SURVIVAL OUTCOMES OF HIV EXPOSED AND HIV INFECTED CHILDREN

by

JANE NAMANGOLWA MUTANGA

(Under the Direction of Christopher C. Whalen)

ABSTRACT

Statement of the Problem: Despite expanded pediatric antiretroviral therapy (ART) coverage, there is paucity of information on effectiveness of the pediatric ART programs in reducing mortality and morbidity among HIV infected children in high HIV burden settings. The **goal** of this research is to evaluate treatment outcomes and generate program relevant information to improve ART outcomes for infants and children in Zambia and similar settings.

Methods: Using a retrospective cohort study design, we abstracted data from medical records of HIV infected children who received ART and HIV exposed infants who had dry blood spots done for HIV diagnosis using DNA-PCR at Livingstone Central Hospital in Zambia. We carried out descriptive analysis and estimated distributions of survival times for the baseline covariates using the Kaplan-Meier (KM) method. Log-rank tests were used to compare the survival curves. Cox Proportional Hazards models were used to estimate the hazard ratios

Results: 3,301 dry blood spot tests were collected from HIV exposed infants and data were abstracted from medical records of 1045 children aged from birth to 20 years who were enrolled in HIV care for 4450-person years between 2003 and 2015. 71 (7%) died,

167 (16%) were lost to follow-up and 216 (20%) were transferred to other health facilities. 17% of the infants tested at birth were HIV infected. MTCT rate declined from 19% in 2009 to 1.4% in 2016. Mortality and loss to follow-up were highest within the first 3 months of treatment (30% and 40% respectively). Younger age at enrollment (<5 years), (HR=3.1 [1.3-6.4]) and WHO stage 4 (HR =4.8 [2.3-10]), were predictive of mortality. Incidence of loss to follow-up declined from 29/100-person years in 2004 to 1.6/100-person years in 2014. Risk factors for loss to follow-up were; 1) lack of disclosure of HIV status to older children at baseline, HR=1.9(1.2-2.9), 2) no phone, HR=2.1(1.6-2.9), 3) starting treatment between 2012 to 2014 HR=5.6 (2.2-14.1).

Conclusion: Birth testing HIV exposed infants is a high yield strategy for early infant diagnosis. Pretreatment screening and engagement in care among children commencing cART needs to be strengthened.

INDEX WORDS: Pediatric HIV, cART, Early Infant Diagnosis, Loss to follow-up, mortality, Survival

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DEDICATION

Dad, I figured out what you meant when you said “stick to your bush”. I miss you, but I know that you are in a better place. Mom, you were right when you said,” if you can think it, then you can do it”. Your words of wisdom, and your love have helped me on my journey. Most of all, thank you for demonstrating through your lives how to love and trust the Lord.

To my brothers and sisters. Your encouragement, support and love is very important to me, I love you and I am blessed to have you in my life.

To my children Mwema and Sepo, and all the precious little ones whom the Lord brings my way; may your dreams be so big and awesome and may you have the love, courage and passion to fly and realize your dreams, because that’s how the world changes and becomes a better place. I love and treasure you little ones, you are world changers, brought into this world with purpose and surrounded by the Lord Jehovah’s favor. May the Lord bless you always.

Simon, thank you for your help, support, encouragement and most of all, your love. You are truly my soulmate.

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CHAPTER 1 : INTRODUCTION

Public Health Significance

Globally, remarkable progress has been made in the fight against HIV. Scale-up of prevention of mother to child transmission of HIV (PMTCT) program coverage that led to a 51% decline in the number of new HIV infections among children aged 0-14 years since 2010. However, in 2016, of 2.1 million (1.7-2.6 million) children aged less than 15 years estimated to be living with HIV globally, only 43% (36%-54%) had accessed combined Anti-Retroviral Therapy (cART). At least 160,000 (100,000-220,000) infants and children were newly infected with HIV worldwide and 88% of these newly infected children live in Sub-Saharan Africa. UNAIDS approximates that over 80% of the people living with HIV globally live in Sub-Sahara Africa (Fig 1-1). Estimates suggest that at least 120,000 children died due to AIDS related [1].

In the absence of cART, 50% of all perinatally HIV-infected children died from HIV related causes before their 2nd birthday [2]. Although it is well established that early identification of children living with HIV and initiation of lifelong treatment is cost effective and lifesaving, there remain critical barriers to scale-up of pediatric ART including complexity of existing approaches to testing and treating children among other challenges [3-5].

In 2014, the joint United Nations program on HIV/AIDS (UNAIDS) launched the ambitious 90-90-90 targets, a strategy to end the HIV/AIDS epidemic. This 3-part strategy aims at the following targets by the year 2020: 1) 90% of all people living with HIV will know their status, 2) 90% of all people diagnosed with HIV will receive sustainable antiretroviral therapy (ART), 3) 90% of all people receiving ART will have viral suppression [6]. These targets focus not just on

expanding treatment access but also on quality of care in terms of retention in care and viral suppression, which are key in optimal HIV outcomes [7]. UNAIDS acknowledges that some populations including children and adolescents have been left behind in the current AIDS response [6]. The 90-90-90 strategy outlines a human rights based approach that prioritizes the vulnerabilities of populations that are experiencing disproportionate risk [6].

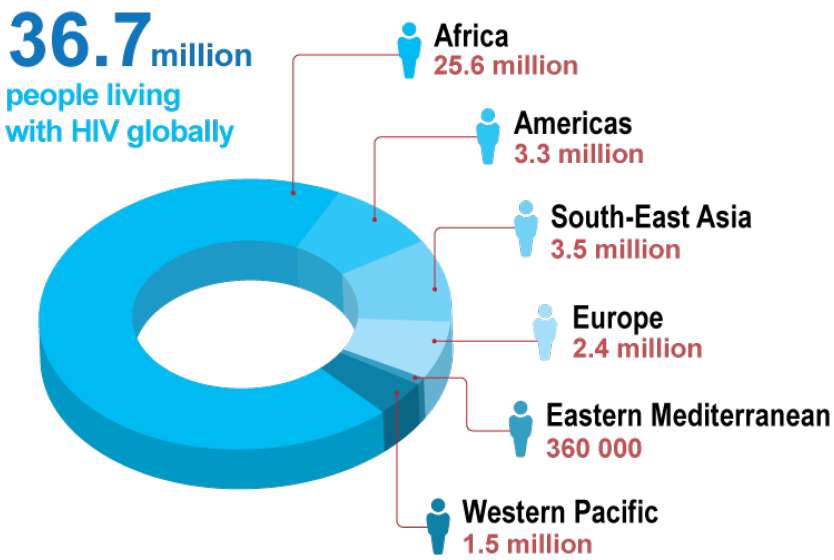


Figure 1-1: People Living with HIV by WHO Region 2016

Combining prevention and treatment is a key strategy for ending the HIV epidemic [8]. Treating HIV infected people results in reduced HIV plasma viral load, which in turn results in decreased risk of transmission. The September 2015 World Health Organization (WHO) guidelines recommended cART for all people living with HIV [9]. Even though the evidence and policy guidelines had been available for three decades, achieving the 90-90-90 targets for children and adolescents by 2020 requires a lot of planning and coordination, monitoring and evaluation of existing programs.

