10-transformed VL (continuous), previous visit(s) adherence, infant's HIV status, log of the time until censored if HIV-uninfected or the time until HIV transmission occurred if HIV-infected, time-dependent breastfeeding status, time-dependent plasma VL, and time-dependent breastmilk VL. Logistic regression was used to impute dichotomous variables and predictive mean matching was used to impute continuous variables.(84) All data analyses were conducted using SAS version 9.3 (SAS Institute, Cary, North Carolina, USA).

**RESULTS**

The baseline characteristics of the mother-infant pairs included in this study were comparable to those in the full BAN cohort.(7) Most mothers were married (92%) with only a primary education (66%) (Table 5.1). The median age was 25 years [interquartile range (IQR): 22 to 29] (Table 5.1). Among those in the maternal ARV arm, most received a boosted protease inhibitor regimen of zidovudine, lamivudine, and lopinavir boosted with ritonavir (70%). At baseline, mothers had a median CD4+ count of 408 per uL (IQR: 301 to 544), median log10 VL of 4.4 copies per ml of blood (IQR: 3.8 to 4.9), and median hemoglobin of 10.6 grams per dl (IQR: 9.7 to 11.5). The infants' median birth weight was 3.0 kg (IQR: 2.7 to 3.2).

Among all mothers randomized to maternal ARVs (n=129), overall mean adherence was 88% [median 0.96, IQR 0.86-1.00] at 2-18 weeks postpartum. Overall median log10 plasma VL was 2.3 copies/ml [IQR: 1.6 to 3.5] at 2-24 weeks postpartum among mothers in the maternal ARV arm. The percentage of mothers with detectable plasma VL generally decreased over time among mothers randomized to maternal ARVs, with 85% (70/82), 77% (86/112), 59% (62/105), 61% (36/59), and 59% (47/80) detectable at 2, 6, 12, 18, and 24 weeks respectively. Between 95-100% of mothers randomized

to the infant NVP arm (n=134) had detectable plasma VL at any given time point between 2-24 weeks postpartum, with a median log10 plasma VL of 4.2 copies/ml [IQR: 3.2 to 4.9].

At 2, 4, 6, 8, 12, 18, and 24 weeks postpartum, the percentage of mothers in the maternal ARV arm with detectable breastmilk VL (>56 copies/ml) was 16% (16/103), 18% (18/98), 12% (12/98), 9% (9/99), 16% (15/92), 16% (12/77), and 15% (10/65) respectively. Overall, median log10 breastmilk VL was 1.7 copies/ml at 2-24 weeks postpartum among mothers in the maternal ARV arm, with 85% (539/632) of specimens having virus detected at levels below the lower limit of quantitation. Among mothers in the infant NVP arm, median breastmilk VL was 1.7 copies/ml [IQR: 1.7-2.4] at 2-24 weeks postpartum, with 15% (16/107) and 37% (41/112) having detectable breastmilk VL at 2 and 4 weeks postpartum, respectively; 51-58% had detectable breastmilk VL at 6, 8, 12, 18 and 24 weeks postpartum.

**Adherence and HIV viral load**

At least one paired maternal adherence and plasma HIV viral load was available for 245 (93%) mothers. Having partial adherence was associated with a 0.82 (95% confidence interval (CI) 0.55-1.09) unadjusted log10 reduction in plasma VL compared to having poor adherence (Table 5.2). Having near perfect adherence was associated with a 0.69 (95% CI 0.45-0.93) unadjusted log10 reduction in plasma VL compared to having poor adherence. Adjusting for nutritional randomization and baseline maternal characteristics, including age, CD4+ count, and plasma VL resulted in similar associations (partial adherence: -0.93, 95% CI -1.19,-0.68; near perfect adherence: -0.75, 95% CI -0.98,-0.52). The association was comparable although slightly reduced when using multiple imputation.

