

INCIDENCE OF MASTITIS AMONG BREASTFEEDING HIV-INFECTED WOMEN IN
MALAWI: ASSOCIATIONS WITH HIV TREATMENT REGIMEN, BREASTFEEDING
STATUS AND VERTICAL HIV TRANSMISSION

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill
in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the
Department of Epidemiology.

Chapel Hill
2016

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ABSTRACT

Sabrina Zadrozny: Incidence of Mastitis Among Breastfeeding HIV-Infected Women In Malawi: Associations with HIV Treatment Regimen, Breastfeeding Status and Vertical HIV Transmission

(Under the direction of Daniel Westreich)

Whether mastitis is associated with HIV transmission among breastfeeding women taking antiretroviral therapy (ART) has important public health implications. We used data from the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study, conducted in Lilongwe, Malawi, to explore the relationship between maternal and infant antiretroviral prophylaxis, breastfeeding behavior, mastitis and HIV transmission. Mother-infant pairs (n = 2369) were randomized to take or not take a nutritional supplement and to one of three treatment groups: maternal antiretroviral therapy (ART), infant nevirapine (NVP) or standard of care.

For aim 1, the 28-week risk of mastitis among 1,472 HIV-infected women delivering infants between 2004 and 2007, was higher in the maternal ART (RD 4.5, 95% confidence interval (CI): 0.9, 8.1) and infant NVP (RD: 3.6, 95%CI: 0.9-6.9) groups compared to standard of care. The hazard of late mastitis (from week 5-28) was also higher for maternal ART (HR: 6.7, 95%CI: 2.0, 22.6) and infant NVP (HR: 5.1, 95%CI: 1.5, 17.5) compared to standard of care.

For aims 2 and 3, 1337 HIV-infected women enrolled in BAN between 2004 and 2007, had breastfeeding data and delivered infants HIV-uninfected by 2 weeks. In aim

2, among women with mastitis (n=97, 7%), one (1%, n=1/97) transmitted HIV to her infant and one infant died. Among women without mastitis (n=1240/1337, 93%), 4% (n=52/1240) transmitted HIV to their infants and 7 infants died.

For aim 3, we fit a marginal structural discrete time proportional hazards model to estimate the effect of time-varying mastitis or breast inflammation on the duration of exclusive breastfeeding and on the duration of any breastfeeding. The duration of exclusive breastfeeding was longer for women with mastitis compared to those without (HR: 1.07; 95%CI: 0.86, 1.34) and women with mastitis stopped breastfeeding sooner (HR: 0.91; 95%CI: 0.69, 1.21) compared to women without mastitis.

The role of mastitis in vertical transmission is a remaining mystery toward eliminating pediatric HIV. Incidence of mastitis and HIV transmission was low, so larger samples or alternate study designs are necessary to investigate the association between mastitis and HIV transmission among breastfeeding women in the era of lifelong ART.

For my mom, who always prioritized my education, even when it meant working
as many as 3 concurrent jobs.

For my kids, whose education I will always prioritize.

ACKNOWLEDGMENTS

I am grateful for time and support from so many people throughout this dissertation journey. Chronologically, my path to UNC Chapel Hill began with Denise Jamieson from the Centers for Disease Control and Prevention (CDC). She connected me with Charles van der Horst at UNC Chapel Hill. At the time, Charlie was the Principal Investigator for the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study. I decided to enroll in the PhD program in Epidemiology at UNC, in part because of the BAN study.

Not all doctoral students in Epidemiology are lucky enough to have had, and retained on their committee, three academic advisors. Audrey Pettifor was my first advisor. When it became clear that I would be able to work on the BAN study, I was lucky enough to become one of the many advisees of Bill Miller. As Daniel Westreich joined the faculty in the Department of Epidemiology at UNC, he became my third, and final advisor.

Daniel was an informal member of my advising committee beginning in 2011 and transitioned to chair my committee in 2013. When I began working with him, I was driven, motivated and productive. After 2 kids and some postpartum medical complications, my productivity waned and I was not the graduate student he initially agreed to mentor. Despite my setbacks and frustrations, he resolved to patiently and

expertly help me navigate through the complexity of rebuilding my reputation and research objectives. He also trusted me. While I had lost my way, he continued to believe that with support and patience, I would find my way back toward productivity. He met me where I was. And, when I regained time, energy and productivity, he matched my pace and adjusted his expectations and challenges accordingly. Second chances are rare in life, but he helped me find a second chance. I am grateful for Daniel's time, patience and encouragement.

Michael Hudgens and Brian Pence rounded out my all-star committee. Michael provided much needed guidance for the BAN study and methodology. Brian Pence agreed to fill a spot on my committee late in the process. He offered calm, incisive and practical advice, as well as an all-access pass to his advisee group meetings. Julie Nelson (Department of Microbiology), Valerie Flax (Department of Nutrition) and Irving Hoffman (Department of Medicine) were not technically members of my committee, but have provided indispensable guidance throughout my graduate studies.

Not everyone is fortunate enough to be paid to learn. Not a day passed during this process where that luxury was forgotten. This indulgence was made possible with financial and collaborative support from several different sources. For my first year in the program, I worked as a research assistant for the NC STAT Program. I was supported in part by the UNC Project, Malawi and the BAN study, which was funded by grants from the Prevention Research Centers Special Interest Project of the Centers for Disease Control and Prevention (SIP 13-01 U48-CCU409660-09, SIP 26-04 U48-DP000059-01, and SIP 22-09 U48-DP001944-01). I also had the pleasure of working as

a research assistant for Dr. Pence on his CNICS Depression study and for Dr. Westreich, as part of his DP2 award.

Dissertating can be a long, lonely journey at times. When it felt like a slog, there were many who provided company, answers, feedback and hilarity. These invaluable, kind people who donated their time include Charlie Poole, who took a chance on me; my epidemiology support group (Kat Tumlinson, Jess Rinsky, Anna Barry Cope, Nadya Belenky, Rushina Cholera, Naman Shah, Anne Butler, Cathy Panozzo, Jonathan Todd and Jessie Buckley); my larger-than-life writing group (Anna Bauer, Dana Pasquale, Melanie Napier and Chris Gray...really, how lucky were we?); the breastfeeding umbrella study team (BUST); and the BWP advisee group, but only after I passed an extensive screening and interview process spearheaded by THE Tiffany Breger.

This document would not be possible without support from my given and chosen family. I would especially like to thank my mom, Susan Fitzpatrick, for being a strong, single mother and an inspiring role model. I am also grateful to my husband, Robert Dow, for all the things. I lost track of the number of dishes, loads of laundry, sick-child days and dinners he handled while I worked in the evenings. Thankfully, he did too. I am also grateful for Dows, the Fitzpatricks and my Carroboro crew for the fun and support they provided along the way. I am also lucky to have the support and friendship of a group of smart and successful science-minded moms, especially Christine Bergeron and Christine M. Page-Lopez. And, while my two sons, Oliver and Arthur Dow did not help with the completion of this dissertation, they provided invaluable perspective to help prioritize my time.

Finally, and most importantly, thank you to the BAN study team, all of the women and infants who participated in the study and the staff from the UNC Project Malawi who took care of them.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ARV	Antiretroviral
ART	Antiretroviral therapy
BAN	Breastfeeding, Antiretrovirals, and Nutrition Study
BF	Breastfeeding
CI	Confidence Interval
CD4	Cluster of Differentiation 4, a glycoprotein on immune cells
DAG	Directed Acyclic Graph
EBF	Exclusive Breastfeeding
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IPCW	Inverse Probability of Censoring Weights
IPTW	Inverse Probability of Treatment Weights
IQR	Interquartile Range
MBF	Mixed Breastfeeding
MTCT	Mother-to-Child Transmission of HIV
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PMTCT	Prevention of mother-to-child transmission of HIV

RNA	Ribonucleic Acid
UNC	University of North Carolina at Chapel Hill
VL	Viral Load
WHO	World Health Organization

CHAPTER 1 – INTRODUCTION & SPECIFIC AIMS

One third to one half of all cases of vertical HIV transmission can be attributed to postnatal transmission through breast milk [1]. While the risk of HIV transmission through breast milk can be eliminated by using formula, breastfeeding is recommended for HIV-infected women in resource-poor settings, despite the transmission risk, due to the prohibitive cost of formula and the increased risks of diarrheal and respiratory illnesses associated with formula feeding [2-4]. Both maternal and infant factors play a role in HIV transmission through breast milk. Mastitis, inflammation of the breast, is a maternal factor associated with vertical HIV transmission [1, 5-9]. Understanding the role of mastitis in vertical transmission is one of the remaining problems in the effort to eliminate pediatric HIV.

The mechanism explaining why mastitis is associated with an increased risk of vertical HIV transmission is unclear. Mastitis triggers an inflammatory response in the breast, which is associated with an increase in breast milk HIV viral load, but the evidence is ambiguous about a causal relationship [7, 10-12]. If elevated HIV RNA levels in breast milk are associated with vertical HIV transmission, antiretroviral treatment (ART) may reduce the effect of mastitis in facilitating HIV transmission through breast milk. However, if mastitis causes infants to have more a susceptible immune system due to compromised gut immunity, managing viral load through ART for

mothers with mastitis will not reduce the risk of vertical HIV transmission through breast milk related to mastitis.

In this study we will describe the incidence of mastitis among HIV-infected women who are ART-exposed and ART-unexposed to understand whether managing viral load with treatment is enough to reduce the association that exists between mastitis and vertical HIV infection in the absence of ART. This study will be the first to assess the association between mastitis and vertical HIV transmission among women on ART compared to women not on ART. In the Breastfeeding, Antiretroviral and Nutrition (BAN) study, based in Lilongwe, Malawi, both mothers and infants took antiretroviral drugs to prevent HIV transmission during breastfeeding. We will assess mastitis incidence among HIV-positive, breastfeeding women who were ART-exposed compared to those who were ART-unexposed. We will also determine whether the association between mastitis and vertical HIV-transmission differs by ART exposure. **We hypothesize that incidence of mastitis will be low among HIV-infected women participating in the BAN study, but mastitis will be associated with vertical HIV transmission in the presence of ART.**

The specific aims of this dissertation are to:

1. Describe the incidence, severity and timing of mastitis among breastfeeding, HIV-infected women who are exposed to ART, whose infants are taking daily nevirapine (NVP) or mother-infant pairs receiving the standard of care.

Hypothesis. The incidence of mastitis will be lower among mothers taking ART compared to mothers not taking ART. *Overview:* We will assess the incidence and timing of mastitis

among a population of breastfeeding HIV-infected women. For women with different treatment exposures (maternal ART, infant NVP and standard care), we will determine whether there is an association between assigned treatment arm and incident mastitis in the first six months of breastfeeding.

2. Determine the association between mastitis and vertical HIV transmission through breast milk, stratified by treatment arm, in a cohort of HIV-infected women during the first six months of breastfeeding.

Hypothesis. Breastfeeding, HIV-infected women with mastitis will transmit HIV to their infants at a higher rate than women without mastitis. *Overview.* For HIV-infected women who are breastfeeding, we will determine whether mastitis affects HIV transmission to infants and whether that relationship differs by treatment group.

3. Investigate whether mastitis or breast inflammation is associated with the duration of exclusive breastfeeding, among a population of HIV-infected women who were all counseled to exclusively breastfeed.

Hypothesis. We hypothesize that the duration of exclusive breastfeeding and the duration of any breastfeeding will be shorter for women with mastitis compared to women without mastitis. *Overview.* While breastfeeding extends the risk period where a mother may transmit HIV to her child, it also provides infants with better immune development, as well as reduced malnutrition, morbidity and mortality for infants born to HIV-infected women in resource-limited countries. We will investigate whether mastitis has any effect on breastfeeding behavior and whether that effect differs by treatment arm.

CHAPTER 2 – BACKGROUND AND SIGNIFICANCE

2.1. Description of the burden, timing and causes of mastitis among breastfeeding women.

Mastitis is inflammation of the breast, generally associated with lactation [13]. The severity of mastitis may vary, ranging from subclinical mastitis, where symptoms may not be detectable, to severe mastitis (sometimes called puerperal mastitis) which results in flu-like symptoms, high fever or the formation of a painful abscess on the breast. It may present unilaterally or bilaterally and ranges in duration from 2-14 days on average [14]. Mastitis may be associated with either the presence of *Staphylococcus* or less often with bacteria from the mother's skin or the infant's nose or mouth [15, 16].

The burden of mastitis varies. Among breastfeeding women, incidence of mastitis varies widely, from 2 to 30%, depending on the population, geographic location and case definition [15, 17-19]. Mastitis incidence is similar between women with HIV and women without HIV [20, 21]. Variation in the burden of mastitis could be attributed to underreporting [16], definitional differences and variations in clinical practice to screen for mastitis.

Timing of mastitis varies over the duration of breastfeeding. Despite differences in definitions, the severity and timing of mastitis incidence appears to be similar for women in different populations and in different regions. Clinical mastitis appears more frequently in the first few months of breastfeeding [15, 16] and may occur repeatedly

over the duration of breastfeeding [16]. Mastitis also occurs during weaning [22, 23], when non-breast milk foods or liquids are introduced.

Primary causes of mastitis are often related to breastfeeding technique or a compromised immune system [17]. Causes of mastitis related to breastfeeding technique include latching difficulties, insufficient drainage, poor education about breastfeeding, blocked ducts, cracked nipples, change in frequency of feedings, use of creams on nipples or starting on the opposite breast on consecutive nursing sessions (i.e., not fully draining the breast) [24-26]. Mixed breastfeeding is one of the more common factors associated with mastitis. Infants do not breastfeed as frequently when they begin eating or drinking other foods or liquids, which may clog ducts resulting in milk stasis. Milk stasis triggers an inflammatory response (i.e., mastitis) in the breast. As many as one in four women cite mastitis as a reason for breastfeeding cessation [19, 27, 28], though these studies were done in developed countries and the relationship between mastitis and cessation of breastfeeding may differ in resource-limited settings where formula feeding is not a safe alternative.

While mastitis may be a cause of breastfeeding cessation, cessation can also cause mastitis, so the temporality of these events is conflated. Factors related to a compromised maternal immune system also cause an increase in mastitis risk. Breastfeeding women may have a compromised maternal immune system from an illness, poor nutrition, stress or fatigue [24, 25]. Poor nutrition, including a deficiency in micronutrients and vitamin E, is associated with higher rates of mastitis [29]. While the mechanism is not clearly understood, women who are not getting sufficient nutrients to sustain themselves will also have trouble producing additional food for their infants,

leading to a problem with their milk supply. Stress or maternal fatigue also compromises the immune system through several mechanisms. Maternal stress may cause immune suppression locally in the mammary glands [30]. Stress and fatigue are also associated with an increase in catecholamines and prolactin, which are related to greater milk production, leading to milk stasis, especially if infants are also stressed.

In clinical practice and research settings, mastitis has been defined in several different ways (**Appendix I**). The Academy of Breastfeeding Medicine definition of mastitis is vague and difficult to operationalize:

“...an inflammation of the breast; this may or may not involve a bacterial infection. Redness, pain, and heat may all be present when an area of the breast is engorged or “blocked”/“plugged,” but an infection is not necessarily present. There appears to be a continuum from engorgement, non-infective mastitis, infective mastitis, to breast abscess.” [31, 32]

Studies that utilized clinical definitions may be based entirely on signs and symptoms, including breast soreness and redness, flu-like aching and a fever over 100.4 F° [16]. Benefits of a clinical definition of mastitis include ease of implementation and identification of mastitis during an exam for an immediate response to relieve pain and discomfort for patients, though subclinical mastitis will often be missed and diagnostic criteria may differ by clinician. When resources are available and mastitis is a condition of interest, lab-based measures of inflammation, like sodium levels (Na⁺), sodium to potassium ratios (Na⁺:K⁺) or leukocyte counts, are often used to identify clinical or

subclinical mastitis in a woman's breast milk [29, 33, 34]. In mature milk of breastfeeding women, mean sodium concentrations are 5-6 mmol/L, with 10-15% variation between days [6]. Women with clinical and subclinical mastitis have higher sodium levels in their breast milk. Using Na⁺ levels or Na⁺/K⁺ ratios in breast milk is a desirable indicator of inflammation, since determining the concentrations of sodium and potassium in breast milk is relatively quick, inexpensive, does not differ between hind- and fore-milk and is not affected by maternal sodium intake [6]. Other benefits of using Na⁺:K⁺ ratios are that this measure accounts for the variation due to different proportions of aqueous and fat fractions as a result of different sampling methods and modest decreases in both electrolytes after months of lactation [34]. Mastitis is difficult to diagnose in resource-limited settings. Sending breastmilk to a laboratory for processing may not be feasible or quick enough to provide the best care in resource-limited settings where patients are often lost to follow-up and clinicians have missed the window of opportunity for taking action. Since the definition of mastitis is not standardized, groups of women may not be comparable.

2.2. Relationship Between Mastitis and Vertical HIV Transmission

For HIV-infected women who are breastfeeding, mastitis is a maternal factor associated with mother-to-child transmission of HIV in the pre-ART era. In the pre-ART era, mastitis is a known, preventable cofactor of postnatal HIV transmission among HIV-positive, breastfeeding women (**Appendix II & Appendix III**). Mastitis incidence is similar between women with HIV and women without HIV [20, 21], but more consequential for women with HIV. Mastitis and other breast health issues are

associated with a higher risk of HIV transmission through breast milk [5, 21]. Women with mastitis have higher concentration of HIV RNA in their breast milk [8] and transmit HIV to their infants more often than women without mastitis, though the increased transmission risk may be higher among women with a high plasma viral load [35]. The association between mastitis and postnatal HIV transmission exists for both subclinical and clinical mastitis (**Appendix III**). Subclinical mastitis may be responsible for 18-21% of all mother-to-child transmission or 50% of all postnatal HIV transmission [36], so detecting subclinical mastitis is beneficial. While mastitis is associated with HIV transmission in general, it is strongly associated with late (≥ 2 months old) HIV transmission [37], which is more likely to be related to changes in the infant feeding pattern, longer duration of exposure to HIV or progression of maternal illness for women who are not treated.

Maternal factors may contribute to the mechanism by which mastitis is associated with breast milk transmission of HIV. For breastfeeding mothers, mastitis results in an increase in inflammatory factors in the breast. Increased HIV viral load in breast milk may accompany this inflammatory response locally or from cell-free virus, leading to an increased risk of vertical HIV transmission through breast milk [37]. HIV in breast milk may originate either systemically or from local, HIV-infected macrophages, lymphocytes and ductal epithelial cells. As part of the immune response, the tissue between the milk secreting cells in the breast becomes more permeable and allows plasma salts and proteins into breast milk. Breast milk becomes saltier, aligning with elevated Na^+ levels and Na^+ to K^+ ratios, which are also associated with several immune and inflammatory mediators (e.g., lactoferrin, SLPI, IL-8 and RANTES) for both

clinical and subclinical mastitis [34, 38]. Increased inflammatory factors in breast milk could facilitate HIV viral replication locally within breast tissue. This theory of increased viral replication in breast milk is supported by a greater genetic distance between milk and plasma HIV in women with elevated sodium levels [10]. Increased tissue permeability may facilitate HIV transmission by causing increased tissue permeability, thereby letting more salts, proteins and perhaps the HIV virus into breast milk from the blood, leading to an increase in breast milk viral load from cell-free virus [refs]. For women with mastitis, the nutritional content of their breast milk changes. Women with mastitis have a reduction in protein, lactose and fat content in breast milk during mastitis compared to women without mastitis [39]. In addition, a change in the nutritional content of breast milk could weaken infants' immune systems and gastrointestinal linings, thereby allowing virus to replicate. The increased transmission risk associated with mastitis may be higher among women with a high plasma viral load [35], so breastfeeding women who have had HIV longer or have acute HIV may also be more likely to transmit if they have mastitis, compared to women who are less healthy.

Infant factors may contribute to the mechanism by which mastitis is associated with breast milk transmission of HIV. Mastitis influences several factors that increase an infant's risk of acquiring HIV through breastmilk. Introducing solid foods can be both a cause and consequence of mastitis. While the ways mixed feeding causes mastitis have been discussed above, the relationship goes both ways. Mastitis may lead women to introduce solid foods sooner than anticipated. The introduction of new foods and liquids when mixed feeding begins disturbs the gut flora and creates an environment in the infant's stomach that may stimulate immune cells or make the gut more permeable,

both resulting in increased susceptibility to HIV infection, though there is some evidence that contradicts the gut permeability mechanism. Gut permeability, assessed by a lactulose/mannitol dual sugar permeability test, did not differ between infants who were exclusively breastfed compared to those whose breastfeeding was mixed with solid foods (Discussed by Filteau, 2003 (TRSTMH), results in Rollins *et al.*, 2001). The combination of a high breast milk viral load, from mastitis, and a more susceptible environment in the infant's gastrointestinal tract, from mixed breast feeding, results in an increased risk of HIV transmission. Another way that mastitis influences infant factors associated with HIV transmission is that mastitis changes the consistency of breast milk. A change in breast milk content could mean that infants have to change the way they suckle and the frequency of feeding [35]. A change in the breast milk content could also mean that there are fewer protective inflammatory factors in breast milk. Infants who are not getting as much fat, protein or protective inflammatory factors from breast milk are gaining less weight and are at higher risk of becoming sick (e.g., diarrhea, colds, malaria). Infants of women with mastitis did not gain weight as well as infants of mothers who did not have mastitis [20]. Smaller and less healthy infants are more susceptible since their gastrointestinal lining may be compromised [35]. Whether maternal or infant ART for treatment or prophylaxis will mitigate these mechanisms is unclear.

2.3. Reducing Vertical HIV Transmission Among Breastfeeding Women Through Primary and Secondary Prevention of Mastitis

Primary prevention of mastitis may reduce the risk of transmitting HIV from mother to child during breastfeeding. Primary prevention of mastitis through proper breastfeeding techniques and methods should be incorporated into perinatal care to reduce incidence of mastitis. Mastitis incidence can be reduced by eliminating or reducing risk factors including: stress, abrupt weaning, inconsistent feeding leading to milk stasis and blocked ducts. Interventions to educate women about breastfeeding techniques have resulted in conflicting outcomes. Training women on breastfeeding techniques does not result in a reduction in mastitis incidence compared to women who did not receive training [40, 41], nor was there any improvement for women using prophylactic antibiotics or topical ointment [41], although mastitis diagnosis focused on severe mastitis, follow-up was not sufficiently long in duration [42] and sample sizes were small for these studies [43]. In Bangladesh, an intervention focused on improving breastfeeding technique was successful in reducing subclinical mastitis [44]. The positive results in Bangladesh could be attributed to a different study design, longer follow up, a different (less severe) definition of mastitis or a lower level of baseline knowledge of breastfeeding among participating women, offering more room for improvement. In addition, providing HIV-infected women with a micronutrient supplement and vitamin E may successfully reduce rates of mastitis in the first few months of breastfeeding [20, 29]. The poor study design and follow-up make it difficult to conclude how best to effectively prevent mastitis.

Mastitis can be easily treated. There are guiding principles for treating mastitis: supportive counseling, effective milk removal, antibiotic therapy and symptomatic

treatment (WHO, 2000 management of mastitis). Women diagnosed with mastitis by a clinician may receive antibiotics to treat breast inflammation if bacterial cell counts are available and indicate an infection, symptoms are severe, a nipple fissure is visible or symptoms do not improve after 12-24 hours of improved milk removal. Physicians treat mastitis with a 10 to 14 day course of antibiotics, including either a penicillinase-resistant form of penicillin, dicloxacillin or a cephalosporin that covers *Staphylococcus aureus* [14, 45]. However, there is conflicting evidence about whether antibiotic treatment effectively improves resolution of mastitis or prevents abscess formation. In addition, the use of broad-spectrum antibiotics by physicians could lead to higher prevalence of antibiotic resistance. Physicians also recommend continuing to express milk from the affected breast and completely draining it, a hot compress, draining milk in the shower to help unplug the duct to keep milk moving. Most HIV-negative patients are also advised to breastfeed during the infection to keep milk flowing and to avoid further milk stasis if possible, though HIV-positive women who are breastfeeding should stop nursing from the affected breast, but continue to express milk to help resolve the inflammation [13, 16].

Whether mastitis is a maternal factor associated with vertical HIV transmission is unknown for breastfeeding women taking ARV's. The association between mastitis and vertical transmission has not been studied among women or infants taking prophylactic medication. If the risk of vertical transmission remains high for women with mastitis who are also receiving treatment, modifications of the current behavioral and treatment recommendations for breastfeeding women will be necessary. If HIV transmission in the presence of mastitis is increased, physicians will be counseled to screen for and

diagnose mastitis and possibly recommend women stop nursing on the infected breast, but continue to express milk (if it is not bilateral mastitis) while nursing on the uninfected breast. When to resume feeding from the infected breast will need to be studied since breast milk viral load remains elevated after mastitis despite treatment with antibiotics [46]. While ART reduces maternal viral load and reduces the risk of HIV transmission (**Appendix IV**), whether ART can reduce breast milk viral load, transmission associated with mastitis or even prevent mastitis by improving the maternal immune response is also unclear. When policies changed to recommend breastfeeding, the question of whether mastitis is associated with HIV transmission among women taking ARV has not been answered yet. If mastitis is associated with a similarly high risk of transmission among women taking ARV as those who are not, then even with the new policies to improve treatment availability, transmission will continue to occur unless resources and interventions begin to focus on mastitis and associated transmission. Even with improved HIV treatment options for breastfeeding mothers [47], women continue to transmit HIV to their infants through breast milk. However, whether mastitis plays a role in residual HIV transmission for treated women or if maternal or infant HIV treatment affects mastitis risk is unclear. Is the association between breast milk viral load and HIV transmission only restricted to women with a high baseline viral load? Whether this increase in risk only applies to women with high plasma viral loads is unclear. The increased transmission risk may be higher among women with a high plasma viral load [35], so women who have had HIV longer or have acute HIV while breastfeeding may also be more likely to transmit.