In 2016, the UNAIDS joined with diverse partners to launch the "Start Free, Stay Free, AIDS Free" a Super-Fast-Track Framework to end AIDS among children, adolescents and young women by 2020 [10] by building on lessons learned from the Global Plan of 2015 [11]. These lesson include; the critical role of country ownership, putting women at the center of policy discourse, strong monitoring and evaluation, good coordination and strong technical assistance and have culminated into components of the Start Free, Stay Free, AIDS Free outlined in (Figure 1.2). These super-fast –track targets were developed to accelerate prevention and treatment of HIV among infants and children and to contribute to ending AIDS by 2030. To achieve these targets, evidence is needed to inform global policy change and ensure better outcomes for infants and children across the HIV cascade [5, 10, 12]. There is a paucity of information on effectiveness of pediatric ART programs in reducing morbidity and mortality as well as addressing obstacles to treatment access and uptake to long-term adherence and retention in care in high HIV burden settings.

HIV Prevention in Infants and Children

The major component of the HIV response for young children is the PMTCT programs. In developing countries, scientific advances were rapidly translated into policy and this resulted in near elimination of new pediatric HIV-infections [13]. In low resource and middle income countries (LMICs) however, it is estimated that 90% of the new HIV infections in children are attributed to MTCT [14]. Between 2005 and 2009, it was estimated that nearly 100,000 new pediatric HIV-infections were averted through PMTCT programs in Sub-Saharan Africa [15]. Although a number of countries in the region have demonstrated success, data from several African countries shows that the number of new pediatric HIV-infections is remains high [15].

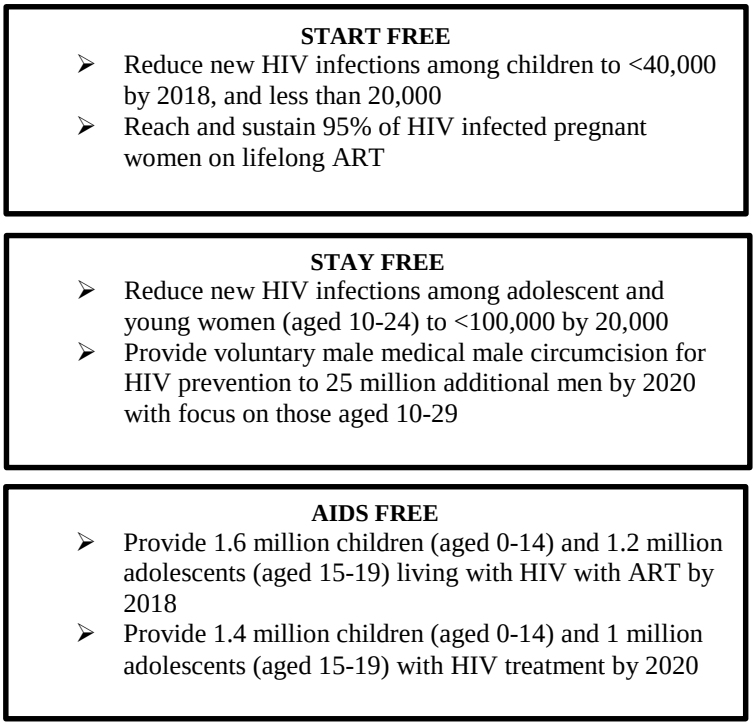


Figure 1-2: A Super-fast-track Framework for Ending AIDS among children, Adolescents and Young Women by 2020

The PMTCT Cascade

The WHO issued a strategy for comprehensive prevention of mother to child transmission of HIV in 2007 based on the United Nations strategy [14]. The approach provides policy guidelines for high burden countries with the goal of eliminating Mother-to-Child Transmission of HIV (eMTCT) by 2020 and has four (4) major themes or prongs as follows (Figure 1:3):

1) **Primary prevention of HIV infection in young women:** HIV prevention is the most efficient strategy to avoid HIV infection in infants. Strengthening primary prevention services such as sex education, delaying age of sexual debut and condom use in the most at risk populations and other strategies to prevent HIV among women. A key component of prong 1 is periodic testing of women of child bearing age including during pregnancy and breastfeeding[16].

2) **Prevention of unintended pregnancies among women living with HIV:** Strengthening of reproductive health services so that women can access HIV counseling, testing and birth control.
3) **Prevention of Transmission of HIV from mother to infant:** Efforts to decrease an HIV-infected woman's risk of disease transmission to her infant can include: 1) Increasing accessibility of antenatal and postnatal care services that offer HIV counseling and testing, 2) ARVs, 3) safe delivery, 4) infant feeding counseling.
4) **Provision of treatment, care and support to HIV positive women, their children and families**: Provision of appropriate care, treatment and support for women living with HIV and their families. The WHO guidelines recommend immediate lifelong CART for all HIV infected adults and children regardless and immunological status [10].

Elimination of MTCT is now a realistic public health goal for resource-limited settings. Research has shown that providing a comprehensive package of care including effective cART for pregnant and breastfeeding women can reduce MTCT to less than 5% in breastfeeding populations and 3% in non-breastfeeding populations [17-20]. In September 2015, the WHO released new guidelines that recommend commencing cART for all HIV-infected pregnant and breastfeeding women and continued lifelong treatment regardless of CD4 count and clinical stage [9]. Treatment of HIV-infected pregnant and breastfeeding women with cART has public health benefits because of three reasons; 1) improves individual health outcomes, 2) prevents mother-to-child transmission of HIV, 3) prevents horizontal HIV transmission to her uninfected sexual partner. This recommendation is referred to as option B+ and was adopted in Zambia in 2013 [21].

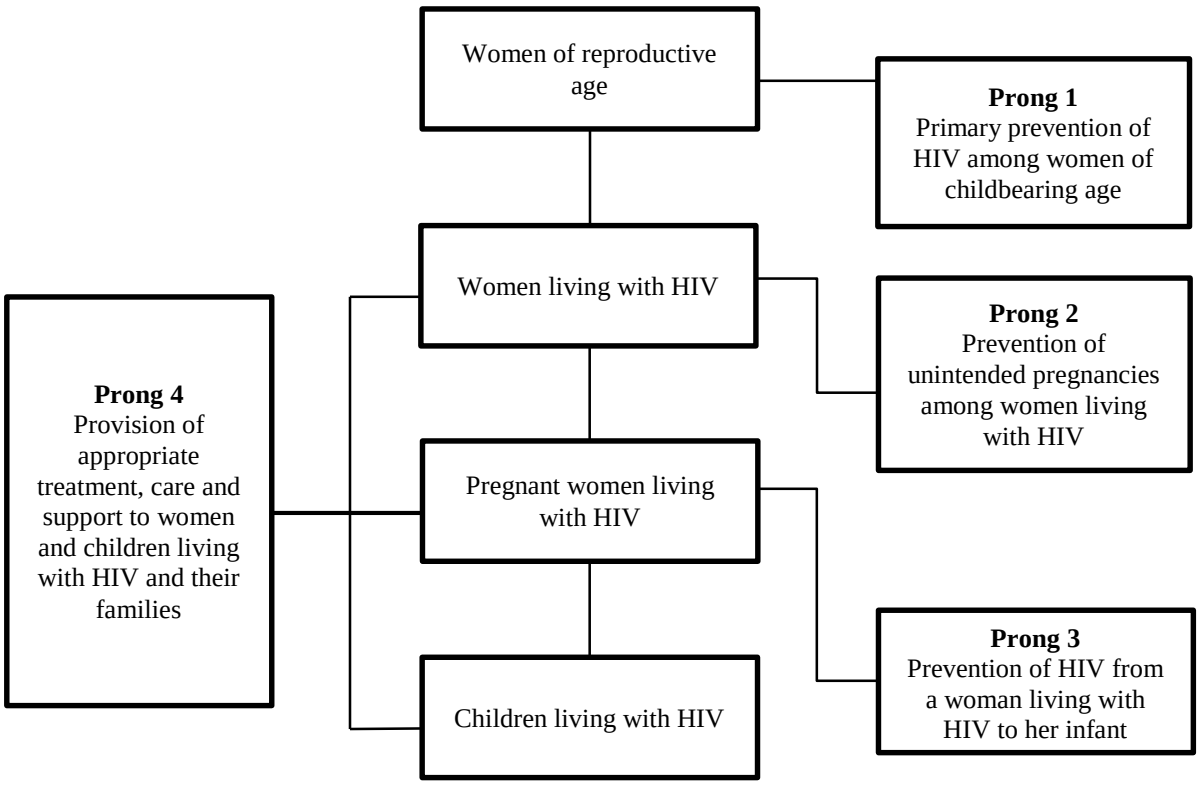


Figure 1-3: Four prong to eliminate mother-to child transmission of HIV and improve maternal health