Maternal adherence had an analogous association with suppression of virus in breastmilk. Among 211 (80%) mothers with paired adherence and breastmilk HIV VL data, having partial adherence was associated with a 76% (95% CI 28-92%) relative reduction in the unadjusted odds of having detectable breastmilk VL, compared to having poor adherence (Table 5.2). Having near perfect adherence was associated with a 62% (95% CI 14-83%) reduction in the unadjusted odds of having detectable breastmilk VL, compared with having poor adherence. Adjustment for potential confounding variables did not appreciably change the odds ratio estimates or precision. The associations remained similar but less precise when using multiple imputation.

**Plasma and breastmilk HIV viral load**

Among the 221 (84%) mothers with ≥1 paired plasma and breastmilk HIV VL, mothers with detectable breastmilk VL typically had detectable plasma VL. Two (<1%) mothers had detectable breastmilk VL and undetectable plasma VL, both at 6 weeks postpartum with breastmilk VL values of 56 and 77 copies/ml. Mothers with a detectable plasma VL had 59 (95% CI 21-169) times the unadjusted odds of having a detectable breastmilk VL, compared with mothers with an undetectable plasma VL (Table 5.3). Adjusting for study arm, baseline maternal CD4+ count, and baseline maternal plasma VL resulted in a consistently strong though slightly attenuated association between detectable plasma and breastmilk VL (OR 40, 95% CI 15-107). Using multiple imputation produced similar findings.

**Plasma viral load and HIV transmission**

At least one plasma viral load between 2-24 weeks postpartum was available for 27 of the 33 (82%) HIV-infected infants. Among these, one transmission event occurred among a mother with undetectable baseline plasma viral load. However, transmission did not occur until 24 weeks postpartum and occurred after 3 reported viral load measures ranging from 8,000-108,000 copies/ml at 6, 12, and 24 weeks postpartum. The remaining 25 mothers of infected infants had a baseline plasma viral load >3500 copies/ml.

Plasma viral load from the time point immediately preceding transmission was available for 21 mother-infant pairs, with viral load ranging from 102 to >636,000 copies/ml. All 27 mothers who transmitted HIV during 2-28 weeks postpartum had ≥1 plasma VL >100 copies/ml.

Complete time-dependent maternal plasma viral load and infant HIV status information was available for 116 mother-infant pairs. Of these, 8 experienced a HIV-1 transmission event between 2-28 weeks postpartum (maternal ARV arm: 6, infant NVP arm: 2). No transmission occurred among mothers with undetectable plasma viral load.

Among the 116 mothers with complete maternal plasma viral load information, 5 had undetectable plasma VL at all measured time points. Of these 5, all also had undetectable breastmilk viral load at all measured time points.

**Breastmilk viral load and HIV transmission**

Complete time-dependent breastmilk VL and infant HIV status were available for 134 (51%) mother-infant pairs. Of these, 15 experienced a HIV transmission event between 2-28 weeks postpartum (maternal ARV arm: 11, infant NVP arm: 4). Eleven of the 15 mothers (73%) had at least one detectable breastmilk VL before transmission occurred, and 8 of the 15 mothers (53%) had a detectable breastmilk VL at the last measured time point before transmission occurred. Having a detectable breastmilk VL was associated with 2.7 (95% CI 1.4, 5.4) times the unadjusted rate of breastmilk HIV transmission, compared with having an undetectable breastmilk VL (Table 5.4). A much stronger though less precise association was estimated after adjusting for study arm and baseline maternal characteristics including age, CD4+ count, hemoglobin, and plasma VL (HR 7.4, 95% CI 3.2-17.1). Using multiply imputed data, detectable breastmilk VL had a similar unadjusted and somewhat diminished adjusted association with HIV transmission (unadjusted HR 2.9, 95% CI 1.0-8.4; adjusted HR 3.8, 95% CI 1.2-12.1).