Whether mastitis is associated with HIV transmission through breast milk among women taking ART has important public health implications. In this study, we will use a definition of mastitis that incorporates a wide spectrum of severity to assess the relationship between mastitis and breastmilk HIV-transmission among women taking ART and ART-naïve women. Using a broader definition of mastitis, including any signs of inflammation or precursors to inflammation, may be more feasible for clinicians to incorporate into care compared to confirmatory diagnosis by a lab in resource-limited settings. A diagnosis based on clinical signs and symptoms would allow diagnosis of less severe, though clinically relevant, cases of mastitis. More accessible identification of mastitis and active prevention could reduce the risk of vertical HIV transmission associated with breastfeeding. Guidelines are shifting in resource-limited settings to make ART more available for treatment and prophylaxis of HIV-infected women throughout pregnancy and breastfeeding. With this shift in care, to reduce pediatric HIV, we need to focus on the causes of vertical transmission that remain in the presence of treatment, which may include mastitis.

CHAPTER 3 – DESCRIPTION OF THE PARENT STUDY AND DATA

3.1. Study Population

All three aims of this dissertation were completed using data from the Breastfeeding, Antiretroviral and Nutrition (BAN) study. The BAN study was a randomized controlled trial among HIV-infected women and their breastfeeding infants. The purpose of BAN was to evaluate (i) antiretroviral prophylaxis for infants or mothers during breastfeeding to prevent HIV transmission, (ii) nutritional supplementation for maternal health, and (iii) the feasibility of exclusive breastfeeding with early, rapid weaning [48]. Enrollment for BAN took place in Lilongwe, Malawi between April 2004 and September 2009 and 2369 mother-infant pairs (**Figure 3.1**) were followed for 48 weeks after delivery (**Figure 3.2**). Both mothers and infants received peripartum antiretroviral prophylaxis, at delivery and for 7 days after birth. Mother-infant pairs who met primary and secondary eligibility criteria [48] were assigned to one of six treatment groups by using a 3 by 2 factorial design and permuted-block randomization. Mother-infant pairs were randomized to one of three antiretroviral arms (maternal ART, daily infant NVP or standard of care treatment) and to take a maternal nutritional supplement or not. Patients and clinical staff were not masked to treatment allocation, but study investigators, including the data management and analysis staff, were masked.

Treatment lasted for the duration of breastfeeding. Women were counseled to exclusively breastfeed for the first 24 weeks, then rapidly wean between 24 and 28 weeks. All women were followed for 48 weeks, barring dropout, death or HIV infection.

3.2. Data Collection in the BAN Study

Demographic data for mother-infant pairs were collected by study nurses using case report forms (CRFs) at delivery. Data collection forms were formatted to be compatible with Teleform data collection tools. Teleforms were scanned in Lilongwe, Malawi and uploaded to a form-specific database in Chapel Hill, NC. Participants were linked to their forms, laboratory specimens, reports and other records only by their unique study ID number. No names are associated with study data. All data forms were kept in a locked file cabinet in Lilongwe, Malawi. Electronic files are password protected and only accessible by authorized study personnel.

For all women, a breast exam was scheduled to be conducted at delivery, 2, 6, 12, 18, 24 and 28 weeks postpartum or if they answered “yes” to a screening question at a non-breast exam visit (**Appendix V**). Infant feeding data were collected during study visits at weeks 4, 8, 12, 18, 21, 24 and 28 after delivery.

Figure 3.1. Enrollment and retention of mother-infant pairs recruited to participate in the breastfeeding, antiretroviral and nutrition (BAN) study

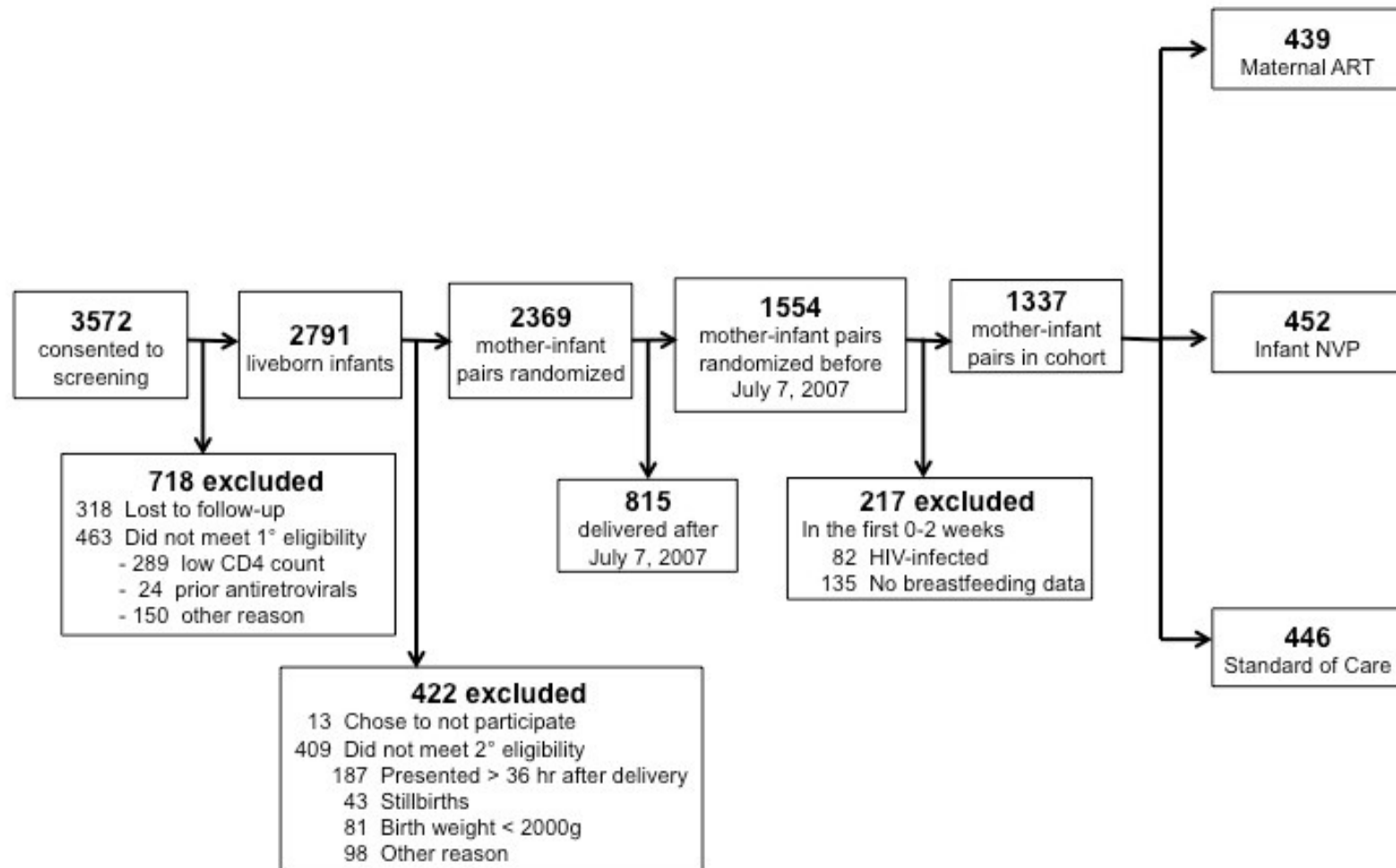
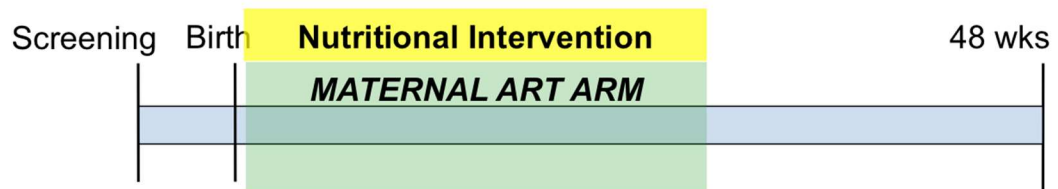
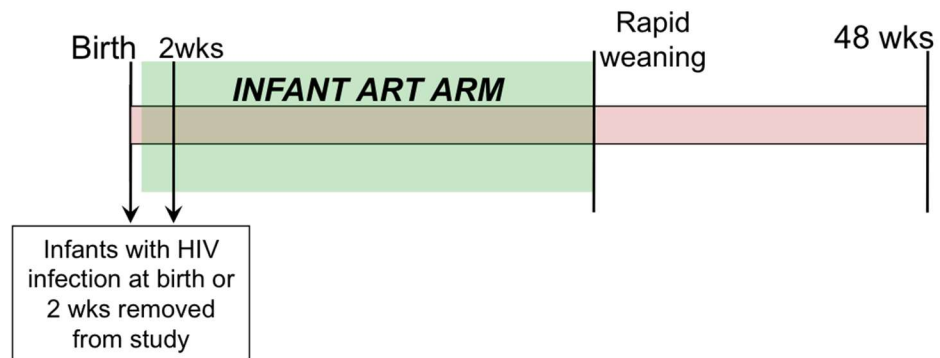


Figure 3.2. Timing of follow-up and intervention for HIV-infected pregnant women and HIV-exposed infants.

HIV-Infected Pregnant Women



HIV-Exposed Infants



3.3. Exposure Assessment

Specific Aim 1: Treatment Assignment Arm. The primary exposure for the first specific aim was randomization to an antiretroviral prophylaxis group. Mother-infant pairs were randomized to three treatment groups, described earlier. Pairs assigned to maternal ART (mART) received combination therapy with three drugs twice daily through 28 weeks—300mg of Zidovudine (ZDV), 150mg of 3TC (lamivudine) and a third

drug. The third drug changed from 200mg nevirapine (NVP) for the first 39 women, 1250 mg nelfinavir for the next 134 women and Kaletra (400mg lopinavir plus 100mg ritonavir) for the remaining women. Infants in the NVP group received a daily dose of NVP that increased according to infant age, ranging from 10 to 30 mg per day. Pairs in the standard of care group did not receive any treatment after 7 days postpartum, consistent with Malawian guidelines at the time.

A secondary exposure was randomization to take or not take a nutritional supplement. Women enrolled in BAN were also randomized to take or not take a nutritional supplement. The nutritional supplement was a high-energy, high protein food supplement that contained 100% of the recommended dietary allowance of micronutrients, except for vitamin A (which is associated with higher rates of vertical HIV transmission) [49].

Specific Aims 2 & 3: Mastitis or Breast Inflammation while Breastfeeding.

The primary exposure for the second and third aim was mastitis or breast inflammation while breastfeeding. At each visit, the exposure variable was dichotomized to indicate whether breastfeeding women had ever had an occurrence of mastitis or breast inflammation. Serious mastitis with a fever among BAN participants was physician-diagnosed and reported as an adverse event (AE). Additional occurrences of mastitis or breast inflammation were identified by the presence of signs or symptoms of breast pathology during the maternal breast exams (**Appendix V**).

Women were categorized as having mastitis or breast inflammation while breastfeeding if they had a diagnosis of severe mastitis or breast infection or had any of

the following breast problems: discolored or shiny, hard, lumpy, hot, painful, tight breasts, tender axilla nodes, cracks, blood, rash, exudate, open or oozing sores on breast or areola. At every visit, women should have been asked several screening questions including, “Since the last visit have you developed any breast pain or discomfort, fissures, lesions, cracks, open or oozing sores on your breasts or areola, itching rash or white patches on your breasts?” If women had signs or symptoms of mastitis or breast inflammation during a visit, we assumed that their signs and symptoms started prior to that visit.

The average case of severe mastitis was assumed to last 2 weeks [39], so the start date for an occurrence of mastitis or breast inflammation was set to two weeks before the study visit. When visits occurred more frequently than every 4 weeks, the start date was set to the midpoint between study visits (i.e., never more than 2 weeks prior to any visit).

Over the course of the BAN study, the breast exam form was increasingly not completed during expected study visits unless breast health issues were indicated at screening and eventually the breast exam form was dropped entirely in July 2007 in the interest of focusing on the primary study objectives. However, even after the breast exam form was dropped, women were still screened for breast pathologies at the start of each visit and clinicians continued to report mastitis as an adverse event and diagnose and address any clinical breast pathologies identified.

A stricter definition of mastitis was used in sensitivity analyses. The narrow definition was intended to identify mastitis occurrences that would have been more likely to be diagnosed at a routine postpartum visit compared to the definition in the primary

analyses. The stricter definition, which included mastitis only (i.e., not breast inflammation), was defined as breasts that, upon exam, had tender axilla nodes or were discolored or shiny, hard, lumpy, hot or painful during exam (**Appendix V**).

3.4. Outcome Assessment

Specific Aim 1: Effect of Antiretroviral Prophylaxis on Mastitis. In the first aim, the outcome was time to first occurrence of mastitis or breast inflammation while breastfeeding. Our outcome measure was an indicator of whether or not women had mastitis or breast inflammation while breastfeeding during a study visit, described above in the exposure assessment for specific aim 2 and 3.

Specific Aim 2: Mastitis and HIV Transmission through Breastfeeding. In the second aim, time to infant HIV infection was the primary outcome and time to infant HIV infection combined with infant death was the secondary outcome. The primary outcome for this study is time to infant HIV transmission by 48 weeks among infants who were HIV-negative at 2 weeks postpartum. A secondary outcome is a combined event including infant HIV infection or infant death from any cause by 48 weeks for infants who were HIV-negative at 2 weeks postpartum. As in previous BAN studies, pairs whose infants were HIV-positive in the first 2 weeks of life were excluded to ensure they had not acquired HIV during labor/delivery. HIV status for infants was determined by PCR assay with Roche Amplicor 1.5 DNA PCR (Roche Molecular

Systems, Pleasanton, CA, USA) at birth and again at 2, 12, 28, and 48 weeks. Infant HIV status was assigned by the principal investigator of the BAN study, who was blinded to study-group assignments. Results that were HIV positive were confirmed by tests of another specimen. The window of infant HIV infection was narrowed by testing infants' dried blood-spot specimens from interim visits. The date of seroconversion was set to be the midpoint between visits where one test was negative and the next was positive. For infants who were lost to follow-up or died before the confirmatory test, a second sample from the last available sample was tested to confirm HIV status.

Specific Aim 3: Duration of Exclusive Breastfeeding & Duration of Any Breastfeeding. At each visit, women were categorized as exclusively breastfeeding, mixed breastfeeding or not breastfeeding. For this study, two outcomes were considered: i) transition from exclusive breastfeeding to either mixed breastfeeding or breastfeeding cessation; or ii) from any type of breastfeeding (i.e., either exclusive or mixed) to no breastfeeding.

Women were considered to be exclusively breastfeeding until any food or drink (including water) other than breast milk was introduced to an infant (**Appendix VI**). The date of cessation of exclusive breastfeeding was set to be the first visit where women indicated that infants ingested a solid or liquid besides breast milk. If breastfeeding continued after the introduction of non-breast milk substance, then breastfeeding status was categorized as mixed until cessation of any breastfeeding. The date of cessation of any breastfeeding was defined as the first visit where women indicated that they had stopped nursing their infants, as long as there was no evidence of breastfeeding in

subsequent visits based on an infant feeding questionnaire. Breastfeeding status was sometimes missing at the time of events, so to avoid loss of information, we assumed infants were still breastfeeding within 1 month of when the mother last reported breastfeeding.

3.5. Covariates

Baseline covariates. To identify a minimally sufficient set of adjustment variables, a directed acyclic graph (DAG) was used to construct a conceptual model for each aim. For the third aim, the following covariates were included in our analyses to block confounding pathways: treatment group randomization (maternal ART, daily infant NVP or standard of care), age (continuous), baseline CD4 count (200-350, 351- 500, >500), baseline plasma viral load (log10 transformed), marital status (married, not married), primiparity (0, ≥ 1) and education (\leq primary, > primary). Nutritional supplement group was assessed as a potential modifier of the effect of mastitis on HIV transmission from mother to child, using an $\alpha=0.15$ threshold interaction term for retention.

Time-varying covariates. Feeding status and plasma viral load are confounders of the effect of mastitis on breast milk transmission of HIV that are also on the causal pathway. Feeding status is a confounder of the relationship between mastitis and transmission of HIV through breastmilk and it is also influenced by a woman's prior exposure status (i.e., having mastitis could result in a change in frequency or exclusivity of breastfeeding). We categorized women as exclusively breastfeeding, mixed

breastfeeding or not breastfeeding. Once a mother introduced food or drink other than breast milk to her infant (including water), we considered the infants to be no longer exclusively breastfeeding (**Appendix VI**). Reported breastfeeding was sometimes missing at the time of events, so to avoid loss of information about events, we assumed infants were still breastfeeding if an event occurred within 1 month of the mother reporting she was still breastfeeding. HIV RNA in maternal blood plasma is another time-varying covariate affected by exposure (i.e., mastitis), which is also a confounder of the association between mastitis and MTCT of HIV through breast milk. Plasma viral load data were only available for 30% of participants so this covariate was not included in the primary analysis, but we conducted a sensitivity analysis among women with plasma viral load results to assess whether plasma viral load was an effect measure modifier.

CHAPTER 4 – RESEARCH DESIGN AND METHODS

4.1. Study Sample

As in previous analyses from the BAN study, mother-infant pairs whose infants tested HIV-positive in the first 2 weeks of life were excluded. We also excluded mothers who delivered after July 14, 2007, when data collection from the breast exam ceased. After this date in July, the breast exam form was dropped. Since our primary outcome (for aim 1) and exposure (for aim 2 & aim 3) were dependent on the breast exam form, we excluded people after the form was no longer used. For treatment and nutritional group analyses, mother-infant pairs were excluded if they did not have a visit with an infant feeding questionnaire (i.e. to identify feeding status) after randomization or if they had breast health issues or mastitis at delivery. For the first aim, we excluded women who had signs or symptoms at delivery since their mastitis was unrelated to exposure to their assigned treatment arm. For all aims, only the first case of mastitis for each mother was counted in the numerator for this aim since there were only seven repeat cases of mastitis in this cohort.

4.2. Statistical Analyses

Specific Aim 1: Treatment Assignment & Mastitis. We estimated the cumulative incidence of the first occurrence of mastitis or breast inflammation while breastfeeding and evaluated the severity of symptoms over time. For all treatment analyses, we compared women taking maternal ART and women whose infants were taking daily NVP to the standard of care group. We assumed adherence to the assigned intervention arm [50].

The proportional sub-distribution hazards model was employed to estimate hazard ratios of mastitis or breastfeeding inflammation while breastfeeding accounting for competing risks of breastfeeding cessation, infant and maternal death [51-53]. The proportional hazards assumption was tested by adding an interaction term between group and time. The proportionality assumption was violated for both treatment and nutritional group analyses ($p < 0.001$), so an interaction term with time and treatment was included in the final models. Since the causes of early and late mastitis differ, the interaction term for time was a dichotomous time indicator variable allowing the treatment effect in the first 4 weeks to differ from weeks 5-28. Cumulative incidence was estimated using the Breslow estimator of the cumulative sub-distribution hazard function [51-53]. In the hazards models, baseline viral load and baseline CD4 count were assessed as potential effect measure modifiers of the relationship between treatment and mastitis, using an $\alpha=0.15$ threshold for interaction term retention. Person-time after 28 weeks from delivery and after July 14, 2007 was treated as (non-informatively) administratively right-censored. Women were considered lost to follow-up if the date of

their last infant feeding questionnaire (to determine breastfeeding status) occurred prior to the end of the 28-week follow-up period.

Sensitivity Analyses. Several sensitivity analyses were conducted. Analyses were repeated using a stricter definition of severe clinical mastitis than in the primary analysis. The narrow definition was intended to identify mastitis occurrences that would have been more likely to be diagnosed at a routine postpartum visit compared to the definition in the primary analyses. We also conducted a sensitivity analysis using a multiple imputation approach to examine the extent of possible bias associated with the assumption that women with missing outcome data did not have mastitis. For the primary analysis missing outcome data were assumed to be missing not at random (MNAR), such that women without mastitis were more likely to be missing breast exam data. In contrast, the multiple imputation analysis assumes data are missing at random, specifically that women without a breast exam would have the same probability of mastitis as women with a similar covariate distribution who had a breast exam. While the latter is unlikely based on our knowledge of BAN protocol implementation, the multiple imputation analysis provides an indication of the sensitivity of the primary analysis to the MNAR assumption. Values of the missing outcome (mastitis) were imputed using covariates selected a priori, including a breast health screening question (any breast health issues, none), treatment arm, nutritional supplement, visit, age (continuous), baseline CD4 count (continuous), baseline plasma viral load (continuous, log₁₀ transformed and if undetectable, set to the lower limit of detection minus one), detectable viral load (detected, not detected), marital status (married, not married) and parity (0, ≥ 1). Analyses were run separately for each dataset and then combined

across datasets to account for uncertainty within- and between- imputations. All analyses were conducted using SAS version 9.4 (Cary, North Carolina).

Specific Aim 2: Mastitis and Infant HIV Infection Through Breastmilk. Given the low number of events, the analyses presented are mainly descriptive. The risk of transmission was calculated for women with and without mastitis or breast inflammation, for each treatment arm using Fisher's exact confidence intervals. As in previous BAN studies [48, 54, 55], mother-infant pairs whose infants were HIV-positive in the first 2 weeks of life were excluded. We additionally excluded mothers who delivered after July 7, 2007, when data collection for mastitis and breast inflammation symptoms was unreliably collected and eventually ceased.

If the incidence of mastitis and infant HIV transmission had been higher in our substudy, we would have estimated the association between mastitis and HIV transmission with a marginal structural model using weighted pooled logistic regression, such that there was one observation per pair for each week in the study. For each person, for each week of follow-up, the analysis plan included calculation of two types of stabilized weights: exposure weights and censoring weights. Inverse probability of exposure weights (IPEW) would assign each individual a weight that indicated the probability of receiving the exposure history they actually experienced. Inverse probability of censoring weights (IPCW) would have been calculated to estimate the probability that each person was not censored during each week they remained in the study. We then would have multiplied the stabilized IPEW and IPCW to create a combined, stabilized weight for every pair, for each week in the study. The combined,

stabilized weights would then be applied to the discrete time proportional hazards model to provide a marginal estimate where the exposure would be the only explanatory variable in the model. We would have used robust variance estimators and independent correlation matrices to address within-subject correlation induced by weights. Stabilized, exposure and censoring weights would be created using logistic regression models by regressing mastitis on time-varying and baseline covariates selected *a priori*.

Specific Aim 3: Mastitis and Breastfeeding Status. To identify a minimally sufficient set of adjustment variables, a directed acyclic graph (DAG) [56] was used to construct a conceptual model of the relationship between mastitis or breast inflammation and duration of exclusive breastfeeding or any breastfeeding (**APPENDIX VII**). For both analyses, the minimally sufficient set of covariates included time-varying infant weight-for-age (continuous, z-score) and any food insecurity. Baseline covariates were treatment group randomization (maternal ART, daily infant NVP or standard of care), age (continuous), baseline CD4 count (200-350, 351- 500, >500), baseline plasma viral load (log10 transformed), primiparity (0, ≥ 1) and education (\leq primary, > primary). Minimally sufficient adjustment sets were similar for both duration of exclusive breastfeeding and duration of any breastfeeding, except exclusive breastfeeding status was included as a time-varying confounder for the duration of any breastfeeding analysis. Nutritional supplement group was assessed as a potential modifier of the effect of mastitis on HIV transmission from mother to child, using an $\alpha=0.15$ threshold interaction term for retention [57].

To estimate the average causal effect of time-varying mastitis or breast inflammation on the duration of exclusive breastfeeding, we fit a marginal structural

model using a weighted pooled logistic regression, such that there was one observation per pair for each week in the study. A second model was fit to estimate the average causal effect of time-varying mastitis or breast inflammation on duration of any breastfeeding. For each person, for each week of follow-up, two types of stabilized weights were calculated: exposure weights and censoring weights. We used inverse probability of exposure weights (IPEW) to assign each individual a weight that indicates the probability of receiving the exposure history they have actually experienced. Using inverse probability of exposure weights adjusts for time-varying confounders affected by prior exposure, thus creating a pseudo-population where the exposure is not associated with measured confounders [58, 59]. Inverse probability of censoring weights (IPCW) were calculated to estimate the probability that each person was not censored during each week they remained in the study. The stabilized IPEW and IPCW were multiplied to create a combined, stabilized weight for every pair, for each week in the study. The combined, stabilized weights were then applied to the Cox proportional hazards regression model to provide a marginal estimate where the exposure is the only explanatory variable in the model. Robust variance estimator and independent correlation matrices were used to address within-subject correlation induced by weights.