Gaps in the PMTCT Cascade

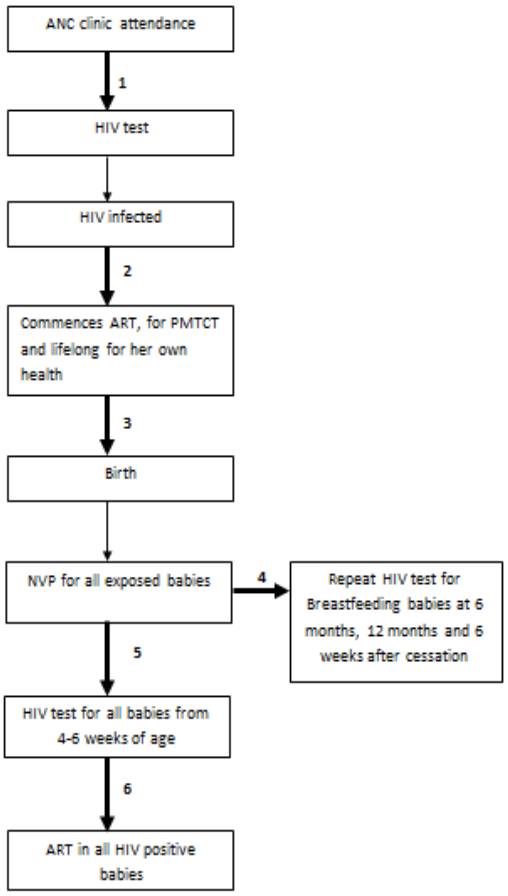
Testing and counseling for HIV, ART and Early Infant Diagnosis (EID) in developing countries takes place in the antenatal clinic (ANC), postnatal clinic (PNC) and under-five clinic settings. Postnatal care, which involves clinical follow-up of a mother for at least 6 weeks after delivery, is conducted as a 'super market approach' in combination with ANC and under-five clinic services in most primary health care settings. PMTCT is a package of care that starts with primary prevention.

For the context of this study we will mainly focus on the third and fourth prongs. The PMTCT cascade (Figure 1.3) identifies a sequence of steps to deliver PMTCT interventions to HIV-infected

women and their infants [22]. Each aspect of the package is important and a deficiency in any of the interventions compromises overall effectiveness, thereby resulting in increased risk for MTCT [23]. Modeling studies have shown that to reduce the number of HIV-infected infants, uptake and retention at each step of the cascade needs to be greater than 90% [24].

In the context of a pregnant woman in Zambia, the first step in the PMTCT cascade is to visit the ANC clinic. The first visit is known as the booking visit and has standard-operating procedures, among them is HIV counseling and testing. The pregnant woman has the option to either agree or decline the test. If she agrees to the test and the HIV test is positive, the pregnant woman enrolls into care and initiates cART. If she tests negative, further counseling continues and all women are advised to retest after three (3) months. Under this strategy, women are encouraged to test with their partners. If the partner is HIV positive (and the mother is HIV negative), the partner is commenced on cART regardless of the CD4+ count as a discordant couple

The Ministry of Health in Zambia recommends that labor and delivery is conducted in health facilities under supervision of skilled midwives and/or doctors, followed by collection of dry blood for DNA-PCR from the baby and administration of antiretroviral drugs for post exposure prophylaxis [25, 26]. HIV-DNA testing is recommended within to be repeated at 6 weeks, 6 months and 9 months of age. Beyond 12 months or 6 weeks after cessation of breastfeeding, rapid HIV tests are recommended to rule out HIV infection. Early Infant Diagnosis is complicated because infants are born with maternal antibodies, which makes serologic tests give false positive results [27]. Virologic tests require specialized infrastructure and equipment, skilled staff and training, which are not readily available in low-resource settings [28].



1) HIV testing is opt-out, meaning women can refuse the test
2) In Zambia 98% of all pregnant women who are tested are commenced on ART, adherence to medication remains an issue, especially for healthy women
3) Skilled delivery is challenging because non-skilled staff run many health facilities in rural areas.
4) Breastfeeding is a child survival strategy in developing countries but the risk of transmission remains high during breastfeeding
5) Only 32% of all HIV exposed babies tested for HIV-infection by 6 weeks. There is need for point of care HIV diagnostics
6) Only babies who test positive can commence ART

Bold arrows represent the steps where attrition is highest. ANC is antenatal clinic. Adapted from Wettstein 2010 [23]

Figure 1-4: Steps in the PMTCT cascade 1: Steps in the PMTCT cascade

HIV transmission can occur through breastfeeding. On the other hand, infants who are not exclusively breastfed in these populations have a high risk of Protein Energy Malnutrition (PEM) and diarrhea. PEM kills over 1 million infants every year [29]. Exclusive breastfeeding reduces the risk of a child developing and dying from PEM and has other benefits for both the mother and child. Exclusive breastfeeding is a child survival strategy in developing countries, even though there is enough evidence showing that there is risk of HIV transmission through breastfeeding [29].

Gaps in the PMTCT cascade occur at different levels. The figure (Figure 1.4) above highlights some of the challenges encountered in the second and third prongs of PMTCT. Apart from the high prevalence of HIV and fewer number of people who know their HIV status, there is a high burden of unintended pregnancies among HIV infected women in many countries [30]. Access to birth control has continued to be a challenge and PMTCT coverage remains low especially in rural areas and densely populated urban areas [31]. PMTCT services in LMICs operate within fragile Mother and Child Health (MCH) services, which fail to deliver basic reproductive health packages. MCH is ill-equipped to deliver the complex PMTCT package [32]. For PMTCT to be a long term intervention, the whole MCH package and infrastructure has to be urgently revisited [31]. Achieving the 90-90-90 targets will mean applying successful interventions already in place and understanding the particular needs of women, children and their families.

Efforts to strengthen health systems and address the human resource for health crisis in Sub-Saharan Africa and other LMICs are constantly implemented. Children in these settings are vulnerable to a variety of health threats and therefore these efforts will not only prevent new pediatric HIV infections but also improve the state of the children's health [32, 33]. There is need to identify, engage and retain all women and infants at risk and minimize loss while enhancing efficiency across the PMTCT cascade. The gains made in this campaign must be measured and documented so that best practices can be replicated in other facilities. We can only know that we have achieved the 90-90-90 target for children when we measure where we are now and keep monitoring and evaluating existing programs.

Success of PMTCT programs in reducing new HIV infections in children provided the first evidence for treatment as prevention, which is now the prevention strategy in HIV programing.

Figure 1:5 is a summary of the PMTCT algorithm in Zambia. This is similar to figure 1.4 except that more detail of the activities at each step is shown.

HIV/AIDS in Zambia

Zambia has a generalized HIV/AIDS epidemic. In 2013 there were over 54,000 new adult infections and 12,000 new pediatric infections [15]. The adult HIV prevalence reached its peak in 2013 at 14.5% and declined to 12.4% by the end of 2014. HIV prevalence in younger women is higher than in younger men (15.1% compared to 11.3%) [34].