## DISCUSSION

We have shown that antiretroviral adherence is associated with reduced breastmilk HIV transmission, at least partly mediated by reduced maternal plasma and breastmilk HIV RNA concentration. In addition, we found a strong association between having detectable maternal plasma and breastmilk HIV RNA, and have shown that breastmilk HIV VL is associated with breastmilk HIV transmission between 2-28 weeks of infant's life among women or infants receiving ARVs. We were able to detect HIV RNA at lower concentrations than several previous studies, but were unable to identify a breastmilk HIV RNA threshold below which transmission did not occur.

Non-adherence to ART has been associated with higher plasma HIV VL in pregnant women. Similarly, we found non-adherence to postpartum maternal ARVs to be associated with significantly higher plasma and breastmilk HIV RNA concentration during the breastfeeding period, and provide the first estimates of the association between maternal antiretroviral adherence and breastmilk HIV VL. In addition, plasma VL has been correlated with breastmilk VL.(29, 41, 43, 85) Using more frequently measured paired plasma and breastmilk specimens, we also found a strong positive association between plasma and breastmilk HIV RNA concentration.

The relative contribution of cell-associated (DNA) versus cell-free (RNA) breastmilk VL toward breastmilk HIV transmission remains unclear. Cell-free virus has been shown to be more important for HIV transmission at later stages of breastfeeding, while cell-associated virus has been found to be more important in the first months of breastfeeding.(79, 80) We assessed the association between HIV RNA levels in breastmilk and resulting HIV transmission using a larger sample of mothers, and more frequent measures of time-dependent breastmilk HIV RNA than previously reported studies. In addition, we used methods to take into account missing adherence and VL, infants lost to follow-up, and infant death. We found a strong association between breastmilk VL and breastmilk HIV transmission at 2-28 weeks of infant's age, suggesting that HIV RNA levels in breastmilk is an important risk factor for breastmilk HIV transmission in the first 7 months of breastfeeding.

The adherence levels and breastmilk HIV RNA concentration found in BAN (among mothers receiving and not receiving maternal ARVs) are similar to levels found in previous studies.(4, 8, 80, 85-87) Making direct comparisons, however, is difficult due to differences in adherence measurement and viral assays, and the variety of thresholds used to define adherence and detectable VL. Most studies

measure adherence using self-report, which is known to be subject to recall and social desirability bias. We used pill count adherence, which is subject to patient manipulation but is thought to have greater validity than self-reported adherence. However, we previously found a consistent association between adherence and breastmilk HIV transmission in BAN using both pill count and maternal self-reported adherence.(82) In addition, the lower limit of RNA quantitation varies by viral assay used, and thresholds for defining detectable viral load have ranged from concentration levels greater than the lower limit of quantitation to concentration levels above the median log10 viral load found among study subjects.

Detectable breastmilk HIV VL and breastmilk HIV transmission occurred despite perfect (100%) maternal ARV pill count adherence, potentially due to imperfection in our adherence measure, the length of time between maternal initiation of antiretrovirals and viral suppression, mastitis, antiretroviral drug resistance, or other unidentified processes. Breastmilk HIV transmission also occurred despite undetectable breastmilk HIV VL, possibly due to persistent cell-associated HIV viral reservoirs in breastmilk which are not eliminated under triple ARV drug pressure. (88-91)

There was inconsistency in the time intervals between scheduled visits, and therefore in the amount of weeks between adherence and viral load measures. In addition, only contiguous visits for which pill count information was collected could be used to measure adherence, resulting in extended time intervals between measures. Adherence was held constant during the interval, and therefore may not reflect the true adherence at the time immediately preceding the VL measure. Similarly, measured VL may not be the true VL at the time immediately preceding transmission. Holding adherence and VL constant during the interval may have resulted in reduced variance. We did not use methods that take

into account interval censoring, however interval censoring methods made little difference in previous BAN analyses.(7)

Slightly stronger associations between adherence and both plasma and breastmilk VL were estimated in the partial adherent group, compared to the near perfect adherence group. The paradoxical result between adherence and VL among those with near perfect (>98%) adherence may be due to measurement error in our adherence measure. However, both adherence categories were associated with reduced plasma and breastmilk HIV-RNA concentration. The likely presence of some adherence measurement error reinforces the difficulty of obtaining accurate adherence estimates even in heavily monitored clinical trials, and the need for simple and accurate adherence measurement tools.