Stabilized, exposure or censoring weights were created using logistic regression models. Numerators for IPEW were calculated as predicted probabilities of exposure, using an intercept only model. Denominators of the IPEW were created by regressing mastitis on time-varying weight-for age (continuous, z-score), time-varying food insecurity status (ever=yes, never=no) and fixed, baseline covariates selected *a priori*, including treatment arm, nutritional supplement, age (continuous), baseline CD4 count

(continuous), baseline plasma viral load (log10 transformed), primiparity (0, ≥ 1) and a restricted cubic spline for time (week). For the IPCW, numerators were calculated as predicted probabilities of censoring, using an intercept only model. Denominators for IPCW were created by regressing censoring status on covariates selected *a priori*, including the above variables and as well as variables for time-varying exposure (ever had mastitis, yes/no) and maternal BMI.

Weighted cumulative incidence curves were calculated to visualize the association between mastitis and duration of exclusive breastfeeding and duration of any breastfeeding. Cumulative incidence curves were adjusted using inverse probability weights for the measured time-varying confounders that are also on the causal pathway. Person-time was censored at HIV transmission to the infant, infant death, maternal death or after 48 weeks of follow-up. Mother-infant pairs were defined as lost to follow-up 4 weeks after the last visit where infant feeding information was available. Person-time after July 14, 2007 (i.e., when breast exam data collection ceased) was (non-informatively) right-censored.

Sensitivity Analyses. In one sensitivity analysis, we used a narrower, more specific definition of exposure (mastitis). Time-varying plasma viral load results were not available for all women enrolled in BAN, but since plasma viral load is a time-varying confounder that may also be on the causal pathway between mastitis or breast inflammation and breastfeeding status, we conducted a sensitivity analysis restricted to the women with time-varying plasma viral load results. IPTW and IPCW were modified to include a restricted cubic spline term for the natural log of the time-varying viral load results in the denominator of the weights.

CHAPTER 5 – EFFECT OF POSTNATAL HIV TREATMENT ON CLINICAL MASTITIS AND BREAST INFLAMMATION IN HIV-INFECTED BREASTFEEDING WOMEN

5.1. Introduction

Mastitis is defined as inflammation of the breast, generally associated with lactation [13]. Mastitis ranges in severity from mild, asymptomatic inflammation that is usually non-infectious in origin, to severe, clinically evident mastitis, which manifests as redness, swelling of the breast, fever or systemic infection. Among all breastfeeding women, incidence varies widely, from 2 to 30% [15, 17-19]. Mastitis is an unwelcome complication for all breastfeeding women, but especially for HIV-infected women. HIV-infected and uninfected women who breastfeed are not differentially affected [20, 21], but HIV-infected women with mastitis are more likely to transmit HIV to their infants compared to women without mastitis if they are not taking antiretroviral therapy (ART) [5, 6, 8, 9, 35, 37, 60].

Mastitis can arise from factors associated with maternal health, infant health or both. Maternal causes of mastitis include poor breastfeeding practices due to insufficient knowledge or education about breastfeeding, blocked ducts, cracked nipples [24-26] or a compromised maternal immune system, which can cause mastitis through systemic mechanisms that increase susceptibility to infection or reduce milk supply in response to poor nutrition, stress and maternal fatigue [17, 24, 25, 29]. Infant factors associated with mastitis include poor latching and inadequate suckling, both of which

could be exacerbated by poor infant health. Some causes of mastitis are difficult to attribute to either maternal or infant origin only, including insufficient breast drainage, change in frequency of feedings and mixed feeding [24-26].

The relationship between mastitis and either maternal ART or daily infant nevirapine (NVP) while breastfeeding has yet to be established. The objective of this study was to describe the incidence, severity, and timing of mastitis among breastfeeding women who are HIV-infected and to evaluate whether maternal ART, daily infant NVP or a nutritional supplement influence patterns of incident mastitis. We hypothesized that the risk of mastitis would be lower for women receiving maternal ART and whose infants are taking NVP, compared to women in the standard of care. We expected that women taking a nutritional supplement that provides energy to support exclusive breastfeeding and provides 100% of their daily allowance of micronutrients would be healthier, so we also hypothesized that mastitis or breast inflammation would be lower for mother-infant pairs taking a nutritional supplement compared to those who were not.

5.2. Methods

Participants and Study Design. We performed a secondary analysis of data from the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study. The BAN study was a randomized controlled trial among HIV-infected women and their breastfeeding infants [48]. Briefly, enrollment for BAN took place in Lilongwe, Malawi between April 2004 and September 2009 and mother-infant pairs were followed for 48 weeks after delivery. Both

mothers and infants received peripartum antiretroviral prophylaxis, at delivery and for 7 days after birth. Mother-infant pairs who met primary and secondary eligibility criteria [48] were assigned to one of six treatment groups by using a 3 by 2 factorial design and permuted-block randomization. Mother-infant pairs were randomized to one of three antiretroviral arms (maternal ART, daily infant NVP or standard of care treatment) and to take a maternal nutritional supplement or not. Treatment lasted for the duration of breastfeeding. Women were counseled to exclusively breastfeed for the first 24 weeks, then rapidly wean between 24 and 28 weeks. All women were followed for 48 weeks, barring dropout, death or HIV infection.

As in previous analyses from the BAN study, mother-infant pairs whose infants tested HIV-positive in the first 2 weeks of life were excluded. We also excluded mothers who delivered after July 14, 2007, when data collection from the breast exam ceased. For treatment and nutritional group analyses, mother-infant pairs were excluded if they did not have a visit with an infant feeding questionnaire (i.e. to identify feeding status) after randomization or if they had breast health issues or mastitis at delivery. All women provided informed consent. The protocol was approved by the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention.

Data Collection and Measurements

Treatment Groups. Mother-infant pairs were randomized to three treatment groups. Pairs assigned to maternal ART (mART) received combination therapy with three drugs twice daily through 28 weeks—300mg of Zidovudine (ZDV), 150mg of 3TC

(lamivudine) and a third drug. The third drug changed from 200mg nevirapine (NVP) for the first 39 women, 1250 mg nelfinavir for the next 134 women and Kaletra (400mg lopinavir plus 100mg ritonavir) for the remaining women. Infants in the NVP group received a daily dose of NVP that increased according to infant age, ranging from 10 to 30 mg per day. Pairs in the standard of care group did not receive any treatment after 7 days postpartum, consistent with Malawian guidelines at the time.

Nutritional Supplement. Women were also randomized to take or not take a nutritional supplement. The nutritional supplement was a high-energy, high protein food supplement that contained 100% of the recommended dietary allowance of micronutrients [49].

Mastitis or Breast Inflammation. Our outcome measure was an indicator of whether women had mastitis or breast inflammation while breastfeeding. Women were categorized as having mastitis or breast inflammation while breastfeeding if they had a diagnosis of clinical mastitis or breast infection reported as an adverse event (AE) or, if during a breast exam, their breasts were discolored or shiny, hard, lumpy, hot, painful or tight, or if they had tender axilla nodes, cracks, blood, rash, exudate, open or oozing sores on breast or areola (**Appendix V**). For all women, a breast exam should have been conducted at 2, 6, 12, 18, 24 and 28 weeks postpartum or if they answered “yes” to a screening question at a non-breast exam visit until July 2007. Based on the BAN protocol and implementation before July 2007, women who did not have a breast exam at an expected visit were assumed to not have mastitis. The average case of severe mastitis was assumed to last 2 weeks [14]. The start date for an episode of mastitis or breast inflammation was set to two weeks before the study visit if visits were 4 or more

weeks apart. If visits were less than 4 weeks apart, the start date was set to the midpoint between the two study visits.

Breastfeeding Cessation Visit. The date of breastfeeding cessation was defined as the first visit where women indicated that they had stopped nursing their infants, as long as there was no evidence of breastfeeding in subsequent visits based on an infant feeding questionnaire (**Appendix VI**).

Statistical Analysis. We estimated the cumulative incidence of the first occurrence of mastitis or breast inflammation while breastfeeding and evaluated the severity of symptoms over time. For all treatment analyses, we compared women taking maternal ART and women whose infants were taking daily NVP to the standard of care group. We assumed adherence to the assigned intervention arm [50].

The proportional sub-distribution hazards model was employed to estimate hazard ratios of mastitis or breastfeeding inflammation while breastfeeding accounting for competing risks of breastfeeding cessation, infant and maternal death [51-53]. The proportional hazards assumption was tested by adding an interaction term between group and time. The proportionality assumption was violated for both treatment and nutritional group analyses ($p < 0.001$), so an interaction term with time and treatment was included in the final models. Since the causes of early and late mastitis differ, the interaction term for time was a dichotomous time indicator variable allowing the treatment effect in the first 4 weeks to differ from weeks 5-28. Cumulative incidence was estimated using the Breslow estimator of the cumulative sub-distribution hazard function [51-53]. Risk differences (RD) were calculated using cumulative incidence estimates

and data were bootstrapped to estimate confidence intervals. In the hazards models, baseline viral load and baseline CD4 count were assessed as potential effect measure modifiers of the relationship between treatment and mastitis, using an $\alpha=0.15$ threshold for interaction term retention. Person-time after 28 weeks from delivery and after July 14, 2007 was treated as (non-informatively) administratively right-censored. Women were considered lost to follow-up if the date of their last infant feeding questionnaire (to determine breastfeeding status) occurred prior to the end of the 28-week follow-up period.

Sensitivity Analyses. Several sensitivity analyses were conducted. Analyses were repeated using a stricter definition of severe clinical mastitis than in the primary analysis. The narrow definition was intended to identify mastitis occurrences that would have been more likely to be diagnosed at a routine postpartum visit compared to the definition in the primary analyses. We also conducted a sensitivity analysis using a multiple imputation approach to examine the extent of possible bias associated with the assumption that women with missing outcome data did not have mastitis. For the primary analysis missing outcome data were assumed to be missing not at random (MNAR), such that women without mastitis were more likely to be missing breast exam data. In contrast, the multiple imputation analysis assumes data are missing at random, specifically that women without a breast exam would have the same probability of mastitis as women with a similar covariate distribution who had a breast exam. While the latter is unlikely based on our knowledge of BAN protocol implementation, the multiple imputation analysis provides an indication of the sensitivity of the primary analysis to the MNAR assumption. Values of the missing outcome (mastitis) were

imputed using covariates selected a priori, including a breast health screening question (any breast health issues, none), treatment arm, nutritional supplement, visit, age (continuous), baseline CD4 count (continuous), baseline plasma viral load (continuous, log10 transformed and if undetectable, set to the lower limit of detection minus one), detectable viral load (detected, not detected), marital status (married, not married) and parity (0, ≥ 1). Analyses were run separately for each dataset and then combined across datasets to account for uncertainty within- and between- imputations. All analyses were conducted using SAS version 9.4 (Cary, North Carolina).

5.3. Results

Among 2369 mother-infant pairs enrolled and randomized to participate in the BAN study, 1554 infants were delivered before July 7, 2007 and screened for the present study. We excluded 82 pairs whose infants were HIV-positive within their first 2 weeks of life, leaving 1472 mother-infant pairs in the cohort for our descriptive analyses of mastitis and breastfeeding signs and symptoms. For analysis by treatment group, our cohort consisted of 1318 mother-infant pairs since we additionally excluded 154 women who did not have at least one follow-up visit where breastfeeding status could be obtained after randomization. Over the 28-week follow-up period, 194 mother-infant pairs were lost to follow-up, 50 infants acquired HIV and 52 pairs experienced a competing event (12 infants died, 1 mother died and 39 stopped breastfeeding).

For 92.8% of visits where a breast exam was supposed to occur, information about the health of women's breasts was available from either a screening question, the breast exam or both. For all visits where a breast exam was supposed to occur, many women (50.8%) did not receive a breast exam. Frequency of missing breast exam data was similar for all treatment arms, over all expected visits. For those who were screened about their breast health history, most (99.8%) who were missing breast exam data reported no breast pain or discomfort at screening.

Most women breastfed for at least 24 weeks, many had two or more previous children (62%), and the median age was 25 (**Table 5.1**). During the 28-week study period, 102 women had at least one occurrence of mastitis or breast inflammation while breastfeeding. We focused on the first occurrence of mastitis or breast inflammation while breastfeeding since only 9 women had repeat occurrences. Most symptoms occurred either in the first few weeks after delivery or near the end of the study period (**Figure 5.1**). The most common symptoms of discomfort associated with breastfeeding identified by study nurses during the breast exam were breasts that were lumpy, hard, cracked, painful, hot, discolored or shiny.

The overall 4-week risk of mastitis or breast inflammation while breastfeeding was 3.8% (95%CI: 2.8, 4.9), while the overall 28-week risk was 7.5% (95%CI: 5.9, 9.0). The 28-week risk of mastitis or breast inflammation while breastfeeding was higher for women in the maternal ART (RD: 4.5, 95%CI: 0.9,8.1) and infant NVP (RD: 3.6 95%CI: 0.3,6.9) groups compared to the standard of care (**Table 5.2, Figure 5.2a**). The overall 4-week risk of severe mastitis while breastfeeding was 2.5% (95%CI: 1.7, 3.4) and was similar between treatment groups at this time. The overall 28-week risk of severe

mastitis was 5.7% (95%CI: 4.3, 7.1) and was also higher for women in the maternal ART (RD: 3.4, 95%CI: 0.4, 6.4) and infant NVP (RD: 3.6, 95%CI: 0.4, 6.8) groups compared to the standard of care.

In the first 4 weeks after delivery, there were no differences in the hazard of mastitis or breast inflammation while breastfeeding between treatment groups (**Table 5.3**). After the first 4 weeks, the hazard of late mastitis or breast inflammation while breastfeeding was higher for women in the maternal ART (HR: 6.7, 95%CI: 2.0, 22.6) and infant NVP (HR: 5.1, 95%CI: 1.5, 17.5) groups compared to the standard of care group (**Table 5.3**). We examined modification of the effect of assigned treatment group on the outcome by baseline viral load (< median, > median) and CD4 count (< 350, > 350). Women in the maternal ART group had a slightly higher hazard of late mastitis (after 4 weeks postpartum) or breast inflammation while breastfeeding if they had a low baseline CD4 count or high baseline viral load; however, in all cases the interaction terms did not meet the a priori criteria ($p < 0.15$) for inclusion. Moreover, confidence intervals were wide and overlapping the point estimates (results not shown). Comparing women taking a nutritional supplement with those who did not, neither the 28-week risk (**Table 5.2**) nor the hazard (**Table 5.3**) of mastitis or breast inflammation while breastfeeding differed between groups.

We conducted several sensitivity analyses. In our primary analysis, we used a definition of mastitis or breast inflammation that was more inclusive compared to signs and symptoms that could be used to diagnose mastitis in clinical practice. As a sensitivity analysis, we repeated all of our analyses with a stricter definition of mastitis, which resulted in a lower overall incidence, but very similar trends over time and

differences between treatment and nutritional groups (**Table 5.3**). From the multiple imputation analyses for missing outcomes, the estimated overall 28-week cumulative incidence of mastitis or breast inflammation while breastfeeding was 12.4% (95%CI: 9.6,15.2). The estimates of the 28-week risk difference of mastitis or breast inflammation obtained from the imputation analysis were similar to the estimates in Table 2: for women taking maternal ART (RD: 5.6, 95%CI: -0.3,12.5) and infant NVP (RD: 3.5, 95%CI: -1.4, 8.4) compared to the standard of care. Compared to the primary analysis, hazard ratios for early mastitis or breast inflammation were similar (maternal ART HR: 1.1, 95%CI: 0.5, 2.2 and infant NVP HR: 1.0, 95%CI: 0.5, 1.9) and hazard ratios for late mastitis or breast inflammation were closer to the null (maternal ART HR: 2.1, 95%CI: 0.7, 5.7 and infant NVP HR: 1.8, 95%CI: 0.7, 5.0).

5.4. Discussion

We evaluated whether maternal ART or daily infant NVP treatment affected the incidence of mastitis or breast inflammation among breastfeeding, HIV-infected women. We hypothesized that women and infants in a treatment arm would have a lower incidence of mastitis or breast inflammation compared to the standard of care. Contrary to our hypothesis, the incidence of mastitis or breast inflammation while breastfeeding was higher among women taking maternal ART and women whose infants took daily NVP compared to women in the standard of care group, where no ART was provided.

The observed higher incidence of mastitis or breast inflammation while breastfeeding in both treatment arms could be due to several reasons. One possible

explanation is that there were biological mechanisms related to maternal or infant treatment that increased women's risk of mastitis or breast inflammation while breastfeeding. Following initiation of maternal ART, women may have experienced a partial recovery of the immune system in response to treatment. With immune reconstitution, which may occur 4-8 weeks after initiation of ART [61-63], women might be more capable of mounting an inflammatory response to milk stasis, clogged ducts, a bacterial infection or other antigens in the breast. This timing coincides with the observed timing of mastitis in this group. For women in the infant NVP group, we hypothesize that the elevated incidence of mastitis or breast inflammation could stem from effects of the introduction of a non-breastmilk substance (i.e., daily NVP). Even though most infants are considered to be exclusively breastfed even if they are taking medication [64], it is conceivable that NVP in infant saliva could irritate the mother's nipple or affect the quality and frequency of feeding, suckling, latch or other factors that could be associated with mastitis or breast inflammation.

Another possible explanation for an increased incidence of mastitis in treatment arms could be that the study may have suffered from ascertainment bias because participants and study staff were not blinded to the treatment arm. Unintentionally, nurses or physicians may have more carefully observed or asked questions about the breast health of mother-infant pairs who were randomized to either the maternal or infant treatment arm. Women in the maternal or infant treatment groups may also have more frequently reported or paid more attention to breast health issues compared to women without a treatment intervention. Missing outcome data are unlikely to be the

cause since breast exams were missing with similar frequency across treatment arms and visits.

In our cohort of HIV-infected, breastfeeding women, the overall 28-week risk of mastitis or breast inflammation for all enrolled women was 7.5%, which was lower than the risk of mastitis in a South African cohort (17%) of HIV-infected, African women using a clinical definition of mastitis similar to ours [21], though the timing of events was similar. Mastitis or breast inflammation occurred more frequently in the first few weeks after delivery and then again when solid foods were introduced. We used a definition of clinical mastitis that was more inclusive than in some other studies since fever was not a necessary symptom. Other cohorts used similar clinical definitions, but estimated point prevalence, only capturing mastitis events at one particular time after delivery (7% in Kenya [65], 9% in Zambia [23], 18% in Kenya [37]). The analysis decision about missing outcome data—that women who were missing breast exam data did not have mastitis—could also be the reason the observed incidence of mastitis was lower than expected among women enrolled in BAN. We believe missing breast exam data were missing not at random and women with missing breast exam data were less likely to have mastitis than women who did have a breast exam. In a sensitivity analysis where missing values for mastitis or breast inflammation were imputed with the same probability as women with observed data and similar covariate distributions, incidence estimates were higher, hazard ratios lower and risk differences did not differ much compared to the primary analyses. Based on the BAN protocol, communication with study staff and screening data, women who were missing breast exam data were less

likely to have breast health issues, so the primary analysis estimates are probably less biased, with the estimates from the multiple imputation analysis serving as bounds.

For women enrolled in BAN, the hazard of mastitis or breast inflammation was highest immediately after delivery, decreased quickly during the first 4 weeks of breastfeeding and stayed low for the remainder of the 28-week follow-up period. The timing of mastitis or breast inflammation in our population was similar to previous estimates where mastitis was most common in the first month after delivery [15, 16]. Several studies, with similar follow-up, identified a spike in mastitis between 14-28 weeks [22, 23] in relation to mixed feeding or weaning, but our study protocol instructed women to rapidly wean between 24-28 weeks and considered completion of breastfeeding cessation to be a competing risk. Women were followed for breastfeeding-related mastitis or breast inflammation until 28 weeks to focus on breastfeeding-related mastitis and consequently, we did not see a late spike in mastitis. While the hazard of mastitis and breast inflammation while breastfeeding decreased at the end of follow-up, individuals who had an event in the last few weeks of follow-up, when solid foods were likely introduced in conjunction with breastfeeding, had more symptoms than those who had mastitis immediately after delivery.

We hypothesized that the effect of maternal ART or infant NVP on mastitis or breast inflammation would be modified by baseline plasma viral load or CD4 count. In our cohort, baseline viral load and CD4 count did not modify the effect of treatment on mastitis or breast inflammation. Incorporating time-varying viral load would help address the question of how treatment influences mastitis more directly. We also did not see an effect of nutritional supplementation on the incidence of mastitis or breast inflammation.

Using sodium potassium ratios to define subclinical mastitis, others observed similar effects of a nutritional supplement on subclinical mastitis [20, 34, 66], with the exception of one instance [67] where healthier (with higher baseline CD4) HIV-infected women who took either of two vitamin supplements (one consisting of vitamin B-complex, C, and E & one consisting of vitamin A + b-carotene) had an increased risk of mastitis. The authors attributed the effect to an inflammatory response restoration, but higher milk production could also have contributed.

Our results may be subject to bias due to misclassification. The definition of mastitis or breast inflammation while breastfeeding was based on a range of clinical signs and symptoms. We conducted sensitivity analyses with a more narrow definition of mastitis and while the 28-week incidence was lower, the trends over time and between treatment groups were similar. On the other end of the spectrum, even mild inflammation of the breast, not just severe, is associated with transmission of HIV through breast milk. In fact, asymptomatic subclinical mastitis is more prevalent and could be responsible for up to 18-21% of all mother-to-child transmission or 50% of postnatal HIV transmission [36]. Breast milk samples to measure sodium/potassium ratios, a measure of breast inflammation that is less susceptible to misclassification, were not available for these analyses.

The BAN study was ideally designed to study the concerns that face HIV-infected, breastfeeding women in the context of lifelong ART in resource-limited settings. Because this was a sub-study of a large, randomized trial, we could safely assume that treatment assignment arms were exchangeable at baseline. BAN also had

good retention for a postpartum study in a resource-limited setting, losing only 12% at 28 weeks [48].

This study was the first to consider whether maternal ART or infant NVP affects incidence of mastitis or breast inflammation for breastfeeding, HIV-infected women. Based on our results, we conclude that mastitis may be more problematic for breastfeeding women taking ART and women whose infants are taking antiretroviral prophylaxis. As the standard of care shifts and women have access to lifelong ART beginning in pregnancy, most women will initiate treatment during pregnancy or be taking ART when they become pregnant, unlike in BAN where women who initiated ART did so after delivery. The effect of earlier initiation of ART during pregnancy on incidence of mastitis will have to be monitored. The role of mastitis in this population is relevant, not just for the comfort and nutrition of nursing mothers and babies, but also as it relates to HIV transmission.

Table 5.1. Characteristics for 1472 women participating in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study in Lilongwe, Malawi (2004-2007)¹

	Overall n=1472	Maternal ART n=487	Infant NVP n=492	Standard of Care n=493
Median (IQR)				
Age	25 (22-29)	26 (22-29)	25 (22-29)	26 (22-29)
Baseline Maternal CD4	443 (328-590)	443 (326-571)	440 (328-600)	446 (333-589)
HIV RNA Viral Load (log10) ³	4.2 (3.7-4.7)	4.3 (3.8-4.7)	4.2 (3.7-4.7)	4.2 (3.6-3.7)
Number (%)				
Primary Education or More	1298 (88%)	429 (88%)	433 (88%)	436 (88%)
Primiparous	180 (12%)	54 (11%)	71 (14%)	55 (11%)
Married	1350 (92%)	451 (93%)	450 (91%)	449 (91%)
Breastfeeding to at least 24 weeks	1154 (78%)	375 (77%)	395 (80%)	384 (78%)
HIV Transmission to Infant ²	74 (5%)	23 (5%)	15 (3%)	36 (7%)
Mastitis Occurrences—count				
1	102 (7%)	38 (8%)	40 (8%)	24 (5%)
2+	9 (1%)	3 (1%)	4 (1%)	2 (1%)

¹ Data are median(interquartile range) or number(%). Abbreviations: antiretroviral therapy (ART), nevirapine (NVP), human immunodeficiency virus (HIV), ribonucleic acid (RNA)

² Infants with inconclusive HIV test excluded.

³ Baseline plasma HIV viral load RNA log10 copies/mL

Table 5.2. Risk differences (%) and 95% confidence limits of mastitis or breast inflammation while breastfeeding by treatment group and nutritional supplement group among women enrolled in the BAN study¹

Weeks After Delivery		4	12	18	24	28
Treatment Group	Maternal ART	-0.5 (-3.9, 2.9)	1.0 (-2.0, 4.0)	2.8 (-0.5, 6.0)	3.8 (0.3, 7.2)	4.5 (0.9, 8.1)
	Infant NVP	0.1 (-3.0, 3.2)	1.4 (-1.6, 4.3)	1.9 (-1.1, 5.0)	2.3 (-0.8, 5.3)	3.6 (0.3, 6.9)
	Standard of Care	0.	0.	0.	0.	0.
Nutritional Supplement	Yes	-0.0 (-2.3, 2.3)	0.7 (-1.9, 3.3)	1.5 (-1.4, 4.3)	1.9 (-1.0, 4.9)	1.5 (-1.7, 4.7)
	No	0.	0.	0.	0.	0.

¹ Risk differences were estimated by contrasting cumulative incidence estimates by group. Data were bootstrapped to estimate confidence intervals. Mastitis or breast inflammation while breastfeeding includes women who had a diagnosis of mastitis or breast inflammation or, who, upon examination, had breasts that were discolored or shiny, hard, tight, lumpy, hot, bleeding/bloody, painful during the exam, or that had tender axilla nodes, cracks, a rash, exudate, or open or oozing sores on breast or areola.