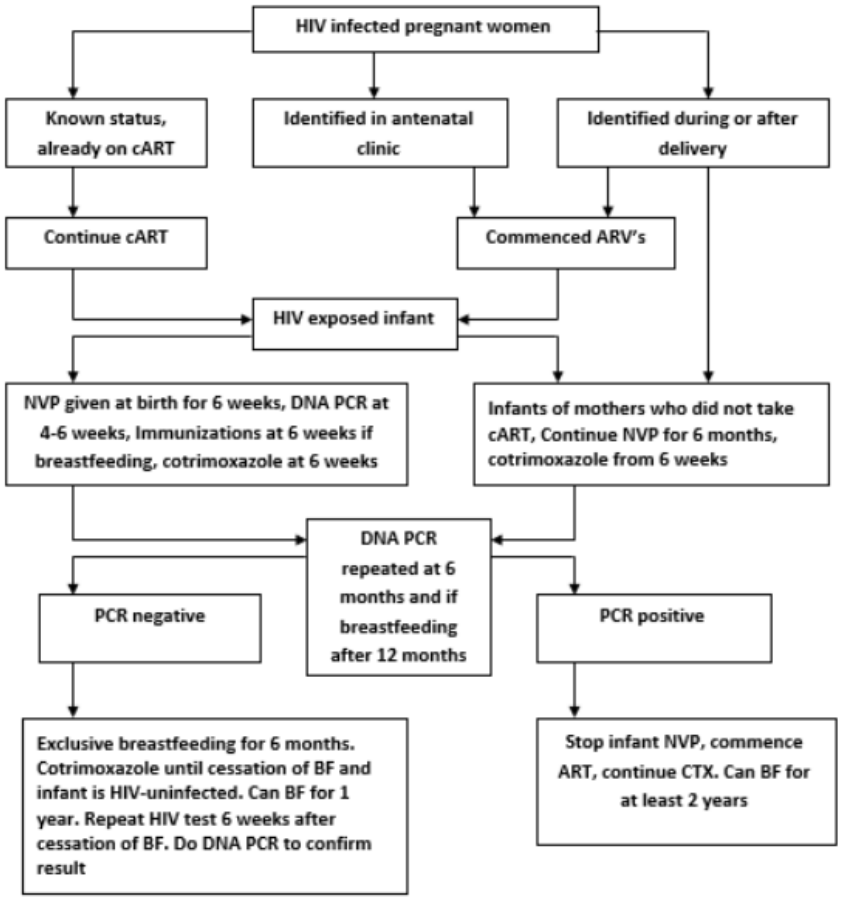
Interestingly, younger women recorded a higher HIV prevalence compared to younger men. This reflects the problem of intergenerational sex in which girls have an earlier age of sexual debut and usually with older men who may already be living with HIV. This additionally reflects that younger girls are less likely to attain education to the level where they get sex education and empowerment that enables them to make good choices [34]. The HIV epidemic in Zambia is driven by heterosexual transmission with 90% of newly infected people reporting not using condoms [34]

Pediatric HIV in Zambia

By the end of 2013, it was estimated that there were over 150,000 children living with HIV in Zambia and only 34% of these were receiving treatment [15]. The pediatric HIV treatment coverage is low as compared to the adult coverage, which is above 60%.

The statistics from the PMTCT program indicate that 91% of women who tested positive during pregnancy received ARVs to reduce MTCT. However, only 37% of infants born to HIV infected women received a virologic test for HIV within 2 months of birth [34]. The estimated child infection rate during delivery was 9% in 2014, which is a drop from 24% in 2009. The

statistics for retention in care are aggregated with those for adults and the gaps in pediatric ART are not adequately studied [34].



Source: PMTCT country report, Ministry of Health Zambia, 2014

Figure 1-5: PMTCT Algorithm in Zambia

The challenges remain however because of the following reasons:

1. Inadequate infrastructure exists in most facilities to provide a comprehensive PMTCT package [34].
2. Tests for early infant diagnosis still have to be sent to central laboratories and the turnaround time is still long with frequent stock-outs of reagents [34].

3. Linkages to long term care for women and their babies after they leave the PMTCT program are not ideal [35].
4. Retention to care is still low with much loss to follow-up [35].
5. Lack of readily available viral load testing [34].
6. Inadequate adolescent health care services [34].

Early Infant Diagnosis in Zambia

Early diagnosis of HIV in infants is crucial so that they can have access to cART and other preventive interventions early. In an effort to ensure that the HIV status of all children attending MCH and immunization clinics is known, HIV test results are documented on the Road-to-Health charts of all children in Zambia. The expectation is that at every contact with a child effort must be made to identify HIV. In cases where the HIV status is marked on the card, the MCH providers check for appropriate interventions. The extent to which this is implemented and monitored is not well documented. Monitoring and evaluation of current approaches is important for quality improvement of systems and for more targeted health systems strengthening.

Currently, dry blood spots are collected from HIV-exposed babies and couriered to the central laboratories in three cities (Lusaka, Livingstone and Ndola) to detect HIV-DNA. Once the laboratory tests are done, they are communicated back to the health workers using Program Mwana [36]. Program Mwana is a short message service that sends text messages to health facilities within Zambia. This software is available for use to deliver early infant diagnostic test results. Use of Program Mwana shortened the turnaround time for the test results from 3 months to less than 2 weeks [36].

There are no specific guidelines for preterm and low birthweight babies and this presents a challenge among the health workers on how to handle this group of babies. Neonates and preterm

babies are a special risk group that requires special attention in order to achieve the 90-90-90 targets [34, 37].

The Case for Adolescents in Zambia

The 2015 Zambia country report showed that 50% of young people aged 15-24 have low risk perception for HIV infection. The reported key indicators were aggregated with adults [34]. Targeted interventions for quality improvement require age-disaggregation of the data. There is very little data on HIV-infected adolescents from Zambia and the other high HIV burden settings.

Meanwhile, as PMTCT programs are being strengthened worldwide, the burden of pediatric HIV is shifting to adolescents aged between 10-19 years old [4]. In 2012, there were an estimated 4 million HIV infected young people aged 15-24 living with HIV worldwide [29]. Adolescents (age 10-19 years) living with HIV are predominantly survivors of perinatal HIV infection with a small proportion of behaviorally infected adolescents and adolescents who were infected through blood transfusions before blood screening became available. Adolescents are at risk of acquiring HIV through sexual transmission especially in settings where sex education is limited, early marriage is common, and sexual debut is at a young age. The younger the age at first sexual activity, the higher the lifetime risk of HIV because of the increased risk of sexual exploitation and lower condom use. Globally, the age of sexual debut has been increasing [38].

By the end of 2013, AIDS related deaths among adolescents increased by 50%, making HIV/AIDS the second leading cause of deaths among adolescents worldwide second to trauma, and the leading cause of death among adolescents in sub-Saharan Africa [39]. The statistics for HIV counseling and testing are very low in this population.

Adolescents and young people living with HIV confront numerous obstacles to meaningful treatment access and favorable health outcomes. These challenges include stigma, discrimination,

problematic laws and policies including need for parental consent to access HIV testing [6]. Adolescents struggle with linkage to care and retention in care as they transition from pediatrics to adult services and often have no access to sexual and reproductive health services. In addition, they have difficulty with disclosure of their HIV status [40]. Furthermore, they struggle to navigate the fragmented health care services on their own especially as they face the complexities of living with HIV as a young person [6]. These challenges and many more need to be addressed in order to provide a comprehensive package of care to this group.

Data on adolescent HIV is scarce globally and there is need to collect age-disaggregated data in order to develop robust surveillance strategies to monitor outcomes for children and adolescents [6, 40].