The limited number of available paired adherence and VL measures among mothers in the maternal ARV arm affected the precision of our estimates, and limited our ability to make conclusions by ARV study arm. Missing adherence and VL measures were multiply imputed. Similar but slightly reduced associations were seen for all contrasts when multiply imputed data were used. No substantial gains in precision were seen when using multiply imputed data, potentially due to the number of outcomes that had to be imputed and the lack of strong auxiliary variables.(92) However, mixed effects models produce valid inferences despite missing data on the dependent variable, provided the data are missing at random. The consistency in our estimates using both complete case and multiply imputed data increase the confidence in our findings.

As we have previously shown, antiretroviral adherence needs to be maintained to maximize efforts to prevent mother-to-child transmission and increase HIV-free survival.(82) However, we now more conclusively show that maintaining antiretroviral adherence alone is not likely to prevent all of breastmilk transmission. The sufficient set of mechanisms that cause breastmilk HIV transmission remain incompletely determined. Provision of lifelong maternal ART to all pregnant and breastfeeding women, with the ARVs currently available, will likely have to be implemented in concert with other prevention interventions to fully eliminate breastmilk HIV transmission.(93)

**Table 5.1.** Baseline characteristics of 263 mother-infant pairs.

| | Total* | |
|---|---|---|
| | **N** | **(%)** |
| Antiretroviral randomization | | |
| Maternal antiretroviral | 129 | (49) |
| Infant nevirapine | 134 | (51) |
| Nutritional randomization | | |
| No supplement | 125 | (48) |
| Received supplement | 138 | (52) |
| Mothers: | | |
| Age (years) | | |
| 15-25 | 144 | (55) |
| 26-35 | 105 | (40) |
| 36-45 | 13 | (5) |
| Education | | |
| Primary school only | 173 | (66) |
| More than primary school | 90 | (34) |
| Married | | |
| No | 21 | (8) |
| Yes | 242 | (92) |
| Parity | | |
| 0 | 36 | (14) |
| ≥1 | 227 | (86) |
| CD4+ count per mm3 | | |
| 200-350 | 95 | (36) |
| 351-500 | 81 | (31) |
| >500 | 87 | (33) |
| Plasma viral load copies/ml | | |
| ≤ 1,000 | 20 | (8) |
| 1,001-10,000 | 60 | (23) |
| > 10,000 | 181 | (69) |

| | Total* | |
|---|---|---|
| | **N** | **(%)** |
| Hemoglobin (g/dl) | | |
| < 11 | 160 | (61) |
| ≥ 11 | 103 | (39) |
| ARV Regimen** | | |
| Nevirapine based | 5 | (4) |
| Nelfinavir based | 29 | (26) |
| Lopinavir/ritonavir based | 78 | (70) |
| Infants | | |
| Sex | | |
| Female | 139 | (53) |
| Male | 124 | (47) |
| Birth weight (kg) | | |
| <2.5 | 27 | (10) |
| ≥2.5 | 236 | (90) |

*Maternal age is missing for 1 mother, baseline plasma viral load is missing for 2 mothers, and ARV regimen is missing for 17 mothers assigned to maternal ARVs