Table 5.3. Sub-distribution hazard ratios of mastitis or breast inflammation while breastfeeding, by treatment group and nutritional supplement group¹

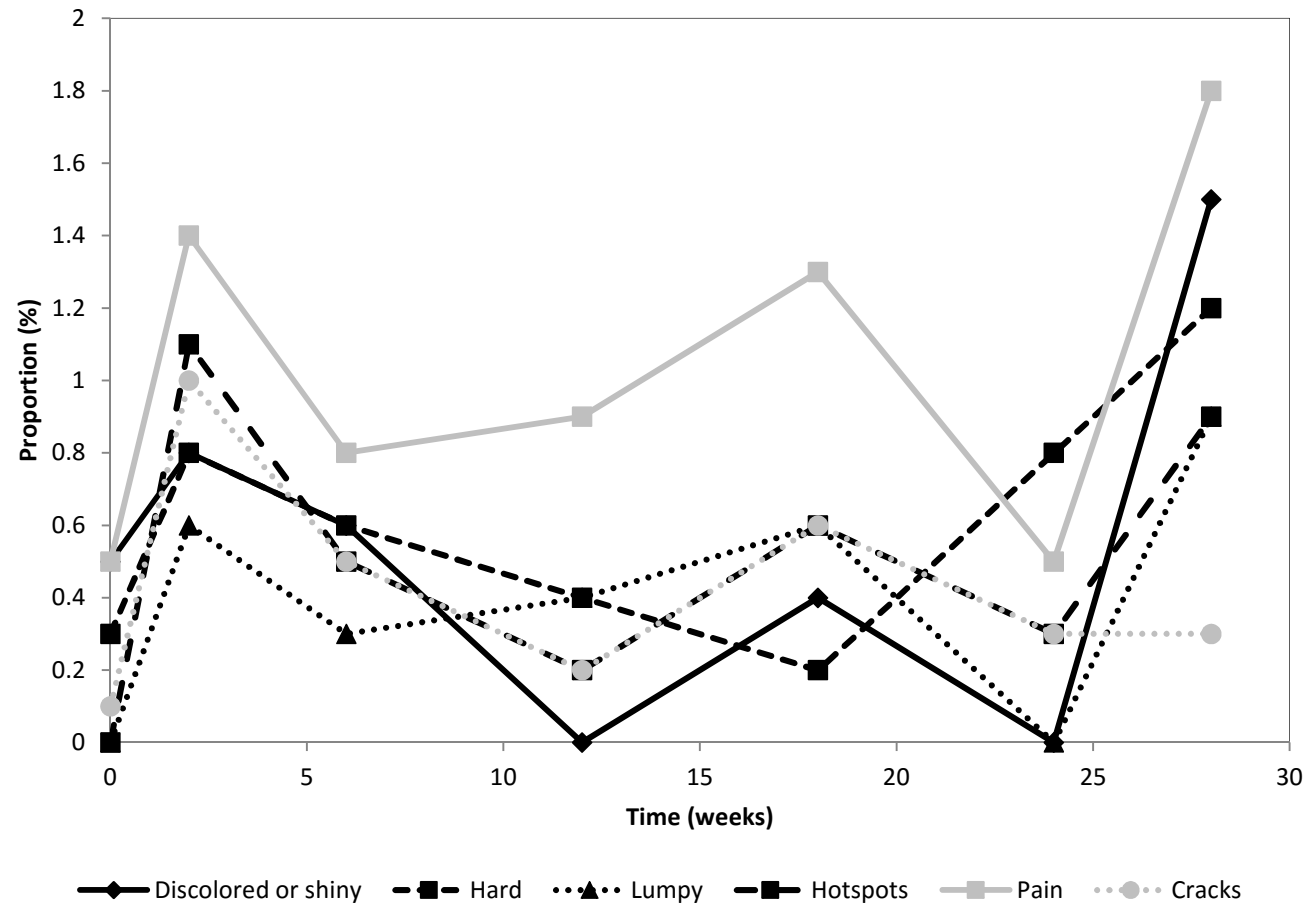
		Early Mastitis (weeks 0 – 4)		Late Mastitis (weeks 5-28)	
		Hazard Ratios	95% CI	Hazard Ratios	95% CI
Primary Analysis: Mastitis or breast inflammation²					
Treatment Group	Maternal ART	0.9	(0.4, 1.8)	6.7	(2.0, 22.6)
	Infant NVP	1.0	(0.5, 1.9)	5.1	(1.5, 17.5)
	Standard of Care	1.		1.	
Nutritional Supplement	Yes	1.0	(0.6, 1.8)	1.5	(0.8, 2.8)
	No	1.		1.	
Sensitivity Analyses: Severe Mastitis³					
Treatment Group	Maternal ART	0.8	(0.3, 1.8)	8.0	(1.8, 35.0)
	Infant NVP	0.9	(0.4, 2.0)	7.7	(1.8, 33.3)
	Standard of Care	1.		1.	
Nutritional Supplement	Yes	1.1	(0.6, 2.3)	1.5	(0.7, 2.9)
	No	1.		1.	

¹ This cohort consisted of 1317 women enrolled in the BAN study between April 2004-July 7, 2007. To estimate the effect of treatment assignment on mastitis, sub-distribution hazard ratios were estimated and an interaction term between treatment group and time (0-4 weeks vs 5-28 weeks) was included.

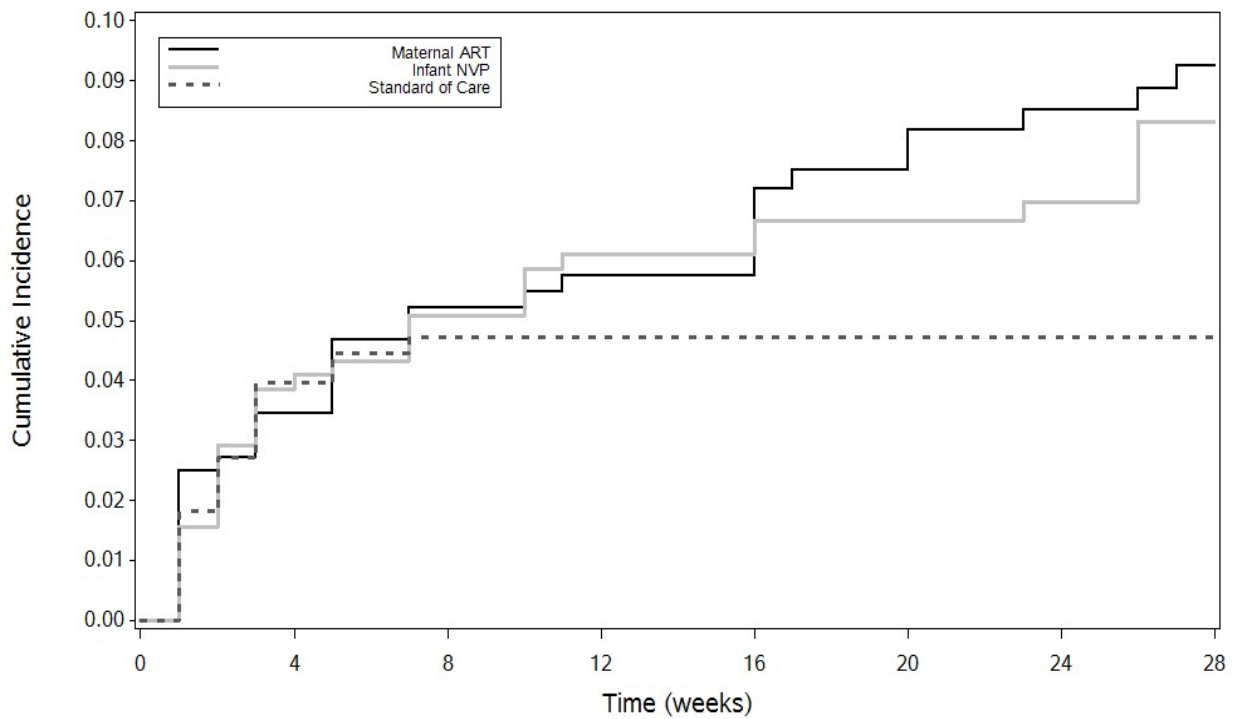
² Mastitis or breast inflammation while breastfeeding includes women who had severe mastitis or breast infection or had any of the following breast problems: discolored or shiny, hard, lumpy, hot, painful, tight breasts, tender axilla nodes, cracks, blood, rash, exudate, open or oozing sores on breast or areola.

³ Severe mastitis is defined as breasts that, upon exam, had tender axilla nodes or were discolored or shiny, hard, lumpy, hot or painful during exam.

Figure 5.1. Prevalence of symptoms of breast inflammation in the first 28 weeks after delivery among HIV-infected breastfeeding women in Lilongwe, Malawi*



5.2a



5.2b

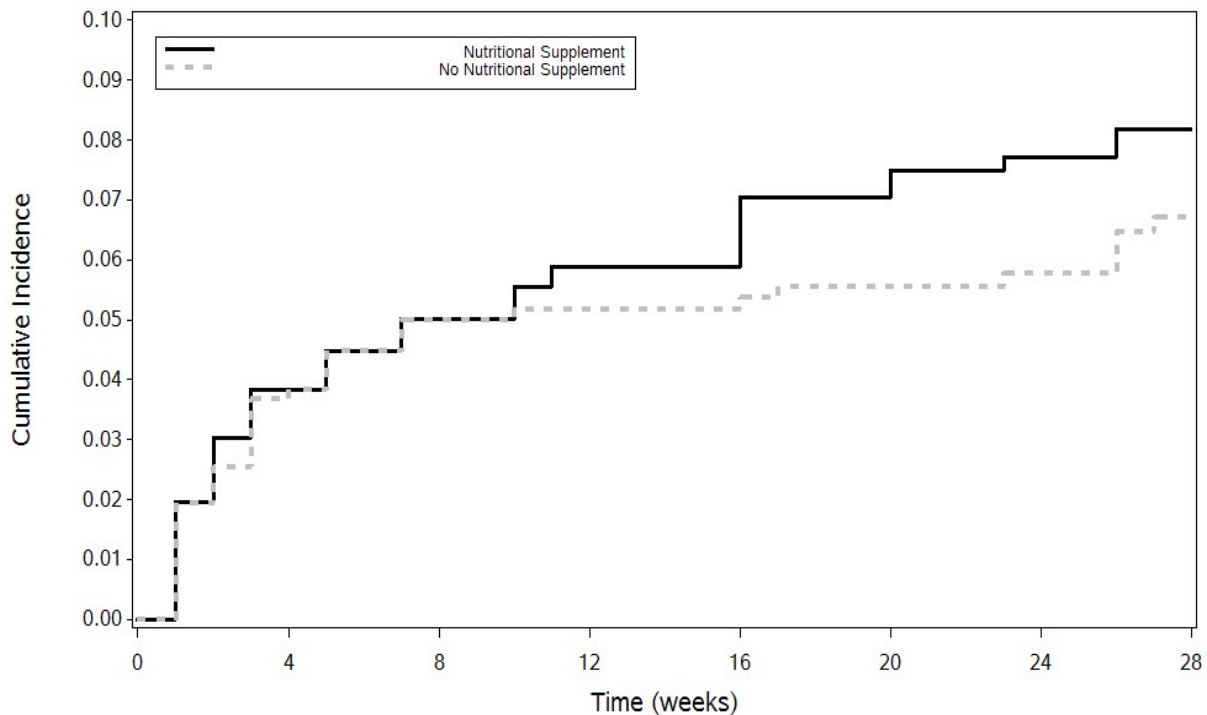


Figure 5.2. Cumulative incidence estimates for mastitis or breast inflammation, comparing 2a) maternal ART (mART), infant NVP (iNVP) and standard of care (CTRL) and 2b) nutritional supplement (NUTR) versus no nutritional supplement (No Nutr).

CHAPTER 6 – MASTITIS AND MOTHER-TO-CHILD TRANSMISSION OF HIV AMONG BREASTFEEDING WOMEN IN MALAWI

6.1. Introduction

Among breastfeeding women, rates of mastitis vary from 2 to 30% [15, 17-19]. The incidence and severity of mastitis is similar for HIV-infected and uninfected women who breastfeed[20, 21], but mastitis has consequences for breastfeeding, HIV-infected women who are not taking antiretroviral therapy (ART) [5, 6, 8, 9, 35, 37, 60]. HIV-infected women with mastitis have higher concentrations of HIV RNA in their breast milk compared to women without mastitis [8] and women with mastitis transmit HIV to their infants more often than women without mastitis [35] [Systematic Review: Zadrozny, et al], though the mechanism is poorly understood.

The mechanisms driving the association between mastitis and HIV transmission may be related to maternal factors, infant factors or both. Maternal factors affecting the relationship between mastitis and HIV transmission include increases in cell-associated virus (CAV) through viral replication localized to the breast tissues [10, 37]; changes in the nutritional content of the milk [5]; and an increase in breast tissue permeability [5], which may allow more cell-free virus (CFV) into breast milk. Infant-related factors include the overall health of the infant, rigorousness of suckling and whether infants are

exclusively breastfed [20, 35]. Whether maternal or infant antiretroviral treatment while breastfeeding mitigates these mechanisms is unclear.

The objective of this study was to investigate the association between mastitis or breast inflammation and HIV transmission through breast milk in a population of HIV-infected, breastfeeding women where some were randomized to ART.

6.2. Methods

We performed a secondary analysis of data from the Breastfeeding, Antiretrovirals, and Nutrition (BAN) randomized controlled study [48]. Briefly, enrollment for BAN occurred in Lilongwe, Malawi between April 2004 and September 2009. Mother-infant pairs who met primary and secondary eligibility criteria were randomized, using a permuted block randomization and two-by-three factorial design, to one of two nutritional supplement arms (lipid-based nutritional supplement or no supplement) and one of three antiretroviral prophylaxis arms (maternal ART, daily infant nevirapine and no additional treatment after 7 days postpartum). All mothers and infants received peripartum single-dose nevirapine and antiretroviral prophylaxis for 7 days after birth. Women assigned to maternal ART (mART) received combination therapy with three drugs twice daily through 28 weeks: 300mg of zidovudine (ZDV), 150mg of lamivudine (3TC) and a third drug (200mg nevirapine (NVP) for the first 39 women, 1250 mg nelfinavir for the next 134 women and Kaletra (400mg lopinavir plus 100mg ritonavir) for the remaining women). In the infant NVP group, infants received a daily dose of NVP that increased according to age, ranging from 10 to 30 mg per day. Pairs assigned to

the standard of care did not receive any additional treatment after the 7 days following delivery, per Malawian guidelines at the time. Further details on BAN are available elsewhere [48, 49, 55].

Mastitis or breast inflammation while breastfeeding was a binary indicator, defined by clinical signs and symptoms identified by physicians or study nurses during a breast exam [Zadrozny, P1]. Women were categorized as having mastitis or breast inflammation while breastfeeding if they had a diagnosis of clinical mastitis or breast infection reported as an adverse event (AE) or, if during a breast exam, their breasts were discolored or shiny, hard, lumpy, hot, painful or tight, or if they had tender axilla nodes, cracks, blood, rash, exudate, open or oozing sores on breast or areola. For all women, a breast exam was scheduled for delivery and at 2, 6, 12, 18, 24, 28, 36 and 48 weeks postpartum or if they answered “yes” to a screening question at a non-breast exam visit until July 2007. Women were categorized as ever having mastitis or breast inflammation while breastfeeding if the start date of an episode of mastitis or breast inflammation preceded infant HIV transmission, loss to follow-up or the end of the study period. Women sometimes were missing breast examination data. Based on the BAN protocol and our knowledge of implementation, women without breast exam data at an expected visit were assumed to not have mastitis.

HIV status for infants was determined by PCR assay with *Roche Amplicor 1.5 DNA PCR (Roche Molecular Systems, Pleasanton, CA, USA)* on whole blood collected at birth and again at 2, 12, 28, and 48 weeks. The HIV infection window was narrowed by testing infants’ dried blood-spot specimens from interim visits. The date of infant HIV

infection was set to be the midpoint between visits where one test was negative and the next was positive.

Given the low number of events, the analyses presented are primarily descriptive. The crude risk of transmission was calculated for women with and without mastitis or breast inflammation and Fisher's exact confidence intervals were calculated for each treatment arm. As in previous BAN analyses [48, 54, 55], mother-infant pairs whose infants were HIV-positive in the first 2 weeks of life were excluded. We additionally excluded mothers who delivered after July 7, 2007, when the data collection form from the breast exam stopped being collected.

All women provided informed consent. The protocol was approved by the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention.

6.3. Results

Among 2369 mother-infant pairs enrolled and randomized to participate in the BAN study, 1554 infants were delivered before July 7, 2007. We excluded 82 mother-infant pairs whose infants were HIV-positive within their first 2 weeks of life (indicating transmission likely occurred during pregnancy or delivery) and 135 who did not have any breastfeeding data, leaving 1337 mother-infant pairs in our cohort. For visits in which a breast exam was scheduled to occur, 54% of women did not have a breast

exam recorded. For 93% of visits where a breast exam was scheduled, either from a screening question, breast exam data or both were available.

The median age at delivery was 26 years old. Most women were married (92%), did not have any secondary education (64%) and did not experience any food insecurity in the past 4 weeks (72%) over the course of follow-up. Patient characteristics were well balanced across women with and without mastitis, except more women randomized to maternal ART or infant NVP eventually had mastitis or breast inflammation compared to the standard of care (**Table 6.1**).

After 28 weeks, 160 mother-infant pairs were lost to follow-up, 8 infants died and 1 mother died (**Figure 6.1**). Ninety-seven (7%) of the 1337 women had at least one occurrence of mastitis or breast inflammation. Between 2 and 28 weeks, 53 infants acquired HIV. Overall incidence of mother-to-child transmission of HIV through breastmilk by 28 weeks was 4%. One woman who had a history of mastitis or breast inflammation transmitted HIV to her infant (1%), and 52 women (4%) without mastitis or breast inflammation transmitted HIV to their infants. Combined incidence of vertical HIV transmission or death was 5%; two (2%) women who had a history of mastitis or breast inflammation had events (1 HIV transmission and 1 infant death) and 59 women (5%) without mastitis or breast inflammation had events (52 HIV transmissions and 7 infant deaths). There was one infant death and one infant HIV-infection among women in the infant NVP group who also had a history of mastitis. In the infant NVP group, the risk of HIV transmission was slightly higher among women with mastitis 2.6% (95%CI: 0.1, 13.5) compared to women without mastitis, 1.7% (95%CI: 0.7, 3.5), but the confidence intervals of each overlapped the point estimates (**Table 6.2**).

6.4. Discussion

The current study was the first to consider mastitis and mother-to child transmission of HIV in a population in which some women were receiving treatment for HIV, which is relevant in this era of lifelong ART. World Health Organization (WHO) guidelines were updated in 2012, to recommend lifelong highly active antiretroviral therapy (HAART) for HIV-infected pregnant women (Option B+) and daily prophylaxis from birth to 4-6 weeks old for their infants, regardless of maternal CD4 count or stage of disease [68]. For breastfeeding women enrolled in BAN, only one woman with a history mastitis or breast inflammation transmitted HIV to her infant. Due to this low incidence of events among exposed women, the study had insufficient power to estimate effects of mastitis or breast inflammation on the risk of HIV transmission or to determine whether effects differed by antiretroviral prophylaxis intervention.

In the pre-HAART era, among breastfeeding women without antiretroviral prophylaxis, mastitis was associated with an increased risk of HIV transmission to infants [Zadrozny, et al (systematic review)]. In the absence of treatment, we would have expected women with mastitis or breast inflammation to have a higher incidence of HIV transmission to their infants. Breastfeeding women enrolled in BAN who were taking maternal ART or women whose infants were taking NVP had a higher incidence of mastitis or breast inflammation [Zadrozny, et al]. In our study, though power was limited, the proportion of vertical HIV transmission events was higher among women without mastitis or breast inflammation, independent of antiretroviral intervention arm. In

our cohort, most women with mastitis were taking ART or had infants taking NVP, and HIV transmission on these intervention arms was low. While it may be presumptuous, given insufficient power, we can speculate that one explanation for the low vertical HIV transmission risk among women with mastitis or breast inflammation is that breast milk viral load was low for women in the maternal ART group, which could have protected women with mastitis or breast inflammation from an increased risk of HIV transmission through breast milk.

In this study, data may be subject to bias due to exposure misclassification for mastitis or breast inflammation. We defined mastitis or breast inflammation using clinical signs and symptoms. Subclinical mastitis is associated with HIV transmission [36], but breast milk samples were not available to measure sodium/potassium ratios, a measure of breast inflammation that includes subclinical mastitis and is less susceptible to misclassification. Misclassification of exposure may also have arose because some women were missing breast exam data and based on the BAN protocol, an analysis decision was made to assume that women who were missing breast exam data, but indicated no breast health problems at the start of the visit, did not have mastitis. We believe women missing breast exam data were missing not at random and were less likely to have mastitis than similar women who did have a breast exam.

In the pre-HAART era, mother-to-child transmission risk was higher for breastfeeding women with mastitis. With effective postnatal HIV therapy [69-71] and better treatment coverage for HIV-infected, pregnant women [47, 72], postnatal HIV transmission risk will be lower. In this subsample of HIV-infected, breastfeeding women, the risk of HIV transmission through breast milk is low even though mother-infant pairs

in the antiretroviral prophylactic group had a higher incidence of mastitis compared to mother-infant pairs who were not taking prophylaxis. The present data lent some assurance that the risk of HIV transmission among mothers and infants receiving antiretroviral therapy was still low, even for women with mastitis. Subsequent studies on this topic would require a larger sample size or alternate study designs, like case-control or case-cohort studies, to ensure sufficient cases to address whether mastitis affects HIV transmission risk among HIV-infected women who are breastfeeding and taking antiretroviral therapy.

Table 6.1. Description of mother-infant pairs by mastitis or breast inflammation status

	Total (n=1337)	Mastitis or Breast Inflammation (n=97)	No Mastitis or Breast Inflammation (n=1240)
Median (IQR)			
Maternal Age	26 (22, 29)	26 (23, 30)	26 (22, 29)
CD4 count	445 (329, 590)	430 (319, 571)	445 (330, 591)
log10 plasma viral load ¹	4.2 (3.7, 4.7)	4.3 (4.0, 4.9)	4.2 (3.7, 5.0)
Hemoglobin (g/dL)	10.7 (10.0, 11.6)	10.6 (9.8, 11.2)	10.8 (10.0, 11.6)
Maternal BMI ²	22.9 (21.3, 25.1)	22.7 (21.3, 24.1)	22.9 (21.3, 25.1)
Weight for age z-score	-0.52 (-1.12, 0.01)	-0.62 (-1.18, 0.01)	-0.52 (-1.13, 0.01)
Exclusive breastfeeding duration ³	27 (25, 27)	27 (25, 28)	27 (25, 27)
Number (%)			
Treatment Group			
Maternal antiretrovirals	439 (33)	35 (36)	404 (33)
Infant daily nevirapine	452 (34)	39 (40)	413 (33)
Standard of care	446 (33)	23 (24)	423 (34)
Nutritional Supplement	670 (50)	55 (57)	615 (50)
Secondary Education	475 (36)	38 (39)	437 (35)
Married	1227 (92)	92 (95)	1135 (92)
Primiparous	149 (11)	8 (8)	141 (11)
Any food insecurity	374 (28)	28 (29)	331 (27)

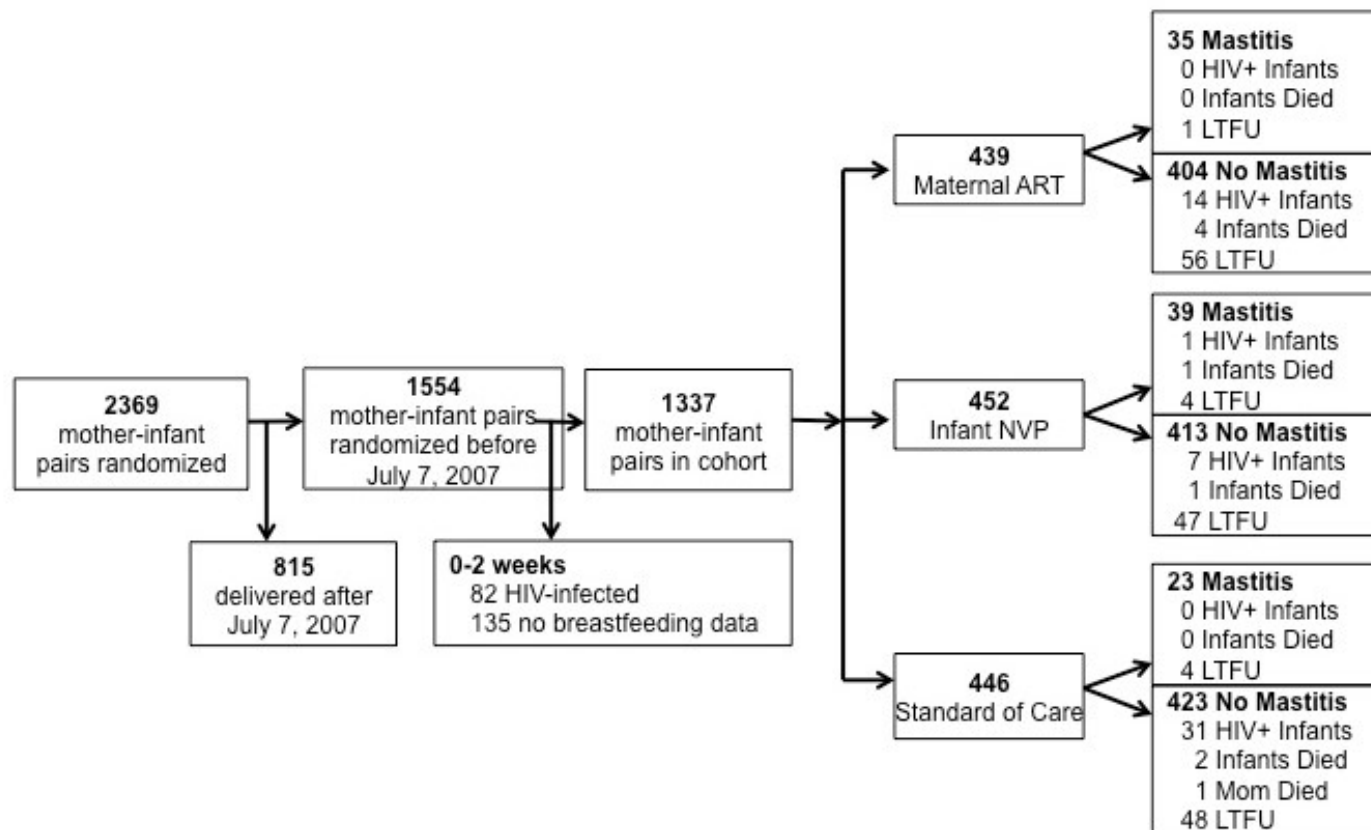
¹ Missing baseline plasma viral load for n=1 woman.² Missing baseline BMI for 2 women.³ 1312 women ever reported exclusively breastfeeding, of whom 58 transmitted HIV to their infants and 1254 did not.

