

IMPLEMENTATION OF PEDIATRIC HIV PREVENTION AND CARE GUIDELINES IN
ROUTINE SETTINGS: OUTCOMES AMONG HIV-EXPOSED INFANTS IN KINSHASA,
DEMOCRATIC REPUBLIC OF CONGO (2007-2013)

Lydia Feinstein

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Approved by:

Frieda Behets

Benjamin H. Chi

Stephen R. Cole

Andrew Edmonds

Annelies Van Rie

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ABSTRACT

Lydia Feinstein: Implementation of Pediatric HIV Prevention and Care Guidelines in Routine Settings: Outcomes Among HIV-exposed Infants in Kinshasa, Democratic Republic of Congo (2007-2013)
(Under the direction of Frieda Behets)

Outcomes of HIV-exposed infants remain inadequately studied, particularly in resource-deprived settings. We conducted an observational study of 1707 mother-infant pairs who received care in a comprehensive HIV program in Kinshasa, DR Congo during 2007-2013. The study resulted in two manuscripts, one which describes temporal changes in the outcomes of HIV-exposed infants and one that assesses the relationship between infant retention in care and the provision of combination antiretroviral therapy (cART) to their HIV-infected mothers.

The first manuscript suggests there have been encouraging improvements over time in the outcomes of HIV-exposed infants but that continued efforts are needed. Accounting for competing risks (e.g. death), we estimated the cumulative incidences of having an initial specimen collected for HIV virologic testing, loss to follow-up (LTFU), HIV transmission, and death through age 18 months, as well as cART initiation among HIV-infected infants through age 24 months. The 18-month cumulative incidence of specimen collection increased from 73% (95% confidence limit [CL]: 68-78%) for infants enrolled in 2007-2008 to 99% (95% CL: 98-100%) for infants enrolled in 2011-2012. The 18-month cumulative incidence of HIV declined from 15% (95% CL: 11-21%) for infants enrolled in 2007-2008 to 8% (95% CL: 6-11%) for infants enrolled in 2011-2012 and death declined from 8% (95% CL: 5-11%) to 3% (95% CL: 2-5%). The 18-month cumulative incidence of LTFU did not improve, with 18-month cumulative

incidences of 18% (95% CL: 14-22%) for infants enrolled in 2007-2008 and 18% (95% CL: 15-21%) for infants enrolled in 2011-2012. Among HIV-infected infants, the 24-month cumulative incidence of cART increased from 61% (95% CL: 43-75%) to 97% (95% CL: 82-100%); the median age at cART decreased from 17.9 to 9.3 months.

In the second manuscript, we show that increasing access to cART for pregnant women could improve retention in care of their HIV-exposed infants. The 18-month cumulative incidence of LTFU was 9% among infants whose mothers had initiated cART by infant enrollment and 19% among infants whose mothers had not yet initiated cART (Gray's *p*-value <0.001). Adjusted for baseline factors, the subdistribution hazard ratio comparing LTFU between the two groups was 2.8 (95% CL: 1.8-4.3).

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral
AZT	Zidovudine
cART	Combination Antiretroviral Therapy
CL	Confidence Limit
DAG	Directed Acyclic Graph
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of Congo
EID	Early Infant Diagnosis
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IQR	Interquartile Range
LTFU	Loss to Follow-up
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PMTCT	Prevention of Mother-to-Child HIV Transmission
RNA	Ribonucleic Acid
TB	Tuberculosis
UNC	University of North Carolina at Chapel Hill
WHO	World Health Organization

CHAPTER 1: SPECIFIC AIMS

Globally, an estimated 1.4 million infants are born each year to women living with human immunodeficiency virus (HIV) [1]. Although the incidence of vertical HIV transmission is declining [1], there remain substantial gaps in the literature on outcomes of HIV-exposed infants and drivers of those outcomes, particularly in routine care settings. The overall goal of this project was to assess implementation of HIV-exposed infant care and key outcomes in Kinshasa, Democratic Republic of the Congo (DRC). We conducted a cohort study of routinely collected clinical data on approximately 1,700 infants who received care between January 2007 and August 2013 in a comprehensive HIV prevention, care and treatment program affiliated with the University of North Carolina (UNC-DRC program). There were two specific aims to the project.

Specific Aim 1: Describe temporal changes in the outcomes of HIV-exposed infants since the implementation of HIV-exposed infant care in Kinshasa.

Guidelines for prevention of mother-to-child transmission of HIV (PMTCT) have developed rapidly, yet little is known about how outcomes of HIV-exposed infants have changed over time. Accounting for competing risks, we estimated the cumulative incidences of early infant HIV diagnosis (EID), HIV transmission, death, loss to follow-up (LTFU), and combination antiretroviral therapy (cART) initiation for infants enrolled during three calendar periods (2007-2008, 2009-2010, and 2011-2012).

Specific Aim 2: Quantify the effect of providing cART to HIV-infected mothers on LTFU of their HIV-exposed infants.

LTFU of HIV-exposed infants is a common problem in most PMTCT programs, with some studies reporting over 70% LTFU [2–11]. LTFU reduces the clinical- and population-level impacts of PMTCT programs [12], opportunities for EID, and the likelihood of early treatment initiation among HIV-infected infants. The literature suggests that receiving cART improves retention among HIV-infected adults [13,14]; as such, we hypothesized that infants whose mothers receive cART experience less LTFU than infants whose mothers do not receive cART.

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CHAPTER 2: INTRODUCTION

Section 2.1. State of the pediatric HIV epidemic

Over 35 million people were estimated to be living with HIV in 2012, with approximately 2.3 million new infections occurring each year [1]. More than three million of the individuals living with HIV are children under 15 years of age [2]. Over 90% of pediatric infections result from mother-to-child transmission during pregnancy, labor, or breastfeeding [3]. Although PMTCT programs have been scaled-up worldwide and the incidence of vertical transmission is declining, an estimated 260,000 children continue to be infected with HIV each year [1]. Pediatric HIV has virtually been eliminated in developed countries. Sub-Saharan Africa, where 90% of all pediatric HIV infections occur, carries a disproportionate burden of the ongoing epidemic [1].

Section 2.2. Challenges faced by HIV-exposed infants and their families

Although there has been a reduction in the size of the pediatric HIV epidemic, a decrease in the number of infants exposed to HIV during pregnancy, labor, and breastfeeding has not occurred. In 2012, 1.3 million infants were born to HIV-infected mothers [4]. In some countries, HIV-exposed infants may account for up to 30% of all births [5]. As access to interventions such as cART is increasing and individuals living with HIV are experiencing healthier and longer lives, the population of HIV-exposed infants will likely grow [6,7].

Regardless of whether they become infected with HIV, HIV-exposed infants face unique challenges compared to HIV-unexposed infants due to the social, economic, and health impacts HIV has on families [4]. Illness within the family may lead to or worsen already existing income loss, food insecurity, and lower educational attainment [4]. HIV-infected women also have an increased rate of adverse birth outcomes, such as preterm birth and low birth weight [8,9]. Furthermore, HIV-exposed infants often experience the death of one or more care-givers, as evidenced by the world's 18 million children who are estimated to have been orphaned due to HIV/AIDS [10].

Moreover, HIV-exposed infants, even if they remain uninfected with HIV, experience increased morbidity and mortality early in life. The elevated morbidity and mortality appears to be in large part driven by the high burden of infectious disease that has been observed in this population [11–15]. The reasons for the increased infectious disease burden remain unclear. Some evidence suggests that HIV-exposure impairs the immune system of exposed infants, increasing their susceptibility to infectious agents [16]. Others point to the potentially increased frequency of exposure to infectious agents that may occur in families affected by HIV, as well as to the potential effect exposure to antiretroviral therapy may have on immune function.

Section 2.3. HIV-exposed infant care in the Democratic Republic of Congo

The HIV epidemic in the DRC is particularly unique. The DRC is an extremely resource-deprived setting with one of the highest burdens of maternal and child mortality in the world [17]. Scale-up of HIV-exposed infant care in the DRC has been extremely challenging due to the aftermath of decades of civil war and a deteriorating socioeconomic infrastructure. Although the estimated two percent prevalence of HIV among women seeking antenatal care in

the capitol city of Kinshasa [18] is relatively low compared to other sub-Saharan African settings, the most current estimate available suggests that fewer than 20% of HIV-infected women in the country currently have access to PMTCT services. Although it is likely that access to PMTCT services has increased in recent years [19], with a population of almost 10 million people in Kinshasa, there is a high unmet need for quality HIV-exposed infant care.

Section 2.4. Importance of early infant diagnosis

Compared to adults, disease progression in HIV-infected children is extremely rapid. If they are not started on cART, one-third of infected infants will die within the first year of life and half will die within the first two years of life [20–23]. As early diagnosis is a prerequisite for early treatment, the World Health Organization (WHO) recommends that HIV-exposed infants be tested for HIV within the first four to six weeks after birth [23]. However, fewer than 15% of exposed infants are currently tested for HIV in the first two months of life [24,25], suggesting that more guidance is needed on how to operationalize current testing recommendations.

EID of HIV-exposed infants by virological testing represented a major paradigm shift and has only been routinely recommended in resource-constrained settings during the past few years [26,27]. Before the availability of virological testing, the HIV infection status of exposed infants could only be confirmed by serology at 18-months of age [28,29]. The implementation of virological testing has allowed children infected with HIV to be identified as early as six weeks of age and immediately started on treatment [24,26]. However, the implementation of DNA PCR testing has presented new challenges, as it means that HIV-exposed infants need to be linked into care earlier than ever before.

Section 2.5. Early infant diagnosis as a care continuum

Although many vertically exposed infants are identified in the antenatal care and delivery setting through testing of HIV-infected pregnant women and new mothers, follow-up of infants and their mothers is poor [30–37]. Exposed infants may be referred for testing, but many mothers do not bring their infants for testing until they show signs or symptoms of illness and many never bring them at all. Several structural barriers to testing have been identified, including cost, distance to testing centers, limited availability of testing sites, waiting time at the clinic, and negative experiences with providers [35,38–40]. Such structural barriers, in conjunction with fatalistic attitudes towards pediatric HIV and fear of stigma and discrimination [41], have contributed to the low numbers of exposed infants being retained in care.

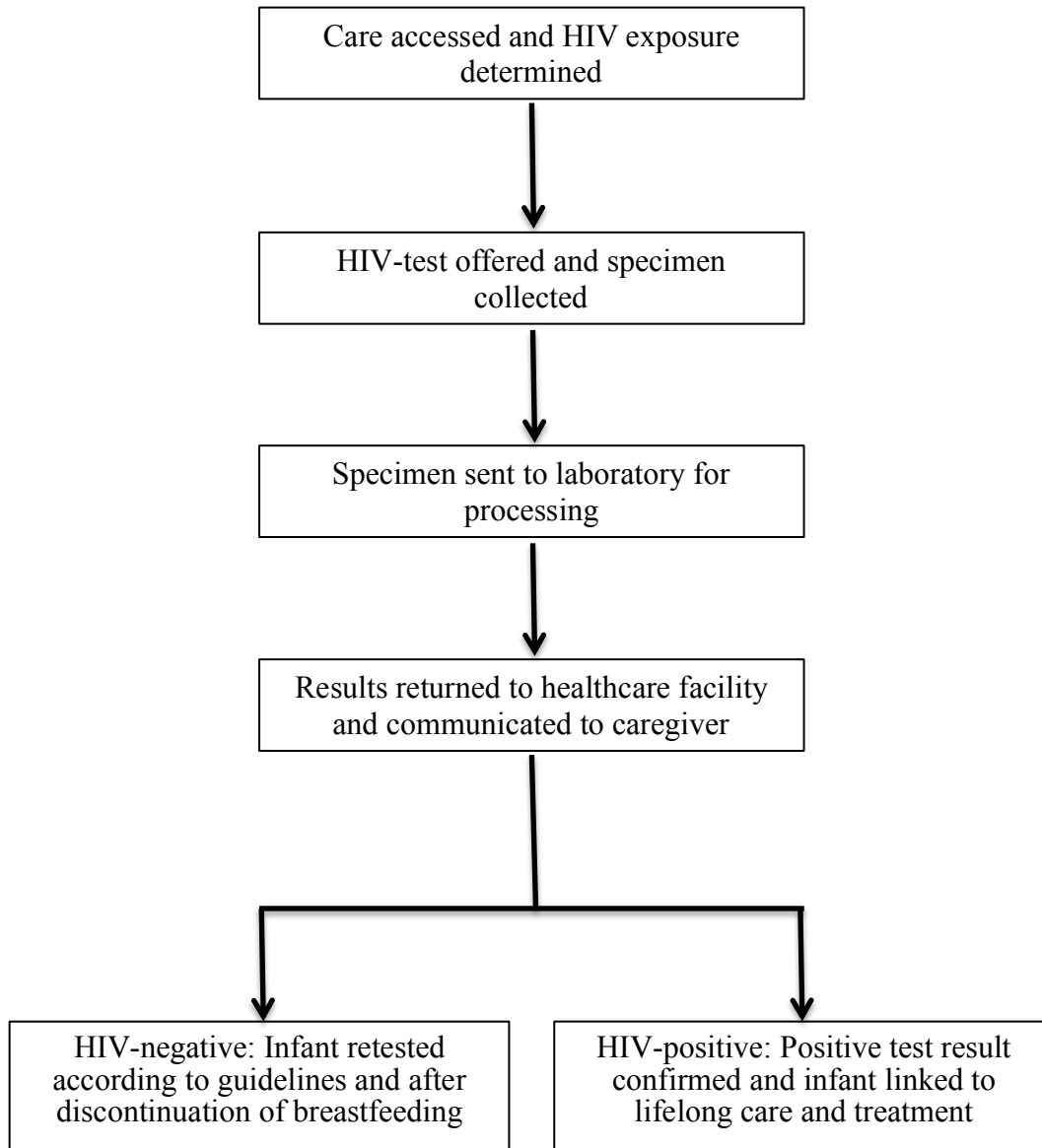
EID is further complicated by the type of assays available for accurate testing of vertically exposed infants. While rapid point-of-care tests to detect HIV antibodies are widely available for older children and adults, they are unreliable for use among HIV-exposed infants because maternal HIV antibodies can persist up to 18 months of age [24,28,29]. Antibody-based tests therefore only confirm HIV exposure in infants and accurate diagnosis in this population requires virological testing. Deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) assays are the most widely available virological test for EID, but few PMTCT programs have laboratory capacity to process the assays onsite [42]. As such, blood samples are often collected and dried on filter paper (dried blood spots) so that they can be sent for testing at an external laboratory [42,43]. Turnaround time for specimen processing takes days in ideal conditions, but realistically may take months in field settings [42]. A new generation of point-of-care infant HIV tests have been evaluated, but are yet to be available in the field.

Thus, there are currently multiple steps involved in diagnosing HIV in exposed infants. As previously summarized by Ciaranello et al., the EID cascade includes: “infant presentation to care, test offer by healthcare professionals, test acceptance by parents/caregivers, specimen processing, result return to healthcare facilities and [result communicated to] parents/caregivers...” [42]. Each of these steps represents a potential loss point in the implementation of EID, with complete implementation depending on multiple structural- and individual-level factors.

Visual depictions of the EID cascade, one of which is shown in Figure 2.1, are useful in conceptualizing EID as a process. Figure 2.1 builds on the cascade published by Ciaranello et al. [42], except that it also includes the important requirement that infants must first access care before they can receive EID. Unlike the cascade by Ciaranello et al., Figure 2.1 also points out the need to reconfirm positive HIV test results, as discussed in the following paragraph.

As the last section in Figure 2.1 alludes to, complete EID generally requires more than a single test due to continued exposure to HIV through breastfeeding after the first test. According to the WHO guidelines, there are three major HIV testing events that are supposed to occur as part of the EID process for children without signs or symptoms suggestive of HIV. These include a first DNA PCR at four to six weeks of age, a serological test around nine months of age followed by a second DNA PCR test if seropositive, and a final serology at 18 months of age or three months after discontinuation from breastfeeding (whichever comes later) [24]. Confirmatory DNA PCR testing is recommended for infants who test positive by DNA PCR due to the possibility of obtaining a false-positive result [24,44]. Additional testing by serology is recommended for infants with signs or symptoms suggestive of HIV, followed by a DNA PCR test if seropositive [24].

Figure 2.1 - Early infant diagnosis cascade.



The above figure builds on the cascade presented by Ciaranello et al. [45].

Continued exposure to HIV after delivery poses a substantial risk, with up to a third of transmissions occurring through breastfeeding [46,47]. However, the benefits of breastfeeding for preventing early childhood morbidity and mortality are also substantial, particularly in resource-deprived settings where safe formula feeding is often problematic due to unstable supplies and a lack of clean water [48–50]. Accordingly, the WHO recommends that HIV-infected mothers in low- and middle- income countries breastfeed through at least 12-months of age, and discourages weaning for the purpose of obtaining an HIV diagnosis [23,24]. As the HIV testing algorithm for exposed infants is dependent on their weaning status, regular follow-up is needed to assess their exposure through breastfeeding.

Finally, additional visits need to occur during the EID process to provide prophylactic regimens such as cotrimoxazole and extended NVP, monitor the child’s health, and to provide other services (e.g. vaccinations). Since it can take a month or longer to obtain the results of a DNA PCR test in resource-constrained settings and it is often unknown ahead of time when exactly the result will be available at the clinic, follow-up visits also provide crucial opportunities to communicate infant HIV test results to caregivers and to initiate cART among infants who are HIV-positive.