**Among mothers randomized to maternal ARV arm

**Table 5.2.** Estimates of adherence as a risk factor for plasma and breastmilk HIV-1 viral load (VL)

| | Complete case | | | | | | Imputed | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unadjusted | | | Adjusted* | | | Unadjusted | | | Adjusted* | | |
| | Beta; Odds Ratio† | (95% CI) | | Beta; Odds Ratio† | (95% CI) | | Beta; Odds Ratio† | (95% CI) | | Beta; Odds Ratio† | (95% CI) | |
| **Maternal plasma VL** | | | | | | | | | | | | |
| ***Maternal and Infant Arm*** | | | | | | | | | | | | |
| *Partial vs poor adherence* | -0.82 | (-1.09, | -0.55) | -0.93 | (-1.19, | -0.68) | -0.45 | (-0.76, | -0.15) | -0.52 | (-0.82, | -0.22) |
| *Near perfect vs. poor adherence* | -0.69 | (-0.93, | -0.45) | -0.75 | (-0.98, | -0.52) | -0.37 | (-0.65, | -0.08) | -0.42 | (-0.70, | -0.15) |
| ***Maternal arm only*** | | | | | | | | | | | | |
| *Partial vs poor adherence* | -0.07 | (-0.45, | 0.30) | -0.08 | (-0.45, | 0.29) | 0.01 | (-0.31, | 0.33) | 0.01 | (-0.31, | 0.33) |
| *Near perfect vs. poor adherence* | 0.09 | (-0.25, | 0.44) | 0.10 | (-0.24, | 0.44) | 0.11 | (-0.19, | 0.41) | 0.12 | (-0.18, | 0.41) |
| **Breastmilk VL** | | | | | | | | | | | | |
| ***Maternal and Infant Arm*** | | | | | | | | | | | | |
| *Partial vs poor adherence* | 0.24 | (0.08, | 0.72) | 0.23 | (0.08, | 0.67) | 0.30 | (0.07, | 1.30) | 0.29 | (0.07, | 1.19) |
| *Near perfect vs. poor adherence* | 0.38 | (0.17, | 0.86) | 0.36 | (0.16, | 0.81) | 0.42 | (0.10, | 1.83) | 0.41 | (0.10, | 1.69) |
| ***Maternal arm only*** | | | | | | | | | | | | |
| *Partial vs poor adherence* | 0.47 | (0.16, | 1.41) | 0.47 | (0.15, | 1.40) | 0.50 | (0.11, | 2.21) | 0.50 | (0.11, | 2.21) |
| *Near perfect vs. poor adherence* | 0.64 | (0.28, | 1.43) | 0.64 | (0.29, | 1.43) | 0.67 | (0.15, | 2.96) | 0.68 | (0.15, | 3.00) |

*Adjusted for: baseline maternal age, baseline maternal CD4 count, baseline log10 plasma viral load, and nutrition randomization

† Beta coefficient (average difference in log10 plasma viral load) presented for mixed linear regression plasma VL results and odds ratio presented for mixed logistic regression breastmilk VL results

**Table 5.3.** Estimates of detectable plasma viral load as a risk factor for detectable breastmilk viral load

| | Complete Case | | | | Imputed | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted | | Adjusted* | | Unadjusted | | Adjusted* | |
| | Odds Ratio | (95% CI) | Odds Ratio | (95% CI) | Odds Ratio | (95% CI) | Odds Ratio | (95% CI) |
| *Maternal and Infant Arm* | | | | | | | | |
| *Detectable vs. Undetectable* | 59 | (21, 169) | 40 | (15, 107) | 43 | (8, 236) | 35 | (7, 169) |
| *Maternal arm only* | | | | | | | | |
| *Detectable vs. Undetectable* | 45 | (16, 124) | 40 | (15, 108) | 41 | (9, 185) | 38 | (9, 168) |

* Adjusted for baseline maternal CD4+ and baseline maternal plasma viral load. Study arm was included as a confounding variable in combined maternal and infant arm models, but was not included in maternal arm only models.