The Purpose of the Study and Scientific Rationale

With the rapid scale-up of ART treatment in resource-constrained settings, the number of children accessing HIV diagnostic and treatment services increased. There is a paucity of information on effectiveness of pediatric ART programs in reducing morbidity and mortality as well as addressing obstacles to treatment access and uptake to long-term adherence and retention in care. Health facilities in resource limited settings like Zambia have been treating HIV exposed and infected children since 2004 with large number of children and extensive experience in pediatric ART. Livingstone Central Hospital alone has over 600 children on cART. However, there is very little research on this population to highlight success, best practices and system successes. In fact, to our knowledge only one paper has been published on the success of provider initiated testing and counseling [41]. We therefore have limited knowledge about the impact of this program.

While there is progress in identifying and treating HIV infected women, identifying and effectively treating HIV infected infants remains a challenge. This is partly due to lack of affordable point of care laboratory tests and health system failures to follow-up HIV exposed children. Additionally, there is poor understanding of the trends in perinatal HIV infection rate in this population and the factors accounting for excess cases in the presence of a functional PMTCT program. There is need to carryout studies to enable better understanding of the PMTCT and pediatric HIV treatment program in Zambia, which will in turn provide data for policy about elimination of mother to child of HIV and pediatric HIV in Zambia. The **goal** of this research is to evaluate treatment outcomes and generate program relevant information to improve ART outcomes for infants and children in Zambia and similar settings. This project will fill critical gaps in the pediatric ART treatment in Zambia and will be accomplished through answering the specific aims that follow.

Gaps in Literature

Few studies have documented the survival patterns of children and adolescents and disaggregated the data according to age groups[42]. The impact on the cascade of care and the magnitude of the prevention benefit of early initiation of cART versus late cART initiation, especially among children and adolescents is known [9]. However, there is little evidence which has demonstrated the effectiveness as opposed to efficacy of this strategy. Because most of the evidence is from clinical trials, where patients are followed stringently there is limited data on optimal service delivery models to ensure rapid identification and cART initiation among infants and children in Zambia and other countries in Africa.

In our previous study we showed that Provider Initiated Counselling and testing improves uptake of cART in the pediatric department at Livingstone General Hospital without adversely

affecting other child survival interventions [41]. To our knowledge, no study has been conducted to describe the clinical outcomes of this large cohort of children living with HIV and identify best practices and gaps in the care, which are most likely similar in most LMICs with a high HIV prevalence

Specific Aims

To address the overarching goal of our study, we compiled routinely collected program data and addressed the following specific aims:

Specific aim 1: To quantify the proportions of early infant diagnosis, including birth testing among HIV exposed infants at Livingstone Central Hospital (LCH), from January 2009 to December 2016.

Specific aim 2: To describe treatment outcomes, measure mortality rates and assess predictors of mortality among children receiving cART over a 10-year period at Livingstone Central Hospital in Zambia.

Specific aim 3: To estimate the annual incidence of loss to follow-up and identify risk factors for loss to follow-up among children on long term treatment with antiretroviral therapy at Livingstone Central Hospital in Zambia.

Study Design

Pediatric HIV is a complicated disease to study because of ethical issues regarding clinical research in children and the fact that 90% HIV infected children globally live in low resource and middle-income settings often under vulnerable circumstances [43]. Although the benefit of cART in children is well-documented and indisputable, the best approach to deliver comprehensive HIV prevention, treatment and care packages to resource-constrained settings remains unclear.

Routinely collected program data have the potential to provide much-needed data to inform policy on the effectiveness of current models of care. We utilized routinely collected program data to understand survival patterns and loss to follow-up among children and adolescents treated at Livingstone Central Hospital.

To address specific aim 1, we implemented a prospective cohort study by compiling routinely collected program data for children who came to LCH for early infant testing of HIV within the first two months of life and later. This enabled us to calculate the perinatal HIV infection by calendar year and to study the uptake of HIV testing at different ages and to assess how the National HIV testing and treating guidelines have been implemented at this health facility.

To address specific aims 1 and 2, we compiled retrospective data from the patient medical records and were able to study their treatment outcomes.

Table 1-1: Overview of study aims

	Aim 1	**Aim 2**	**Aim 3**
Study population	HIV exposed infants at LCH, Zambia	HIV infected children on cART at LCH	
Study design	Prospective Cohort	Retrospective Cohort	Retrospective Cohort
Research Theme	Uptake of early infant diagnosis	Mortality of infants and children on cART	Retention in care for children on cART
Research priority area	**Testing**: Interventions to ensure timely HIV diagnosis	**Treatment**: Impact of HIV infection and ART on short and long-term outcomes	**Service delivery**: Strategies to improve access to uptake of and retention in care and factors that impact their success
Data sources	Early infant diagnosis testing registers	Patient Medical records	
Outcome Variables	Proportions of infants tested at birth, 6 weeks and beyond. Proportion HIV infected perinatally	Number died and associated risk factors	Number lost to follow-up and associated risk factors

Organization of the study

Chapter 2 is the literature review, Chapter 3 outlines the methods used in this research. Aims 1, 2 and 3 are in Chapters 3, 4 and 5. Each of the chapters is in manuscript style with an abstract,

background, methods, results, discussion, conclusions and references. Chapter 7 is the synthesis chapter that summarizes the major findings.

Ethical considerations

This study was approved by the Zambia National Health Research Authority and the institutional review boards at Macha Research Trust and the University of Georgia. We conducted secondary analysis of anonymized routinely collected program data. Hence, informed consent was waived.

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CHAPTER 2 : LITERATURE REVIEW

This chapter focuses on critical review of HIV infection in children and its prevention strategies. HIV/AIDS is a new disease and therefore, a brief history is presented to understand the milestones that have been achieved so far and therefore discuss the current gaps. This is followed by review of the biology and natural history of HIV infection in children. Since prevention of mother to child transmission of HIV (PMTCT) is the most effective prevention strategy in children, we discuss this in depth so that we can understand how it works and why we still have gaps especially in LMICs.

Finally, we present a review of the critical literature in pediatric HIV.

Main themes in this review:

1. History of HIV in children
2. History of PMTCT
3. Limitations in PMTCT and pediatric HIV
4. Challenges in ART provision for mothers and children

Methods for literature search

We performed a search for articles on pediatric HIV and prevention of mother to child transmission of HIV. The following databases were searched: PubMed, EMBASE, web of science, Medline, Global health, WHO Global health library, WHO clinical trials registry, NIH clinical trials registry, google scholar and the Cochrane Library. The search terms used in the various databases included “Pediatric”, “infant”, “baby”, “adolescent” “HIV”,” AIDS”, “PMTCT”, “MTCT”, “eMTCT”, “HAART”, “ART”, “ARVs”, “mortality”, “death”, “loss to follow-up”. We

included research articles, policy documents and journal letters. We now review literature from 1981 to August 2016.

What is HIV

Human immunodeficiency virus (HIV) is a lentivirus of the Retroviridae family. Long incubation periods characterize Lentiviruses and their ability to deliver a large amount of viral RNA into host cell DNA. The unique feature of lentiviruses is that they have the ability to infect non-dividing cells, which is a very efficient method of gene delivery [1].

HIV can be divided into two major types, HIV type 1 (HIV-1) and HIV type 1 (HIV-2). HIV-1 is the most widespread and is responsible for the global HIV pandemic [2]. We will therefore focus on HIV-1.

AIDS was first recognized as a disease in 1981 after a sudden increase in cases of pneumocystis Carinii pneumonia (PCP) and Kaposi sarcoma among men who have sex with men in Los Angeles and New York [3-5]. Within a few months, an elderly man and two younger men with severe hemophilia died of PCP [6]. These patients had no homosexual contact and were not intravenous drug users. They all had received lyophilized clotting factor concentrate which was derived from the blood of at least 1,000 different donors and was known for transmitting hepatitis viruses [6].