Table 6.2. Proportion of vertical HIV transmission incidents between 2 and 28 weeks, by treatment group and mastitis status, among breastfeeding, HIV-infected women in the Breastfeeding, Antiretrovirals and Nutrition (BAN) Study

Treatment Group		N	Infant HIV		Crude Risk	95% CI	Infant HIV or Death		Crude Risk	95% CI
			Infant HIV				Infant HIV or Death			
Maternal ART	Mastitis or Breast Inflammation*	35	0		0.0%	(0.0, 10.0)	0		0.0%	(0.0, 10.0)
	No Mastitis or Breast Inflammation	404	14		3.5%	(1.9, 5.7)	18		4.5%	(2.7, 7.0)
Infant NVP	Mastitis or Breast Inflammation	39	1		2.6%	(0.1, 13.5)	2		5.1%	(0.6, 17.3)
	No Mastitis or Breast Inflammation	413	7		1.7%	(0.7, 3.5)	8		1.9%	(0.8, 3.3)
Standard of Care	Mastitis or Breast Inflammation*	23	0		0.0%	(0.0, 14.8)	0		0.0%	(0.0, 14.8)
	No Mastitis or Breast Inflammation	423	31		7.3%	(5.0, 10.3)	33		7.8%	(5.4, 10.8)

*one-sided, 97.5% confidence intervals were calculated for strata without events.

Figure 6.1. Breakdown of HIV transmission events, infant deaths, maternal deaths and loss-to-follow-up (LTFU) by exposure to mastitis or breast inflammation and by treatment assignment group, among women enrolled in the breastfeeding antiretroviral and nutrition (BAN) study.



CHAPTER 7 – MASTITS AND DURATION OF BREASTFEEDING IN THE BREASTFEEDING IN THE BREASTFEEDING, ANTIRETROVIRALS, AND NUTRITION (BAN) STUDY

7.1. Introduction

Breastfeeding is complicated for HIV-infected women whose infants are HIV-uninfected at birth. Breastfeeding extends the risk period where a mother may transmit HIV to her child, but in resource-limited settings, it also provides infants with better immune development, as well as reduced risk of malnutrition, morbidity and mortality for infants [3, 73-76]. For these HIV-exposed infants, breastfeeding is a necessary source of nutrition. In low and middle-income countries, where a safe water supply may not be available, the World Health Organization recommends that HIV-infected women with newborn infants should exclusively breastfeed for the first 6 months and continue breastfeeding until babies are 12 months old, in conjunction with an extended medication regimen [77, 78].

While breastfeeding is common in many resource-limited settings, convincing women to exclusively breastfeed is a more difficult case to make. An exclusively breastfed infant is one who is breastfed and no other liquid or solid from any other source enters the infant's mouth [64]. Mixed breastfeeding is a term that describes breastfeeding in conjunction with food or a non-breast milk liquid. Exclusively breastfeeding is not common in most sub-Saharan African countries and for those

women who do exclusively breastfeed, the duration is generally less than the recommended 6 months [79], despite the benefits of exclusive breastfeeding compared to mixed feeding. Based on demographic health surveys, exclusive breastfeeding for infants under 4 months is low in Malawi (11%), Zimbabwe (16%), Kenya (17%) and Zambia (27%) [80]. Compared to mixed feeding, infants who are HIV-exposed but uninfected have a lower risk morbidity and mortality if they are exclusively breastfed [4, 81]. Exclusive breastfeeding is also associated with a reduced risk of HIV-infection for infants who are HIV-exposed [4, 82-84].

One theory about how exclusive breastfeeding is associated with a reduced risk of HIV transmission is that exclusive breastfeeding reduces the risk of mastitis, which in turn reduces the risk of HIV transmission through breast milk. The mechanism behind the association between mastitis, exclusive breastfeeding and HIV transmission may be related to maternal or infant factors [5, 10, 20, 35, 37]. The theory that predominates is that exclusive breastfeeding prevents breast engorgement and milk stasis, which reduces periods of epithelial permeability in breast tissue [35, 80, 85]. In addition, the temporality of the relationship between mastitis and exclusive breastfeeding is entangled, so it is unclear whether women stop exclusively breastfeeding because they have mastitis or if mastitis is a consequence of a change in breastfeeding behavior. Both mechanisms may work in a feedback loop as well.

The relationship between mastitis and breastfeeding is complex. Among breastfeeding women, mastitis may be both a cause and consequence of a change in breastfeeding status. The present study focuses only on mastitis or breast inflammation for breastfeeding women (i.e., not associated with complete cessation of breastfeeding).

The objective of this study was to investigate whether mastitis is associated with the duration of exclusive breastfeeding or time to cessation of breastfeeding, among a population of HIV-infected women who were all counseled to exclusively breastfeed. We hypothesize that the duration of exclusive breastfeeding and the duration of any breastfeeding will be shorter for women with mastitis compared to women without mastitis.

7.2. Methods

Participants and Study Design. We performed a secondary analysis of data from the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study [48]. Briefly, enrollment for BAN took place in Lilongwe, Malawi between April 2004 and September 2009 and mother-infant pairs were followed for 48 weeks after delivery. Both mothers and infants received peripartum antiretroviral prophylaxis, at delivery and for 7 days after birth. Mother-infant pairs who met primary and secondary eligibility criteria were assigned to one of six treatment groups by permuted-block randomization. Participants were randomized to maternal ART, daily infant NVP or standard of care treatment and each mother-infant pair was also randomized to take or not take a nutritional supplement. Women were counseled to exclusively breastfeed for the first 24 weeks, then abruptly wean between 24 and 28 weeks. All women were followed for 48 weeks, barring dropout, death or HIV infection.

As in previous analyses from the BAN study [48, 50, 54, 55], mother-infant pairs whose infants tested HIV-positive in the first 2 weeks of life were excluded so infants who may have acquired HIV during labor and delivery were not part of the analysis. In

addition, mother-infant pairs were excluded if they did not have a visit with an infant feeding questionnaire (i.e. to identify breastfeeding status) after randomization. All women provided informed consent. The protocol was approved by the Malawi National Health Science Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention.

Data Collection and Measurements.

Mastitis or breast inflammation while breastfeeding. Mastitis or breast inflammation was the exposure of interest. It was operationalized as a time-varying variable that was dichotomized to indicate whether or not women had ever had mastitis or breast inflammation while breastfeeding. Mastitis or breast inflammation was defined by clinical signs and symptoms identified by physicians or study nurses during a breast exam [Zadrozny, P1]. Women were categorized as having mastitis or breast inflammation while breastfeeding if they had a diagnosis of clinical mastitis or breast infection reported as an adverse event (AE) or, if during a breast exam, their breasts were discolored or shiny, hard, lumpy, hot, painful or tight, or if they had tender axilla nodes, cracks, blood, rash, exudate, open or oozing sores on breast or areola. For all women, a breast exam was scheduled for delivery and at 2, 6, 12, 18, 24, 28, 36 and 48 weeks postpartum or if they answered “yes” to a screening question at a non-breast exam visit until July 2007. To capture the elevated inflammatory response before signs and symptoms of mastitis were present, signs and symptoms were assumed to start prior to that visit. The average case of severe mastitis was assumed to last 2 weeks

[14], so the start date for an occurrence of mastitis or breast inflammation was set to two weeks before the study visit. When visits occurred more frequently than every 4 weeks, the start date was set to the midpoint between study visits (i.e., never more than 2 weeks prior to any visit). Women were sometimes missing data from a breast exam visit, so based on the BAN protocol and our knowledge of implementation, women who did not have a breast exam at an expected visit were assumed to not have mastitis if they reported no breast health issues at the start of the visit when a breast exam was supposed to occur.

Breastfeeding Status. For this study, two outcomes were considered: i) transition from exclusive breastfeeding to not exclusive breastfeeding, which included either mixed breastfeeding or breastfeeding cessation (**Figure 7.1**); or ii) transition from any type of breastfeeding (i.e., either exclusive or mixed) to no breastfeeding (**Figure 7.2**).

At every visit, women were categorized as exclusively breastfeeding, mixed breastfeeding or not breastfeeding. Women were considered to be exclusively breastfeeding until any food or drink (including water, cow's milk, and formula) other than breast milk was introduced to an infant (**Appendix VI**). The date of cessation of *exclusive* breastfeeding was set to be the first visit where women indicated that infants ingested a solid or liquid besides breast milk. If breastfeeding continued after the introduction of non-breast milk substance, then breastfeeding status was categorized as mixed until cessation of any breastfeeding. The date of cessation of *any* breastfeeding was defined as the first visit where women indicated that they had stopped nursing their infants, as long as there was no evidence of breastfeeding in subsequent visits based on an infant feeding questionnaire. Breastfeeding status was sometimes missing, so to

avoid loss of information, if breastfeeding data status was missing we assumed infants were still breastfeeding for 4 weeks after the mother last reported breastfeeding.

Statistical Analysis. To identify a minimally sufficient set of adjustment variables, a directed acyclic graph (DAG) [56] was used to construct a conceptual model of the relationship between mastitis or breast inflammation and duration of exclusive breastfeeding or any breastfeeding. For both analyses, the minimally sufficient set of covariates included time-varying infant weight-for-age (cubic z-score) and a variable indicating whether families ever experienced food insecurity during follow-up. Baseline covariates were treatment group randomization (maternal ART, daily infant NVP or standard of care), age (cubic), baseline CD4 count (cubic), baseline plasma viral load (restricted cubic spline of the natural log of viral load), primiparity (0, ≥ 1) and education (\leq primary, $>$ primary). Minimally sufficient adjustment sets were similar for both duration of exclusive breastfeeding and duration of any breastfeeding, except exclusive breastfeeding status was included as a time-varying confounder for the duration of any breastfeeding analysis. Nutritional supplement group and treatment group was assessed as a potential effect measure modifier of the relationship between mastitis on HIV transmission from mother to child, using an $\alpha=0.15$ threshold interaction term for retention [57].

To estimate the average causal effect of time-varying mastitis or breast inflammation on the duration of exclusive breastfeeding, we fit a marginal structural model using a weighted pooled logistic regression model, such that there was one observation per pair for each week in the study. A separate model was fit to estimate

the average causal effect of time-varying mastitis or breast inflammation on duration of any breastfeeding. For each person, for each week of follow-up, two types of stabilized weights were calculated: treatment (or in this case, exposure) weights and censoring weights. We used inverse probability of treatment weights (IPTW) to assign each individual a weight that indicates the probability of receiving the treatment history they have actually experienced [58] (**Figure 7.3**). Using IPTW creates a pseudo-population where the exposure is not associated with measured confounders [58, 59], and allows us to control for time-varying confounders affected by prior exposure. Inverse probability of censoring weights (IPCW) were calculated to estimate the probability that each person was not censored during each week they remained in the study (**Figure 7.3**). The stabilized IPTW and IPCW were multiplied to create a combined, stabilized weight for every pair, for each week in the study [86]. The combined, stabilized weights were then applied to the Cox proportional hazards regression model to provide a marginal estimate where the exposure is the only explanatory variable in the final model. Robust variance estimator and independent correlation matrices were used to address within-subject correlation induced by weights.

Stabilized, treatment and censoring weights were estimated using logistic regression models. Numerators for IPTW were calculated as predicted probabilities of exposure, using an intercept-only model. Denominators of the IPTW were created by regressing mastitis on time-varying weight-for age z-score modeled using a linear, quadratic and cubic term, time-varying food insecurity status (ever=yes, never=no) and fixed, baseline covariates selected *a priori*, including treatment arm, nutritional supplement, primiparity (0, ≥ 1), a linear, quadratic and cubic term for both continuous

age and baseline CD4 count, and a restricted cubic spline for time (weeks) and the natural log of baseline plasma viral load. For the IPCW, numerators were calculated as predicted probabilities of censoring, using an intercept only model. Denominators for IPCW were created by regressing censoring status on covariates selected *a priori*, including the above variables as well as variables for time-varying exposure (ever had mastitis, yes/no) and baseline and time-varying maternal BMI (restricted cubic spline).

To assess time-varying effect measure modification by exclusive breastfeeding for the analysis with duration of any breastfeeding as the outcome, we added exclusive breastfeeding to the numerator of the exposure weights, included exclusive breastfeeding as a variable in the weighted marginal structural model and added an interaction term between exclusive breastfeeding and the exposure to the weighted marginal structural model.

Weighted cumulative incidence curves were calculated to visualize the association between mastitis and duration of exclusive breastfeeding and duration of any breastfeeding. Cumulative incidence curves were adjusted using inverse probability weights for the measured time-varying confounders that are also on the causal pathway. Person-time was censored at HIV transmission to the infant, infant death, maternal death or after 48 weeks of follow-up. Mother-infant pairs were defined as lost to follow-up 4 weeks after the last visit where infant feeding information was available. Person-time after July 14, 2007 (i.e., when breast exam data collection ceased) was (non-informatively) right-censored.

Sensitivity Analyses. In one sensitivity analysis, we used a narrower, more specific definition of exposure (mastitis). Time-varying plasma viral load results were not

available for all women enrolled in BAN, but since plasma viral load is a time-varying confounder that may also be on the causal pathway between mastitis or breast inflammation and breastfeeding status, we conducted a sensitivity analysis restricted to the women with time-varying plasma viral load results. IPTW and IPCW were modified to include a restricted cubic spline term for the natural log of the time-varying viral load results in the denominator of the weights. [missing data analysis]

7.3. Results

Among 2369 mother-infant pairs enrolled and randomized to participate in the BAN study, 1554 infants were delivered before July 7, 2007 and screened for the present study. We excluded 82 pairs whose infants were HIV-positive within their first 2 weeks of life and 135 that did not have any breastfeeding data, leaving 1337 mother-infant pairs in our cohort. While women were exclusively breastfeeding, 167 pairs were lost to follow-up, 92 women had at least one occurrence of mastitis or breast inflammation, 49 infants were HIV-infected, 5 infants died and 1 mother died. When duration of any breastfeeding was the outcome, which sometimes resulted in a longer time before the event, an additional 28 mother-infant pairs were lost to follow-up, 6 women had at least one occurrence of mastitis or breast inflammation, 5 more infants were HIV-infected and one more infant died.

For all visits where a breast exam was scheduled, many women (54%) did not receive a breast exam. Frequency of missing breast exam data was similar across all expected visits. For 93% of visits where a breast exam was scheduled, information

about the health of women's breasts were available from either from a screening question, from the breast exam or from both.

The mean age of women at the time of enrollment in the BAN study was 26 years old. Most women were married (92%), did not have any secondary education (64%) and did not experience any food insecurity (28%) over the course of follow-up (**Table 7.1**). The mean duration of exclusive breastfeeding was 27 weeks and the mean duration of any breastfeeding was 30 weeks. The duration of exclusive breastfeeding was slightly longer for women with mastitis or breast inflammation compared to those without (HR: 1.07; 95%CI: 0.86, 1.34). In contrast, women with mastitis or breast inflammation stopped breastfeeding completely slightly sooner (HR: 0.91; 95%CI: 0.69, 1.21) compared to women without mastitis or breast inflammation (**Table 7.2 & Figure 7.4**). The effect of mastitis or breast inflammation on duration of exclusive breastfeeding and duration of any breastfeeding did not differ by level of treatment group or nutritional supplement group. The relationship between mastitis and breast inflammation to complete cessation of breastfeeding was not modified by exclusive breastfeeding status over time.

Several sensitivity analyses were conducted (**Table 7.2**). Using a narrower, more specific definition of exposure (mastitis) for all analyses resulted in estimates that were very similar to the primary analyses. Time-varying plasma viral load results were missing for 720 women (54%). Since plasma viral is a time-varying confounder that may also be on the causal pathway for the relationship between mastitis or breast inflammation and breastfeeding status, we conducted a sensitivity analysis restricted to the 617 women with time-varying plasma viral load results. Estimates that were also

adjusted for time-varying viral load were slightly closer to the null compared to the estimates utilizing the full sample.

7.4. Discussion

In conducting this secondary analysis of data from the BAN cohort, we have an opportunity to understand breastfeeding behavior among HIV-infected, breastfeeding women where women were given uniform recommendations—to breastfeed for 24 weeks then wean between 24 and 28 weeks. Mastitis and exclusive breastfeeding are both associated with HIV transmission. To better understand the role of mastitis among HIV-infected women, we hypothesized that women with mastitis or breast inflammation would stop exclusive breastfeeding and stop breastfeeding altogether sooner than women without mastitis or breast inflammation. However, in our cohort of breastfeeding, HIV-infected women enrolled in BAN, mastitis or breast inflammation did not have an effect on the duration of exclusive breastfeeding or the duration of any breastfeeding.

Based on findings from our study, mastitis was not associated with a change in breastfeeding behavior among women enrolled in BAN, but mastitis has previously been associated with breastfeeding cessation. As many as one in four women cite mastitis as a reason for breastfeeding cessation [19, 27, 28], but prior investigations into the relationship between mastitis and the duration of breastfeeding have been conducted in resource rich locations and were designed to identify factors associated with early cessation of exclusive breastfeeding and early cessation of any breastfeeding [19, 27, 28], rather than assess the specific relationship between mastitis and exclusive

breastfeeding cessation. The relationship between mastitis and cessation of breastfeeding may differ in resource-limited settings where formula feeding is not a safe alternative.

While we investigated mastitis as a potential cause of cessation of exclusive breastfeeding, the temporality of mastitis and breastfeeding behaviors is entangled so a transition in breastfeeding behavior, from exclusive to mixed or complete cessation, may cause mastitis. When infants begin eating or drinking other foods or liquids they do not breastfeed as frequently, which may clog ducts and lead to milk stasis. Milk stasis triggers an inflammatory response (i.e., mastitis) in the breast. Women who did not exclusively breastfeed had a higher risk of breast problems and mastitis compared to untreated women who exclusively breastfed their infants in the Zambia Exclusive Breastfeeding (ZEBS) trial [23].

The relationship between treatment arm and breastfeeding status was previously investigated among women enrolled in BAN [55]. We did not observe a difference in the effect of mastitis on exclusive breastfeeding. We expected to see an effect since breastfeeding duration and mastitis both differed by treatment arm among women enrolled in BAN. Mastitis or breast inflammation was more common among women in the maternal ART and infant NVP group compared to the standard of care [Zadrozny, P1]. At 32 weeks, fewer women in the maternal ART and infant NVP group were exclusive or breastfeeding at all compared to women in the standard of care group.

There were several issues with the definition of mastitis or breast inflammation. By using a marginal structural model to estimate hazard ratios, the estimand compared a pseudo-population of women who always had mastitis or breast inflammation to

women who never did. While this contrast does not represent a realistic scenario, it is intended to represent an etiologic contrast.

The duration of exclusive breastfeeding in our cohort was longer than in other similar cohorts [87], with the exception of women enrolled in the Kesho Bora randomized controlled study. Women in the Kesho Bora RCT study [88] were counseled similarly to women enrolled in BAN—to exclusively breastfeed then rapidly wean over a 2-week period with complete cessation before infants reached 6 months old. Median duration of any breastfeeding was similar, but slightly longer among women enrolled in BAN (30 weeks, IQR: 27-31) compared to women enrolled in the Kesho Bora (21 weeks, IQR: 9-25). In the Kesho Bora trial, prophylaxis was started prenatally and continued during breastfeeding for women in the antiretroviral group, but mastitis and its relationship to breastfeeding behavior was not investigated among these women.

Women who more were more inclined to exclusively breastfeed may have also been more inclined to participate in the BAN study. This potential selection bias could contribute to the longer duration of exclusive breastfeeding compared to other studies of HIV-infected breastfeeding women. Estimates may have been affected by reporting bias since women were counseled to breastfeed exclusively for the first 24 weeks, then rapidly wean by 28 weeks. If their true breastfeeding behavior differed from the provided guidance, women may have misreported their breastfeeding status to appear compliant with the guidance they were given. This social desirability bias could have worked in two ways. Women may have reported that they stopped exclusively breastfeeding and stopped breastfeeding entirely 24-28 weeks after delivery, even if they had not stopped breastfeeding since they were counseled to cease breastfeeding entirely at that time.

Women may have reported exclusive breastfeeding at a study visit even if they had introduced solid foods, water, formula or cow's milk. Misclassification of exclusive breastfeeding after solid foods were introduced could bias our estimate toward the null.

Since women enrolled in BAN were counseled to exclusively breastfeed and attended more frequent postpartum care than most women would after delivery, generalizability to other HIV-infected, postnatal women may be limited. However, since exclusive breastfeeding has so many benefits, these aspects of BAN offer hope that education and additional care can successfully influence women to extend the duration of exclusive breastfeeding.

Table 7.1. Characteristics of HIV-infected, breastfeeding women and infants at study entry and during follow-up⁴

	Baseline (n=1337)	Person-weeks (n=39,570)
Number (%)		
Treatment Group		
Maternal antiretrovirals	439 (33)	
Infant daily nevirapine	452 (34)	
Standard of care	446 (33)	
Nutritional Supplement	670 (50)	
Some secondary education	475 (36)	
Married	1227 (92)	
Primiparous	149 (11)	
Any food insecurity ⁵	140 (10)	7516 (19.0)
Median (IQR)		
Maternal Age	26 (22 – 29)	
CD4 count	445 (329 – 590)	
log10 plasma viral load ⁵	4.2 (3.7 – 4.7)	
Hemoglobin (g/dL)	10.7 (10.0 – 11.6)	
Maternal BMI ⁶	22.9 (21.3 – 25.1)	22.8 (20.7, 24.3)
Weight for age z-score	-0.52 (-1.12 – 0.01)	-0.52 (-1.15, 0.15)
Exclusive breastfeeding duration ^{7,5}	27 (25 – 27)	33,793 (85.4%)
Any breastfeeding duration ⁵	30 (27 – 31)	

⁴ Values are number(%) or median (IQR). Numbers describe characteristics of mothers, except the weight for age z-score. Most data are baseline measurements except duration of exclusive breastfeeding, time to breastfeeding cessation and any food insecurity, which were collected over the duration of follow-up.

⁵ Missing baseline plasma viral load for n=1 woman.

⁶ Missing baseline BMI for 2 women.

⁷ 1312 women ever reported exclusively breastfeeding.

⁵ For time-varying BMI and weight for age z-score, values are median (IQR) for all person-weeks. Time-varying food insecurity is the number of person-weeks in which women ever experienced any food insecurity in the 4 weeks prior to a study visit and the proportion over all person-weeks. At baseline, exclusive breastfeeding describes the duration and IQR, but the person-week column includes the number of person-weeks and proportion of all person-weeks contributed for each category.