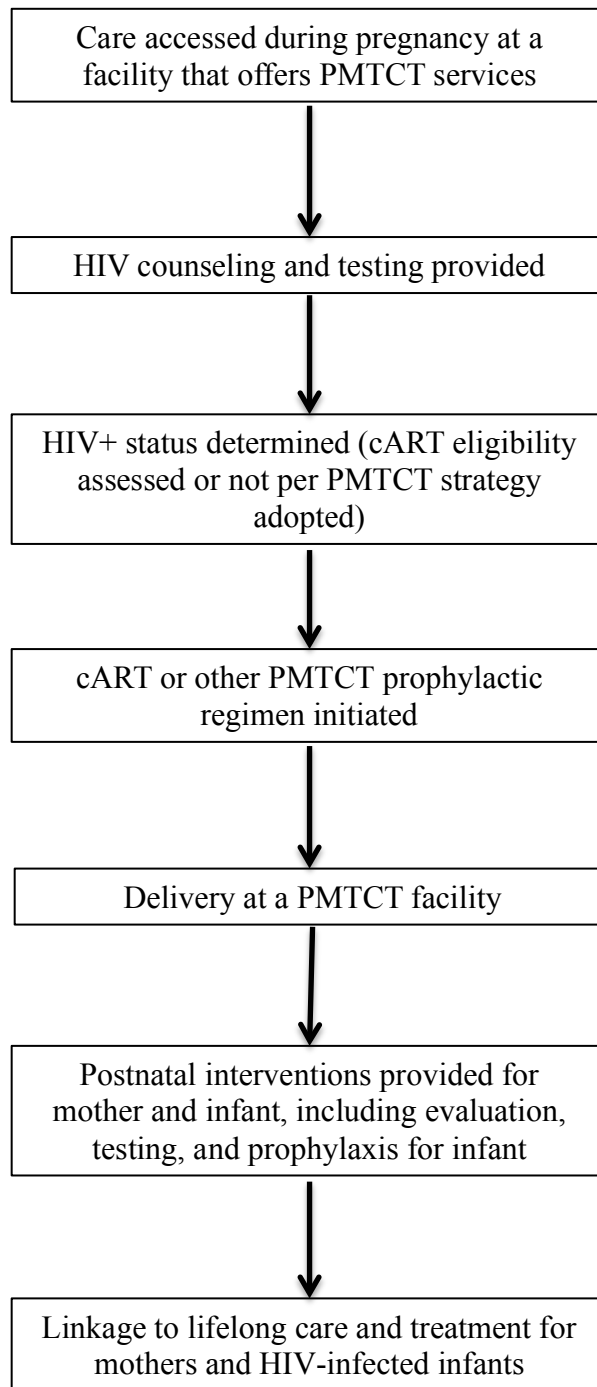
Section 2.6. Early infant diagnosis as part of the evolving prevention of mother-to-child HIV transmission landscape

The EID process is best understood within the greater framework of what has been referred to as the “PMTCT cascade,” a series of services that begins with women accessing antenatal care and continues postpartum through the HIV status determination of exposed infants and the successful linkage of HIV-infected mothers and children to lifelong HIV care and treatment. Several versions of the PMTCT cascade have been depicted and are often tailored by

programs to reflect particular PMTCT care protocols. For reference, one version of the PMTCT cascade is shown in Figure 2.2. Figure 2.2 builds upon the cascade published by Wettstein et al. [51], incorporating the new recommendation that countries adopt Option B+, as discussed in more detail below. The new recommendation allows us to simplify the cascade by Wettstein et al. by removing the need to assess for cART eligibility before initiating pregnant women on cART.

As depicted in Figure 2.2, HIV testing of exposed infants is downstream to many essential steps in PMTCT programming. It is important to consider the EID process within the context of the greater PMTCT cascade in the evaluation of HIV-exposed infant care because, since the implementation of infant virological testing, other parts of the PMTCT landscape have also continued to evolve rapidly. For example, in its 2010 guidelines the WHO changed its recommendations for the period of breastfeeding from six months to at least 12 months, which had major implications for the length of exposed infant care [52]. Perhaps the most rapidly evolving aspect of the PMTCT landscape has been the guidelines for providing antiretroviral (ARV) drug regimens for reducing the risk of vertical transmission.

Figure 2.2 - Prevention of mother-to-child HIV transmission cascade.



Abbreviations: PMTCT, prevention of mother-to-child HIV transmission; cART, combination antiretroviral therapy.

The above figure builds on the the cascade presented by Wettstein et al. [51]

Changes in the recommendations of ARVs for PMTCT have been substantial and implementation has varied drastically between programs. Commonly used PMTCT drug interventions include simplified short-course ARV prophylaxis for mothers during pregnancy and for exposed infants immediately after birth, as well as cART for immunologically or clinically eligible mothers [53]. In many resource-deprived settings, the only available drug intervention is single-dose nevirapine (NVP), which has been shown to reduce vertical HIV transmission to eight percent of exposed infants [54]. More effective combination regimens can reduce transmission to below two percent [53].

In its 2010 PMTCT guidelines, the WHO recommended that programs implement one of two regimen options for mothers not eligible for cART for their own health (i.e. maternal CD4 count >350 cells/mm³ and WHO clinical stage I or II) and their HIV-exposed infants [52]. The so-called options “A” and “B” are described in detail Table 2.1 below. Moreover, both options A and B include a short-course ARV regimen for cART-ineligible women starting during pregnancy and continuing until one week after delivery (option A) or until one week after all exposure to breast milk has ended (option B). Both options also include a short-course ARV regimen for exposed infants starting from birth and continuing until four to six weeks of age (options A and B) and until one week after all exposure to breast milk has ended (option A only). With regards to preventing vertical transmission of HIV, they were presented by the WHO as equally effective options.

In April 2012, the WHO released an update to the comprehensive 2010 guidelines in which it recommended cART for all pregnant women, regardless of previously established eligibility criteria (Option B+) [55]. As the 2010 guidelines required programs to distinguish between treatment and prophylaxis based on CD4 count or clinical staging criteria, the updated

recommendations were expected to be simpler to implement from a programmatic prospective while also preventing transmission to serodiscordant partners and subsequent children.

In addition to requiring mothers and their exposed infants be linked into care earlier, the new PMTCT interventions require more complex follow-up and service delivery. Although policy makers and program implementers have called into question the operational feasibility and sustainability of such efforts in resource-deprived settings, few programs have actually published their program's implementation experience and clinical results. As such, many of the current WHO guidelines for PMTCT, including the recent recommendation to implement lifelong cART for all pregnant women (option B+) in large part because of its expected programmatic advantage compared to short-course regimens (options A and B), are based on theoretical feasibility and efficacy rather than on demonstrated results in real-world PMTCT settings [55].

Table 2.1 - Three strategies for prevention of mother-to-child HIV transmission.

	Maternal regimen		Infant regimen	
	cART eligible (CD4 <350)	cART ineligible (CD4 <350)	Breastfeeding	Not breastfeeding
Option A	Lifelong cART	AZT from 14 weeks gestation until onset of labor, single-dose NVP at onset of labor, and AZT/3TC from labor until 7 days postpartum	<u>No maternal cART</u> Daily NVP from birth until the cessation of breastfeeding <u>Maternal cART</u> Daily NVP from birth until 4-6 weeks of age	Daily NVP from birth until 4-6 weeks of age
Option B	Lifelong cART	cART from 14 weeks gestation until delivery or 1 week after the cessation of breastfeeding	Daily NVP or AZT from birth until 4-6 weeks of age	Daily NVP or AZT from birth until 4-6 weeks of age
Option B+	Lifelong cART	Lifelong cART	Daily NVP or AZT from birth until 4-6 weeks of age	Daily NVP or AZT from birth until 4-6 weeks of age

Strategies as outlined by the World Health Organization [55]

Abbreviations: cART, combination antiretroviral therapy; AZT, zidovudine; NVP, nevirapine; 3TC, lamivudine

Section 2.7. Retention and combination antiretroviral therapy

The complex follow-up required to care for HIV-exposed infants through the care continuum necessitates the need to focus on retention efforts. A crucial goal is to keep exposed infants in care until their final HIV status can be determined so that they can be started on treatment as soon as possible if a positive status is confirmed. However, the literature indicates consistently high attrition from exposed infant care under a variety of programmatic settings [30–37]. For example, a large study of PMTCT program effectiveness in South Africa reported that one-third of infants never returned for a follow-up visit and that almost 70% were LTFU by four months of age [30]. Another study in Kenya found that seven percent of exposed infants were LTFU by three months of age and another 27% by 18 months of age [36]. Attrition is a major concern for programs attempting to scale-up PMTCT interventions because, even with the availability of efficacious ARV regimens, only a marginal impact on population-level vertical transmission can be achieved if program retention remains low [56].

The adult HIV literature indicates that patients receiving cART are less likely to be LTFU compared to patients not on cART [57,58]. Since infants are dependent on their caregivers to bring them to care, this evidence suggests that providing cART to caregivers may also play a role in the retention of their infants. Limited evidence from two studies suggests that receiving maternal cART may improve the proportion of HIV-exposed infants who receive EID. The first study, which included 217 mother-infant pairs from Mozambique, found that mothers who received cART were more likely to ever bring their infant for a virological test compared to women who did not receive cART, with an odds ratio of 3.15 (95% CL: 1.02-9.73) controlling for self-reported socio-demographic factors [59]. In a second analysis that included the 105 infants who received a test, the authors reported that mothers who received cART brought their

infant for a test 2.63 (95% CL, 0.26-5.00) months later than women who did not receive cART. Although the study provides preliminary evidence for an association between providing mothers with cART and retention of their exposed infants, a more appropriate methodological approach and a larger sample size would provide more accurate and precise results, respectively. In the first analysis, the authors used logistic regression without a clearly defined risk period that did not incorporate the longitudinal nature of their data. By excluding infants who never received a test from their second analysis, the authors limited the interpretation of their finding. A comparison of Kaplan-Meier estimates of time to first PCR test for the entire study population (including those who never received a test) would have been more informative.

A second study that included 1587 HIV-exposed infants in Cameroon, examined the association between multiple predictors and having an incomplete EID process (a complete EID process was defined as receiving a DNA PCR test and returning to receive the result) by seven months of age [60]. Controlling for clinical site, maternal education, time since maternal HIV diagnosis, mode of delivery, and multiple birth status, the authors found that women who received no prophylaxis or a short-course prophylaxis were more likely to have an incomplete EID process compared to women who received cART (OR [95% CL] 2.3 (1.2-4.1) and 1.4 (0.9-2.1) for “No prophylaxis” and “Short-course prophylaxis” versus “cART,” respectively). Although this study provides additional evidence for an association between maternal cART and retention of HIV-exposed infants from a large study population, the authors used logistic regression that did not fully utilize the longitudinal information available to them.

Section 2.8. Motivation for the dissertation project

Moreover, the body of literature on outcomes of HIV-exposed infants and our understanding of what drives those outcomes has increased substantially over the past decade. With each new discovery, new guidelines for care are developed. HIV program implementers strive to adopt new guidelines as they become available and to provide the best care they can for HIV-exposed infants. However, changes in the standard of care have been substantial and shifting away from old practices to implement new recommendations is typically an extensive process. Implementation in resource-deprived settings is particularly challenging, as generally clinics are understaffed, workforces are inadequately trained, resources are severely limited, and information systems are poor. Implementation fidelity, or the extent to which interventions are implemented as intended by those who designed them, can act as an important mediator of the relationship between a program and its intended outcomes [61]. For this reason and to inform future scale-up of priority program interventions, it is thus imperative to understand past implementation successes and challenges. Although many current PMTCT interventions are known to improve outcomes of HIV-exposed infants in trial settings, there is still a substantial gap in our understanding about how HIV program implementers have been able to improve outcomes of HIV-exposed infants in field settings. The purpose of Aim 1 was to fill this gap in the literature by describing how key clinical and programmatic outcomes of HIV-exposed infants have changed over time in a real world setting in Kinshasa.

As discussed in more detail above, one outcome of HIV-exposed infants that has been particularly challenging to improve has been retention in care of HIV-exposed infants. There is evidence indicating that adults on cART have better retention than adults not yet on cART. Prior studies also suggest that providing mothers with cART improves EID among their HIV-exposed

infants. Since HIV-exposed infants are dependent on their caregivers to bring them for clinic visits, we hypothesized that providing caregivers with cART could also improve retention of their HIV-exposed infants. Specifically, the goal of Aim 2 was to quantify the effect of providing cART to HIV-infected mothers on reducing LTFU among their HIV-exposed infants. Whether or not programs should provide cART to all HIV-infected mothers regardless of previously established eligibility criteria is an important debate that is occurring right now. The results of Aim 2 could highlight an important collateral benefit countries should consider when deciding which strategy to adopt.

There has been substantial progress in the research of HIV-exposed infants and the factors that affect their care. However, continued efforts are needed. By addressing the two project aims, we hope to better understand the impact PMTCT programs are having on HIV-exposed infant outcomes and to identify one strategy that could be implemented to improve program retention among HIV-exposed infants.

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CHAPTER 3: METHODS

Section 3.1. Overview of study design

We conducted a cohort study of prospectively collected clinical data. The observational design allowed us to evaluate the implementation and outcomes of HIV-exposed infant care, as well as the effect of maternal cART on retention of HIV-exposed infants under real-world conditions. A randomized study design would not have been appropriate in this situation, as established PMTCT guidelines made it unethical to randomize the intervention that was assessed in Aim 2. Compared to a randomized design, the observational nature of the study also increased the generalizability of the results to similar programmatic settings. As described in more detail below, the measurements that were considered in this study were routinely collected and entered into a clinical database. Therefore, a cohort study that utilized information on the entire clinic population was as feasible and cost-effective as a case-control study that would have utilized only a sample of this information. Utilizing all available information likely increased the precision of study estimates.

Section 3.2. Study setting/population

The data used for this study came from an ongoing clinical cohort of 1,707 HIV-exposed infants who received family-centered care in an HIV prevention, care, and treatment program

affiliated with UNC between January 1, 2007 and July 30, 2013. The infants received care at one of two centralized sites, Bomoi Healthcare Center and Kalembe Lembe Pediatric Hospital. January 2007 corresponds approximately to the point in time when HIV-exposed infants became eligible for enrollment in the UNC-DRC program as index patients.

Program background

The UNC-DRC program began in 2003 as a partnership between UNC, the Kinshasa School of Public Health and the DRC National Acquired Immune Deficiency Syndrome (AIDS) Program to scale-up HIV prevention, care, and treatment programming in the DRC. By the close of the UNC-DRC program at the end of July 2013, the program in Kinshasa consisted of a network of 90 maternities providing PMTCT services, 32 tuberculosis (TB) clinics providing HIV voluntary testing and counseling, and one primary health center and one pediatric hospital providing family-centered comprehensive HIV/AIDS prevention, care, and treatment services. The program was designed to identify patients through the increased use of HIV testing in PMTCT, TB, routine, and pediatric care, and then link them to a full continuum of services, including counseling, testing, and treatment for family members, nutritional and psychosocial support, and treatment of HIV disease and opportunistic infections.

The UNC-DRC program attempted to provide care for HIV-exposed infants in accordance with WHO or national guidelines and updated its clinical protocol at least yearly (the evolution of these guidelines is described in more detail in Chapter 4). However, due to logistical challenges, implementation sometimes lagged behind protocol updates. The UNC-DRC protocol included routine HIV virological testing for HIV-exposed infants over the entire 2007-2013 follow-up period, with initial testing at six weeks of age and repeat testing at nine

months of age. All positive results were supposed to be confirmed by virological testing on a second specimen. However, until the end of 2009, HIV ribonucleic acid (RNA) assays were the only HIV virological tests available at the national laboratory in Kinshasa and stock outs were frequent. Processing of HIV DNA PCRs was implemented at the national laboratory in November 2009, by which time the availability of virological tests had become more or less stable. Dried blood spots were intermittently collected before November 2009 in hopes that testing by DNA PCR would soon be available and occasionally stored samples were sent to national laboratories in other countries for analysis; however, results were rarely available for clinical decision-making before HIV infection could be confirmed by serology.

Before 2011, all HIV-infected mothers identified at an affiliated maternity and their HIV-exposed infants were referred for follow-up care at one of the two centralized care and treatment sites. Decentralization of pre-cART follow-up began in April 2011 at four maternities and expanded to include all maternities by the end of the year. In the decentralized model, pregnant women who were eligible for cART for their own health and their exposed infants were referred to a centralized care and treatment site for follow-up care, including PMTCT services. Women who were not yet eligible for cART had the option of receiving follow-up care for themselves and their exposed infants at the level of the maternity.

In accordance with the DRC national guidelines, HIV-positive pregnant women who enrolled into care at a UNC-DRC prevention, care, and treatment site received cART if they were eligible for their own health. Before the implementation of Option A in 2010, mothers who were not yet eligible for cART received a PMTCT prophylactic regimen of single-dose NVP. Once the national guidelines changed to include Option A, mothers who presented for care during pregnancy who were not yet eligible for cART began to receive zidovudine (AZT)

prophylaxis from as early as 14-weeks gestation up to seven days postpartum. However, the AZT drug supply was inconsistent and until recently was only offered at centralized care and treatment sites. Furthermore, it takes several weeks for individuals on cART or AZT prophylaxis to achieve undetectable viral loads and women in resource-deprived settings such as Kinshasa often present for care late in pregnancy or at delivery. Therefore, some women continued to receive a prophylactic regimen of single-dose NVP on its own or in addition to AZT even after the national guidelines changed.

The UNC-DRC program also provided prophylactic regimens to HIV-exposed infants to prevent vertical transmission. Before the national guidelines changed, they received only single-dose NVP at birth. Extended NVP was implemented as part of Option A, meaning that exposed infants were eligible to receive a daily prophylactic regimen with NVP from birth until six weeks after weaning or for six days after birth if they were not breastfed. Due to challenges in implementation, some HIV-infected women or their exposed infants received no prophylactic regimen.

Section 3.3. Inclusion and exclusion criteria

Per the program protocol, all infants born to HIV-infected women enrolled at one of the UNC-DRC affiliated programs or referred from Pre-School Pediatric consult at participating maternities were eligible for enrollment at one of the two centralized care and treatment sites. Enrollees were classified as ‘HIV-exposed infants’ if they were <18 months of age at the time of enrollment and born to a confirmed HIV-infected mother. The maternal HIV-infection status was confirmed in the biological mother or in the infants <18 months of age with a positive HIV antibody test.

The inclusion criteria were different for each specific aim. For Aim 1, we started with the population of HIV-exposed infants enrolled between January 1, 2007 and July 31, 2012. Infants enrolled after 18 months of age and those who could not be linked to a mother also receiving care in the UNC-DRC program by the time of infant enrollment were excluded. For Aim 2, we started with the population of HIV-exposed infants enrolled between January 1, 2007 and December 31, 2011. Infants enrolled after 18 months of age and infants not linked to a mother enrolled in the UNC-DRC program by the time of infant enrollment were excluded, as were infants whose mothers were enrolled in the UNC-DRC program before their most recent pregnancy. The rationale for limiting Aim 2 to infants whose mothers were newly enrolled into care during their most recent pregnancy is that we hypothesized that duration of maternal enrollment in care was both an important modifier and confounder of the relationship between maternal cART and infant LTFU.

Section 3.4. Ethical considerations

Written parental informed consent for the UNC-DRC program was obtained for all infants and written informed consent was obtained for all mothers. All research was approved by the Ethics Committee of the Kinshasa School of Public Health and the University of North Carolina at Chapel Hill Institutional Review Board. Published results contain no information that would facilitate the identification of individual patients.

Section 3.5. Data collection and quality assurance

Data collection

In the UNC-DRC program, patient information was recorded by clinic staff at each visit on a simplified patient chart. After the visit, the simplified charts were given to the on-site data entry team and the information was entered into an electronic database, usually on the same day. The visit schedule for exposed infants and protocol of scheduled services for each visit is described in Table 3.1 below. Moreover, visits were scheduled to occur every four weeks from the first visit at two weeks of age through 18 weeks of age and then every three months thereafter. Additional visits and laboratory tests were scheduled as clinically needed. The database was closed for final analysis at the end of July 2013.

Table 3.1 - Summary of clinic visits for HIV-exposed infants up to 18 months of age.