Shortly afterwards, AIDS was reported in an infant who had received blood transfusions and had no family contact with AIDS [7]. This was followed by diagnosis of AIDS in infants born to women at high risk for acquisition of HIV infection, i.e. intravenous drug users and women of Haitian origin and in women who had sexual contact with bisexual men or intravenous drug users[8-10] [11]. A sexual network study showed how these first cases were linked by sexual contact and this provided the first evidence that the cause of AIDS is sexually transmitted [12].

The additional cases among people who had received blood transfusions, and infants of at risk women provided evidence that AIDS is caused by an infectious agent which is transmissible through blood and from a mother to her unborn child [5]. In 1982, AIDS was reported among 34 migrants from Haiti, most of whom were heterosexual and further investigations provided evidence for heterosexual transmission [13, 14].

A vertical mode of transmission from a mother with AIDS via the placenta during pregnancy became more evident as more cases of AIDS in children were reported [15, 16].

The HIV virus was first isolated in the laboratory in 1983 [17, 18]. Development of an antibody blood test followed to detect HIV viral antibodies in HIV-infected individuals. The HIV test soon became very useful for screening donated blood for transfusion and for screening pregnant women in the high-risk groups. The identification of the viral etiologic agent and development of the diagnostic test was a game changer as it led to advances in understanding of HIV/AIDS.

How is HIV/AIDS Transmitted?

Early epidemiologic studies clearly demonstrated that the HIV virus is transmitted through homosexual and heterosexual contact. HIV is present in the blood, breast milk and semen of infected individuals. HIV transmission occurs when a sufficient dose of these fluids gets into the bloodstream of another person.

HIV is transmitted through unscreened blood transfusions and blood contact such as use of contaminated needles, which places intravenous drug users at high risk. HIV is transmitted from an infected mother to her unborn infant during pregnancy, labor, and delivery and through breastmilk. HIV prevention guidelines are available and focus on these modes of transmission. HIV prevention guidelines have helped avert millions of new HIV infections.

Table 2-1: Modes of HIV transmission

1.	Unprotected vaginal or anal intercourse
2.	From infected mother to her child either during pregnancy, labor or breastfeeding
3.	Sharing unsterilized needles as in IV drug users or tattoos
4.	Occupational exposure and infected blood transfusions though now very rare

HIV replication

Once the HIV infection occurs, the virus targets the CD4 T-cells. HIV uses an enzyme called reverse transcriptase to convert its RNA into DNA. HIV gradually attacks the human immune system and specifically targets the T-helper cells, of the white blood cell line. Gradual depletion of the T-helper cells (CD4 T-cells) results in the Acquired Immune Deficiency Syndrome (AIDS) [17, 18]. AIDS impairs the human immune system, making the patient susceptible to opportunistic infections that soon result in death [9].

There is currently no cure for HIV. Antiretroviral medications block HIV viral replication by interfering with different stages of the HIV replication cycle, therefore causing viral suppression [19]. Use of antiretroviral drug therapy in HIV-infected individuals results in HIV plasma viral suppression and the virus can be maintained below the limits of viral detection for prolonged periods of time [19]. Thus morbidity and mortality for HIV-infected people on cART are dramatically improved [20].

Natural history of HIV in children

The primary source of HIV infection in children is MTCT, which accounts for at least 90% of all HIV infections in children. The overall risk for MTCT throughout pregnancy and delivery in non-breastfeeding populations in the absence of any intervention varies from 14% to 32% versus

25% to 48% among breastfeeding populations in resource poor settings [21, 22]. This rate depends on mother's viral load, duration of exposure (i.e. duration of rupture of membranes, duration of labor, duration of breastfeeding) [23]. Evidence from research has been implemented in developed countries and this has resulted in virtual elimination of pediatric HIV to as low as 1% to 2% or less [24, 25]. Pediatric HIV is therefore largely a disease affecting children in LMICs [25, 26].

The clinical course of prenatal HIV infection in children is highly variable especially in the presence of ARVs. Survival patterns studied before ARVs became available described a bimodal pattern of disease [27, 28]. This pattern estimated that 10-20% of infants experienced rapid disease progression and developed AIDS by 4 years of age, while 80-90% would survival for a median of 9-10 years [27]. Children who developed AIDS early (at least within the first 2 years of life) are early progressors and those who survive beyond 10 years of age are called long-term non-progressors or long-term survivors [28, 29]. The reasons for this bimodal pattern are very heterogeneous but in African populations, this has been associated with time when the HIV transmission occurred, the virulence of the HIV strain and the infectious dose transmitted from the mother to her unborn child [28]. Infants born to mothers with advanced HIV disease are at increased risk of rapid disease progression as are infants born to mothers who were infected during the pregnancy [30]. Other factors include genetic makeup of the individual and co-infection with other viruses [29]. The plasma viral load of the mother predicts the risk of HIV transmission but not the timing of the transmission event [31].

In perinatally infected infants, the virus replicated very rapidly in the presence of the immature immune system to reach several millions of copies per microliter of plasma within the first 1-2 months. The decline in viral load is very slow during the first 2 years of life. Infants with very high viral loads are at increased risk of rapid disease progression [32]. A pooled analysis done

in sub-Saharan Africa found that in the absence of ART, at least 50% of HIV infected children die by their 2nd birthday [33]. Other studies suggested rapid disease progression followed by death in prenatally infected infants who commenced ART after 6 weeks of age [34, 35].

Combination antiretroviral therapy dramatically reduces mortality and morbidity in HIV infected children who commence treatment early [36]. Data from systematic reviews shows that virologic suppression in children on cART for at least 2 years was 60-70% [37]. Early treatment with potent cART has significantly improved survival of perinatally HIV infected children and diminished the likelihood of developing AIDS or dying during childhood [38].

HIV Diagnosis in Children

Early identification of infected infants is crucial so that they can have access to timely interventions [32]. Diagnosing HIV in early infancy requires HIV DNA or RNA detection by molecular PCR assays rather than the serological assays used in older children and adults [39]. This is because of the passive in utero transfer of maternal HIV immunoglobulin G. The HIV antibodies persist in the bloodstream until a mean age of 10 months. This implies that HIV antibodies will be detected in peripheral blood even though the infant is not HIV infected and this limits the use of HIV antibody testing [39].

Virologic testing with molecular assays can also yield false negative results in infants and newborns because of low levels of circulating virus especially when the transmission occurred during delivery. Another reason for a false negative result is impaired B-cell function which results in profound hypogammaglobinemia [39]. Virologic testing requires higher levels of technology, training and cost than is available in low resource settings [40]. A study from a rural area Zambia highlighted the long turnaround time for HIV test results [40]. However, mobile technology has been harnessed to innovate quicker ways of communication among the various levels of care [41].

History of PMTCT

PMTCT remains the most effective strategy to eliminate pediatric HIV. This has been effective in the developed countries but LMICs are still lagging behind due to complex health systems failures and other complexities [42]. Perhaps the best way to understand where we are and how we got here is to examine the literature.