**Table 5.4.** Estimates of detectable breastmilk HIV viral load as a risk factor for breastmilk HIV-1 transmission

| | Complete Case | | | | Imputed | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted* | | Adjusted** | | Unadjusted* | | Adjusted** | |
| | Hazard Ratio | (95% CI) | Hazard Ratio | (95% CI) | Hazard Ratio | (95% CI) | Hazard Ratio | (95% CI) |
| **DICHOTOMOUS VIRAL LOAD** | | | | | | | | |
| *Maternal and Infant Arm* | | | | | | | | |
| *Detectable vs. Undetectable* | 2.7 | (1.4, 5.4) | 7.4 | (3.2, 17.1) | 2.9 | (1.0, 8.4) | 3.8 | (1.2, 12.1) |
| *Viral load by study arm* | | | | | | | | |
| *Maternal ARV: Detectable vs. Undetectable* | 5.6 | (2.5, 12.7) | 7.8 | (3.1, 19.2) | 4.8 | (1.5, 15.8) | 4.4 | (1.3, 15.2) |
| *Infant NVP: Detectable vs. Undetectable* | 3.9 | (0.8, 18.0) | 6.0 | (1.2, 31.4) | 2.8 | (0.5, 15.0) | 2.9 | (0.5, 17.7) |

**Combined maternal and infant arm models contained no confounding variables. Viral load by study arm models contained study arm and an interaction term between breastmilk viral load and study arm.*

***Adjusted for continuous baseline maternal age, baseline maternal CD4+, baseline maternal hemoglobin level, and baseline maternal plasma viral load. Study arm was included as a confounding variable in combined maternal and infant arm models. Viral load by study arm models included an interaction term between breastmilk viral load and study arm.*

## CHAPTER SIX: CONCLUSIONS

Postpartum HIV transmission through breastfeeding accounts for up to 44% of infant HIV infections.(23) ARVs can be safely given throughout breastfeeding to reduce HIV transmission risk, as demonstrated in clinical trials. However, transmission still occurs despite the use of efficacious drug regimens. Transmission may partly be explained by suboptimal adherence to prescribed ARV regimens. The purpose of this dissertation was to 1) estimate adherence to postpartum ARVs, 2) quantify the effect of postpartum adherence on breastmilk HIV transmission, and 3) evaluate breastmilk HIV RNA concentration as a mediating factor between ARV adherence and breastmilk HIV transmission.

We showed that while on average postpartum adherence remained high, between 22-40% of mother-infant pairs had intermittent periods of non-adherence. We further showed that non-adherence to antiretroviral regimens is strongly associated with increased breastmilk HIV transmission, and that adherence needs to be maintained throughout the breastfeeding period to maximize efforts to prevent transmission and improve infant HIV-free survival. In addition, we found the association between antiretroviral adherence and breastmilk HIV transmission to be at least partly mediated by reduced breastmilk HIV RNA concentration. Non-adherence, however, did not explain all of the transmissions that occurred, suggesting that other biological mechanisms not affected by ARV use may also be at play. This research adds to the scant literature on postpartum ARV adherence and adherence-related mechanisms of breastmilk HIV transmission.

The strong relationship between postpartum ARV adherence and HIV transmission has several important practical implications. Transmission outcomes seen in closely monitored clinical trials with relatively high mean ARV adherence are used to produce global modeling and local PMTCT program effectiveness estimates of vertical HIV transmission rates. However, adherence is likely to be lower outside of a clinical trial setting, potentially resulting in larger numbers of HIV-infected infants in need of ART than projected. In addition, most PMTCT studies have stopped postpartum ARVs at or before 6 months, with recommended concurrent breastfeeding cessation. However, HIV-positive mothers are now encouraged to continue breastfeeding for the first 12 months of life to avoid malnutrition and an increased risk of serious infant infections due to unsafe feeding practices.(32) Estimates of adherence and transmission probabilities among women receiving ARVs through one to two years of breastfeeding are needed in order to more accurately estimate transmission rates and forecast pediatric ART needs. Therefore, validating model projections with program data will be needed, which will require increased focus and support of national monitoring and evaluation systems.