Procedure	2 wk	6 wk	10 wk	14 wk	18 wk	6 mo	9 mo	12 mo	15 mo	18 mo
Obtain informed consent	X									
Obtain personal, social and demographic information	X									
Assess psychosocial support needs and disclosure of mother/family	X	X	X	X	X	X	X	X	X	
Counsel mother on medication adherence & breastfeeding	X	X	X	X	X	X	X	X	X	X
Distribute bed nets to patient and household members	X									
Physical examination	X	X	X	X	X	X	X	X	X	X
Assess weight, length, head circumference	X	X	X	X	X	X	X	X	X	X
Assess for active TB/STI/malaria/OIs	X	X	X	X	X	X	X	X	X	X
PMTCT prophylaxis	X ¹	X ¹	X ¹	X ¹	X ¹	X ¹	X ¹	X ¹	X ¹	X ¹
Provide OI prophylaxis		X	X	X	X	X	X	X	X	x
Assess toxicity of OI prophylaxis		X	X	X	X	X	X	X	X	x
Immunizations		X	X	X			X		X	
PCR3		X					X			
HIV serology									X	X
Full blood count, differential, total lymphocyte count		X								X

Adapted from the UNC-DRC program protocol, Version OCT2011

1. Protected breastfeeding was recommended for HIV-infected women for the first 12 months of life. When replacement feeding was acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding was recommended. In practice, some mothers might have continued to breastfeed beyond the infant's first 12 months, in which case counseling and ARVs were continued until one week after the cessation of breastfeeding.

Quality assurance

Monitoring and evaluation, including an explicit attention to data and service quality, were part of the core operations of the UNC-DRC program. These efforts are worth mentioning here because they were the inspiration for Aim 1 of this study and because they facilitated to a great extent our ability to assess both aims of this dissertation. Monitoring and evaluation tools can be used to summarize programmatic data to improve delivery of patient service and guide implementation efforts. For monitoring and evaluation tools to be effective, they should facilitate the summary of raw data in a meaningful way and the timely dissemination to program designers and other stakeholders [1].

In the UNC-DRC program, routine data and service quality checks were run every two weeks in EpiInfo Software by program staff in Kinshasa. The software also produced an automated list of patients who did not receive essential services. Following the circulation of the quality report, each indicator and action list was reviewed by a quality assurance committee at both sites and priority improvement efforts were set. The results of these efforts were highlighted in a poster presentation at the AIDS 2012 conference in Washington, DC [2].

Section 3.6. Analytic approach

Outcome: Aim 1

Aim 1 was a descriptive analysis that examined the impact of program interventions for HIV-exposed infants enrolled into the UNC-DRC program between January 1, 2007 and June 30, 2012. Among all HIV-exposed infants, we estimated the 18-month cumulative incidences of the following outcomes:

- 1) Specimen collection for initial HIV virological testing
- 2) Confirmed HIV infection
- 3) Death
- 4) Loss to follow-up

In the UNC-DRC program, confirmatory testing of all positive virological test results was performed on a second specimen. Infants were considered LTFU on their last visit date if they did not yet have a confirmed HIV status (positive or negative) and were deactivated from care with the reason documented as LTFU or if more than six months passed since they were last seen in the clinic. The cutoff of six months was chosen based on empirical evidence from adult HIV treatment programs, which suggests that a cutoff of six months from the last attended clinic visit results in the least amount of outcome misclassification [3]. In the UNC-DRC program, infants were deactivated for LTFU following three tracking attempts after a missed visit.

Among HIV-exposed infants with confirmed HIV infection by 18 months of age, we also estimated the 24-month cumulative incidence of a fifth outcome:

- 5) Initiation of cART

Exposure: Aim 1

Aim 1 was primarily a descriptive analysis, thus we did not attempt to assess specific exposure-outcome relationships. To assess the impact of program interventions over time on the five outcomes of interest, we compared estimates of the cumulative incidences between calendar periods that infants were enrolled into care (2007-2008, 2009-2010, and 2011-2012).

Covariates: Aim 1

To understand how characteristics of the study population changed over time, we also described the distributions of infant and maternal age at infant enrollment into care, whether or not infants and their mothers received prophylactic drug regimens, breastfeeding status (breastfeeding at enrollment or not), infant growth status at enrollment (stunted or not, underweight or not), duration of maternal enrollment in HIV care at the time of infant enrollment, maternal enrollment status (mother newly enrolled into HIV care during most recent pregnancy or not), and maternal cART status (receiving cART by infant enrollment or not).

There was some missing data on infant growth status. Therefore, we used the first available weight and length/height values measured within a month of enrollment. Infants were considered underweight (stunted) if they had a weight-for-age (height-for-age) Z-score more than two standard deviations below the median value for a given age group and sex. Z-scores were derived from the WHO Child Growth Standards [4] using the WHO Anthro software (version 3.2.2, January 2011) and macro for SAS.

We hypothesized that outcomes for infants whose mothers were newly enrolled into care during their most recent pregnancy would be different than for infants whose mothers were enrolled into care before their most recent pregnancy. Since the proportion of infants whose mothers were previously enrolled into care increased over calendar time, we were concerned that any changes we observed could be contributed to this increasing proportion. We therefore decided to stratify the Aim 1 analyses by maternal enrollment status (mother enrolled before most recent pregnancy or mother newly enrolled). This was done for all outcomes except for infant cART initiation. For this outcome, there were too few HIV-infected infants born to mothers who were enrolled into care before their most recent pregnancy to stratify on maternal

enrollment status. Because gestational age at enrollment of the mother was not reliably collected in the UNC-DRC program and there were no routine quality improvement efforts in place to improve assessment and data collection for this variable, we defined “newly enrolled” as being enrolled at any time during the nine months before infant birth up to infant enrollment.

Statistical analysis: Aim 1

Standard descriptive statistics were used to characterize infants and their mothers at infant enrollment into care. Medians with interquartile ranges (IQRs) were reported for continuous variables and counts with proportions for were reported for categorical variables. Descriptive statistics were estimated overall and by infant enrollment period.

For the outcome LTFU, the timescale for analysis was days from enrollment in care and infants without a clinic visit beyond the enrollment visit were assigned one day of follow-up. In all other analyses, follow-up time was defined as days from birth. The rationale for using a different timescale for the LTFU than was used in the rest of the follow-up analyses is that infants cannot be lost from care if they are not first enrolled. Thus, the natural origin for LTFU is enrollment into care. We also wanted to be able to interpret the timescale for this analysis in a way that would allow us to directly answer the question “How long are infants enrolled in care before they are LTFU?”. For the other outcomes, the more pressing questions to answer are: “At what age are infants tested?”, “At what age are HIV infections being confirmed?”, “At what age are infants dying?”, and “At what age are infants started on cART?” This is because HIV disease progression in young children is very rapid and early mortality is high if HIV-infected infants do not initiate cART.

Regardless of the timescale used, follow-up concluded when the first of the following occurred: the event of interest, a competing event, or censoring [5]. Competing risks arise in time-to-event analyses when individuals can experience events that preclude them from ever experiencing the event of interest [5]. Competing events varied by analysis and are summarized in Table 3.2.

Table 3.2 - Competing risks for outcomes in Aim 1.

Outcome	Competing risk(s)	Censoring event
Specimen collection for initial virologic HIV test	Death; Graduation from care; Confirmation of HIV-negative status by serology	LTFU; Transfer of care to another facility; Administrative
Confirmed HIV infection	Death; Graduation from care	LTFU; Transfer of care to another facility; Administrative
Death	Graduation from care	LTFU; Transfer of care to another facility; Administrative
LTFU	Death; Graduation from care	Transfer of care to another facility; Administrative
cART initiation	Death	LTFU; Transfer of care to another facility; Administrative

Graduation from care from care was defined as being deactivated from care after a confirmed HIV-negative status or transition to ongoing HIV care if an HIV-positive result was confirmed. Administrative censoring occurred on the date the dataset was closed for final

analysis (July 31, 2012) or when infants aged-out of the risk period of interest (19 months of age in the analyses that included all HIV-exposed infants and 25 months of age in the analysis of cART initiation among HIV-infected infants).

We also conducted two sensitivity analyses to assess the extent to which informative censoring may have biased our estimates of the 18-month cumulative incidence of confirmed HIV infection. In the first sensitivity analysis, we determined if extending follow-up to 24 months changed our estimate of the cumulative incidence of confirmed HIV infection. In the second sensitivity analysis, we determined the extreme bounds [6,7] of the cumulative incidence function under different assumptions about those who were LTFU. The upper bound assumed all infants who were LTFU were confirmed HIV-positive on the day they were LTFU. The lower bound assumed all infants who were LTFU contributed the full amount of person-time (18 months) before graduating from care.

Outcome: Aim 2

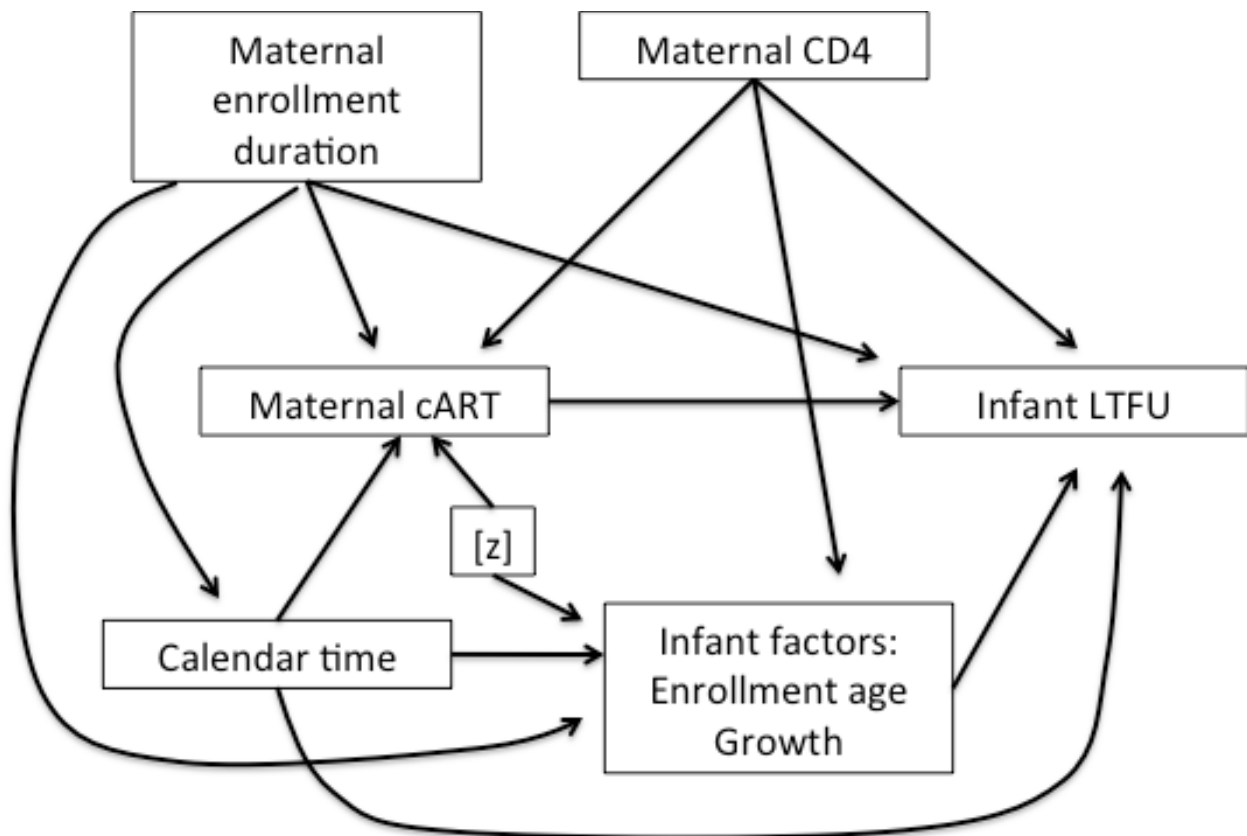
The primary outcome of interest for Aim 2 was being lost from care before confirmation of an HIV-positive or HIV-negative status. In the UNC-DRC program, infants were deactivated for LTFU following three tracking attempts after a missed visit. Infants were considered LTFU on their last attended clinic visit date if they were documented as LTFU or if more than six months passed since they were last seen in the clinic. We chose to add a second mechanism by which infants could be considered LTFU because the routinely implemented mechanism for deactivation LTFU infants often lagged considerably. The second criteria helped classify infants who were likely LTFU but not yet documented as such, helping to avoid outcome misclassification. The cutoff of six months was chosen based on empirical evidence from adult

HIV treatment programs, which suggests that a cutoff of six months from the last attended clinic visit results in the least amount of outcome misclassification [3].

Exposure: Aim 2

The exposure of interest was baseline maternal cART status. Infants were considered “exposed” if their mothers had not yet initiated cART by the time of infant enrollment into care and “unexposed” if their mother initiated cART before infant enrollment. The Directed Acyclic Graph (DAG) [8] in Figure 3.1 below further explains how we will assess the exposure.

Figure 3.1 - Directed Acyclic Graph depicting the effect of providing HIV-infected mothers with combination antiretroviral therapy on loss to follow-up of their HIV-exposed infants.



Covariates: Aim 2

As the DAG analysis in Figure 3.1 suggests, the adjustment set for estimating the effect of baseline maternal cART status on infant LTFU includes calendar time, maternal enrollment duration, maternal CD4 count, infant growth status, and infant age. Additional detail on these covariates and their relationship with the primary exposure and outcome of interest is provided below:

- 1) Maternal CD4: In accordance with DRC national guidelines, pregnant and breastfeeding women only received cART if they were eligible for their own health. Maternal CD4 count is thus associated with the primary exposure (maternal cART status). Furthermore, we hypothesized that women who feel sicker are more likely to return for care and therefore their HIV-exposed infants are less likely to be LTFU. In other words, maternal CD4 count may be both 1) a cause of the exposure and 2) a cause of the outcome and should be treated as a confounder in analyses of the primary exposure-outcome relationship. This covariate was modeled with Stone and Koo's additive splines constrained to be linear in the tails, with knots at the 5th, 35th, 65th, and 95th percentiles¹⁶ or categorized with a cut point at 350 (for stratified analyses).
- 2) Calendar time at infant enrollment into care: Since the proportion of women who received cART increases over calendar time, this covariate is associated with the primary exposure (maternal cART status). Although efforts to reduce infant LTFU increased over the course of the UNC-DRC program, it is unclear whether the outcome itself improved. Regardless, other infant outcomes (such

as positive infant HIV test results) on the causal path from maternal cART status to infant LTFU may have improved over calendar time. Thus, we treat calendar time at infant enrollment as a potential confounder in the Aim 2 analyses. This covariate was modeled with Stone and Koo's additive splines constrained to be linear in the tails, with knots at the 5th, 35th, 65th, and 95th percentiles¹⁶.

- 3) Maternal enrollment duration: The longer mothers are enrolled, the more likely they are to initiate cART. In addition, infants of mothers who are stable and in care are less likely to be LTFU than infants of all newly enrolled mothers. Thus, maternal enrollment duration is likely an important confounder and modifier of the relationship between maternal cART status and infant LTFU. Based on this assumption, we made two decisions in regards to the analysis. First, we excluded infants whose mothers were enrolled before their most recent pregnancy. Although we would have liked to stratify the primary analyses by this covariate (mother previously enrolled or newly enrolled), the number of infants of previously enrolled mothers who were LTFU was too small to generate stratified estimates. Even after limiting our analysis to newly enrolled mothers, maternal cART status remained associated with maternal enrollment duration, with mothers on cART more likely to be enrolled longer than mothers not yet initiated on cART. Therefore, we also adjusted for maternal enrollment duration. This covariate was modeled with Stone and Koo's additive splines constrained to be linear in the tails, with knots at the 5th, 35th, 65th, and 95th percentiles¹⁶.

- 4) Infant factors: We hypothesized that infant growth status and infant age at the time of enrollment would likely be associated with the primary outcome. Although we did not hypothesize that these covariates would be directly associated with maternal cART, it is likely that there may be another unmeasured factor that affects whether mothers get cART and also infant growth and infant enrollment age (for example, socioeconomic status). As we expected the association of these covariates with the outcome to be relatively strong, we opted to adjust for them in the analysis. Both were modeled with Stone and Koo's additive splines constrained to be linear in the tails, with knots at the 5th, 35th, 65th, and 95th percentiles¹⁶.

Statistical analysis: Aim 2

Descriptive statistics were used to generate the distributions of baseline characteristics of the study population overall and by exposure group (maternal cART by infant enrollment or no maternal cART by infant enrollment). For the primary analyses, we conducted competing risk analyses described by Fine and Gray [5,9]. First, we estimated the 18-month cumulative incidence of LTFU stratified by the primary exposure (maternal cART status) and the baseline covariates described in the previous section. 95% confidence intervals were generated for all estimates to assess precision. To quantify the effect of providing HIV-infected mothers with cART on LTFU of their HIV-exposed infants, we implemented the subdistribution proportional hazards model of Fine and Gray [5,9] using the SAS macro %PSHREG [10]. For comparison, we also provided estimates of the cause-specific hazard ratio estimated from traditional Cox regression models, which are still more widely implemented in the literature. Multiple

imputation using Markov Chain Monte Carlo simulation was used to impute missing values for covariates [11,12]. Five imputed datasets were generated. The estimates that were generated from each model are summarized below:

- 1) Unadjusted subdistribution hazard ratio.
- 2) Subdistribution hazard ratio adjusted for baseline factors (calendar time, maternal CD4 count, maternal enrollment duration, infant WAZ, and infant age) and estimated from a model that excluded infants with missing data (complete case analysis).
- 3) Subdistribution hazard ratio adjusted for baseline factors (calendar time, maternal CD4 count, maternal enrollment duration, infant WAZ, and infant age) and estimated from a model that used multiple imputation to impute missing covariate data.
- 4) Unadjusted cause-specific hazard ratio.
- 5) Cause-specific hazard ratio adjusted for baseline factors (calendar time, maternal CD4 count, maternal enrollment duration, infant WAZ, and infant age) and estimated from a model that excluded infants with missing data (complete case analysis).
- 6) Cause-specific hazard ratio adjusted for baseline factors (calendar time, maternal CD4 count, maternal enrollment duration, infant WAZ, and infant age) and estimated from a model that used multiple imputation to impute missing covariate data.

In these analyses, follow-up began at enrollment of the infant and continued until the first of the following occurred: the event of interest (LTFU), a competing event (death or graduation from care), or a censoring event (18 months of age or July 31, 2013). Graduation from care was defined as being deactivated from care after confirmation of an HIV-negative status or transfer to ongoing HIV care and treatment following confirmation of an HIV-positive status. All analyses were conducted in SAS 9.3 (SAS Institute, Inc., Cary, North Carolina).