1980's

Development of the HIV antibody test in 1984 for identification of HIV in the peripheral blood stream confirmed Mother to child transmission of HIV (MTCT) [17, 18, 43]. The newly developed antibody test was used mainly to screen blood for transfusion and not for routine screening of pregnant women. The first recommendations for PMTCT were issued by the U.S Public health service in 1985 [44]. By this time there was evidence that HIV is transmissible from an infected mother to her unborn child through 3 different routes: 1) prenatally through the placenta, [45] 2) through labor and delivery,[16, 45] 3) through breastfeeding [46]. The major recommendations advised that pregnant women in high-risk groups require counselling and testing for HIV whenever they encounter a healthcare setting. The HIV testing guidelines recommended repeating the HIV tests every 2-3 months for pregnant women at high-risk of HIV. Breastfeeding was discouraged based on research findings indicating presence of free HIV virus in breastmilk [46]. The rate of perinatal transmission was at this time approximated to be 65% [44, 47]. At this time, evidence emerged that pregnancy increases an infected women chances of progressing to AIDS [48]. It was therefore additionally recommended that women with negative test results but who engage in high risk behaviors should be counselled on risk reduction and advised to delay pregnancy until they are no longer at risk. Women with a positive test should be counselled on their risk of AIDS and the risk of perinatal and sexual transmission of HIV [44].

In 1987, azido thymidine (AZT) was approved for treatment of AIDS patients not as a cure but to prolong survival of HIV infected patients as demonstrated in clinical trials [49, 50]. However, this was only in adults with AIDS and not in children or pregnant women because of safety concerns. AZT was licensed for use in children in the USA in 1990 [51]. However, there were significant safety concerns. Four years later, there was sufficient evidence regarding safety of AZT in pregnant women after a larger clinical trial done in the USA [52]. AZT however was not available in low and middle income for several years because of the high cost of treatment and the complexity of administering the treatment to pregnant women that involved intravenous administration [53].

Meanwhile in the developing world, heterosexual transmission of HIV was identified as the major mode of transmission and the HIV burden among pregnant women and children was not clearly understood. The Global AIDS program at the World Health Organization (WHO) was established in 1987 with the mandate to organize and coordinate global HIV/AIDS prevention efforts [5, 54].

1990's

There was a steep increase in HIV/AIDS incidence and mortality globally between 1990-2000 [55]. By the end of 1990, there were an estimated 8-10 million people living with HIV worldwide, with only approximately 300,000 reported cases, by the end of 1999, there were an estimated 33 million people living with HIV and 14 million deaths since the start of the epidemic [55].

In the USA, HIV prevention guidelines expanded from selective testing of high-risk women to voluntary counseling and testing for all pregnant women. This led to the universal opt

out antenatal HIV testing with patient notification [42]. This extended to all low and middle-income countries through the WHO guidelines.

Results of the 076 AZT Clinical trials for use of single dose Nevirapine (NVP) compared to AZT for MTCT found that NVP reduced transmission by 50% compared to AZT, which reduced transmission, by 67% [52, 56]. Based on these findings, single dose NVP was adopted as a cheaper and more feasible approach compared to AZT. AZT was expensive and complex to administer. Single dose NVP was however soon found to be problematic because the HIV virus developed resistance, especially when administered to mothers with very high viral loads [57].

2000's

During this period, HIV/AIDS became a public health crisis and the number one cause of death in Sub-Saharan Africa and the fourth leading cause of death globally. Life expectancy in sub-Sahara Africa dropped from 62 years to 47 years on average [58, 59]. There was urgent need for progress in HIV prevention, treatment, research, and funding.

Antiretroviral drugs became more affordable in 2000 when UNAIDS negotiated with pharmaceutical companies to reduce the price. The United Nations incorporated specific goals to reduce HIV/AIDS, Malaria, and TB. The first rapid test was developed in 2002 and this made it possible to provide point-of-care HIV counseling and testing with quick results [59]. Point-of-care rapid testing enabled provision of HIV testing in LMICs.

In 2003, President George W Bush created the President's Emergency Fund for AIDS Relief (PEPFAR). PEPFAR provided 15 million dollars a year in countries with high HIV burden and this made it possible for several Sub-Saharan countries to have antiretroviral therapy programs [60, 61].

The concern regarding single dose Nevirapine resistance became more apparent and further research provided evidence that use of combination antiretroviral throughout pregnancy and breastfeeding reduces MTCT by as much as 97% [62, 63]. Breastfeeding was recommended for LMICs where breast milk substitutes were not affordable, feasible, sustainable and safe [64]. However, conflicting evidence from clinical trials suggested that breastfeeding increases MTCT [65, 66]. Other clinical trials showed overall increased child survival as a result of breastfeeding and use of ARV's [67, 68].

2010-2015

Current evidence is that breastfeeding is the cornerstone of infant survival in LMICs even in the face of HIV/AIDS [53, 69]. The WHO recommends exclusive breastfeeding for all HIV-infected pregnant women in LMICs and infant formula only in cases where it is affordable, accessible, feasible and safe. The WHO 2015 guidelines recommend provision of lifelong combination ART for all pregnant and breastfeeding women regardless of CD4 count or WHO stage. HIV exposed infants should receive post exposure prophylaxis based on the mothers ART regimen and feeding choice [20].

2016

Test and Treat for all. The WHO recommended a test and treat approach

Limitations in PMTCT

The current mode of pediatric HIV care is vertical with heavy reliance on the PMTCT programs for identification of HIV-infected infants. This approach has had many challenges, broadly: 1) Preventing new HIV infections in infants, 2) identifying and engaging HIV infected children into care

1. Preventing new HIV infections in infants

This involves a pregnant mother getting an HIV test in the ANC and accessing ART throughout the duration of pregnancy, delivery, breastfeeding and lifelong [42]. Although option B+ implementation in many countries has resulted in dramatic increases in the number of pregnant women starting ART, significant gaps exist in retention and engagement in care [70, 71]. In LMICs, ANC coverage is below 100% and late presentation to ANC is frequent. In the African setting, this is compounded by cultural beliefs that discourage any early public acknowledgment of pregnancy. The best outcomes are for women who attend ANC early in their pregnancy and receive a more comprehensive package of care.[71] In the case of women who go for ANC, the HIV testing is not mandatory as some women decline HIV testing and therefore cannot access ART [72].

Retention and engagement in care for women who commenced ART has been documented to be low [73]. The PMTCT cascade is complicated by virologic resistance, which develops when women collect the medications but fail to adhere. Resistant HIV strains are transmissible to the baby. This creates complexity when the HIV-infected baby commences ART because they have been infected with a resistant virus that will already be resistant to the standard first line regimens [74].

The approach to diagnosis, care and treatment of HIV infected children should be enhanced beyond PMTCT and incorporated into other child survival platforms [75]. There are various individual, structural, and health systems factors that contribute to whether a woman and her baby can access and stay in care [70, 76, 77].

The major factors described by women living with HIV include: distance and cost of transportation to health facilities, high out of pocket costs, poor treatment by the health facility

staff, inconvenient facility hours, long wait times at the health facilities and self-transfer to other health facilities [42, 74, 76]. Social and cultural factors play a role in these barriers. From a health systems perspective, offering postnatal care to the mother-baby pair has remained a challenge largely because PMTCT is embedded into the weak MCH, which is constrained by low workforce, lack of infrastructure and poor record keeping. In fact patient tracing is complicated because the multiple registers are not linked despite the explosion of electronic and mobile health technologies [76, 78].

Identifying and engaging HIV infected infants into care

Two recent developments offer hope to reduce the burden of pediatric HIV; firstly, the commitment to eliminate mother to child transmission of HIV by identifying all HIV-infected pregnant women and providing them with lifelong antiretroviral therapy (option B+) [20, 79]. Second is the evidence that early ART in infants can limit damage to the developing immune system and limit formation of the latent reservoir [80-82].

Identifying children with HIV infection has been a limitation in the PMTCT cascade. Only 37% of infants born to mothers who went the PMTCT program in Zambia in 2014 got a test within the first 2 months of life [83]. Retention to care wanes during the breastfeeding period and a smaller proportion ever come back for testing after 12 months of age. There are delays in initiating ART on infants who have a positive test because of the long turnaround time for the DNA PCR to get to the laboratory and back to the health facility [84].