Partly to prevent vertical HIV transmission, WHO now recommends that all pregnant and breastfeeding women be initiated on lifelong ART, and receive the same first-line drug regimen (tenofovir, lamivudine, and efavirenz) as non-pregnant adults.(32) The benefits of such an approach are many. PMTCT programs have been plagued by complicated protocols, with various ARV prophylaxis options being offered depending on when HIV diagnosis is made, how many weeks the mother receives ARVs, and the severity of maternal disease. In contrast, this one size fits most approach (in some circumstances, ART is stopped after breastfeeding cessation) greatly simplifies treatment instructions to health care workers, and treatment messaging to women. In addition, it simplifies national-level drug forecasting. Initiating all pregnant and breastfeeding women on ART regardless of immunological status

also removes the barrier that the lack of CD4 testing created in so many resource-limited settings, and ensures that all women with acute infection and advanced disease are immediately initiated on treatment. Immediate treatment initiation can improve maternal health by delaying disease progression, and can reduce transmission risk to both the mother's sexual partners and infant.

Roll-out of current WHO recommendations to extend ARV prophylaxis through breastfeeding and beyond depends on health facilities' ability to retain patients in HIV care, and on patient adherence to lifelong ART. Retention and adherence in PMTCT settings have been long-standing obstacles to the success of national PMTCT programs. Malawi was the first country to implement lifelong ART to all pregnant and breastfeeding women. On average, 17% of women initiated on lifelong ART were lost to follow-up by 6 months after initiation.(94) The lost to follow-up rate was as high as 58% by 6 months post-ART initiation at some high-volume facilities, although some women may have sought care at another clinic. Facilities that offered adherence counseling beyond that required by national guidelines experienced lower rates of early lost to follow-up.(94)

Introducing maternal ART at higher CD4+ counts without simultaneous improvements in health system performance (i.e.: access to ANC services, HIV counseling and testing, and ART) resulted in limited reductions in the modeled number of HIV-infected infants by six weeks of age.(95) Neither postpartum transmission nor acute maternal infections were included in the model, and provision of ART to all pregnant women regardless of CD4+ count was not included as a prophylaxis option. Including breastmilk transmission events and provision of ART to all HIV-infected breastfeeding mothers regardless of CD4+ count would likely reveal to an even greater extent the negative impact that health

system inefficiencies occurring through years of breastfeeding transmission risk have on achieving PMTCT program goals.

Countries that require CD4+ counts as part of ART eligibility criteria for pregnant and breastfeeding women have necessitated between 9-16+ visits per pregnancy in order for mothers and infants to receive comprehensive care (Table 6.1). Each clinic visit has time and financial costs for the mother, making it easy to understand why so many pregnant women, mothers, and children are lost to follow-up at each stage of the PMTCT cascade. The number of required clinic visits for a mother and infant to receive comprehensive care needs to be reduced, without sacrifices to quality of care or overall morbidity and mortality. Adherence seems to be improved with increased counseling, requiring a balance between creating a system with minimal clinic visit requirements yet regular adherence counseling opportunities. This may require more effective clinic-based adherence messages that have a longer lasting effect on pill-taking behavior, or simple and effective non-clinic-based adherence interventions that can be implemented over mobile devices (i.e.: text message) or by home visits from community partners. Being able to predict who will likely be non-adherent would help in developing tailored and targeted adherence interventions, and to offer an adherence intervention before transmission occurs. We found no baseline covariates to be consistently associated with non-adherence in our study, suggesting that prediction of non-adherence may be challenging. Consequently, a more generic intervention that is simple and can be applied to everyone may be required in some settings. Due to changes in adherence seen over time, adherence interventions need to be offered throughout one to two years of breastfeeding. To ensure maximal benefit, a variety of effective adherence interventions will likely be needed to ensure patients do not become desensitized to messaging.