Figure 7.1. Patterns of breastfeeding, the transitions to cessation of exclusive breastfeeding are highlighted by red boxes

Stopped *Exclusive* Breastfeeding

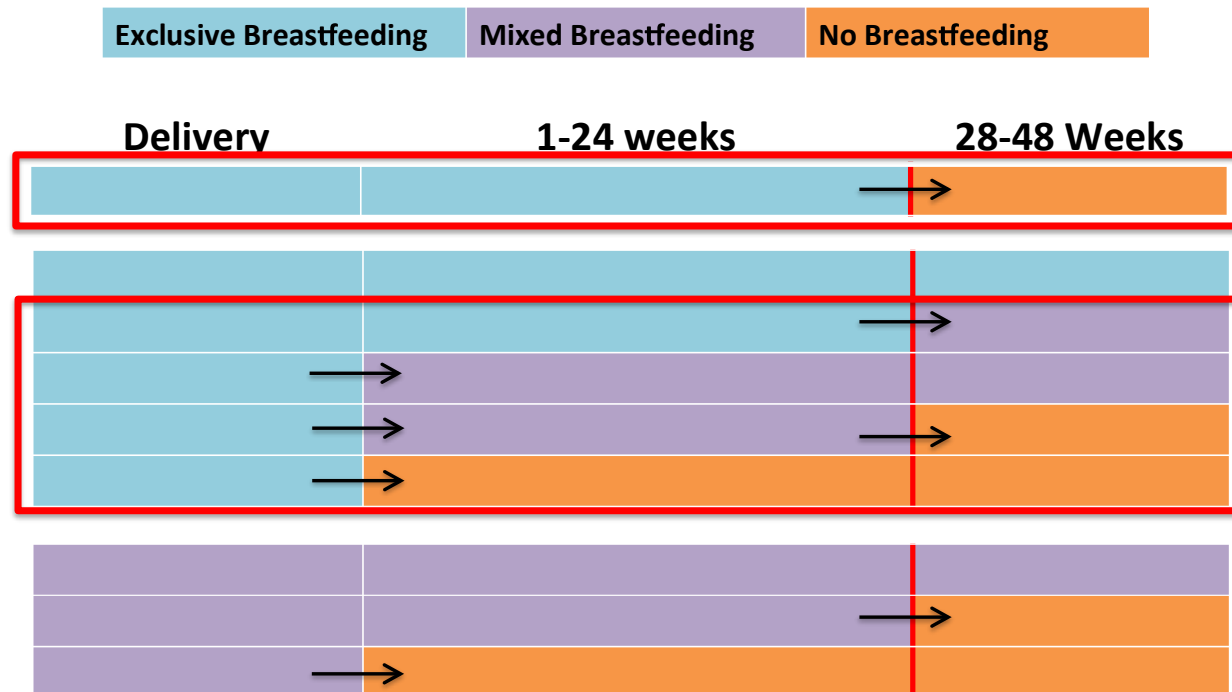


Figure 7.2. Patterns of breastfeeding, transitions to complete cessation of any breastfeeding are highlighted by red boxes

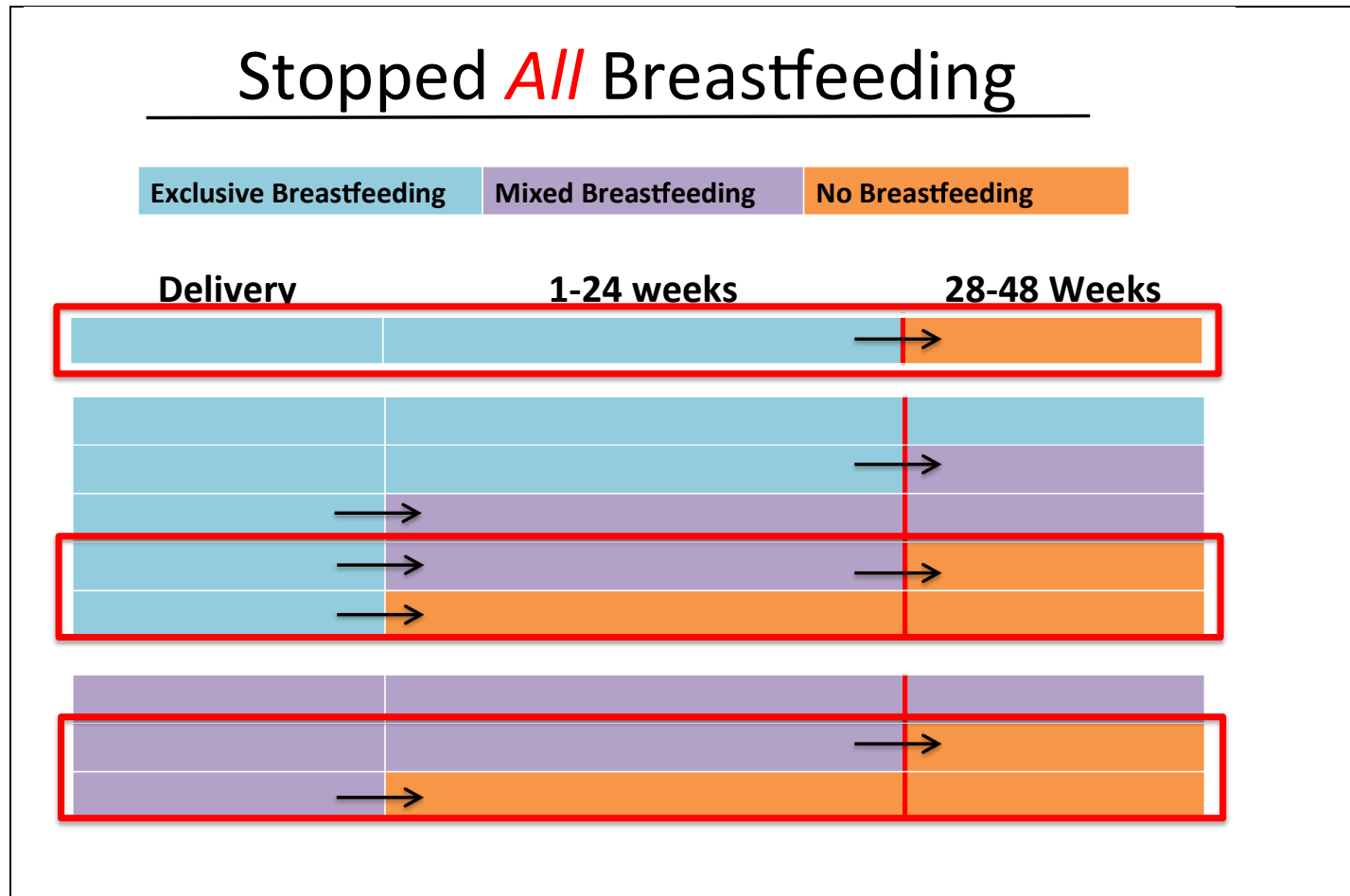
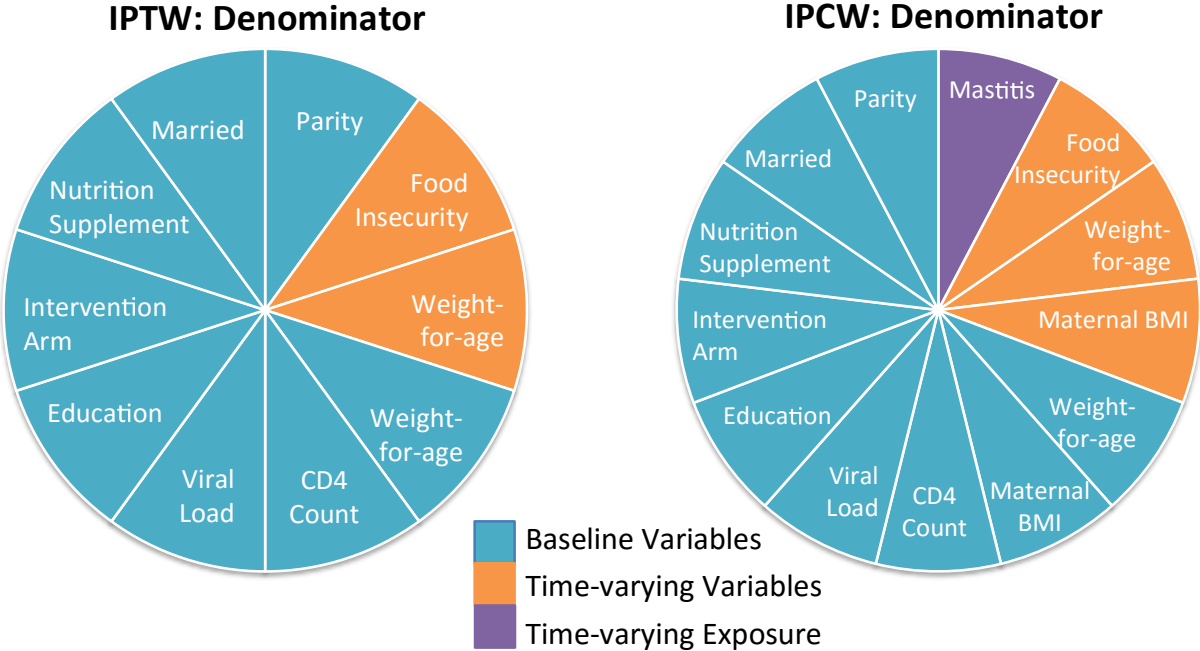


Figure 7.3. Covariates used to calculate inverse probability of treatment and censoring weights.



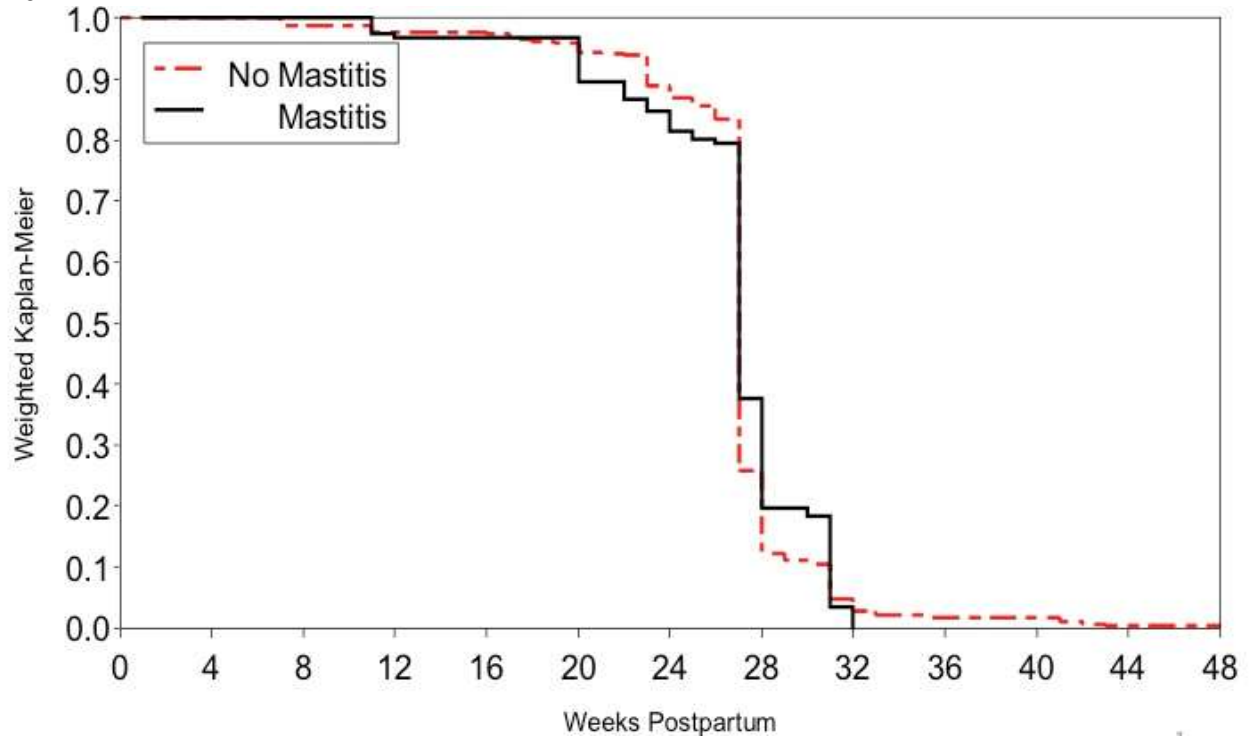
*Weights were stabilized with a restricted cubic spline for time and, for the IPCW, the time-varying exposure (Mastitis).

Table 7.2. Hazard ratios from a marginal structural cox model estimating the effect of mastitis on duration of exclusive breastfeeding and any breastfeeding among all mother-infant pairs enrolled in the BAN study in Lilongwe, Malawi between 2004-2007

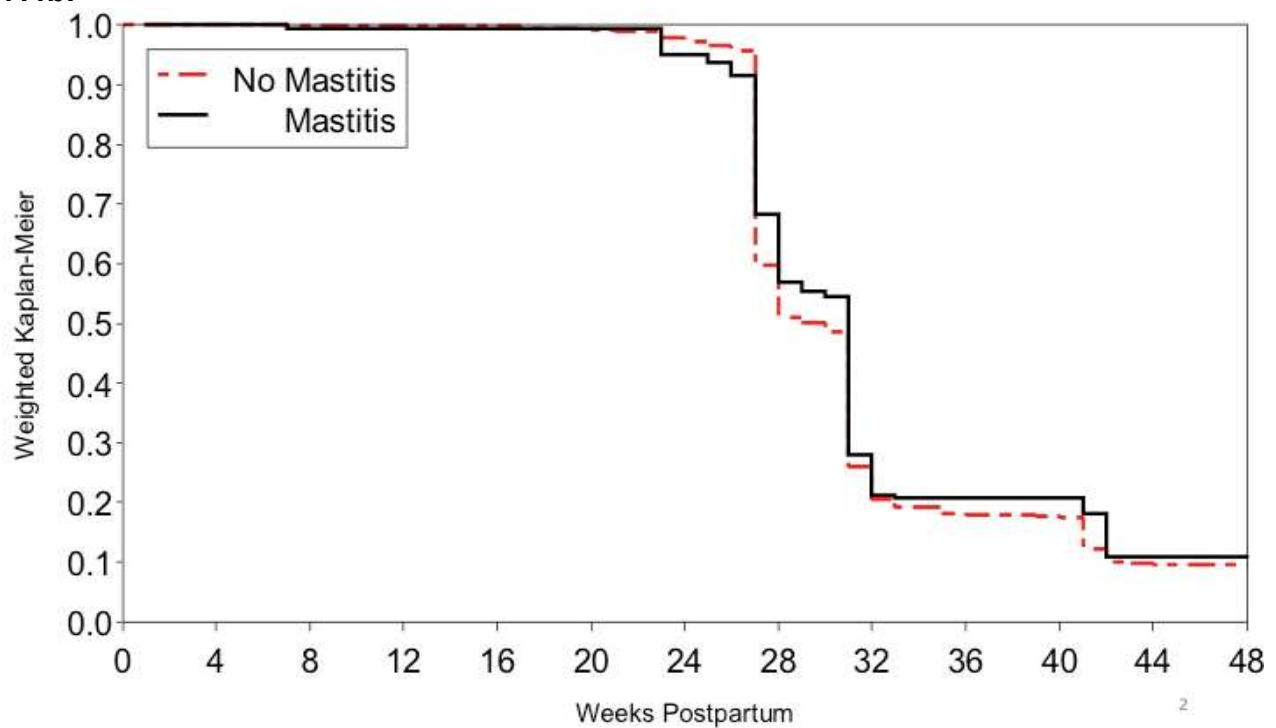
				MSM HR (95%CI)	
				Cessation of Exclusive Breastfeeding	Cessation of Breastfeeding
Primary Analysis					
Mastitis or Breast Inflammation		Yes		1.07 (0.86, 1.34)	0.91 (0.69, 1.21)
		No		1.0	1.0
Sensitivity Analyses					
Sensitive definition	Severe Mastitis	Yes		1.19 (0.93, 1.53)	0.90 (0.66, 1.23)
		No		1.0	1.0
Time-varying VL Weight	Mastitis or BI	Yes		1.08 (0.77, 1.50)	0.94 (0.61, 1.43)
		No		1.0	1.0

Figure 7.4. Weighted, adjusted Kaplan-Meier curves estimating the effect of a) mastitis or breast inflammation on time to cessation of exclusive breastfeeding; and b) mastitis or breast inflammation on time to cessation of any breastfeeding.

7.4a.



7.4b.



CHAPTER 8 – DISCUSSION

In the pre-ART era, mastitis was associated with an increased risk of vertical HIV infection through breast milk. WHO guidelines were updated in 2012 to include an option for lifelong treatment (Option B+) regardless of maternal CD4 count or stage of disease progression. The guidelines were modified to recommend initiation of lifelong highly active antiretroviral therapy (HAART) for HIV-infected pregnant women and daily prophylaxis from birth to 4-6 weeks old for their infants [68]. Implementation of these WHO treatment recommendations resulted in greater access to treatment [72] and lower plasma and breast milk viral loads [69-71].

This dissertation research was designed to explore the role of mastitis in the context of lifelong ART, among a population of breastfeeding, HIV-infected women, where mother-infant pairs were randomized to: i) maternal ART; ii) infants NVP; or iii) the standard of care in Malawi at the time, where mother-infant pairs were untreated after 2 weeks. Ours was the first study to consider whether maternal ART or infant NVP affects incidence of mastitis or breast inflammation for breastfeeding, HIV-infected women. It was also the first investigation into the relationship between mastitis and HIV transmission through breast milk in a population where some women and infants were taking antiretroviral therapy.

8.1. Summary of Findings

The objective of this work was to estimate the incidence of mastitis or breast inflammation among HIV-infected women enrolled the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study, and to examine associations with treatment group, postnatal vertical HIV transmission and duration of exclusive and any breastfeeding.

In the first aim, we estimated the incidence, severity and timing of mastitis among breastfeeding, HIV-infected women and compared mastitis incidence among women who were exposed to ART, whose infants were taking daily NVP and mother-infant pairs receiving the standard of care. We hypothesized that incidence of mastitis would be lower among women taking maternal ART compared to women whose infants were taking NVP and compared to women in the standard of care arm. The overall 28-week risk of mastitis or breast inflammation for all enrolled women was 7.5% and occurred more frequently in the first few weeks after delivery and then again when solid foods were introduced. The low overall incidence of mastitis was unexpected, but could be a function of a population of women that was mainly multiparous; had been counseled to exclusively breastfeed; and had frequent postpartum care visits. Contrary to our hypothesis, the incidence of mastitis or breast inflammation while breastfeeding was higher among women taking maternal ART and women whose infants took daily NVP compared to women in the standard of care group, where no ART was provided.

The observed higher incidence of mastitis or breast inflammation while breastfeeding in both treatment arms could be explained by several factors. One possible explanation is that women taking maternal ART may have experienced a

partial recovery of the immune system in response to treatment, explaining the difference in late mastitis (after 4 weeks) as opposed to early mastitis (in the first 4 weeks). Considering mastitis or breast inflammation among women in the infant NVP group, the elevated incidence of mastitis or breast inflammation could stem from effects of the introduction of a non-breastmilk substance (i.e., daily NVP). Even though most infants are considered to be exclusively breastfed if they are taking medication [64], it is conceivable that NVP in infant saliva could irritate the mother's nipple or affect the quality and frequency of feeding, suckling, latch or other factors that could be associated with mastitis or breast inflammation. Another possible explanation for observing a higher incidence of mastitis in treatment arms compared to the standard of care could be that the study may have suffered from ascertainment bias because participants and study staff were not blinded to the treatment arm.

For the second aim, we hypothesized that mastitis would be associated with vertical HIV transmission, and the association would be high, even in the presence of maternal ART. This was the first study to consider the relationship between mastitis and mother-to child transmission of HIV in a population in which some women were receiving treatment for HIV, which is relevant in the era of lifelong ART. For breastfeeding women enrolled in BAN, only one woman who ever had mastitis or breast inflammation transmitted HIV to her infant. Due to the low incidence of events among exposed women, the study had insufficient power to estimate effects of mastitis or breast inflammation on the risk of HIV transmission or to determine whether effects differed by treatment group.

For the third aim, we explored the complex relationship between mastitis or breast inflammation and breastfeeding. Among breastfeeding women, mastitis may be both a cause and consequence of a change in breastfeeding status. In this work, we explored breastfeeding behavior when mastitis or breast inflammation may be a possible cause of breastfeeding changes by looking at mastitis that temporally preceded a change in breastfeeding behavior. In BAN, women with mastitis were counseled to continue to express milk from the affected breast, but continue breastfeeding from the uninfected breast. We were interested in whether mastitis affected the duration of exclusive breastfeeding or the duration of any breastfeeding since nutrition from breastmilk is particularly important for HIV-exposed infants in resource-limited settings. We hypothesized that women with mastitis or breast inflammation would have a shorter duration of exclusive breastfeeding and a shorter duration of any breastfeeding compared to women without mastitis or breast inflammation, but these data did not support those hypotheses. In our cohort, mastitis or breast inflammation did not have a significant effect on the duration of exclusive breastfeeding or the duration of any breastfeeding.

8.2. Limitations

The BAN study was ideally designed to study how postpartum antiretroviral therapy may affect breastfeeding-related concerns that face HIV-infected women in resource-limited settings, which is especially relevant in the context of lifelong ART. This research makes use of an existing, already completed randomized-controlled trial.

Because this was a sub-study of a large, randomized trial, we were able to safely assume that treatment assignment arms were exchangeable at baseline. BAN also had good retention for a postpartum study in a resource-limited setting, losing only 12% at 28 weeks [48]. Despite the appropriateness of BAN for answering these questions, results may not be generalizable to populations where women receive antenatal treatment; are not counseled to exclusively breastfeeding for 24 weeks; and who are not attending frequent postpartum care visits and taking nutritional supplements.

Based on our results, breast-feeding women taking ART and women whose infants are taking antiretroviral prophylaxis have a higher risk of mastitis. The role of mastitis among breastfeeding, HIV-infected women is relevant to address for the comfort and nutrition of nursing mothers and babies. As the standard of care shifts and women have access to lifelong ART beginning in pregnancy, most women will initiate treatment during pregnancy or be taking ART when they become pregnant, unlike in BAN where women who initiated ART did so after delivery. Whether initiating ART during pregnancy affects the incidence of mastitis will have to be monitored.

Missing outcome data are unlikely to be the cause of our findings since breast exams were missing with similar frequency across treatment arms and visits. The definition of clinical mastitis that we used was based on a range of clinical signs and symptoms, but did not include fever, so it was more inclusive than definitions of clinical mastitis used in prior studies. As a consequence, our results may be subject to bias due to misclassification of mastitis or breast inflammation. We conducted sensitivity analyses with a stricter definition of mastitis (i.e., without inflammation) and while the 28-week incidence was slightly lower, the trends over time and between treatment

groups were similar. On the other end of the spectrum, even mild inflammation of the breast is associated with transmission of HIV through breast milk. In fact, asymptomatic subclinical mastitis is more prevalent and could be responsible for up to 18-21% of all mother-to-child transmission or 50% of postnatal HIV transmission [36]. Breast milk samples to measure sodium/potassium ratios, a measure of breast inflammation that is less susceptible to misclassification, were not available for these analyses.

The possible ascertainment bias by treatment groups for breast pathologies, mentioned above, may have been an additional source of bias. Participants and study staff were not blinded to the treatment arm, so nurses or physicians may have more carefully, though unintentionally, observed or asked questions about the breast health of mother-infant pairs who were randomized to either the maternal or infant treatment arm. Women in the maternal or infant treatment groups may also have more frequently reported breast pathologies or paid more attention to breast health issues compared to women without a treatment intervention. An increase in ascertainment and in increase in reporting of breast pathologies among women in treatment arms would bias the effect of treatment on mastitis or breast inflammation up and away from the null.

Women in BAN reported exclusive breastfeeding and any breastfeeding for longer periods of time compared to other cohorts of HIV-infected breastfeeding women. Since women enrolled in BAN were counseled to exclusively breastfeed and attended more frequent postpartum care than most women would after delivery, generalizability to other HIV-infected, postnatal women may be limited. However, since exclusive breastfeeding has so many benefits, these aspects of BAN offer hope that education

and additional care can successfully influence HIV-infected women to extend the duration of exclusive breastfeeding.

For our third aim, breastfeeding data may have been affected by reporting bias since women were counseled to breastfeed exclusively for the first 24 weeks, then rapidly wean by 28 weeks. If their true breastfeeding behavior differed from the provided guidance, women may have misreported their breastfeeding status to appear compliant with the guidance they were given. This social desirability bias could have worked in two ways. Women may have reported that they stopped exclusively breastfeeding and stopped breastfeeding entirely 24-28 weeks after delivery, even if they had not stopped breastfeeding since they were counseled to cease breastfeeding entirely at that time. Women may have reported exclusive breastfeeding at a study visit even if they had introduced solid foods, water, formula or cow's milk. Misclassification of exclusive breastfeeding after solid foods were introduced could bias our estimate toward the null.

Also for aim 3, we used a marginal structural model to estimate hazard ratios. The estimand compared a pseudo-population of women who always had mastitis or breast inflammation to women who never did. While this contrast does not represent a realistic scenario, it is intended to represent an etiologic contrast. Women with mastitis have a reduction in protein, lactose and fat content in breast milk during mastitis compared to women without mastitis [5]. Most women with mastitis experience a return to baseline sodium levels 2 weeks after the inflammatory episode passes, though the nutritional content of breast milk does not always return to pre-mastitis consistency and continues to contain a lower level of fat and protein for long periods after mastitis or breast inflammation. The assumption that women who are exposed are exposed for the

duration of breastfeeding is plausible if having mastitis changes the nutritional content of breast milk and a change in milk content influences feeding patterns.

8.3. Public Health Impact

This research provides insight into several questions about the role of mastitis among breastfeeding, HIV-infected women, where some were treated and some were not. Guidelines are shifting in resource-limited settings to make ART more available for treatment and prophylaxis of HIV-infected women throughout pregnancy and breastfeeding. With this shift in care, to reduce pediatric HIV, we need to focus on the causes of vertical transmission that remain in the presence of treatment, which may include mastitis.