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CHAPTER 4: TEMPORAL CHANGES IN THE OUTCOMES OF HIV-EXPOSED INFANTS IN KINSHASA, DR CONGO DURING A PERIOD OF RAPIDLY EVOLVING GUIDELINES FOR CARE (2007-2013)

Section 4.1. Introduction

Globally, an estimated 1.4 million infants are born to HIV-infected pregnant women each year [1]. The guidelines for care of HIV-exposed infants have evolved rapidly in recent years and ambitious goals for controlling the pediatric HIV epidemic have been set [2,3]. Although the incidence of pediatric HIV is declining [1], we still know little about how programmatic and clinical outcomes of HIV-exposed infants have changed over time.

A major evolution in the care of HIV-exposed infants occurred with the implementation of early infant diagnosis (EID) by virological testing, which was first recommended by the World Health Organization (WHO) in 2007 [4]. Previously, HIV infection in exposed infants could only be confirmed by serology at 18 months of age [5,6]. EID is needed to ensure timely combination antiretroviral therapy (cART) initiation. Without cART, a third of HIV-infected infants will die in the first year of life [7–9].

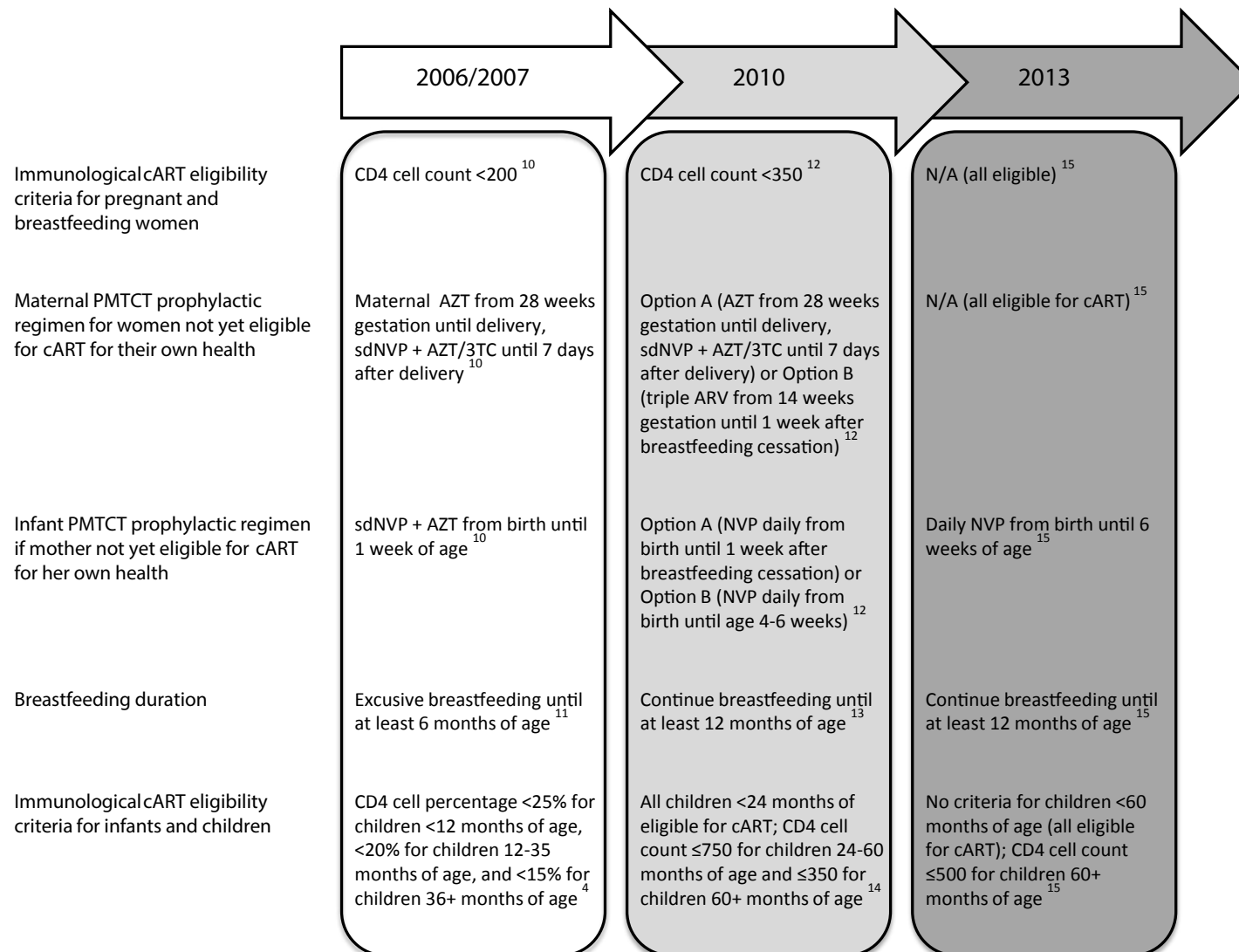
Since the scale-up of EID, other parts of the prevention of mother-to child HIV transmission (PMTCT) landscape have also evolved rapidly (Figure 4.1) [4,10–15]. Mounting evidence on the importance of breastfeeding for preventing HIV-exposed infant mortality [16–18] led the WHO to increase the recommended breastfeeding period from six months (2006 recommendation [11]) to at least 12 months (2010 recommendation [13]). Due to the increased risk period for vertical HIV transmission through breastfeeding [19,20], the duration and

complexity of antiretroviral prophylactic regimens in the 2010 guidelines also increased [12]. By 2012, the WHO endorsed lifelong cART for all pregnant women [21].

Evaluating and reporting outcomes in routine care settings is critical to demonstrate the scalability of recommended interventions for PMTCT and to assure quality care is being provided [22–24]. One study of 561 infants who received care between 2009 and 2012 in the Kilimanjaro Region of Tanzania reported 10% mother-to-child HIV transmission [25], despite the provision of prophylactic regimens that were expected to reduce vertical transmission to below 5% [12]. Another study of 311 mother-infant pairs in Malawi was able to reduce transmission to 3%, but 14% of infants died by 24 months of age [26].

These examples from the field highlight that, despite best efforts to implement current guidelines, PMTCT programs do not always achieve intended outcomes for HIV-exposed infants. Our understanding of the impact that PMTCT programs have had on HIV-exposed infant outcomes is incomplete, in part because guidelines often change before the impacts of previous guidelines have been assessed. The goal of this study was to describe how key clinical and programmatic outcomes of HIV-exposed infants have changed over time in Kinshasa, Democratic Republic of Congo (DRC), under evolving guidelines for care. Beginning with the implementation of HIV-exposed infant care in Kinshasa, we describe temporal trends in the timing of EID, HIV transmission, death, and loss to follow-up (LTFU), as well as cART initiation among infants identified as HIV-infected. To our knowledge, this is the first study to assess if the implementation of new guidelines has coincided with improvements in these outcomes for HIV-exposed infants over time.

Figure 4.1 - Evolution of World Health Organization guidelines for prevention of mother-to-child transmission of HIV.



Section 4.2. Methods

Study population

The data for this study come from a family-centered HIV prevention, care, and treatment program implemented at two sites (one primary healthcare center and one pediatric hospital) in Kinshasa, with technical assistance provided by the University of North Carolina at Chapel Hill (UNC-DRC program). The two centralized sites provided comprehensive care (including PMTCT) to HIV-positive women identified through routine HIV testing at 90 maternities and their newborn infants, former patients at 32 tuberculosis clinics, and HIV-positive children, as well as HIV-positive first-line family members of these individuals. Routinely collected data from HIV-exposed infants enrolled between January 2007 and June 2012 and their mothers were linked to construct a cohort of mother-infant pairs. Mother-infant linkages were constructed using routinely assigned unique patient codes. Each individual's record contained their own patient code, as well as a list of patient codes for their family members also receiving care in the UNC-DRC program. Infants enrolled after 18 months of age and infants not linked to a mother enrolled in the UNC-DRC program by the time of infant enrollment were excluded.

Routine care and clinic procedures

In accordance with WHO guidelines, the UNC-DRC protocol included EID by virological testing over the entire study period, with initial testing at six weeks of age and confirmatory virological testing of all positive results performed on a second specimen. However, until the end of 2009, HIV RNA assays were the only HIV virological tests available at the national laboratory in Kinshasa and stock outs were frequent. HIV DNA PCR testing was implemented in November 2009, by which time the availability of virological tests had

stabilized. Specimens for DNA PCR testing were collected on dried blood spots, which were transported to the national laboratory on a daily basis in vehicles provided by the UNC-DRC program. Information on the turnaround time for specimen processing was not available. All HIV testing was provided free of charge.

PMTCT prophylactic regimens, cART, and breastfeeding support were provided according to current WHO guidelines. Before 2010, the only available PMTCT prophylactic regimen for mothers not yet eligible for cART (CD4 cell count >200) and their infants was single-dose nevirapine, which was provided according to the 2006 WHO guidelines for programs with limited capacity [10]. Mothers were encouraged to exclusively breastfeed for six months and then wean rapidly [11]. The “Option A” strategy was implemented in the DRC following the release of the 2010 WHO guidelines [12]. Mothers not yet eligible for cART (CD4 cell count >350) received zidovudine prophylaxis through pregnancy and peripartum nevirapine with a zidovudine-lamivudine “tail” around delivery. Their infants received extended nevirapine until one week after the cessation of breastfeeding. Mothers were encouraged to continue breastfeeding for at least 12 months [13].

Clinic visits for HIV-exposed infants were scheduled to occur every four weeks from the first visit at two weeks of age through 18 weeks of age and then every three months thereafter. Infants <18 months of age were deactivated from care following a negative HIV virological or serological test result obtained more than three months after the cessation of breastfeeding. Infants 18 months of age or older were deactivated from care following the cessation of breastfeeding and a negative serological test. HIV-infected infants were eligible to receive lifelong care and treatment in the UNC-DRC program. In 2007, infants <18 months of age with a CD4 cell percentage <20, infants 18-24 months of age with a CD4 cell percentage <15, and all

infants with a WHO clinical stage 3 or 4 were eligible for cART [4]. Between 2008 and 2010, infants 12-24 months of age with a CD4 cell percentage <20 and all infants <12 months of age were eligible to receive cART [27]. Starting in 2010, all infants <24 months of age were eligible for cART [14].

Definitions and statistical analysis

Demographics of infants and their mothers at infant enrollment were characterized using standard descriptive statistics. Infants were considered underweight or stunted if they had a weight-for-age or height-for-age Z-score more than two standard deviations below the median value for a given age group and sex. Z-scores were derived from the WHO Child Growth Standards [28] using the WHO Anthro software (version 3.2.2, January 2011) and macro for SAS. Growth status was based on the first available weight and height values measured within a month of enrollment.

Among all HIV-exposed infants, we estimated the 18-month cumulative incidences of specimen collection for initial HIV virological testing, confirmed HIV infection, death, and loss to follow-up (LTFU). For HIV-exposed infants with confirmed HIV infection, we also estimated the 24-month cumulative incidence of cART initiation. In the LTFU analysis, follow-up time was defined as days from enrollment in care and infants without a clinic visit beyond the enrollment visit were assigned one day of follow-up. Infants were considered LTFU on their last attended clinic visit date following three failed tracking attempts after a missed appointment or if more than six months passed since they were last seen in the clinic. In all other analyses, follow-up time was defined as days from birth. Follow-up concluded when the first of the following occurred: the event of interest, a competing event, or censoring [29].

Competing risks arise in time-to-event analyses when individuals can experience events that preclude them from ever experiencing the event of interest [29]. Our study included multiple outcomes of interest, and competing events varied by analysis. Death was treated as a competing event in the analyses of specimen collection for HIV virological testing, confirmed HIV infection, LTFU, and cART initiation. Graduation from care (i.e. deactivation from care after a confirmed negative status or transition to lifelong HIV care if positive) was treated as a competing event in the analyses of LTFU, confirmed HIV infection, and death. In the analysis of first specimen collection for HIV virological testing, a confirmatory negative diagnosis by serology was also treated as a competing event. LTFU and transfer of care to another facility were treated as censoring events. Administrative censoring occurred on the date the dataset was closed for final analysis (August 2013) or when infants aged-out of the risk period of interest (19 months of age in the analyses that included all HIV-exposed infants and 25 months of age in the analysis of cART initiation among HIV-infected infants).

To assess how infant outcomes changed over time, we compared cumulative incidence estimates and 95% confidence limits (CL) between infants enrolled in three time periods (2007-2008, 2009-2010, and 2011-2012). A *p*-value was obtained for Gray's test for equality of the cumulative incidence function [30] using the SAS %CIF macro (version 1.0, March 2012) [31]. Since all HIV-infected women enrolled in the UNC-DRC program were eligible to receive lifelong care, the proportion of infants whose mothers were newly enrolled into care during their most recent pregnancy declined over the study period. Because outcomes among infants whose mothers were enrolled into care before their current pregnancy may be different than those whose mothers were newly enrolled, we stratified comparisons of the cumulative incidence functions by maternal enrollment status (newly enrolled during most recent pregnancy or

previously enrolled) for all outcomes except infant cART initiation. For this outcome, the number of HIV-infected infants whose mothers were previously enrolled was too small to allow for stratified analyses.

Sensitivity analyses

To determine the cumulative incidence of HIV transmission in our study population, we only counted HIV infections that were confirmed before 19 months of age. One infant with an initial positive test result died before the positive status could be confirmed. There were 17 additional infants confirmed positive who were treated as administratively censored. If it is uninformative, administrative censoring should not bias estimates of the cumulative incidence [32]. To check this assumption, we compared our original estimate of the 18-month cumulative incidence of confirmed HIV infection to the 24-month cumulative incidence. Extending follow-up captured 9 additional events that were not observed in the original analysis due to administrative censoring.

We also assessed potential selection bias induced by informative censoring due to LTFU by assessing the extreme bounds [32,33] of the overall 18-month cumulative incidence of HIV. The upper bound was estimated assuming all infants who were LTFU instead were confirmed HIV-positive on the day they were LTFU. The lower bound was estimated assuming all infants who were LTFU were instead confirmed HIV-negative and “graduated” from care at 18 months of age.

Finally, we wanted to know if implementation of PMTCT services differed between the two sites included in the study. To assess for differences, we compared the cumulative incidence

functions for each of the outcomes of interest stratified by site of care. All analyses were conducted in SAS 9.3 (SAS Institute, Inc., Cary, North Carolina).

Ethics statement

Written parental informed consent for the UNC-DRC program was obtained for all infants and written informed consent was obtained for all mothers. All research was approved by the Ethics Committee of the Kinshasa School of Public Health and the University of North Carolina at Chapel Hill Institutional Review Board.

Section 4.3. Results

Study population characteristics

Among 1908 HIV-exposed infants enrolled during the study period, 1707 were linked to a mother receiving care in the UNC-DRC program and included in the analysis. At enrollment, infants were a median of 2.6 weeks of age (interquartile range [IQR]: 2.1-6.4) and almost all (90.6%) were breastfeeding (Table 4.1). 336 (20.3%) infants were underweight and 381 (23.1%) were stunted at enrollment, with little change over time. The proportion of infants who failed to receive a PMTCT prophylactic regimen declined from 29.7% for infants enrolled in 2007-2008 to 7.7% for infants enrolled in 2011-2012.

At infant enrollment, mothers were a median of 31.1 years of age (IQR: 27.0-34.7) and had been enrolled in the UNC-DRC program for a median of 94 days (IQR: 23-168) (Table 4.1). Overall, 1331 (78.0%) of mothers received a PMTCT prophylactic regimen or cART during their pregnancy and 475 (27.8%) had initiated cART by the time of infant enrollment.

Table 4.1 - Characteristics of HIV-exposed infants and their mothers at infant enrollment into care, by calendar period, Kinshasa, Democratic Republic of Congo.

	Year of infant enrollment into care			
	2007-2012 (N=1707)	2007-2008 (N=335)	2009-2010 (N=730)	2011-2012 (N=642)
Infants				
Median age in weeks (IQR)	2.6 (2.1-6.4)	2.9 (2.1-9.7)	2.4 (2.1-6.0)	2.6 (2.1-6.0)
Gender [N (%)]				
<i>Female</i>	872 (51.1)	175 (52.2)	375 (51.4)	322 (50.2)
<i>Male</i>	835 (48.9)	160 (47.8)	355 (48.6)	320 (49.8)
PMTCT regimen [N (%)]				
<i>No</i>	243 (16.7)	76 (29.7)	119 (20.9)	48 (7.7)
<i>Yes</i>	1210 (83.3)	180 (70.3)	451 (79.1)	579 (92.3)
Breastfeeding [N (%)]				
<i>No</i>	123 (9.4)	29 (13.1)	58 (13.1)	36 (5.6)
<i>Yes</i>	1182 (90.6)	193 (86.9)	384 (86.9)	605 (94.4)
Cotrimoxazole prophylaxis [N (%)]				
<i>No</i>	86 (5.0)	19 (5.7)	28 (3.8)	39 (6.1)
<i>Yes</i>	1621 (95.0)	316 (94.3)	702 (96.2)	603 (93.9)
Underweight ^a [N (%)]				
<i>No</i>	1317 (79.7)	248 (79.2)	564 (79.1)	505 (80.5)
<i>Yes</i>	336 (20.3)	65 (20.8)	149 (20.9)	122 (19.5)

	Year of infant enrollment into care			
	2007-2012 (N=1707)	2007-2008 (N=335)	2009-2010 (N=730)	2011-2012 (N=642)
Stunted ^b [N (%)]				
<i>No</i>	1268 (76.9)	233 (75.6)	545 (76.5)	490 (77.9)
<i>Yes</i>	381 (23.1)	75 (24.4)	167 (23.5)	139 (22.1)
Mothers				
Median age in years (IQR)	31 (27-35)	31 (28-35)	31 (27-34)	31 (27-35)
Median days enrolled (IQR)	94 (23-168)	75 (32-129)	89 (0-154)	117 (23-531)
Newly enrolled during most recent pregnancy [N (%)]				
<i>No</i>	296 (17.3)	20 (6.0)	94 (12.9)	182 (28.3)
<i>Yes</i>	1411 (82.7)	315 (94.0)	636 (87.1)	460 (71.7)
PMTCT regimen [N (%)] ^c				
<i>No</i>	376 (22.0)	76 (22.7)	176 (24.1)	124 (19.3)
<i>Yes</i>	1331 (78.0)	259 (77.3)	554 (75.9)	518 (80.7)
cART initiated [N (%)] ^d				
<i>No</i>	1232 (72.2)	279 (83.3)	563 (77.1)	390 (60.7)
<i>Yes</i>	475 (27.8)	56 (16.7)	167 (22.9)	252 (39.3)

Abbreviations: IQR, Interquartile range; PMTCT, prevention of mother-to-child HIV transmission; cART, combination antiretroviral therapy. Covariate totals may not add up to the column totals due to missing data.

^a Underweight defined as weight-for-age Z-score ≤ -2 .

^b Stunted defined as height-for-age Z-score ≤ -2 .

^c Includes any maternal regimen (described in more detail in the methods section), including maternal cART if initiated before delivery.