We have shown that accurately and consistently measuring adherence is difficult even in a clinical trial with intensive monitoring and follow-up. Self-reported adherence measurements, thought to be the easiest and most affordable way to measure adherence, were missing for a large number of patients. Pill count and bottle weight adherence are thought to be more accurate than self-reported adherence. However, we found some pill count and bottle weight adherence values less than zero and more than 100 percent, signaling some measurement error even with measurements that are not thought to be as subject to social desirability bias. Accurately and consistently measuring adherence to ART throughout breastfeeding and beyond, in both highly regulated clinical trials and 'real-world' health facility settings, is crucial information for global, national, and facility-level PMTCT programs. Therefore, innovative, simple, and feasible adherence measurement tools are urgently needed.

Poor antiretroviral adherence can lead to antiretroviral drug resistance. Protease inhibitors have a higher genetic barrier to resistance, as several mutations are required before resistance develops.(36) A single mutation is enough to confer high-level resistance to NNRTI-based regimens.(96) In addition, NNRTIs typically have a longer half-life than PIs, resulting in potential mono-therapy and development of drug resistance if patients are non-adherent for extended periods of time.(36) A protease inhibitor-based ARV regimen was prescribed to most (80%) of the mothers randomized to maternal ARVs in our cohort study. However, the WHO recommended first line regimen for pregnant and breastfeeding women contains a NNRTI backbone (efavirenz) and no protease inhibitors, making adherence crucial to the success of initiating all pregnant and breastfeeding women on ART. Among mother-infant pairs with complete adherence information in our study, 71% were non-adherent during at least one time interval. The intermittent intervals of sub-optimal (<90%) adherence found in our study, in combination with the high percentage of mothers lost to follow-up in some PMTCT programs, make resistance a valid concern for operationalizing current WHO guidance. Accurately and consistently measuring adherence to ART

will help to ascertain if a patient's virological failure is due to non-adherence (signaling a needed adherence intervention) or drug resistance (signaling a needed ARV regimen switch).

Currently available ARV drugs have not successfully eliminated cell-associated viral reservoirs in breastmilk. Cell-associated virus has been associated with higher breastmilk transmission, especially during the first months of breastfeeding when breastmilk contains more cells. Provision of maternal ARVs to all pregnant and breastfeeding women is therefore unlikely to eliminate all transmission unless new ARV drugs that eliminate cell-associated reservoirs are developed.(93)

In conclusion, with ARVs we can reduce vertical and sexual transmission by >96 percent.(74, 97) Operational research has simultaneously shown us that effective provision of ARV prophylaxis or treatment to pregnant and breastfeeding women is hampered by health system constraints and societal norms. In order to make breastfeeding safe for all infants, and to fully eliminate new infant HIV infections, we must answer the difficult operational question of how to implement lifelong efficacious drug regimens with the same rigor, hope, and creativity that is used to answer the scientific question of how to biologically prevent transmission.

**Table 6.1.** Example of a list of visits a HIV-infected pregnant woman/mother needs to make in order to receive comprehensive care*

| Visit number | Service provided |
|---|---|
| 1 | 1st ANC visit |
| 2 | CD4 collection |
| 3 | CD4 result (if lower than ART threshold then go to ART clinic for evaluation) |
| 4 | 1st of 2 pre-treatment counseling appointments |
| 5 | 2nd of 2 pre-treatment counseling appointments |
| 6 | Initiate treatment |
| 7 | Return for hemoglobin test 2 weeks post-ART initiation |
| 8 | 2nd ANC visit |
| 9 | 3rd ANC visit |
| 10 | Delivery |
| 11 | 1 week post-natal exam |
| 12 | 6 week post-natal exam and early infant HIV diagnosis |
| 13 | Return for infant HIV test results |
| 14 | 4 month immunization visit |
| 15 | 9 month immunization visit |
| 16 | Multiple returns for repeat infant HIV testing, drug refills, and ART management appointments |

*Based on actual visit schedule in a sub-Saharan African country, 2009

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