In this study, to assess the relationship between mastitis and breastmilk HIV-transmission among women taking ART and ART-naïve women, we defined mastitis using signs and symptoms that represent a varying spectrum of breast pathologies identified during a breast exam. Using a broader definition of mastitis, including any signs of inflammation or precursors to inflammation, may be more feasible for clinicians to incorporate into care compared to confirmatory diagnosis by a lab in resource-limited settings. A diagnosis based on clinical signs and symptoms would allow diagnosis of less severe, though clinically relevant, cases of mastitis. If mastitis continues to be a risk factor for vertical HIV transmission in the era of lifelong ART, counseling to prevent mastitis paired with identification of a wide range of clinical breast pathologies that might indicate inflammation could reduce the risk mastitis.

The World Health Organization recommended HIV-infected, breastfeeding women with mastitis or breast inflammation stop breastfeeding, though continue to express milk, from the affected breast until there are no further clinical signs or symptoms of mastitis or breast inflammation. While the incidence of mastitis and postpartum HIV transmission were low, our study suggests that the current recommendations for HIV-infected women with mastitis are effective as long as women are taking maternal ART or infants are taking daily NVP while breastfeeding. As long as women and clinicians identify mastitis and follow the recommendations, mastitis does not appear to interfere with the effectiveness of ART in reducing MTCT of HIV.

8.4. Future Directions

The role of mastitis among HIV-infected, breastfeeding women is an important question that can benefit from research in several directions. One of our primary objectives in initiating this work was to compare the risk of HIV transmission through breast milk among women with and without mastitis. However, the low incidence of mastitis or breast inflammation resulted in insufficient power and limited our ability to estimate differences in risk of HIV transmission through breastmilk comparing women with and without mastitis or breast inflammation. If standardized data about clinical breast pathologies could be collected and pooled in countries implementing lifelong ART, this question becomes feasible to answer.

With effective postnatal HIV therapy [69-71] and better treatment coverage for HIV-infected, pregnant women [47, 72], many more women will have access to

treatment. In this environment, HIV transmission risk in the postnatal period will be lower. Subsequent studies would need to be approached differently to understand whether mastitis affects HIV transmission risk among HIV-infected women who are breastfeeding and taking antiretroviral therapy. For example, additional studies addressing the relationship between mastitis and postpartum HIV transmission would require a larger sample of women. One way to construct a larger sample would be to use administrative data. Alternate study designs, like case-control or case-cohort studies, might be more appropriate to ensure sufficient cases, though could make the question of temporality more difficult to disentangle.

The mechanism responsible for the association between mastitis and postpartum vertical HIV transmission is poorly understood and the temporality of the association is also unclear. Are women with high plasma or breast milk HIV viral load more susceptible to mastitis? Or, do women with mastitis experience an upregulation or influx of virus in breast milk? In this study, we also explored the effects of maternal or infant treatment on exclusive breastfeeding. Another biological question that arose was how the nutritional and fat content of breast milk changes when women take ART. Clarity on the biological mechanisms and temporality of events are needed to untangle the complex relationships between mastitis, maternal antiretroviral therapy, breast milk viral load, breastfeeding behavior and breast milk transmission of HIV.

8.5. Conclusions

Mastitis is a source of discomfort and pain for women postpartum, in a time of extreme adjustment. Although the relationship between mastitis and vertical HIV transmission was well established in the pre-ART era, the recommendation for lifelong ART necessitates that the role of mastitis should be explored among breastfeeding, HIV-infected women who are treated with maternal ART. While the incidence of mastitis or breast inflammation among HIV-infected women was low in our sample, the incidence of mastitis is likely higher among a population that does not have access to regular care and treatment, as provided to women enrolled in the BAN study. In the context of lifelong ART, in addition to continuing to explore the role of mastitis in HIV transmission through breastmilk, the role of mastitis among HIV-infected individuals remains important because the current WHO guidelines recommend women stop breastfeeding from the affected breast if they have mastitis. Given the numerous benefits of exclusive breastfeeding, if overall incidence of mastitis is reduced and mastitis is not associated with HIV transmission if breastfeeding HIV-infected women are taking ART, then it may be important to re-evaluate the recommendation to change or interrupt breastfeeding behavior if HIV-infected women experience mastitis while breastfeeding.

APPENDIX I – PRIOR DEFINITIONS OF MASTITIS

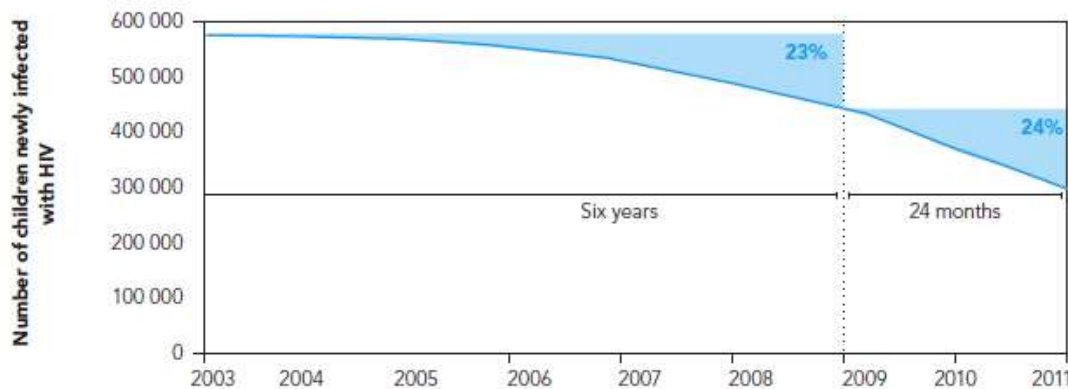
TYPE OF DIAGNOSIS	DEFINITIONS	NOTES
Clinical		
Mastitis: History or Physical Exam	At least two of the following signs or symptoms were present: breast erythema (redness), increased breast tension not relieved by breastfeeding, maternal fever, pain in the breast and lumps in the breast tissue [138-140].	This definition was used as the gold standard and compared to concentrations of bacteria in breast milk. Cronbach's alpha score on contact day three was 0.79 [4,10]. Scores were added to create a Severity Index (SI), range 0 (least severe) to 19.
	If part of the breast was swollen and hard, with inflammation of the overlying skin; mother complains of severe pain and fever and feels ill [141, 142].	
	At least two breast symptoms (pain, redness, lump) and at least one of fever or 'flu-like symptoms [43].	
	A breast with one or many resistances, localized redness, swelling, pain and, in addition, had influenza-like symptoms with or without fever [143].	
	Fever, malaise, tender, reddened breast, and antibiotics prescribed by the physician [42].	
	A physician's diagnosis of mastitis in the medical record, but the diagnostic criteria, signs and symptoms were not specified [11, 144, 145].	

TYPE OF DIAGNOSIS	DEFINITIONS	NOTES
Breast Health Problem: History or Physical Exam	Breast disease (cracked nipples, bleeding nipples, mastitis, or breast abscess) was assessed by history and physical examination at every maternal postpartum visit, and breast milk samples were obtained from breast-feeding mothers at 3-month intervals [37].	Joint outcome
	Breast health problems included: painful nipple, cracked nipple, bleeding nipple, engorgement, blocked milk duct, breast thrush, nipple oozing pus, breast oozing pus, and mastitis/abscess [141, 142].	Women who had not exclusively breast-fed their infants had more breast health problems than were women who had exclusively breast-fed (time dependent variable; adjusted odds ratio, 1.46; 95% CI, 1.13–1.87; P=0.003).
	A breast complication was defined as the presence of any of the following: engorgement, red/shiny breast, sore/swollen nipples, blocked duct, cracked/bleeding nipples, white patches/Candida, or current pain [23].	
Laboratory Definitions		
SubClinical Mastitis: Laboratory Testing	Elevated Na ⁺ at concentrations of >12 mmol/liter [5, 10, 11, 38].	Na ⁺ concentration is > 3 standard deviations above the mean for normal human milk at 1 month of lactation, determined by ion-selective electrodes, fat-free samples.[5, 38]
	<u>Na⁺/K⁺ Ratio:</u> Continuous variable, log-transformed [20] Na/K ≤ 0.6, Normal; [7, 20] 0.6 < Na/K ≤ 1.0, Mildly raised; [8, 20, 146] Na/K > 1.0, Severely raised [11, 20]	The geometric mean milk Na/K ratio and the proportion of women with Na/K Ratio > 1.0 in one or both breasts were significantly higher among HIV-infected than among uninfected women.[146]
	Milk leukocyte counts ≥1 million cells/ml [11, 12, 46]	

APPENDIX II – BREAST MILK HIV TRANSMISSION

Postnatal transmission during breastfeeding accounts for 1/3 to 1/2 of all infant HIV cases. New pediatric HIV infections have decreased 43% between 2003 and 2011. In 2011, approximately 330,000 children were newly infected with HIV; 90% of these children lived in sub-Saharan Africa [89].

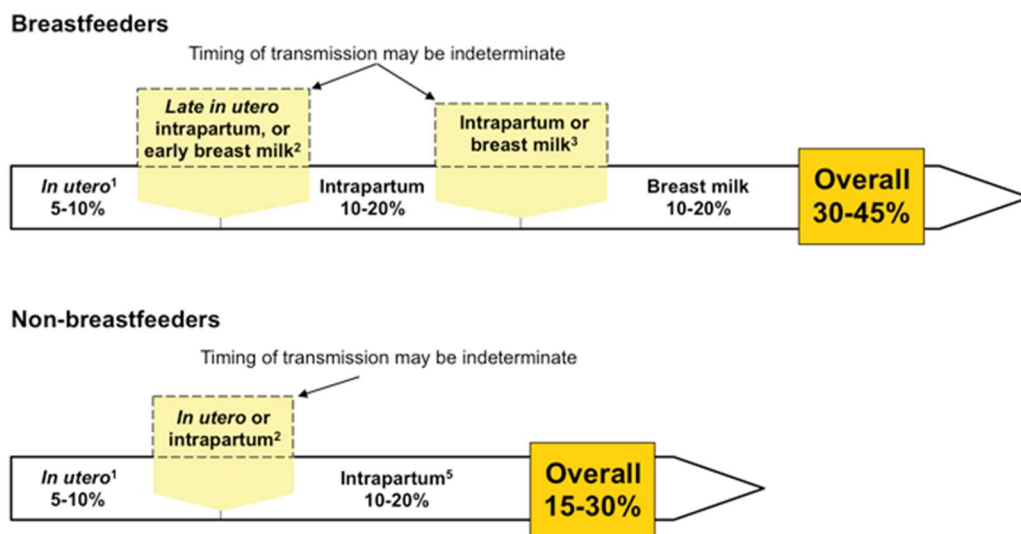
Figure A.1. New HIV infections among children 0-14 years old, between 2003-2011[90]



In the developed world, rates of perinatal HIV transmission have decreased to around 2% among HIV-infected women, which is due in part to the availability of maternal antiretroviral prophylaxis (ARV) during pregnancy, cesarean delivery and using formula instead of breastfeeding [91-94]. However, cesarean delivery and replacement feeding with formula are not cost-effective, sustainable or safe options for HIV-infected mothers in resource limited settings. As a result, the rate of vertical HIV transmission in sub-Saharan Africa is 10 to 15 times higher. In resource-limited settings where

breastfeeding is recommended, the risk of an HIV-infected woman transmitting HIV to her infant is 30-45% without treatment [95]. Postnatal transmission during breastfeeding accounts for 1/3 to 1/2 of all infant HIV cases, which is an overall risk of up to 20%, depending on the duration of breastfeeding [89, 96, 97]. Approximately 26% of children who acquire HIV through breastfeeding will die within one year of transmission [98].

Figure A.2. Timing and absolute rates for mother-to-child HIV-1 transmission among breastfeeders and non-breastfeeders [96]



¹ Detection of HIV-1 RNA or DNA in infant blood <48 hours after delivery diagnostic of in utero transmission;

² Detection of HIV-1 RNA or DNA in infant blood 48 hours—14 days after birth consistent with in utero, intrapartum or early breast milk transmission;

³ Detection of HIV-1 RNA or DNA in infant blood 14 days—1 month after birth most consistent with intrapartum or breast milk transmission [13];

⁴ Infants with negative HIV-1 RNA assays at 1 month of age who subsequently have positive assays most likely to be infected via breast milk;

⁵ Infants with negative HIV-1 RNA assays at 14 days of age who subsequently have positive assays most likely to be infected intrapartum

The World Health Organization recommends that HIV-infected women should breastfeed for up to one year despite the risk of HIV transmission through breast milk. An unsafe water supply prevents use of replacement formula feeding in

resource-limited settings and formula should not be used unless it is acceptable, feasible, affordable, sustainable and safe (AFASS) [77]. While breastfeeding extends the risk period where a mother may transmit HIV to her child, it also provides infants with better immune development, as well as reduced morbidity and mortality for infants born to HIV-infected women in resource-limited countries. Breastfeeding is associated with reduced risk of malnutrition, infant morbidity and mortality, compared to formula feeding in resource-limited countries [3, 73-76]. For these HIV-exposed infants, breastfeeding is a necessary source of nutrition. Where safe water is available, balancing cultural norms and nutritional needs with the prevention of HIV transmission from mother to infant remains an issue. Considering the cultural, logistic and health situation in resource-limited settings, the World Health Organization (WHO) guidelines on HIV and infant feeding [77] encourage HIV-infected women to breast-feed for at least 12 months in conjunction with an extended medication regimen [77, 78].

Infant factors such as low birth weight, preterm birth and a compromised gastro-intestinal barrier are related to vertical HIV transmission through breast milk. Both infant and maternal factors contribute to an increased risk for postnatal vertical HIV transmission. Infant factors that are associated with a higher transmission risk include low birth weight, preterm birth [37] and a compromised gastro-intestinal barrier related to mixed feeding or illness. Some infant factors may be difficult to prevent, like low birth weight and preterm birth, but others may be easier to prevent by not abruptly weaning and prolonging introduction of solid foods or non-breast milk liquids. Infants who are born early or with a low birth weight may be more susceptible to postnatal HIV transmission since they are likely to have a less well-developed immune

system compared to infants born at term. Abrupt weaning or beginning mixed breast feeding in the first six months of life is another factor that may lead infants to be more susceptible to HIV infection [99, 100]. In Zambia and Malawi, early abrupt weaning was detrimental to HIV-exposed but uninfected infants, resulting in higher transmission rates and infant mortality [3, 74]. If weaning (either abruptly or too soon, i.e., before 6 months) or mixed breastfeeding before 6 months occurs prior to the establishment of sufficient immune functioning, the mucosal barrier in the gastrointestinal tract of infants may deteriorate. In Zimbabwe, rates of postnatal HIV transmission were 5.1, 6.7, and 10.5 infections per 100 child-years of breast-feeding for infants exclusively breastfed, predominantly breast-fed (feeding breast milk and other nonmilk liquids), and mixed breastfed, respectively [82]. A damaged mucosal barrier in the intestines of infants creates an environment that facilitates replication of the HIV virus and is associated with higher risk of HIV-infection for infants [3, 101]. When mothers decide to stop breastfeeding, WHO guidelines recommend a slow introduction to mixed feeding (i.e., food or drink that is not breast milk) by a gradual cessation of breastfeeding over a 1 month period, which will prevent the breakdown of the mucosal barrier [77].

Maternal factors including plasma viral load, breast-milk viral load, disease progression, breastfeeding duration, mastitis, treatment availability and adherence are related to vertical HIV transmission through breast milk. Maternal factors that are associated with an increased risk of vertical transmission include maternal plasma viral load, maternal breast-milk viral load, overall health of the mother (i.e, disease progression), breastfeeding duration [37], mastitis,[37] treatment availability and adherence. Most maternal factors associated with vertical HIV transmission through

breast milk are related to having a high plasma viral load or high breast milk viral load [102-106]. An increase in maternal viral load may result from advanced HIV disease, undetected sero-conversion during pregnancy [107], limited treatment availability, poor adherence to treatment or mastitis. Risk of HIV transmission can be reduced by managing plasma viral load and potentially, though this is not as clear, breast milk viral load, with ARV treatment, though treatment will not eliminate all risk of HIV transmission. The mechanisms for postnatal HIV transmission among women taking treatment are different than treatment naïve women and may include treatment adherence and mastitis.

The risk of postnatal HIV transmission from mother to child through breast milk is reduced if either the mother or infant is taking ART for the duration of breastfeeding. In several studies, women were treated for an extended perinatal period to reduce the risk of transmission during pregnancy and delivery, but in October, 2000, the post-exposure infant prophylaxis study in South Africa began to explore treatment for infants for an extended period of time (6 weeks) during breastfeeding [108]. In July, 2003, the Kisumu Breastfeeding Study was the first to enroll patients into a trial to explore extended prophylaxis for mothers during breastfeeding (6 months) [106]. While there were many other studies and they varied in the type (i.e., single dose NVP or multiple drug regimens), target (i.e., mother or infant) and duration of treatment, each has provided support for reducing risk of postnatal HIV transmission by either maternal or infant prophylaxis for the duration of breastfeeding. Postnatal maternal treatment is important to reduce the risk of transmission since ART effectively manages disease progression by reducing viral load, which is associated with a reduced risk of vertical

transmission through breast milk [69-71]. For women who initiated treatment during or after delivery (i.e., they did not initiate treatment during antenatal care), infant prophylaxis is preferred since maternal viral load may not be managed by treatment by the time breastfeeding begins [48, 109]. While maternal prophylaxis is effective at reducing viral load and the risk of HIV transmission, it does not completely eliminate the risk of postnatal transmission, so for women taking ART, when viral load is low, vertical HIV transmission may still occur. In addition, infant exposure to treatment through breastmilk or direct treatment may result in the development of drug resistance or adverse events related to drug toxicity. Both maternal and infant prophylaxis is associated with the development of ARV drug resistance among infants who become HIV-infected [110-116]. While infant prophylaxis effectively reduces the risk of HIV transmission, drug toxicities associated with prophylaxis for infants may also be harmful, but the frequency and severity of those adverse events are unclear. Despite the potential for HIV transmission, adverse events related to drug toxicity and developing drug resistance, the WHO recommends breastfeeding for 12 months with an extended treatment regimen for the duration of breastfeeding in resource-limited settings due to the benefits of breastfeeding for reducing infant mortality and the questionable sanitation of water sources for replacement formula feeding.

The World Health Organization recommends several treatment options for HIV-infected breastfeeding mothers to reduce HIV transmission through breast milk. In 2010, the WHO recommended lifelong treatment to pregnant women who met the criteria for treatment and also offered two short-term treatment options (Option A and Option B) for pregnant women who were ineligible for treatment at the time [77].

With the release of the programmatic update to the WHO guidelines for the use of antiretroviral drugs [78], more women in resource-limited settings will have access to ART while breastfeeding (**Table 1**). The WHO programmatic update offers three options for PMTCT programs. All recommend treatment or prophylaxis for women during pregnancy, regardless of CD4 count and for all infants for at least 4-6 weeks of life. Option A is reliant on an initial CD4 count to determine whether pregnant women should initiate ART. For Option B and Option B+, women initiate ART regardless of their CD4 count. The newest, Option B+, is more inclusive than the previous two options. It includes a recommendation to start pregnant women who are HIV-infected on triple ARV's continued for life and daily prophylaxis for infants from birth to 4-6 weeks old. Option B+ offers many logistic and health benefits including alignment with the launch of the *Treatment 2.0* initiative, which is a simplification of treatment regimens in order to improve access, optimize treatment and reduce supply issues [78, 117, 118]. Option B+ also offers HIV-negative partners of HIV-infected women protection from transmission [119] and the lifelong ART option protects against MTCT during pregnancy and delivery for future children. In addition, starting women on a simple, consistent regimen over the course of their life will reduce the viral load and health flux resulting from treatment interruption and resumption [120, 121].

Table A1.1. World Health Organization recommendations for reducing HIV transmission through breast milk.

Woman receives:			
	Treatment (for CD4 count ≤350 cells/mm3)	Prophylaxis (for CD4 count >350 cells/mm3)	Infant receives:
Option A	Triple ARVs starting when diagnosed, <i>continued for life</i>	<i>Antepartum:</i> AZT starting as early as 14 weeks gestation <i>Intrapartum:</i> at onset of labour, sdNVP and first dose of AZT/3TC <i>Postpartum:</i> daily AZT/3TC through 7 days postpartum	Daily NVP from birth through 1 week beyond complete cessation of breastfeeding; or, if not breastfeeding or if mother is on treatment, through age 4–6 weeks
Option B	<i>Same initial ARVs for both^b:</i> Triple ARVs starting when diagnosed, <i>continued for life</i>	Triple ARVs starting as early as 14 weeks gestation and continued intrapartum and through childbirth if not breastfeeding or until 1 week after cessation of all breastfeeding	Daily NVP or AZT from birth through age 4–6 weeks regardless of infant feeding method
Option B+	<i>Same for treatment and prophylaxis^b</i> Regardless of CD4 count, triple ARVs starting as soon as diagnosed, ^c <i>continued for life</i>		Daily NVP or AZT from birth through age 4–6 weeks regardless of infant feeding method

Adapted from the World Health Organization [78]

Note: “Triple ARVs” refers to the use of one of the recommended 3-drug fully suppressive treatment options.

^a Recommended in WHO 2010 PMTCT guidelines

^b True only for EFV-based first-line ART; NVP-based ART not recommended for prophylaxis (CD4 >350)

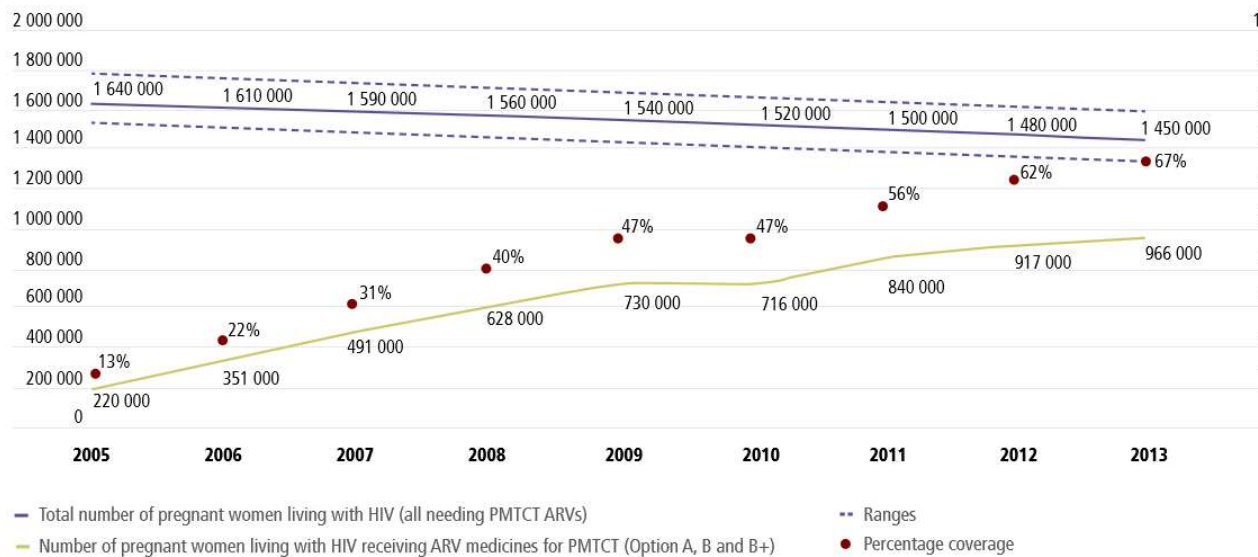
^c Formal recommendations for Option B+ have not been made, but presumably ART would start at diagnosis.

Treatment availability has been a barrier to reducing postnatal HIV

transmission risk. For women known to be HIV-positive during pregnancy, necessary treatment to prevent vertical HIV transmission has not been available. While perinatal antiretroviral treatment and prophylaxis is recommended to prevent MTCT of HIV, in Malawi in 2007, only 30% of pregnant women living with HIV received antiretrovirals [122]. In 2010, only 50% of HIV-positive pregnant women in sub-Saharan Africa received adequate perinatal treatment to prevent mother-to-child transmission during pregnancy [123]. Since the WHO guidelines only recently changed in 2012 to recommend breastfeeding through 12 months with extended treatment regimens for the duration of breastfeeding, coverage data for women receiving postnatal treatment is limited. The goal is that simplification of treatment regimens (*Treatment 2.0*) and the change in the recent WHO guidelines may also improve access and availability of treatment to pregnant and breastfeeding women. If these programs are successful and more women have access to treatment, the focus of preventing vertical HIV transmission will need to shift to a focus on reducing causes of transmission among women taking treatment.

Figure A.3. Number of pregnant women living with HIV in low & middle-income countries and the number and percentage of those women receiving ARV drugs for PMTCT of HIV, 2005-2013

Fig. 3.2. Number of pregnant women living with HIV in low- and middle-income countries and the number and percentage of those women receiving ARV drugs for PMTCT of HIV, 2005–2013



Single-dose nevirapine is included in the data for 2005 to 2009.

Sources: Global AIDS Response Progress Reporting (WHO/UNICEF/UNAIDS) and validation process for the number of pregnant women living with HIV receiving ARV drugs for PMTCT, and UNAIDS 2013 estimates for the number of pregnant women living with HIV.