^d Includes maternal cART initiated before infant enrollment.

Outcomes of HIV-exposed infants

The 18-month cumulative incidence of having a specimen collected for initial virological HIV testing increased over the study period, with 99% (95% CL: 98, 100%) of infants enrolled in 2011-2012 having a specimen collected by 18 months of age (Table 4.2). Among infants whose mothers were newly enrolled into care, the cumulative incidence of specimen collection by two months of age was 28% (95% CL: 24, 34%) for infants enrolled in 2007-2008, 49% (95% CL: 45, 53%) for infants enrolled in 2009-2010, and 63% (95% CL: 59, 68%) for infants enrolled in 2011-2012.

Among 1411 infants with newly enrolled mothers, 135 infants were confirmed HIV-positive, 62 died, and 252 were LTFU over 14300 person-months of follow-up (Table 4.2). The 18-month cumulative incidence of HIV declined from 16% (95% CL: 11, 22%) for infants enrolled in 2007-2008 to 11% (95% CL: 8, 16%) for infants enrolled in 2011-2012 (Table 4.2, Figure 4.2) and death declined from 8% (95% CL: 5, 12%) to 3% (95% CL: 2, 5%) (Table 4.2, Figure 4.3). The 18-month cumulative incidence of LTFU did not improve over calendar time, with 18-month cumulative incidences ranging from 16% (95% CL: 13, 19%) to 22% (95% CL: 18, 26%) (Table 4.2, Figure 4.4).

Among 296 infants whose mothers enrolled before their current pregnancy, seven infants were confirmed HIV-positive, 11 died, and 24 were LTFU over 3949 person-months of follow-up (Table 4.2). The 18-month cumulative incidence of HIV declined from 5% (95% CL: 0, 21%) for infants enrolled in 2007-2008 to 1% (95% CL: 0, 3%) for infants enrolled in 2011-2012 (Table 4.2, Figure 4.2). Death also declined over calendar time, with 18-month cumulative incidences of 5% (95% CL: 0, 21%) for infants enrolled in 2007-2008 and 3% (95% CL: 1, 7%)

for infants enrolled in 2011-2012 (Table 4.2, Figure 4.3). LTFU increased from 0% (95% CL: 0, 0%) to 9% (95% CL: 5, 14%) (Table 4.2, Figure 4.4).

Table 4.2 - Follow-up of HIV-exposed infants in Kinshasa, Democratic Republic of Congo, by calendar period at infant enrollment into care and enrollment status of the mother (newly enrolled during pregnancy or previously enrolled).

	Overall			Newly Enrolled			Previously Enrolled		
	2007-08	2009-10	2011-12	2007-08	2009-10	2011-12	2007-08	2009-10	2011-12
HIV-exposed infants (N)	335	730	642	315	636	460	20	94	182
First specimen collection									
<i>Person-months</i>	1435	1943	694	1265	1692	483	170	251	211
<i>N</i>	220	604	612	212	522	435	8	82	177
<i>18-month cumulative incidence (95% CL)</i>	0.73 (0.68, 0.78)	0.89 (0.86, 0.91)	0.99 (0.98, 1.00)	0.76 (0.70, 0.80)	0.88 (0.85, 0.91)	0.99 (0.98, 1.00)	0.40 (0.19, 0.60)	0.89 (0.80, 0.94)	0.99 (0.96, 1.00)
<i>Median weeks to collection (IQR)^a</i>	12.3 (4.9-63.1)	4.9 (3.7-27.9)	4.0 (1.3-4.9)	9.9 (4.7-50.0)	4.9 (3.7-32.7)	4.0 (0.1-5.0)	. ^d (30.7-.) ^d	4.7 (3.9-8.6)	4.3 (3.7-4.9)
<i>Median age in weeks at collection (IQR)^b</i>	30.3 (8.1-.) ^d	8.3 (6.7-39.3)	7.0 (6.6-10.4)	25.4 (8.0-71.4)	9.0 (6.9-39.4)	7.3 (6.6-12.3)	. ^d (39.3-.) ^d	7.3 (6.6-12.0)	6.9 (6.4-7.6)

	Overall			Newly Enrolled			Previously Enrolled		
	2007-08	2009-10	2011-12	2007-08	2009-10	2011-12	2007-08	2009-10	2011-12
Final outcomes									
<i>Person-months</i>	3425	6446	8378	3227	5459	5614	198	987	2764
<i>Confirmed HIV (N)</i>	39	67	36	38	62	35	1	5	1
<i>18-month cumulative incidence (95% CL)</i>	0.15 (0.11, 0.21)	0.12 (0.09, 0.15)	0.08 (0.06, 0.11)	0.16 (0.11, 0.22)	0.13 (0.10, 0.16)	0.11 (0.08, 0.16)	0.05 (0.00, 0.21)	0.06 (0.02, 0.12)	0.01 (0.00, 0.03)
<i>Death (N)</i>	23	33	17	22	28	12	1	5	5
<i>18-month cumulative incidence (95% CL)</i>	0.08 (0.05, 0.11)	0.05 (0.04, 0.07)	0.03 (0.02, 0.05)	0.08 (0.05, 0.12)	0.05 (0.03, 0.07)	0.03 (0.02, 0.05)	0.05 (0.00, 0.21)	0.06 (0.02, 0.12)	0.03 (0.01, 0.07)
<i>LTFU^c (N)</i>	59	103	111	59	97	96	0	9	15
<i>18-month cumulative incidence (95% CL)</i>	0.18 (0.14, 0.22)	0.15 (0.13, 0.18)	0.18 (0.15, 0.21)	0.19 (0.15, 0.23)	0.16 (0.13, 0.19)	0.22 (0.18, 0.26)	0.00 (0.00, 0.00)	0.1 (0.05, 0.17)	0.09 (0.05, 0.14)

	Overall			Newly Enrolled			Previously Enrolled		
	2007-08	2009-10	2011-12	2007-08	2009-10	2011-12	2007-08	2009-10	2011-12
<u>HIV-infected infants (N)</u>	39	67	36						
Person-months	364	387	151						
Died before cART (N)	6	8	1						
LTFU before cART (N)	5	3	1						
cART (N)	21	56	34						
<i>24-month cumulative</i>	0.61	0.88	0.97						
<i>incidence (95% CL)</i>	(0.43,	(0.77,	(0.82,						
	0.75)	0.94)	1.00)						
Median months from	12.1	4.9	3.8						
enrollment to cART (IQR) ^a	(5.0-.) ^d	(3.0-	(1.2-6.3)						
		11.8)							
Median age in months at	17.9	9.2	9.3						
cART (IQR) ^b	(10.8-.) ^d	(5.1-	(5.5-						
		17.2)	13.5)						

Abbreviations: CL, confidence limits; IQR, interquartile range; LTFU, loss to follow-up; cART, combination antiretroviral therapy

^a Estimated from the cumulative incidence function using time since enrollment as the time scale.

^b Estimated from the cumulative incidence function using age as the time scale.

^c Individuals were considered lost to follow-up if they were deactivated with the reason documented as lost to follow-up or if they were last seen more than six months before the administrative end of the study.

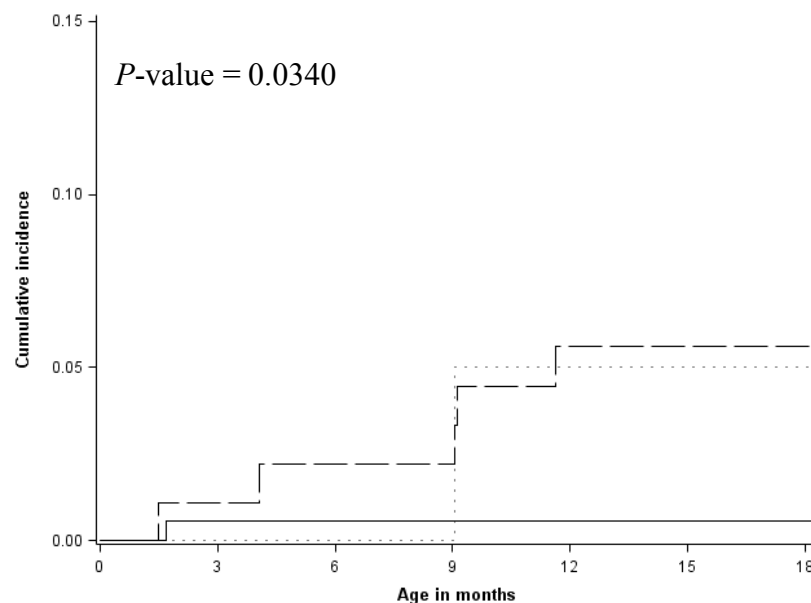
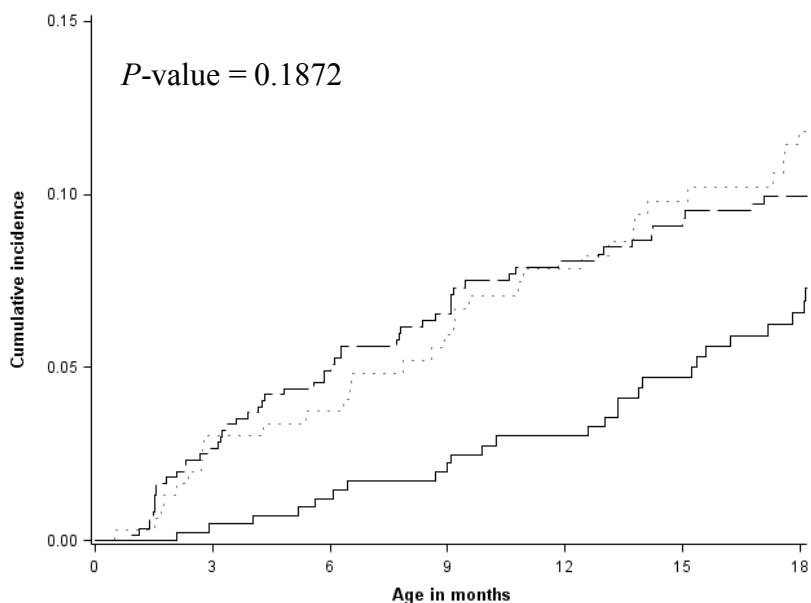
^d The cumulative incidence in this period was less than 0.5 (median) or 0.75 (upper quartile range) by the end of follow-up.

Figure 4.2 - 18-month cumulative incidence functions of confirmed HIV infection among HIV-exposed infants in Kinshasa, Democratic Republic of Congo.

64

Mother newly enrolled

Mother previously enrolled

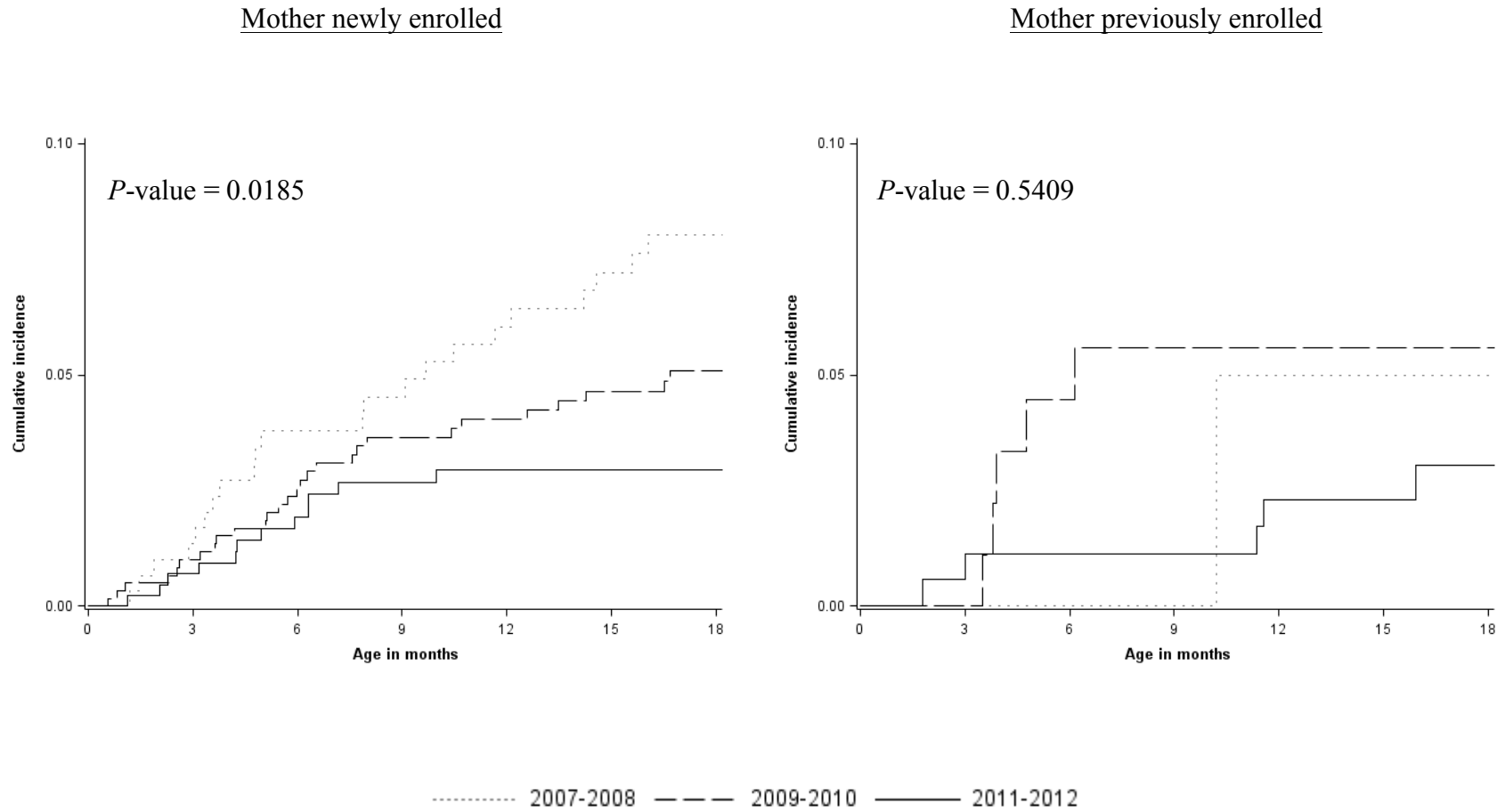


----- 2007-2008 - - - - 2009-2010 ——— 2011-2012

* Cumulative incidence functions are plotted by calendar period at infant enrollment into care and enrollment status of the mother (enrolled before current pregnancy or newly enrolled). *P*-values are for Gray's Test for equality of the cumulative incidence functions.

Figure 4.3 - 18-month cumulative incidence functions of death among HIV-exposed infants in Kinshasa, Democratic Republic of Congo.

65

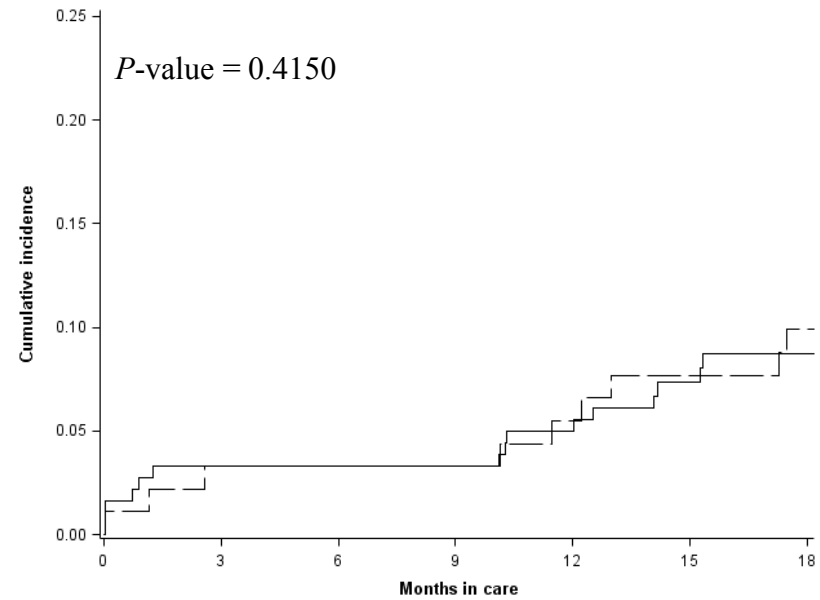
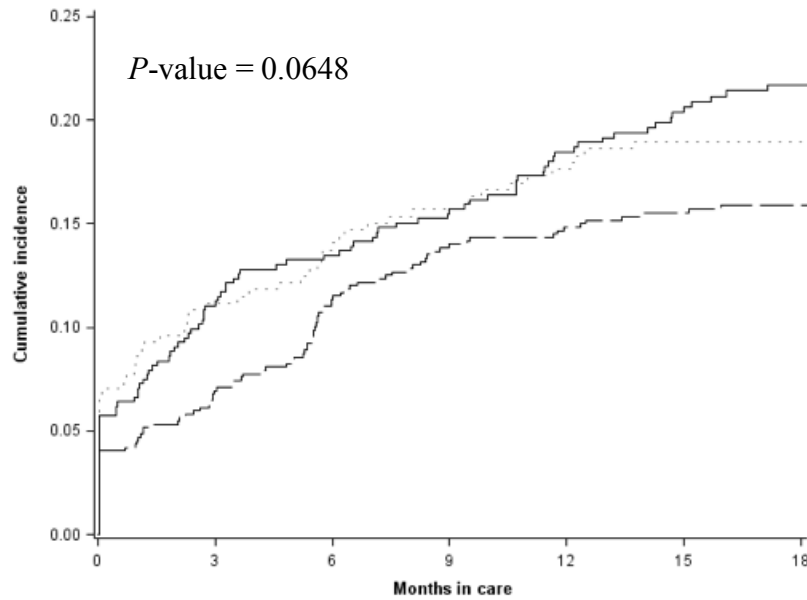


* Cumulative incidence functions are plotted by calendar period at infant enrollment into care and enrollment status of the mother (enrolled before current pregnancy or newly enrolled). *P*-values are for Gray's Test for equality of the cumulative incidence functions.

Figure 4.4 - 18-month cumulative incidence functions of loss to follow-up among HIV-exposed infants in Kinshasa, Democratic Republic of Congo.

Mother newly enrolled

Mother previously enrolled



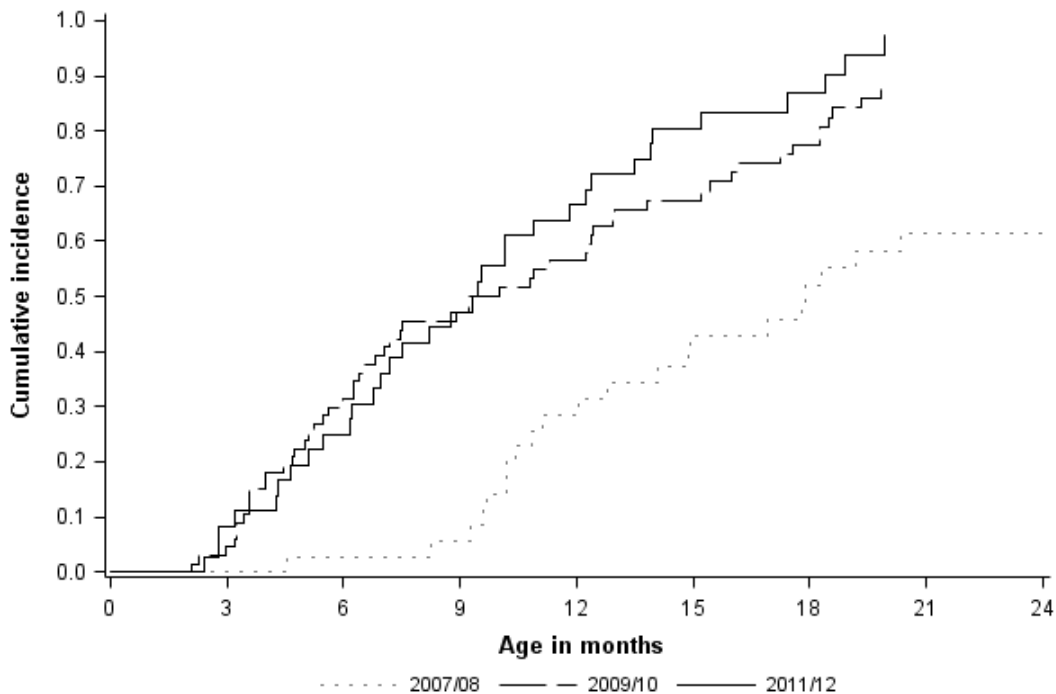
..... 2007-2008 - - - 2009-2010 ——— 2011-2012

* Cumulative incidence functions are plotted by calendar period at infant enrollment into care and enrollment status of the mother (enrolled before current pregnancy or newly enrolled). *P*-values are for Gray's Test for equality of the cumulative incidence functions.

cART initiation among HIV-infected infants

Among the 142 infants with confirmed HIV infection by 18 months of age, the median age at enrollment into care was 6.4 weeks (IQR: 2.3-32.0 weeks) and the median age at specimen collection for initial virological HIV testing was 17.3 weeks (IQR: 7.3-46.0 weeks). The 24-month cumulative incidence of starting cART increased over time, from 61% (95% CL: 43, 75%) for infants enrolled in 2007-2008 to 97% (95% CL: 82, 100%) for infants enrolled in 2011-2012 (Table 4.2, Figure 4.5). The median time between enrollment and cART initiation declined from 12.1 to 3.8 months, and the median age at cART initiation decreased from 17.9 to 9.3 months.

Figure 4.5 - 24-month cumulative incidence function of combination antiretroviral therapy initiation among HIV-infected infants in Kinshasa, Democratic Republic of Congo.



* Cumulative incidence functions are plotted by calendar period at infant enrollment into care. The *P*-value is for Gray's Test for equality of the cumulative incidence functions.

Sensitivity analyses

Extending follow-up to 24 months of age did not appreciably change the cumulative incidence of confirmed HIV compared to the 18-month estimate, suggesting administrative censoring was uninformative in the original analysis. Among infants whose mothers were newly enrolled, the 24-month cumulative incidence of HIV was 15% (95% CL: 11, 19%) for infants enrolled in 2007-2008, 12% (95% CL: 10, 15%) for infants enrolled in 2009-2010, and 12% (95% CL: 9, 16%) for infants enrolled in 2011-2012. Among infants whose mothers were previously enrolled, the 24-month estimate was 5% (95% CL: 0, 21%) for infants enrolled in 2007-2008, 7% (95% CL: 3, 14%) for infants enrolled in 2009-2010, and 1% (95% CL: 0, 03%) for infants enrolled in 2011-2012.

Our second sensitivity analysis suggested that our estimates of the 18-month cumulative incidence of HIV may be susceptible to bias due to LTFU. Among all infants, the 18-month cumulative incidence of HIV was 11% (95% CL: 9, 13%). Assuming all infants who were LTFU were instead confirmed HIV-positive, the estimate was 26% (95% CL: 24, 28%). Assuming all LTFU infants were HIV-negative and “graduated” from care at 18 months of age, the estimate was 9% (95% CL: 8, 11%).

There appeared to be some differences in infant outcomes between the two sites included in the analysis. The only differences that were statistically significant (i.e. Gray’s p -value < 0.05) were specimen collection for HIV virological testing and confirmed HIV infection. At the pediatric hospital, the 18-month cumulative incidence of specimen collection was 82% (95% CI: 78, 85%) and the 18-month cumulative incidence of HIV was 12% (95% CI: 8, 16%). At the primary care center, the 18-month cumulative incidence of specimen collection was 94% (95%

CI: 93, 96%) and the 18-month cumulative incidence of confirmed HIV was 11% (95% CI: 9, 14%).

Section 4.4. Discussion

Our program's experience in Kinshasa shows that improvements over time can be achieved in a routine care setting under evolving guidelines for care. Among infants whose mothers were newly enrolled during their most recent pregnancy, we observed declines in both vertical HIV transmission and infant death. The 18-month cumulative incidence of HIV was 30% lower among infants enrolled in 2011-2012 than among infants enrolled in 2007-2008. The 18-month cumulative incidence of death declined by 60%. Among HIV-infected infants, we also observed an increase in the proportion initiating cART, with over 95% of infants enrolled in 2011-2012 initiating cART by 24 months of age.

However, despite these achievements, our results also reveal areas for improvement. Among infants in the 2011-2012 cohort whose mothers were newly enrolled, the proportion with confirmed HIV infection at 18 months of age was still over 10% and our sensitivity analyses suggested that this may be an underestimate if those who were LTFU were at higher risk of HIV than those who were retained in care. Effective interventions can reduce transmission to below 5% in breastfeeding populations [34]. In our study, about 20% of mothers had not received a PMTCT prophylactic regimen or cART by delivery. Furthermore, most HIV-infected infants enrolled in 2011-2012 still did not have their positive status confirmed until after nine months of age and 21% of all infants died or were LTFU before their HIV status could be determined. The proportion of infants presenting to care in poor health also remained high throughout the study period, with about 20% underweight and 20% stunted overall.

EID by virological testing did not become consistently available in Kinshasa until about halfway through the study period. An important limitation to our study is that the UNC-DRC program did not routinely collect information on how much time passed between specimen collection for virological testing and when results became available for clinical decision-making. Although the 18-month cumulative incidence of specimen collection increased to almost 100% over the study period, many infants failed to have a specimen collected by the 4-6 week target [15]. By two months of age, about 40% of infants with newly enrolled mothers still had not had a specimen collected. This figure leaves ample room for improvement, but is substantially higher than that reported for the country as a whole – a recent UNAIDS report estimated that only 3% of HIV-exposed infants in the DRC received a virological test for HIV by two months of age [1].

Diagnosing HIV in infected infants as early as possible is crucial because up to a third may die in the first year of life without cART [7–9,14]. We observed that the median months from enrollment in care to cART initiation decreased from 12 months among infants enrolled in 2007-2008 to 4-5 months for infants enrolled in later periods, likely due to the increased availability of EID. However, half of HIV-infected infants enrolled in 2011-2012 had not initiated cART by 9 months of age and over a quarter had not initiated cART by 12 months of age. There are likely several reasons why infants continue to experience delayed cART initiation despite the availability of EID. In our setting, one reason may have been that HIV-infected infants enrolled in care later than the population of all HIV-exposed infants. Although median age at enrollment was only three weeks greater, over a quarter of HIV-infected infants enrolled in care after 32 weeks of age.

The number of studies describing obstacles to EID have increased [35–38], but a recent meta-analysis found that only one study traced HIV-infected infants from enrollment in PMTCT care to cART initiation [39]. That study, which included 202 HIV-infected infants in Malawi, observed that although the delay in cART initiation declined over time, the overall proportion of HIV-infected infants initiating cART did not improve [38]. It is crucial that PMTCT programs routinely record individual-level information at each step of the test-and-treat cascade and future research should assess where and why bottlenecks to the provision of early cART exist. Initiating the EID process at birth, particularly for high-risk infants, may also decrease delays in cART initiation [40].

Research has shown that infant nevirapine, a component of the PMTCT strategies Options A, B, and B+, can select non-nucleoside reverse transcriptase inhibitor mutations in the majority of infants who become infected with HIV despite prophylaxis [41]. Diagnosing HIV in infected infants early reduces exposure to nevirapine prophylaxis and thus the potential for HIV drug resistance. Given existing challenges to EID implementation in many settings, future studies should assess how HIV drug resistance and other treatment outcomes of HIV-infected infants change with the implementation of new PMTCT guidelines.

Unlike other outcomes assessed in this study, LTFU did not improve over calendar time and even appeared to increase among infants whose mothers enrolled in HIV care before their current pregnancy (although the increase was not statistically significant). The new consolidated WHO guidelines [15], which recommend interventions to improve program retention, may reduce obstacles to continued engagement in care. The UNC-DRC program increased its efforts to track patients who missed appointments over the study period, but it does not appear that these efforts led to a reduction in the proportion of infants who were LTFU. Research suggests that

infants are less likely to be retained in care if their caregivers perceive them as healthy and more likely to be retained in care if they are identified as HIV-infected [42,43]. Thus, it is possible that the implementation of EID and the communication of initial negative test results, as well as the implementation of more effective prophylactic regimens, also affected the proportion of infants who were LTFU. In addition, increased tracking efforts likely led to infants being classified as LTFU more quickly in later periods than in earlier periods, potentially leading to some degree of bias with regards to ascertaining infant mortality or HIV infection status.

Most of the LTFU we observed occurred in the first six months after infants enrolled, with about 5% never returning for a follow-up visit. A recent meta-analysis that assessed the magnitude of LTFU of HIV-exposed infants along the PMTCT cascade also reported high LTFU early in follow-up, with 4-75% LTFU by three months of age [44]. Interventions to improve retention should be implemented at the first contact. Point-of-care services may also improve retention [45,46].

We observed that infants whose mothers were newly enrolled into care generally had worse outcomes than infants whose mothers who were already enrolled in care when they became pregnant. Retaining mothers in care beyond the pregnancy and postnatal period may improve outcomes among subsequent HIV-exposed infants. As cART has been associated with reduced LTFU among HIV-infected adults [47,48], increasing access to lifelong cART for pregnant women could facilitate retaining mothers in care.

It is important to note that the infants included in this study and their mothers received PMTCT and cART services at centralized sites providing comprehensive HIV care. PMTCT services may not be delivered as effectively at sites in Kinshasa providing decentralized services [49]. The sites in this study provided routine care services, were integrated into the existing

healthcare system, and were supervised by the government. In addition, they served a patient population that was likely representative of other patient populations in Kinshasa. However, technical assistance of the caliber provided by the UNC-DRC program is not routinely available in most settings. Quality assurance was an explicit program priority and may have contributed to the improvements we observed [50].

We believe our program's results are more or less representative of what can be achieved by other programs that provide technical assistance to clinics delivering PMTCT services. However, specific implementation challenges will likely vary between settings. Even within our own program, we observed differences in some infant outcomes that suggested there were differences between the two sites in how effectively EID and other PMTCT services were implemented. It is important that programs assess outcomes disaggregated by clinic in order to assure quality improvement activities are appropriately targeted, even if aggregated program results are ultimately disseminated.

Another major evolution in the care of HIV-exposed infants is occurring now with the worldwide scale-up of the 2013 WHO guidelines that endorse lifelong cART for all pregnant women (Option B+) [15]. Although PMTCT programs should continue to adopt new guidelines as they become available, they should also pay close attention to the quality of care provided and routinely monitor intended program outcomes. We observed encouraging improvements in the outcomes of HIV-exposed infants in Kinshasa, but there remains abundant progress to be made. Modifiable barriers to delivering interventions in routine care settings need to be identified and addressed, and we should continue to evaluate our progress over time.

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CHAPTER 5: PROVIDING COMBINATION ANTIRETROVIRAL THERAPY TO HIV-INFECTED MOTHERS DECREASES LOSS TO FOLLOW-UP AMONG THEIR HIV-EXPOSED INFANTS IN KINSHASA, DR CONGO: A COHORT STUDY

Section 5.1. Introduction

Although prevention of mother-to-child HIV transmission (PMTCT) programs have been scaled up worldwide, an estimated 260,000 children continue to be infected with HIV each year [1]. The ongoing pediatric HIV epidemic and associated mortality is driven in part by the overwhelming number of HIV-exposed infants who are lost to follow-up (LTFU) from PMTCT care [2–10]. A recent meta-analysis of 11 studies conducted in sub-Saharan Africa estimated that 34% of HIV-exposed infants are lost from care by three months of age, with some settings reporting over 70% LTFU [11]. Despite the availability of antiretroviral regimens that can reduce vertical HIV transmission to below five percent [12], only a marginal impact on population-level transmission can be achieved if program retention remains low [13].

At the clinical level, ensuring HIV-exposed infants are retained in care is necessary to administer HIV tests, provide prophylactic drug regimens, monitor breastfeeding, and provide other services such as vaccinations. LTFU of HIV-exposed infants also impedes early initiation of combination antiretroviral therapy (cART) for HIV-infected infants. Early cART initiation is critical because, without treatment, a third of infants will die within the first year of life and one half within two years [14–17].

Despite the importance of infant retention for increasing the population-level impacts of PMTCT interventions, providing clinical interventions, and improving outcomes such as early

cART initiation for HIV-infected infants, few modifiable risk factors for infant LTFU have been identified. There is evidence that adult patients receiving cART are less likely to be LTFU than patients not on cART [18,19]. As HIV-exposed infants are dependent on their caregivers to bring them to care, this evidence also suggests that provision of cART to HIV-infected caregivers may play a role in the retention of their HIV-exposed infants. The goal of this study was to quantify the effect of maternal cART on LTFU of HIV-exposed infants receiving PMTCT care in Kinshasa, Democratic Republic of Congo (DRC). We hypothesized that HIV-exposed infants whose mothers receive cART experience less LTFU than infants whose mothers do not receive cART.

Section 5.2. Methods

Study population

We used data from HIV-exposed infants who received care between January 1, 2007 and July 31, 2013 in a family-centered HIV prevention, care, and treatment program implemented at two sites in Kinshasa affiliated with the University of North Carolina at Chapel Hill (UNC-DRC program). The two centralized sites provided comprehensive care (including PMTCT services) to HIV-positive women identified through routine HIV testing and their newborn infants at up to 90 maternities, former patients at 32 TB clinics, and HIV-positive children, as well as the HIV-positive first-line family members of these individuals. Enrollees were classified as ‘exposed infants’ if they were <18 months of age at the time of enrollment and did not yet have a confirmed HIV-positive diagnosis. HIV exposure was confirmed by a positive HIV antibody test in the mother or in the infant at <18 months of age.

We linked routinely collected data from HIV-exposed infants with data from their mothers to construct a cohort of mother-infant pairs. So that all infants could experience the entire 18-month follow-up period, we only included infants who were enrolled before January 1, 2012. Infants enrolled after 18 months of age and those who could not be matched to a mother receiving care in the UNC-DRC program by the time of infant enrollment were excluded. Since duration of maternal enrollment in care may be an important confounder and modifier of the relationship between maternal cART status and infant LTFU, we also opted to exclude infants whose mothers enrolled in the UNC-DRC program before their most recent pregnancy.

Clinic visits for HIV-exposed infants were scheduled to occur every four weeks from the first visit at two weeks of age through 18 weeks of age and then every three months thereafter, with additional visits scheduled as clinically needed. Infants were considered confirmed HIV-negative and deactivated from care if they received a negative HIV virologic or serologic test result obtained more than three months after the cessation of breastfeeding. HIV-exposed infants diagnosed with HIV were eligible to receive ongoing HIV care and treatment in the UNC-DRC program [20,21]. For infants <18 months of age, HIV was diagnosed by virologic testing, with initial testing at six weeks of age and confirmatory virologic testing of all positive results performed on a second specimen. DNA PCR assays were implemented in Kinshasa in November 2009. Before that time, HIV RNA assays were the only available virologic HIV test and stock outs were frequent. Additional details on the program background and services provided to HIV-exposed infants are published elsewhere [20–22].

Definitions and statistical analysis

Baseline was defined as infant enrollment in the UNC-DRC program and the outcome of interest was LTFU before final confirmation of an HIV-positive or -negative status. Infants were considered LTFU on their last attended clinic visit date following three failed tracking attempts after a missed appointment or if more than six months passed since they were last seen in the clinic. The cutoff of six months was chosen based on empirical evidence from adult HIV treatment programs, which suggests that a cutoff of six months from the last attended clinic visit results in the least amount of outcome misclassification [23]. Infants without a follow-up visit beyond the enrollment visit were assigned a survival time of one day, as has been done in previous studies of LTFU in order to avoid excluding these infants from the analyses entirely [24].

The primary exposure was baseline maternal cART status. The reference group included infants whose mothers initiated lifelong cART on or before the day of infant enrollment. cART was provided in accordance with World Health Organization (WHO) guidelines for pregnant and breastfeeding women in effect at the time. Before 2010, all women with a CD4 cell count <200 cells/mm³ were eligible to receive cART [25]. In 2010, the immunological cutoff increased to 350 cells/mm³ [26].

Demographics of infants and their mothers at infant enrollment were characterized using standard descriptive statistics. Infants were considered underweight if they had a weight-for-age Z-score (WAZ) and stunted if they had a height-for-age Z-score (HAZ) more than two standard deviations below the median value for a given age group and sex. Z-scores were derived from the WHO Child Growth Standards [27] using the WHO Anthro software (version 3.2.2, January 2011) and SAS macro. Growth status was based on the first available weight or height values

measured within a month of enrollment. Maternal CD4 counts were based on the first available measurement within three months of infant enrollment.

Accounting for competing risks [28], the 18-month cumulative incidence of LTFU was estimated within strata of maternal cART status and baseline covariates. To quantify the effect of maternal cART on LTFU, we used the SAS macro %PSHREG [29] to implement the proportional subdistribution hazards model of Fine and Gray [28,30]. Crude and covariate-adjusted hazard ratios (HR), as well as corresponding 95% confidence limits (CL), were generated. In these analyses, follow-up began at enrollment of the infant and continued until the first of the following occurred: the event of interest (LTFU), a competing event (death or graduation from care), or a censoring event (18 months of age or August 2013). Graduation from care was defined as being deactivated from care after confirmation of an HIV-negative status or transfer to ongoing HIV care and treatment following confirmation of an HIV-positive status. Because the Cox proportional hazards model is more widely implemented than the Fine and Gray model, we also provide cause-specific HRs estimated from a Cox model for comparison to the subdistribution HRs estimated from the Fine and Gray model in the primary analysis.