Making lifelong treatment available to all HIV-infected women who are pregnant or breastfeeding (Option B+) will change HIV policies and priorities. In July 2011, the Ministry of Health in Malawi implemented Option B+ and began to offer lifelong ART to HIV infected women who were pregnant or breastfeeding, regardless of CD4 count and/or clinical staging [72, 124]. Women with early HIV infection immediately receive treatment for themselves as well as for prevention of mother to child transmission. The availability of lifelong treatment for all pregnant or breastfeeding women through Option B+ offers the possibility of a dramatic reduction in the incidence

of HIV among children [69]. Already, implementation of this program has led to a 6-fold increase in the number of pregnant and breastfeeding HIV-infected women starting ART in Malawi [72]. With better treatment availability, resources for reducing postnatal vertical HIV transmission will need to be re-allocated to focus on the factors associated with transmission that are not managed by medication. For women who have access to treatment, adherence to ART and mastitis are the suspected factors associated with the remaining vertical transmission through breast milk. Understanding the role of maternal factors, like mastitis and adherence as well as infant factors, like initiation of mixed breastfeeding, becomes more important in reducing transmission risk.

APPENDIX III – MASTITIS & HIV TRANSMISSION

Study	Year	Location	Mastitis Prevalence	Result	Notes
Semba, et al. Human Immunodeficiency Virus Load in Breast Milk, Mastitis, and Mother-to-Child Transmission of Human Immunodeficiency Virus Type 1	1999	Blantyre, Malawi	16.4%	<p>Women with elevated Na⁺ concentrations consistent with mastitis had fewer CD41 lymphocytes and higher plasma HIV-1 load, breast milk HIV-1 load, breast milk lactoferrin level, and rates of mother-to-child transmission of HIV-1 at 6 weeks and 12 months compared with women with normal breastmilk Na⁺ concentrations.</p> <p>Elevated Na⁺ concentration was associated with elevated risk of mother-to-child transmission of HIV-1 by 6 weeks in a multivariate logistic regression model including maternal plasma VL and maternal CD4 count (OR=2.38; 1.26–4.42, p<0.0008) and 12 months of age (2.31;1.23–4.26, p<.01).</p>	<p>Observational study. Authors did not estimate the total effect of mastitis on transmission; instead they estimated the independent association of breast milk viral load and mastitis on transmission. No way to distinguish perinatal transmission with postnatal transmission. Relied only on elevated sodium concentrations, rather than clinical symptoms for diagnosis. Used “multivariate” logistic regression.</p>

Study	Year	Location	Mastitis Prevalence	Result	Notes
John GC, et al. Correlates of Mother-to-Child Human Immunodeficiency Virus Type 1 (HIV-1) Transmission: Association with Maternal Plasma HIV-1 RNA Load, Genital HIV-1 DNA Shedding, and Breast Infections	2001	Nairobi, Kenya	11%	<p>Breast-feeding (OR, 1.7; 95% CI, 1.0–2.9) and mastitis (relative risk [RR], 3.9; 95% CI, 1.2–12.7) were associated with increased transmission overall.</p> <p>Mastitis (RR, 21.8; 95% CI, 2.3–211.0) and breast abscess (RR, 51.6; 95% CI, 4.7–571.0) were associated with late transmission (occurring 12 months postpartum).</p>	Definition of mastitis was vague. Authors adjusted for viral load, which is on the causal pathway, in the model to determine if mastitis was associated with HIV transmission. Used breast infections as a time-dependent covariate in a case-control study with cumulative incidence sampling to estimate a RR. Authors only adjusted for plasma VL, despite using a different exposure variable than was randomized. Ran multivariate analysis.
Willumsen, et al. Breastmilk RNA viral load in HIV-infected South African women: effects of subclinical mastitis and infant feeding	2003	South Africa	Subclinical Mastitis: 23.8%, 19.0%, and 22.9% at weeks 1, 6 and 14.	A raised milk Na ⁺ /K ⁺ ratio was significantly associated with raised milk RNA viral load at 1 week (RD=0.237;0.034, 0.439), 6 weeks (RD=0.329;0.124, 0.534) and 14 weeks (RD=0.252;0.071, 0.433).	Small sample size. Outcome was breastmilk viral load, not HIV transmission since the sample size was too small. Longitudinal study with three time points and breast milk samples. They used linear regression.

Study	Year	Location	Mastitis Prevalence	Result	Notes
Gomo, et al. Subclinical mastitis among HIV-infected and uninfected Zimbabwean women participating in a multimicronutrient supplementation trial	2003	Zimbabwe		The odds ratio for mild or severe subclinical mastitis was 2.82 (95% CI 0.96-8.26, P= 0.07) in the HIV-infected women comparing women not taking micronutrient supplements to those who did.	Outcome: Mastitis, maternal HIV is an exposure. Participants were selected based on whether they had sufficient milk samples before 3 months, though authors discussed differences btw those selected and not. Sampling unclear. Case-control study with cumulative incidence sampling, not randomly based on the exposure distribution in the original study. Adjusted for height, weight and gestational age in the estimate of the effect of micronutrients on mastitis. Authors conclude that elevated Na/K ratios are associated with low infant weight gain, but might be that infants aren't eating enough, leading to milk stasis, a common cause of mastitis.

Study	Year	Location	Mastitis Prevalence	Result	Notes
Bland, et al. Breast Health Problems Are Rare in Both HIV-Infected and HIV-Uninfected Women Who Receive Counseling and Support for Breast-Feeding in South Africa	2007	KwaZulu Natal, South Africa	Mastitis was rare: 0.5-1%, but other breast infections were slightly more frequent—up to 3.5%.	<p>Women who had not exclusively breast-fed their infants were more likely to experience any of the breast health problems than were women who had exclusively breast-fed their infants (time-dependent variable; adjusted odds ratio, 1.46; 95% confidence interval, 1.13–1.87; $P=0.003$)</p> <p>HIV-infected women who experienced any serious breast health problem (i.e., bleeding nipple, pus oozing from a nipple or breast, or mastitis/abscess) were 3.55 times (95% confidence interval, 0.86–14.78 times; $P=.08$) more likely to transmit HIV postnatally to their infant.</p>	Mastitis is an exposure, though was evaluated as an outcome (where exclusive breastfeeding was the exposure).
Lunney, et al. Associations between Breast Milk Viral Load, Mastitis, Exclusive Breast-Feeding, and Postnatal Transmission of HIV	2010	Zimbabwe ZVITAMB O	29.1%, 18.4%, and 12.7%, at week 6 and months 3 and 6 resp	<p>Mastitis was associated with breast milk HIV load, and this effect increased with increasing maternal plasma HIV load.</p> <p>Mastitis was associated with postnatal transmission only when maternal plasma HIV load was high (13.7 log₁₀ copies/mL).</p>	

APPENDIX IV – ANTIRETROVIRAL & BREAST MILK HIV TRANSMISSION

Study & Location(s)	Years	Postpartum ARV**	ARV Duration	Mother-to-Child Transmission (MTCT) Rate and Efficacy
PETRA trial South Africa, Tanzania, Uganda [125]	Jun 1996 to Jan 2000	AP/IP/PP ZDV + 3TC vs IP/PP ZDV + 3TC vs IP only ZDV + 3TC vs placebo	1 week mother and infant	MTCT was 5.7% at 6 weeks for AP/IP/PP ZDV + 3TC, 8.9% for IP/PP ZDV + 3TC, 14.2% for IP-only ZDV + 3TC, and 15.3% for placebo (efficacy compared with placebo: 63%, 42%, and 0%, respectively). MTCT was 14.9% at 18 months for AP/IP/PP ZDV + 3TC, 18.1% for IP/PP ZDV + 3TC, 20.0% for IP-only ZDV + 3TC, and 22.2% for placebo (efficacy compared with placebo: 34%, 18%, and 0%, respectively).
HIVNET 012 trial Uganda† [126]	Nov 1997 to Apr 1999	sdNVP vs ZDV	Infant only, 72 hours (sdNVP) vs. 1 week (ZDV)	MTCT was 11.8% in NVP arm vs. 20.0% in ZDV arm at 6–8 weeks (42% efficacy); 15.7% in NVP arm vs. 25.8% in ZDV arm at 18 months (41% efficacy).
SAINT trial South Africa† [127]	May 1999 to Feb 2000	sdNVP within 48 hours of birth, mother and infant vs. ZDV + 3TC (1 week), mother and infant	Mother and infant, 48 hours (sdNVP) vs 1 week (ZDV+3TC)	MTCT was 12.3% in sdNVP arm vs. 9.3% in ZDV + 3TC arm at 8 weeks (difference not statistically significant, P = 0.11).
DITRAME Plus (ANRS 1201.0) Ivory Coast [128]	Sept 1995 to July 2003	sdNVP + ZDV	Infant only, 1 week	MTCT was 6.5% (95% CI, 3.9%–9.1%) at 6 weeks; MTCT for historical control group receiving short ZDV (98% breastfed) was 12.8%.
NVAZ trial Malawi†† [129]	Apr 2000 to Jan 2002	Neonatal sdNVP vs. sdNVP + ZDV	sdNVP with or without ZDV for 1 week, infant only	MTCT was 15.3% in sdNVP + ZDV arm and 20.9% in sdNVP-only arm at 6–8 weeks. MTCT rate at 6–8 weeks among infants who were HIV uninfected at birth was 7.7% and 12.1%, respectively (36% efficacy)

Study & Location(s)	Years	Postpartum ARV**	ARV Duration	Mother-to-Child Transmission (MTCT) Rate and Efficacy
Postnatal NVP + ZDV Malawi† [130]	April 2000, to Mar 2003	Neonatal sdNVP vs. sdNVP + ZDV	sdNVP with or without ZDV for 1 week, infant only	MTCT was 16.3% in NVP + ZDV arm and 14.1% in sdNVP only arm at 6–8 weeks (difference not statistically significant). MTCT rate at 6–8 weeks among infants who were HIV uninfected at birth was 6.5% and 16.9%, respectively.
Post-exposure Infant Prophylaxis South Africa†‡[108]	Oct 2000 to Sept 2002	Neonatal sdNVP vs. ZDV for 6 weeks	sdNVP vs. ZDV for 6 weeks	For breastfed infants only, MTCT was 12.2% in sdNVP arm and 19.6% in ZDV arm (P = 0.03).
Mashi Botswana [131, 132]	Mar 2001 to Oct 2003	Initial: short-course ZDV with/without maternal and infant sdNVP and with/ without breastfeeding Revised: shortcourse ZDV + infant sdNVP with/without maternal sdNVP and with/ without breastfeeding; women with CD4 Tlymphocyte (CD4-cell) counts <200 cells/mm ³ receive combination therapy	2 nd randomization Breastfeeding + ZDV (infant) 6 months + sdNVP, infant only vs. Formula feeding + ZDV (infant) 4 weeks + sdNVP, infant only	Initial design: In formulafeeding arm, MTCT at 1 month was 2.4% in maternal and infant sdNVP arm and 8.3% in placebo arm (P = 0.05). In breastfeeding + infant ZDV arm, MTCT at 1 month was 8.4% in sdNVP arm and 4.1% in placebo arm (difference not statistically significant). Revised design: MTCT at 1 month was 4.3% in maternal + infant sdNVP arm and 3.7% in maternal placebo + infant sdNVP arm (no significant difference; no interaction with mode of infant feeding). MTCT at 7 months was 9.1% in breastfeeding + ZDV arm and 5.6% in formula feeding arm; mortality at 7 months was 4.9% in breastfeeding + ZDV arm vs. 9.3% in formula-feeding arm; HIV-free survival at 18 months was 15.6% breastfeeding + ZDV arm vs. 14.2% formulafeeding arm.

Study & Location(s)	Years	Postpartum ARV**	ARV Duration	Mother-to-Child Transmission (MTCT) Rate and Efficacy
SWEN Uganda, Ethiopia, India† [133]	Ethiopia: Feb 2001 - Oct 2006 India: Aug 2002– Mar 2007 Uganda: Jul 2004– Jan 2007	sdNVP vs. NVP for 6 weeks	Infant sdNVP vs. NVP 6 weeks	Postnatal infection in infants uninfected at birth: MTCT at 6 weeks was 5.3% in sdNVP arm vs. 2.5% in extended NVP arm (risk ratio 0.54, P = 0.009). MTCT at 6 months was 9.0% in sdNVP arm vs. 6.9% in extended NVP arm (risk ratio 0.80, P = 0.16). HIV-free survival was significantly lower in extended NVP arm at both 6 weeks and 6 months of age.
PEPI-Malawi Trial Malawi† [134]	Apr 2004 to Aug 2007	sdNVP + ZDV for 1 week (control) vs. two extended infant regimens (NVP or NVP/ZDV) for 14 weeks	Infant sdNVP + ZDV for 1 week (control) vs. control + NVP for 14 weeks vs. control + NVP/ZDV for 14 Weeks	Postnatal infection in infants uninfected at birth: MTCT at age 6 weeks was 5.1% in control vs. 1.7% in extended NVP (67% efficacy) and 1.6% in extended NVP/ZDV arms (69% efficacy). MTCT at age 9 months was 10.6% in control vs. 5.2% in extended NVP (51% efficacy) and 6.4% in extended NVP/ZDV arms (40% efficacy). No significant difference in MTCT between the extended prophylaxis arms; however, more hematologic toxicity with NVP/ZDV.
MITRA Tanzania [135]	Aug 2001 to Aug 2003	Infant 3TC for 6 months (observational)	Maternal ZDV/3TC for 1 week; infant 3TC for 6 months	MTCT at age 6 months was 4.9% (postnatal MTCT between ages 6 weeks and 6 months was 1.2%).
Kisumu Breastfeeding Study (KiBS) Kenya [106]	July 2003 and Nov 2006	Maternal triple-drug prophylaxis (observational)	Maternal ZDV/3TC/NVP (NFV if CD4-cell count >250 cells/mm ³) for 6 months; infant sdNVP	MTCT at age 6 months was 5.0% (postnatal MTCT between ages 7 days and 6 months was 2.6%).
MITRA-PLUS Tanzania [136]	April 2004 to June 2006	Maternal triple-drug prophylaxis (observational)	Maternal ZDV/3TC/NVP (NFV if CD4-cell count >200 cells/mm ³) for 6 months; infant ZDV/3TC for 1 week	MTCT at age 6 months was 5.0% (postnatal MTCT between ages 6 weeks and 6 months was 0.9%), not significantly different from 6 months infant prophylaxis in MITRA

Study & Location(s)	Years	Postpartum ARV**	ARV Duration	Mother-to-Child Transmission (MTCT) Rate and Efficacy
Kesho Bora Multi-African [88]	Jun 2005 to Aug 2008	Arm 1: Maternal ZDV/3TC/LPV/r for 6 months; infant sdNVP + ZDV for 1 week Arm 2: Maternal ZDV/3TC for 1 week; infant sdNVP + ZDV for 1 week	6 months (mom) and 1 week (infant) vs 1 week (mom and infant)	MTCT at birth was 1.8% with maternal triple-drug prophylaxis Arm 1 and 2.5% with ZDV/sdNVP Arm 2, not significantly different. In women with CD4-cell counts 350–500 cells/mm ³ , MTCT at birth was 1.7% in both arms. MTCT at age 12 months was 5.4% with maternal triple-drug prophylaxis Arm 1 and 9.5% with ZDV/sdNVP (with no further postnatal prophylaxis after 1 week) Arm 2 (P = 0.029).
Mma Bana Botswana[137]	July 2006 and May 2008	Maternal triple-drug prophylaxis (compares 2 regimens) in women with CD4-cell counts >200 cells/mm ³	Arm 1: Maternal ZDV/3TC/ABC for 6 months; infant sdNVP + ZDV for 4 weeks Arm 2: Maternal ZDV/3TC/LPV/r for 6 months; infant sdNVP + ZDV for 4 weeks	MTCT at age 6 months overall was 1.3%: 2.1% in ZDV/3TC/ABC Arm 1 and 0.4% in ZDV/3TC/LPV/r Arm 2 (P = 0.53).
BAN Malawi† [48, 55]	Apr 2004 to Jan 2010	Postpartum maternal tripledrug prophylaxis vs. infant NVP in women with CD4-cell counts ≥250 cells/mm	Arm 1 (control): Maternal DV/3TC for 1 week; infant sdNVP + DV/3TC for 1 week Arm 2: Control, then maternal ZDV/3TC/LPV/r for 6 months Arm 3: Control, then infant NVP for 6 months	Postnatal infection in infants uninfected at age 2 weeks: MTCT at age 28 weeks was 5.7% in control Arm 1; 2.9% in maternal triple-drug prophylaxis Arm 2 (P = 0.009 vs. control); 1.7% in infant NVP Arm 3 (P <0.001 vs. control). MTCT at age 48 weeks was 7.0% in control Arm 1; 4% in maternal triple-drug prophylaxis Arm 2 (P = 0.0273 vs. control); 4% in infant NVP Arm 3 (P = 0.0027 vs. control). No significant difference between maternal triple-drug prophylaxis Arm 2 and infant NVP Arm 3 (P = 0.12 at 28 weeks and P = 0.426 at 48 weeks).

Study & Location(s)	Years	Postpartum ARV**	ARV Duration	Mother-to-Child Transmission (MTCT) Rate and Efficacy
HPTN 046 South Africa, Tanzania, Uganda, Zimbabwe [116]	Jun 2008, to Mar 2010	Postpartum prophylaxis with 6 weeks vs. 6 months of infant NVP	All infants received daily NVP until 6 weeks. Arm 1: Daily infant NVP from 6 weeks to 6 months Arm 2: Daily infant placebo from 6 weeks to 6 months	<p>In infants uninfected at age 6 weeks, the 6-month infant HIV infection rate was 1.1% (0.3%–1.8%) in the extended NVP Arm 1 and 2.4% (1.3%–3.6%) in the placebo Arm 2 (P = 0.048).</p> <p>At infant randomization at age 6 weeks, 29% of mothers in each arm were receiving a triple-drug ARV regimen for treatment of HIV.</p> <p>For mothers receiving tripledrug ARV regimens at the time of randomization, in infants uninfected at age 6 weeks, the 6-month infant HIV infection rate was 0.2% and not statistically different between extended NVP Arm 1 (0.5%) and placebo Arm 2 (0%).</p> <p>For mothers with CD4-cell counts >350 cells/mm³ who were not receiving triple-drug ARV regimens, in infants uninfected at age 6 weeks, the 6-month infant HIV infection rate was 0.7% (0%–1.5%) in the extended NVP Arm 1 and 2.8% (1.3%–4.4%) in the placebo Arm 2 (P = 0.014).</p>
<p>* This table has been modified from the table 3 of the US Perinatal Guidelines, 2012 and was restricted to studies where infants were breastfed.</p> <p>** In all of these studies except where noted, women were also given antenatal and intrapartum ARV.</p> <p>† No antepartum ARV</p> <p>‡ No intrapartum treatment</p> <p>Key to Abbreviations: 3TC = lamivudine, ABC = abacavir, AP = antepartum, ARV = antiretroviral, CDC = Centers for Disease Control and Prevention, CI = confidence interval, IP = intrapartum, IV = intravenous, LPV/r = lopinavir/ritonavir, MTCT = mother-to-child transmission, NFV = nelfinavir, NVP = nevirapine, PP = postpartum, sd = single-dose, ZDV = zidovudine</p>				

APPENDIX V – DEFINING MASTITIS IN THE BAN STUDY

Mastitis or Breast Inflammation

A diagnosis of mastitis from an adverse event report; OR

An affirmative answer to one of the following questions for either breast, after the clinician evaluates or palpates the mother's breast during the breast exam:

- | | |
|------|-----------------------------------------------------------------|
| BE4 | Are there any cracks on tip of nipple? |
| BE5 | Is there any bleeding/dried blood on nipple? |
| BE6 | Is there nipple rash? |
| BE7 | Is there nipple exudate? |
| BE9 | Are breasts discolored or shiny?* |
| BE10 | Are there any open or oozing sores on breast? |
| BE11 | Are there any open or oozing sores on areola and adjacent area? |
| BE14 | Does breast feel hard?* |
| BE15 | Does breast feel lumpy?* |
| BE16 | Are lumps tender?* |
| BE17 | Does breast feel hot?* |
| BE18 | Does exam cause pain?* |
| BE19 | Are axilla nodes tender?* |

Breastfeeding Cessation

Breastfeeding cessation was indicated if women provided the answer that follows to each of these questions

- | | |
|------|------------------------------------------------------------------------|
| IFQ1 | "Did you breastfeed the baby since last visit?" (0=No) |
| IFQ3 | Since the last visit, have you <u>tried</u> to stop breastfeeding your |
| 8 | baby?" |
| | (3=No, stopped before last visit) |

And women consistently indicated that they were not breastfeeding by providing the indicated answer to any of the following questions:

- | | |
|------|-------------------------------------------------------------------------------------------------------------|
| IFQ1 | Did you breastfeed the baby since last visit?" (0=No) |
| IFQ4 | Does your baby breastfeed at night?" (0=No) |
| IFQ7 | Since the last visit, did your <i>baby</i> receive anything to drink or eat other than breast milk?" (0=No) |

* Indicates items used to define severe mastitis

APPENDIX VI – DEFINING EXCLUSIVE BREASTFEEDING IN THE BAN STUDY

Exclusive Breastfeeding

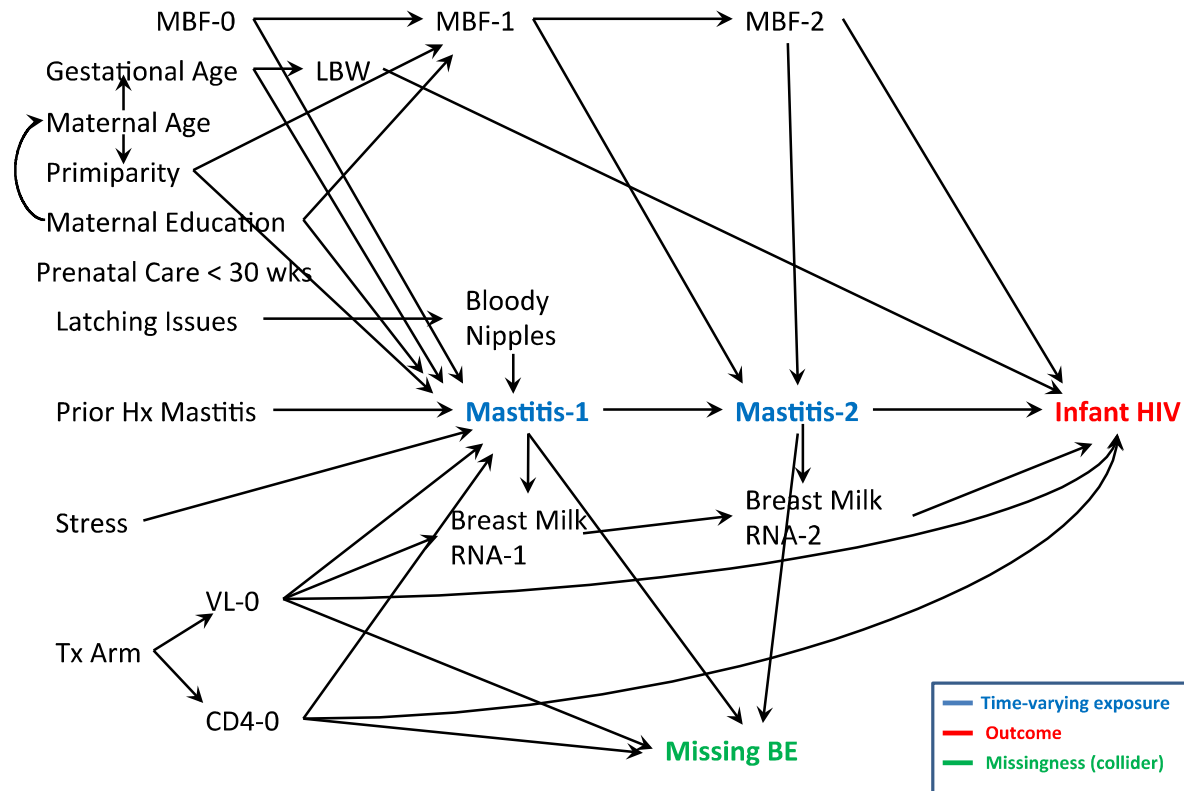
If women are not mixed feeding and have not ceased breastfeeding, determined by the specified answer to one of the questions.

- IFQ1 Did you breastfeed the baby since last visit?" (1=Yes)
IFQ4 Does your baby breastfeed at night. (1=Yes)
IFQ3 Since the last visit, have you tried to stop breastfeeding your
8 baby? (2=No, mother still breastfeeding)

AND

- IFQ7 Since the last visit, did your *baby* receive anything to drink or eat other than breast milk? (0=No)
-

APPENDIX VII – DIRECTED ACYCLIC GRAPH (DAG)



Infant HIV = transmission of human immunodeficiency virus (HIV) from mother to child through breastfeeding
 Tx Arm = treatment assignment arm for the breastfeeding antiretroviral and nutrition study (BAN),
 VL-# = baseline HIV RNA viral load (log10 copies per mL) in plasma,
 CD4-# = number of CD4 cells per cubic millimeter of blood,
 Breast milk RNA-# = HIV RNA viral load (log10 copies per mL) in breast milk at each visit after delivery,
 Hx = History, LBW = low birth weight, MBF = mixed breast feeding status at each visit, BE = breast exam form

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