Potential confounding factors were assessed via a directed acyclic graph [31] and included the baseline variables infant age, infant WAZ, maternal age, maternal CD4 count, maternal enrollment duration, and time since the beginning of the study (defined as days between January 1, 2007 and the date of infant enrollment). The covariates were modeled with Stone and Koo's additive splines constrained to be linear in the tails, with knots at the 5th, 35th, 65th, and 95th percentiles [32]. Missing values for covariates were imputed [33] using the sequential regression method described by Raghunathan et al. [34] and implemented in the IVEware 0.2

software system (Institute for Social Research, University of Michigan, Ann Arbor, Michigan). Five imputed datasets were generated. To assess whether our results were sensitive to imputation assumptions, we also conducted a complete case analysis. A Firth correction [35] was applied because of the small number of events that occurred among some subgroups in stratified analyses. All analyses were conducted in SAS 9.3 (SAS Institute, Inc., Cary, North Carolina).

Secondary analyses

The current PMTCT strategies recommended by the WHO (Options B and B+) imply providing cART to all HIV-infected women regardless of previously established immunological criteria. Therefore, it is also important to know whether providing HIV-infected mothers with cART reduces LTFU of their HIV-exposed infants regardless of the health status of the mother. To inform this question, we secondarily assessed the HR for the effect of maternal cART on infant LTFU stratified by maternal CD4 cell count (<350 or 350+) at infant enrollment.

Our primary hypothesis was that maternal cART would decrease LTFU of HIV-exposed infants by increasing retention of their mothers. To further explore this hypothesis, we conducted a secondary analysis to assess LTFU of mothers during the period of infant follow-up. We estimated the cumulative incidence of maternal LTFU during the period of infant enrollment, as well as the subdistribution hazard ratio for the effect of maternal cART on maternal LTFU. In these analyses, baseline was defined as enrollment of the infant and follow-up continued until the event of interest (maternal LTFU), a competing event (death), or a censoring event (end of infant follow-up or transfer of care to another facility).

Ethics statement

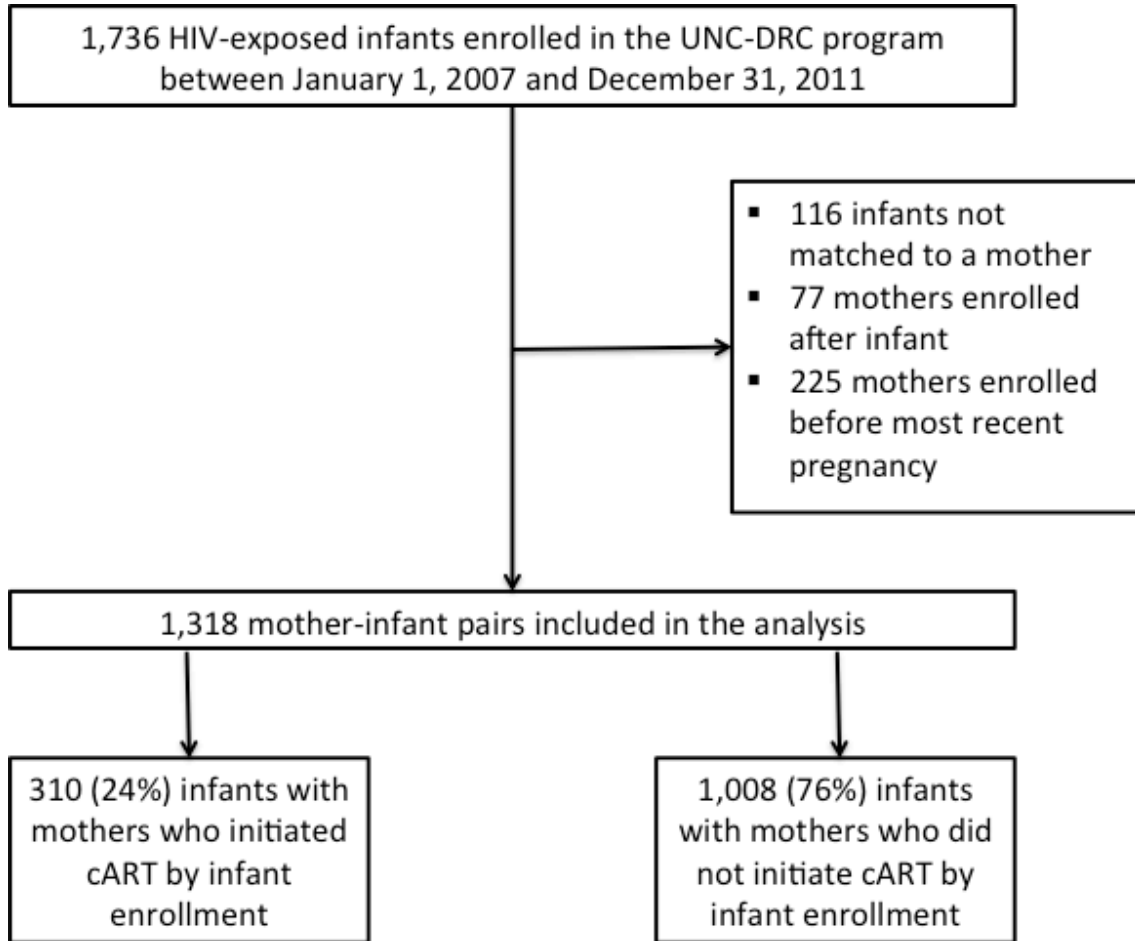
Written parental informed consent for the UNC-DRC program was obtained for all infants and written informed consent was obtained from all mothers. All research was approved by the Ethics Committee of the Kinshasa School of Public Health and the University of North Carolina at Chapel Hill Institutional Review Board.

Section 5.3. Results

1,736 HIV-exposed infants enrolled into care during the study enrollment period, of which 1,318 were included in the analysis after exclusion criteria were applied (Figure 5.1). Infants included in the analysis were enrolled at a median age of 2.6 weeks (interquartile range [IQR]: 2.1-6.9 weeks), at which time 89% were breastfeeding, 21% were underweight, and 24% were stunted (Table 5.1). 1,008 (76%) infants had mothers who had not yet initiated cART by infant enrollment (Figure 5.1).

Mothers were a median age of 31 years (IQR: 27-34 years) and had been enrolled in the UNC-DRC program for a median of 72 days (IQR: 0-126 days) at the time of infant enrollment (Table 5.1). The median enrollment duration was longer for mothers who initiated cART by infant enrollment than those who had not initiated cART (109 days versus 56 days). Baseline maternal CD4 count also differed between the exposure groups, with a higher proportion of mothers who initiated cART having a CD4 cell count <350 cells/mm³ than mothers who had not initiated cART (55% versus 30%). Mothers who initiated cART had been receiving cART for a median of 65 days (IQR: 32-102 days) by the time of infant enrollment.

Figure 5.1 - Flowchart depicting the study population.



Abbreviations: UNC, University of North Carolina at Chapel Hill; DRC, Democratic Republic of Congo; cART, combination antiretroviral therapy.

Table 5.1 - Characteristics of HIV-exposed infants and their mothers at infant enrollment into care, by maternal combination antiretroviral therapy status, Kinshasa, Democratic Republic of Congo.

	Maternal cART (N=310)	No Maternal cART (N=1,008)	Overall (N=1,318)
Median infant age in weeks (IQR)	2.3 (2.1, 3.3)	2.7 (2.1, 9.4)	2.6 (2.1, 6.9)
Calendar year at infant enrollment			
2007	15 (4.8)	112 (11.1)	127 (9.6)
2008	30 (9.7)	158 (15.7)	188 (14.3)
2009	65 (21.0)	279 (27.7)	344 (26.1)
2010	68 (21.9)	224 (22.2)	292 (22.2)
2011	132 (42.6)	235 (23.3)	367 (27.8)
Infant gender [N (%)]			
<i>Female</i>	156 (50.3)	513 (50.9)	669 (50.8)
<i>Male</i>	154 (49.7)	495 (49.1)	649 (49.2)
Any PMTCT regimen [N (%)]			
<i>Yes</i>	307 (99.0)	786 (86.1)	1093 (89.4)
<i>No</i>	3 (1.0)	127 (13.9)	130 (10.6)
Breastfeeding			
<i>Yes</i>	222 (90.6)	628 (89.0)	850 (89.4)
<i>No</i>	23 (9.4)	78 (11.0)	101 (10.6)

	Maternal cART (N=310)	No Maternal cART (N=1,008)	Overall (N=1,318)
Infant underweight			
<i>Yes</i>	57 (19.1)	210 (21.5)	267 (21.0)
<i>No</i>	241 (80.9)	765 (78.5)	1006 (79.0)
Infant stunted			
<i>Yes</i>	66 (22.2)	236 (24.3)	302 (23.8)
<i>No</i>	231 (77.8)	737 (75.7)	968 (76.2)
Median maternal age in years [IQR]	32 (29-35)	30 (26-34)	31 (27-34)
Median days mother enrolled [IQR]	109 (74-141)	56 (0-115)	72 (0-126)
Median days mother on cART	65 (32-102)	N/A	N/A
Maternal CD4 count			
<350 <i>cells/mm</i> ³	163 (54.9)	278 (29.6)	441 (35.7)
350+ <i>cells/mm</i> ³	134 (45.1)	661 (70.4)	795 (64.3)

Abbreviations: cART, combination antiretroviral therapy; IQR, interquartile range; PMTCT, prevention of mother-to-child HIV transmission

Overall, 238 infants were LTFU over 13,221 person-months of follow-up and the 18-month cumulative incidence of LTFU was 19% (95% CL: 16, 21%). Many infants (5% [95% CL: 4, 7%]) never returned for a follow-up visit after enrolling in care, 9% (95% CL: 8, 11%) were LTFU within three months, and 13% (95% CL: 11, 15%) were LTFU within six months. The 18-month cumulative incidence of LTFU was 9% (95% CL: 6, 13%) among infants whose mothers had initiated cART by baseline and 22% (95% CL: 19, 24%) among infants whose mothers had not initiated cART by baseline (Gray's test for equality p -value <0.0001) (Table 5.2, Figure 5.2).

An assessment of the 18-month cumulative incidence of LTFU within strata of baseline covariates suggested that older infant enrollment age, infant growth stunting, younger maternal age, and shorter maternal duration in care were associated with increased LTFU (Table 5.2). However, these associations were not statistically significant at an alpha level of 0.05. There did not appear to be differences in the 18-month cumulative incidences of LTFU between strata of other covariates, including maternal CD4 cell count.

Figure 5.2 - 18-month cumulative incidences of loss to follow-up among HIV-exposed infants in Kinshasa, Democratic Republic of Congo, plotted by maternal combination antiretroviral therapy (cART) status at infant enrollment into care.

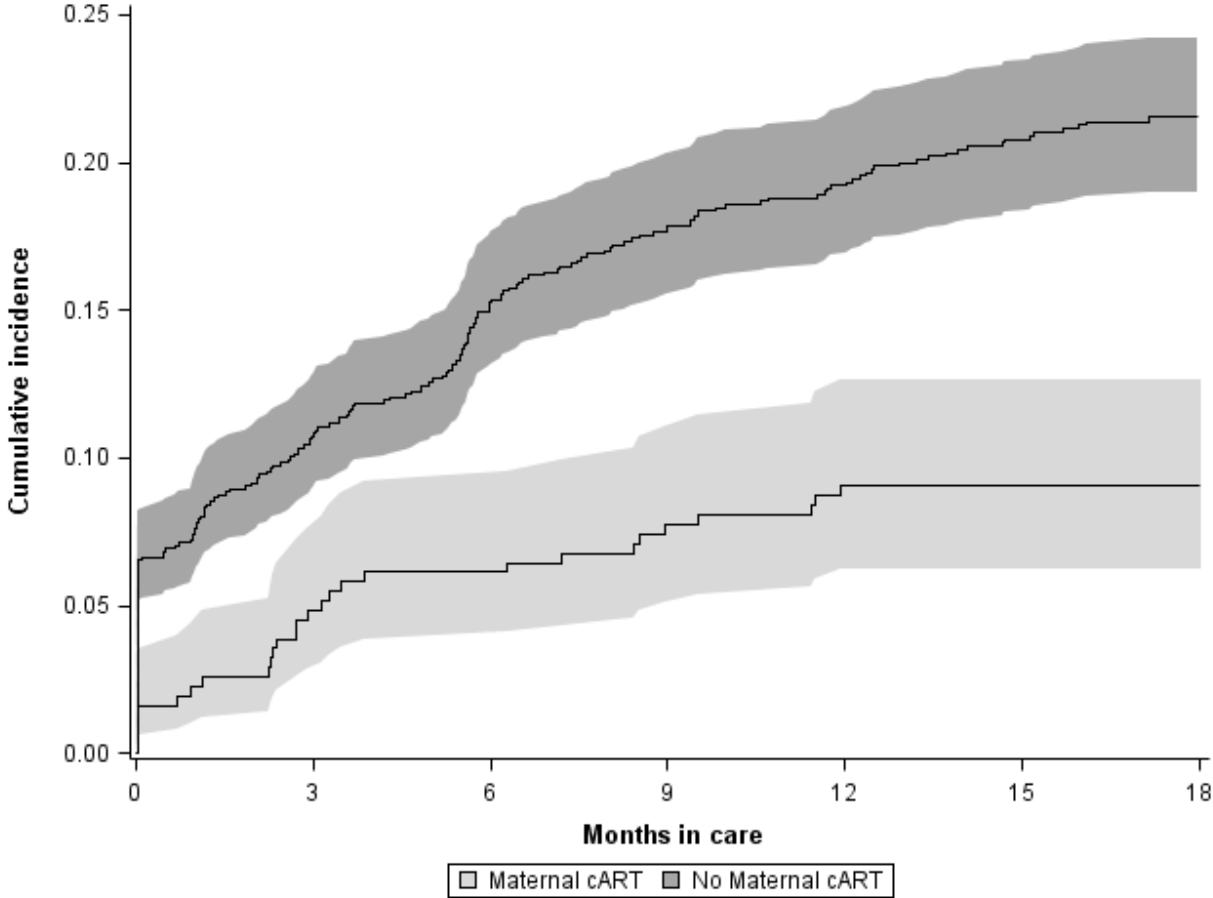


Table 5.2 - 18-month cumulative incidence of loss to follow-up^a within strata of maternal combination antiretroviral therapy status and baseline covariates, Kinshasa, Democratic Republic of Congo.

	Cumulative incidence	95% Confidence limit
Maternal cART by infant enrollment		
<i>Yes</i>	9.1	(6.2, 12.6)
<i>No</i>	21.5	(19.0, 24.2)
Age at infant enrollment		
<i><4 weeks</i>	17.3	(14.8, 19.9)
<i>4+ weeks</i>	20.6	(17.0, 24.6)
Calendar year at infant enrollment		
<i>2007-2008</i>	18.5	(14.4, 23.0)
<i>2009-2011</i>	18.5	(16.2, 21.0)
Gender		
<i>Female</i>	19.0	(16.1, 22.1)
<i>Male</i>	18.0	(15.1, 21.1)
Breastfeeding		
<i>Yes</i>	18.6	(16.2, 20.9)
<i>No</i>	17.9	(10.2, 25.7)
Underweight		
<i>Yes</i>	19.6	(14.8, 24.4)
<i>No</i>	18.2	(15.8, 20.6)
Stunted		

	Cumulative incidence	95% Confidence limit
<i>Yes</i>	21.1	(16.4, 25.8)
<i>No</i>	17.7	(15.3, 20.2)
Age of mother at infant enrollment		
<i><30 years</i>	20.7	(17.5, 24.0)
<i>30+ years</i>	16.8	(14.1, 19.6)
Enrollment duration of mother at infant enrollment		
<i><3 months</i>	19.9	(17.2, 22.9)
<i>3+ months</i>	16.5	(13.5, 19.8)
Maternal CD4 count at infant enrollment		
<i><350 cells/mm³</i>	17.7	(13.9, 21.5)
<i>350+ cells/mm³</i>	18.9	(16.2, 21.7)

Abbreviations: cART, combination antiretroviral therapy; PMTCT, prevention of mother-to-child transmission of HIV

^a Infants were considered LTFU on their last attended clinic visit date following three failed tracking attempts after a missed appointment or if more than six months passed since they were last seen in the clinic.

In the unadjusted Fine and Gray subdistribution hazards model, the HR comparing LTFU among infants whose mothers did not receive cART by infant enrollment to those whose mothers did receive cART by infant enrollment was 2.48 (95% CL: 1.71, 3.74) (Table 5.3). Without imputing missing data, the covariate-adjusted HR was 3.20 (95% CL: 2.00, 5.33). After multiple imputation was used to account for missing covariate data, the covariate-adjusted HR was 2.76 (95% CL: 1.79, 4.26). Using Cox regression, the unadjusted HR was 2.73 (95% CL: 1.88, 4.12), the covariate-adjusted HR excluding infants with missing covariate data was 3.27 (95% CL: 2.05, 5.46), and the covariate-adjusted HR using multiple imputation for missing data was 2.91 (95% CL: 1.93, 4.56).

In stratified analyses, we observed a protective effect of providing HIV-infected mothers with cART on LTFU of their HIV-exposed infants among both maternal CD4 groups (<350 and 350+) (Table 5.3). The effect appeared to be stronger among infants whose mothers had a CD4 cell count 350 and above (HR: 3.96 [95% CL: 1.73, 9.06]) at the time of infant enrollment compared to infants whose mothers had a CD4 cell count below 350 (HR: 2.00 [95%CI: 1.16, 3.46]), but the difference was not statistically significant.

Table 5.3 - Estimated effect of maternal combination antiretroviral therapy on loss to follow-up of HIV-exposed infants in Kinshasa, Democratic Republic of Congo.

	LTFU (N) ^a	Person-months of follow-up	Subdistribution hazard ratio	95% Confidence limit
<u>Unadjusted Fine and Gray model</u>				
No maternal cART	210	9426	2.48	(1.71, 3.74)
Maternal cART	28	3795	1.	
<u>Covariate-adjusted Fine and Gray model, complete case^b</u>				
No maternal cART	175	8616	3.20	(2.00, 5.33)
Maternal cART	21	3548	1.	
<u>Covariate-adjusted Fine and Gray model, multiple imputation^c</u>				
No maternal cART	210	9426	2.76	(1.79, 4.26)
Maternal cART	28	3795	1.	
<u>Covariate-adjusted Fine and Gray model, multiple imputation, stratified by maternal CD4^{d,e}</u>				
CD4 <350, no maternal cART	60	2230	2.00	(1.16, 3.46)
CD4 <350, maternal cART	20	1911		

CD4 350+, no maternal cART	150	7196	3.96	(1.73, 9.06)
CD4 350+, maternal cART	8	1884		

Abbreviations: cART, combination antiretroviral therapy; LTFU, loss to follow-up

^a Infants were considered LTFU on their last attended clinic visit date following three failed tracking attempts after a missed appointment or if more than six months passed since they were last seen in the clinic.

^b Fine and Gray subdistribution proportional hazards model adjusted for baseline variables, including infant age, infant WAZ, maternal age, maternal CD4 count, maternal enrollment duration, and calendar time. Infants with missing covariate data (N=122) were excluded from the analysis.

^c Fine and Gray subdistribution proportional hazards model adjusted for baseline variables, including infant age, infant WAZ, maternal age, maternal CD4 count, maternal enrollment duration, and calendar time. Missing data were imputed.

^d Fine and Gray subdistribution proportional hazards model adjusted for baseline variables, including infant age, infant WAZ, maternal age, maternal enrollment duration, and calendar time. Missing data were imputed.

^e Loss to follow-up counts and person-months of follow-up are the average from 5 imputed datasets.

During the infant follow-up period, the overall cumulative incidence of maternal LTFU was 26% (95% CL: 24, 28%). The cumulative incidence of maternal LTFU was 13% (95% CL: 10, 17%) among mothers who initiated cART by infant enrollment and 30% (95% CL: 27, 33%) among mothers who had not yet started cART by infant enrollment (Gray's test for equality p -value <0.0001). Adjusted for the same baseline factors as in the infant LTFU analysis, the subdistribution hazard ratio for the effect of providing HIV-infected mothers on maternal LTFU was 2.04 (95% CL: 1.43, 2.91).

Section 5.4. Discussion

Although it is clear that LTFU of HIV-exposed infants is an ongoing problem, research has yet to identify clear implementation strategies to improve infant retention. In a large cohort of HIV-exposed infants in Kinshasa, DRC, we found that infants whose mothers had not yet initiated cART by infant enrollment were more than twice as likely to be LTFU than infants whose mothers had initiated cART. These results suggest that providing cART to HIV-infected mothers could lead to improvements in the implementation of PMTCT care for HIV-exposed infants, including early infant diagnosis (EID) and timely cART initiation for those that are positive.

To our knowledge, this is the first study to report the cumulative incidence of infant LTFU within strata of maternal cART status and the first to provide an estimate of the causal effect of maternal cART on LTFU of HIV-exposed infants. Two prior studies suggested that maternal cART is predictive of successful EID of HIV. The first study, which included 217 mother-infant pairs from Mozambique, found that mothers who received cART were more likely to ever bring their infant for a virological test compared to women who did not receive cART,

with an odds ratio of 3.15 (95% CL: 1.02-9.73) controlling for self-reported socio-demographic factors [36]. A larger study that included 1,587 HIV-exposed infants in Cameroon examined the association between multiple predictors and having an incomplete EID process by seven months of age [37]. Controlling for clinical site, maternal education, time since maternal HIV diagnosis, mode of delivery, and multiple birth status, the authors found that women who received no prophylaxis (OR: 2.3 [95% CL: 1.2-4.1]) or short-course prophylaxis (OR: 1.4 [95% CL: 0.9-2.1]) were more likely to have an incomplete EID process compared to women who received cART.

While those prior studies provide evidence for an association between maternal cART and EID, HIV-exposed infant care involves more than a single testing event due to continued exposure to HIV through breastfeeding [38]. Regular follow-up of HIV-exposed infants is needed to administer additional HIV tests, communicate test results to caregivers, provide prophylactic drugs, and monitor breastfeeding. An important strength of our study is that we assessed attrition through the entire HIV-exposed infant care cascade. Our findings suggest that there is a strong beneficial effect of maternal cART on infant retention and that it persists beyond the initial HIV testing event. We observed that most infants who were LTFU were last seen at the clinic in the first six months of infant enrollment. Other studies have also described high rates of LTFU within the first few months [11]. To have the greatest impact on LTFU of HIV-exposed infants, HIV-infected mothers should be started on cART as early as possible.

Other strengths of our study are the large sample size, relatively long follow-up time, and use of competing risk analyses. Failure to account for competing risks, which are common in longitudinal studies, has been shown to overestimate the risk of experiencing the event of interest, particularly when the risk of experiencing the competing event is high [39]. Whether or

not providing HIV-infected mothers with cART predicts LTFU of their HIV-exposed infants is a function of both the effect of maternal cART on infant LTFU and the effect of maternal cART on the competing events (e.g. death) [28]. We chose to use the subdistribution hazards model described by Fine and Gray in our primary analysis over a traditional Cox regression model because doing so allowed us to interpret the hazard ratio as a measure of risk without the added assumption that the event of interest (LTFU) was independent of the competing events (death and graduation from care). Since competing risks are common in routine care settings, we consider the subdistribution HR more informative for policy decisions about PMTCT strategies than the cause-specific HR from a Cox model. However, as the distribution of competing events may vary between populations, we chose to additionally report cause-specific HRs. In our study, the subdistribution and cause-specific HRs were similar.

A limitation of our study is that we were only able to estimate the association between maternal cART and infant LTFU among infants whose mothers newly enrolled into care. We chose to exclude infants whose mothers were enrolled before their most recent pregnancy because we assumed that the effect of providing maternal cART on infant LTFU would be different for infants of mothers who were stable and in care compared to the population of infants of all newly enrolled mothers. Although ideally we would have stratified our results by maternal enrollment duration (newly versus previously enrolled), the number of events among the 225 infants whose mothers were previously enrolled was too few (21 LTFU in 2,894 person-months of follow-up) to generate stratified estimates. As having a mother who is stable and in care appears to result in low infant LTFU, providing cART to these mothers would not likely reduce infant LTFU to the degree observed among infants of all newly enrolled mothers.

It is also important to note that the mother-infant pairs in this study received PMTCT services at centralized sites providing family-centered comprehensive HIV care. The effect of maternal cART on infant LTFU may not be as strong in settings where infants do not receive care at the same site as their mothers. Although our results are also not directly generalizable to programs providing decentralized care, we suspect that a similar reduction in infant LTFU could be achieved by providing newly enrolled mothers cART in that setting. Future studies should assess the effect of maternal cART on infant LTFU in decentralized care. In addition, we excluded 116 infants who could not be matched to a mother also receiving care in the UNC-DRC program. Some of these mothers may have been receiving HIV care at a different healthcare facility, but it is more likely that most of the excluded infants were orphans. The orphan status of HIV-exposed infants should be routinely documented so that research can be conducted on how to provide optimal PMTCT care for HIV-exposed infants who may have lost one or more caregiver.

Since DRC was an “Option A” country during the study period, our referent group consisted of infants whose mothers were eligible for cART based on established immunologic or clinical criteria. On the other hand, the comparison group primarily consisted of infants whose mothers were not yet eligible for cART. We attempted to address this by controlling for maternal CD4 count in the analysis, but residual confounding may remain if there are additional differences affecting care-seeking behavior that we did not account for. We also examined stratified effect estimates and saw a strong protective effect of maternal cART on infant LTFU in both the low and high maternal CD4 groups. This suggests that providing HIV-infected mothers with cART irrespective of previously established immunological criteria (Options B and B+) may also improve infant retention. Unfortunately, the association between maternal cART and

infant retention will be difficult to assess in settings where all mothers receive cART due to the lack of a clear comparison group. Given that most countries have already or are moving towards implementing Options B or B+, it is important that we assess the impact that maternal cART has on infant outcomes while data are still current from Option A countries.

Little is known about other factors that contribute to LTFU among HIV-exposed infants. There is evidence to suggest that addressing structural barriers including cost, transportation, waiting time, and service quality may improve retention in the PMTCT setting [7,36,40–43]. Addressing issues involving fatalistic attitudes around pediatric HIV and reducing fear of stigma and discrimination may also be important [44]. Covariates in this study that appeared to predict infant LTFU were older infant enrollment age, infant growth stunting, younger maternal age, and maternal enrollment duration. Estimating the causal effects of these covariates on infant LTFU was beyond the scope of this analysis and should be explored in future studies.

Despite the fact that LTFU of HIV-exposed infants is an ongoing public health problem, it remains inadequately studied. One reason for this is that quality individual-level data from HIV-exposed infants are not widely available from field settings. The UNC-DRC program routinely collected prospective individual-level data on HIV-exposed infants and linked data between infants and their mothers. Such linkages are often difficult or impossible to obtain retrospectively, limiting the number of studies that are able to construct cohorts of mother-infant pairs in order to assess the impact that maternal factors have on infant outcomes. Routine quality improvement activities implemented at the UNC-DRC sites, including active tracking of patients who missed clinic appointments, also assured that the data included in the analyses were of high quality [45]. It is likely that the active tracking procedures also contributed to the relatively low proportions of LTFU we observed.

In conclusion, providing HIV-infected mothers with cART could increase retention in care of their HIV-exposed infants. This is an important collateral benefit that countries should consider as they make decisions around the provision of cART for all pregnant and breastfeeding women (Options B/B+) [46]. As LTFU remains an important barrier to delivering optimal care for HIV-exposed infants, explicit interventions to improve retention of HIV-exposed infants should be prioritized, particularly in settings where cART is not provided to all mothers.

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CHAPTER 6: CONCLUSIONS

Section 6.1. Summary of findings

We conducted an observational study of mother-infant pairs receiving PMTCT care in a comprehensive HIV prevention, care, and treatment program in Kinshasa, DRC between 2007 and 2013. There were two aims to the dissertation project. To fulfill the first aim, we produced a manuscript that described temporal changes in the outcomes of HIV-exposed infants since the implementation of HIV-exposed infant care in Kinshasa. To fulfill the second aim, we produced a manuscript that assessed the effect of providing cART to HIV-infected mothers on reducing LTFU among their HIV-exposed infants.

For Aim 1, we assessed five primary outcomes among 1707 HIV-exposed infants: first specimen collection for an HIV virologic test, confirmed HIV infection, death, LTFU, and cART initiation (HIV-infected infants only). Overall, we observed encouraging trends. The proportion of infants who had a specimen collected for an HIV virologic test by 18 months of age increased from 73% among infants enrolled during the first study period (2007-2008) to 99% among infants enrolled in the last study period (2011-2012). Likewise, the median age at specimen collection improved drastically, from 30 weeks among infants enrolled during 2007-2008 to 7 weeks among infants enrolled during 2011-2012. We also observed improvements in the outcomes confirmed HIV infection and death over time. The proportion with confirmed HIV infection by 18 months of age decreased by half, from 15% infected among those enrolled in the first study period to 8% among those enrolled in the last study period. Death declined from 8%

to 3%. The proportion of HIV-exposed infants who were LTFU did not improve over the study enrollment period, hovering between 13-15%. Although the proportion of HIV-exposed infants who were LTFU was relatively low compared to what has been observed in other settings, the stagnant trend in this outcome highlights the need for continued efforts to improve retention in PMTCT programs.

For the 129 HIV-infected infants assessed in the analysis, the proportion of infants who initiated cART by 24 month of age increased from only 61% to 97%. The median age at cART initiation declined from 18 month to 9 months. While this is an important improvement, 10 months is still relatively late for HIV-infected infants to initiate cART. Additional bottlenecks to the provision of early cART need to be identified and addressed.

We examined trends stratified by maternal enrollment status (enrolled in HIV care prior to most recent pregnancy or not) because of hypothesized differences in care-seeking behavior between these groups. Overall, we observed few negative outcomes among the group of infants whose mothers were enrolled in HIV care before their most recent pregnancy compared to the group of infants whose mothers were newly enrolled.

For Aim 2, we assessed LTFU among 1318 mother infant pairs, a subset of the mother-infant pairs included in Aim 1. Overall, 19% of infants were LTFU within 18 months of enrolling in care. We observed that infants whose mothers had not yet initiated cART by infant enrollment were more than twice as likely to be LTFU than infants whose mothers had initiated cART by infant enrollment. Accounting for competing risks (i.e. death and graduation from care), the 18-month cumulative incidence of LTFU was only 9% among infants whose mothers were on cART and 22% among infants whose mothers had not yet initiated cART. Adjusted for infant and maternal factors at baseline, the subdistribution hazard ratio was 2.76 (95% CL: 1.79,

4.26). For both the exposure (no maternal cART) and referent group (maternal cART), we observed that most LTFU occurred soon after infant enrollment. Five percent of infants never returned to care after their first clinic visit and 13% of infants were LTFU within the first six months. To have the greatest impact on infant LTFU, HIV-infected mothers should be provided with cART as soon as possible.

Section 6.2. Public health impact

There are several public health messages that the results of this dissertation project point to. A detailed assessment of the public health implications of each aim of the dissertation appears in the discussion sections of Chapter 4 (Aim 1) and Chapter 5 (Aim 2). In this section, we synthesize the key public health impacts of the dissertation project.

Aim 1

In the manuscript “Temporal changes in the outcomes of HIV-exposed infants in Kinshasa, DR Congo during a period of rapidly evolving guidelines for care (2007-2013),” we analyzed individual-level data on HIV-exposed infants and their mothers to assess changes in infant outcomes over time in a family-centered HIV program in Kinshasa, DRC. During the evaluation period (2007-2013), EID was scaled-up and guidelines for PMTCT evolved rapidly – the recommended breastfeeding period increased, prophylactic drug regimens became more complex, and criteria for initiating cART became more inclusive. Our results demonstrate that it is possible to achieve improvements in the outcomes of HIV-exposed infants over time in field conditions, but that high rates of HIV transmission, death, and program attrition, as well as

delayed cART initiation and diagnosis of HIV infections, continue despite implementation of revised PMTCT guidelines.

The results of Aim 1 are unique and significant for several reasons. First, to our knowledge, this is the first paper to provide a comprehensive assessment of how programmatic and clinical outcomes of HIV-exposed infants have changed over time in a resource-deprived setting. Evaluating and reporting outcomes in field conditions are critical to demonstrate the scalability of recommended interventions and to assure that quality care is being provided. Although HIV programs have implemented new PMTCT guidelines as they become available and the incidence of pediatric HIV is declining, we have little knowledge about the extent to which intended program outcomes for HIV-exposed infants have been achieved. As PMTCT programs are gearing up to implement the new 2013 consolidated guidelines [1], our evaluation is particularly timely.

In addition to filling a substantive gap in the literature, Aim 1 was also novel because we employed epidemiological methods that appropriately estimated the outcomes of interest in the presence of competing risks. Competing risk analyses are still uncommon in the literature, particularly in applied research studies. Failure to account for competing risks, which are common in longitudinal studies, has been shown to substantially bias estimates in many instances. We also provide results from sensitivity analyses to support our main findings. Thus, the manuscript for Aim 1 has clinical, programmatic, and methodological impact as well as local and global relevance.

Aim 2

In the manuscript “Providing Combination Antiretroviral Therapy to HIV-infected Mothers Decreases Loss to Follow-up Among Their HIV-exposed Infants in Kinshasa, DR

Congo: A Cohort Study,” we analyzed individual-level data from a large cohort of mother-infant pairs who received services for PMTCT in Kinshasa, DRC between 2007 and 2013. We assessed the impact of providing cART to HIV-infected mothers on reducing LTFU among their HIV-exposed infants. Our results demonstrated that infants whose mothers do not receive cART are more than twice as likely to be LTFU than infants whose mothers do receive cART.

The manuscript for Aim 2 is unique and significant for several reasons. First, to our knowledge, this is the first study to report the cumulative incidence of infant LTFU within strata of maternal cART status and the first to provide an estimate of the causal effect of maternal cART on LTFU of HIV-exposed infants. The magnitude of LTFU among HIV-exposed infants in most PMTCT programs is overwhelming, with some studies reporting over 70% LTFU [2]. Despite the importance of infant retention for increasing the impact of PMTCT programs, opportunities to provide early infant HIV diagnosis, and access to early cART initiation for HIV-infected infants, few modifiable risk factors for infant LTFU have been identified. Our findings suggest that there is a strong beneficial effect of maternal cART on infant retention and that it persists beyond the initial HIV testing event. This is an important collateral benefit for countries to consider as they decide whether to provide cART for all pregnant and breastfeeding women (Options B/B+), as was recently recommended by the World Health Organization [1]. Thus, the results of this analysis are particularly timely.

In addition to filling a substantive gap in the literature, the Aim 2 analysis was also novel because we employed epidemiological methods that appropriately estimated the effect of maternal cART on infant LTFU in the presence of competing risks (e.g. death). By accounting for competing risks, we did not have to make the assumption that the event of interest (LTFU) was independent of the competing events, as is required in order to interpret the hazard ratio

from a Cox proportional hazards model as a measure of risk. Although competing risks are common in longitudinal studies, competing risk analyses are still rare in the literature. This is likely because statistical software that readily provides estimates from such analyses are not widely available. We also provide results from sensitivity analyses to support our main findings. Thus, like Aim 1, the manuscript for Aim 2 has clinical, programmatic, and methodological impact as well as local and global relevance.

Section 6.3. Future research directions

There have been substantial advances in our understanding of outcomes among HIV-exposed infants in recent years, but continued research efforts are needed. An important strength of our study is that we assessed infant outcomes through the entire HIV-exposed infant care cascade. A recent meta-analysis found that only one study traced HIV-infected infants from enrollment in PMTCT care to cART initiation [3]. One reason for this is that quality individual-level data from HIV-exposed infants is not widely available from field settings. Even less frequently available is information that can be used to link data from infants with data from their mothers.

The limited availability of quality individual-level data from HIV-exposed infants and their mothers means that HIV-exposed infant outcomes remain inadequately studied, particularly in field settings. To remedy this, PMTCT programs should be encouraged to routinely record individual-level information at each step of the test-and-treat cascade and future research should assess if intended program outcomes for HIV-exposed infants are being achieved and where bottlenecks to the provision of quality care exist. Explicit guidelines on how to implement high quality routine data collection and monitoring for HIV-exposed infants should be developed.

Although implementation research is increasingly being recognized as a priority research area, such questions will be difficult to address without first increasing the availability of quality data.

This study focused on outcomes of HIV-exposed infants receiving family-centered care at centralized sites providing comprehensive HIV prevention, care and treatment. In this regard, two future research directions seem particularly important to highlight. First, given the trend towards decentralization of HIV services, it has become increasingly important to understand the impact decentralization has on outcomes of HIV-exposed infants. Second, future research should seek to better understand the effect family-centered care (as opposed to care provided at a facility different than where other HIV-affected family members are receiving their care) has on HIV-exposed infant outcomes. In our study, we excluded infants who could not be matched to a mother also receiving care in the UNC-DRC program. Some of these mothers may have been receiving HIV care at a different healthcare facility, but it is more likely that most of the excluded infants were orphans. More research is needed on how to provide optimal PMTCT care for HIV-exposed infants who may have lost one or more caregiver.

Most PMTCT programs struggle to maintain HIV-exposed infants in care through the entire exposure period, which depends on breastfeeding cessation and typically lasts until about 12-24 months of age. Thus, the follow-up period assessed in this study (18 months) is considered “relatively long” by most current standards. However, HIV exposure is only one of many challenges faced by children living in families affected by HIV. More research is needed to describe outcomes of HIV-exposed infants across the life course and to understanding the relationship between the unique biological, social, economic, and environmental determinants of health that affect this growing population.

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