Retention and engagement in care for infants and their mothers continues to be a challenge in most countries and several models have been adopted including retaining the mother-baby in postnatal care for at least 2 years, using community health workers and peers to keep track of the mother-baby pair and integrating EID into routine EPI programs [85-87].

PMTCT though the cornerstone of MTCT cannot by itself eliminate pediatric HIV.[71] Health systems must have a comprehensive approach to identify and treat pediatric infections, but must now focus on retention and engagement to care and treatment [88]. Integration of early infant diagnosis (EID) in the expanded program for immunization (EPI) of children in Zambia demonstrated that the EPI will not be affected by the EID [85]. Other innovations like SMS for delivering results to peripheral health facilities, community health workers to engage mothers in care etc. need to be tailored to the needs of the individual sites [89]. However, there is a wide array of challenges in implementing these innovations including fragile health infrastructure which is already constrained to carry out the already existing programs like EPI, lack of qualified human resources in most health facilities, challenges in records keeping, and inconsistent commodity and drug supply [76].

Identifying and engaging HIV infected children and adolescents into care

Global statistics indicated that less than half of all children living with HIV are diagnosed and actively engaged in care [90]. There are still challenges to identifying children who fall through the PMTCT cracks. Routine HIV testing of children remains a challenge in most places. In Zambia, the HIV status is indicated on the road to health chart of every child who goes to EPI, however the impact of this intervention has not been measured, hence we cannot make any conclusions on how effective this has been. Other programs have tried to improve pediatric case finding through provider initiated counseling and testing for inpatients, malnutrition patients, tuberculosis and urgent care cases. Pediatric case finding has been increased by testing children and adolescents in primary care in Zimbabwe [91]. However, this model has proven to be challenging because of the extra resources required to carry out HIV counseling and testing in these setting [92-95].

Testing the children of adults living with HIV would improve case finding in children and adolescents, but stigma still surrounds this and there are no policy guidelines in this area. Studies have been done and provided evidence that testing pediatric contacts of adults with HIV improved pediatric case finding [96]. Table 2 below summarizes the key approaches from identifying children and adolescents for PEPFAR [76, 97].

Table 2-2: Strategies to improve pediatric case finding and antiretroviral therapy

Area		**Description**
Cross-cutting strategies	Health workforce	Increase number and capacity of health workers to provide pediatric services, and engage community lay workers
	Service delivery	Integration of services based on the needs and capacity of each site
	Supply chain	Improve drug and commodity logistics management
	Monitoring and evaluation	Data collection and documentation of current best practices. Routine measurement of key indicators
Strategies to improve HIV testing	Early infant diagnosis	Point of care diagnostic testing, testing at birth and innovative ways of communicating results back to providers and patients
	Testing of older children and adolescents	Policy guidance on routine testing of older children in health settings and other settings where adolescents congregate. Policy change on consent process for adolescents on HIV testing
Strategies to improve HIV treatment		More research on drug formulations for children. Age appropriate disclosure and retention to care interventions

Adapted from [76] who adapted from "strategies for identifying and linking HIV infected infants, children and adolescents to HIV care" http://www.pepfar.gov/documents/organization/244347.pdf

Public Health Implications of HIV in Adolescents

At the end of 2014, it was reported that there were 2.1 million adolescents aged 10-19 years old living with HIV [98]. One sixth of all new HIV infections were in adolescents aged 15-19, making it the number one cause of death among adolescents in Sub-Saharan Africa [99]. At least 85% of adolescents living with HIV globally are in sub-Saharan Africa [90]. Globally, the AIDS related deaths have reduced in all age groups due to treatment options except in the age group 10-19 years old. AIDS related deaths in this age group have in fact increased by 50% between 2000 and 2013 [100].

Adolescents are a mix of survivors of prenatally acquired HIV and behaviorally acquired HIV infection. This population together faces the same challenges in terms of access to care, treatment and linkage to support services. Adolescence is a critical time in a person's life during which they establish their identity, autonomy, master abstract thought, negotiate independent decision making, manage and master education and employment challenges and encounter intimate relationships [101]. For the HIV infected youth, the additional challenges of disclosure of their HIV status and taking charge of their own care and treatment as well reality of having to negotiate life with a chronic condition (HIV) have to be dealt with.

Adolescents struggle with disclosure of their status as a result of stigma in the environments where they live, work or go to school [26]. Programs and policies targeting adolescents have been absent from HIV programs as the focus has been on adults and children [99]. In Zambia for instance, there is an absence of youth friendly services [83].

Adherence to treatment is challenging in adolescents especially if disclosure of their HIV status was not handled adequately [102]. Generally, adolescents benefit from the once daily dosages that have made adult treatment easier. Adherence to medication and care has to be supported in this age group because of the challenges that they encounter in their daily lives which can adversely affect their self-efficacy to take medication [103].

Transitioning from pediatric to adult care is an important linkage point that can result in adolescents being lost to follow-up [104]. There needs to be emphasis on adolescent friendly services which need structures that will be safety nets during this transition period. Clinics that offered reproductive health services, support groups and other programs were found to have better retention to care in sub-Saharan Africa [25].

Adolescents who have lived with HIV for a longer time are prone to chronic co-morbidities and there needs to be an integrated approach to managing these complications and documenting them as they arise [105].

Transition efforts have to be focused and youth friendly services need to be optimized to ensure that adolescents are well managed and benefit from the improvements in care that are currently available [97]. Otherwise this group will remain a neglected reservoir of new HIV infections in the highest burden countries.

Summary of Gaps in Literature

1. Research priorities include assessing the incidence of short- and long-term severe adverse events as a result of increased exposure to ART, barriers to and enablers of adherence and long-term retention in care and the impact on the cascade of care and the magnitude of the prevention benefit of early initiation of ART, especially among key populations and adolescents [20].
2. How earlier ART affects retention, adherence and HIV drug resistance among adolescents with less advanced disease requires further investigation. Improved age disaggregation of existing cohort and surveillance data is needed to improve understanding of adolescent-specific issues and needs.
3. How earlier ART affects retention, adherence and potential HIV drug resistance among children with less advanced disease needs to be investigated further. Optimal service delivery models to ensure rapid identification and ART initiation among infants and children also need to be investigated. Strategies are needed to provide an integrated package of care to reduce overall child mortality.

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CHAPTER 3 : METHODS

Pediatric HIV is a complicated disease to study because of ethical issues regarding clinical research in children and because over 90% HIV infected children globally live in low resource and middle-income settings often under vulnerable circumstances [1]. Although the benefits of cART in children are well-documented, best approaches to deliver comprehensive HIV prevention, treatment, and care packages to resource-constrained settings remain unclear. Routinely collected program data have the potential to provide much-needed data to inform policy on the effectiveness of current models of care.

This study utilizes routinely collected program data to understand uptake of early infant diagnosis, survival patterns and loss to follow-up among children and adolescents treated at LCH, Zambia. The first part the study is a prospective cohort study of infants who had dry blood spots for HIV DNA-PCR testing and the second part utilizes data collected from medical records of patients receiving ART. We begin with description of the retrospective cohort study followed by description of the prospective cohort study.

Retrospective Cohort Study

Specific aims and study design

We will address specific aims 2 and 3 using a retrospective cohort study as follows:

Specific aim 2: To describe treatment outcomes, measure mortality rates and assess predictors of mortality among children receiving cART over a 10-year period at Livingstone Central Hospital in Zambia.

Specific aim 3: To estimate the annual incidence of loss to follow-up and identify risk factors for loss to follow-up among children on long term treatment with antiretroviral therapy at Livingstone Central Hospital in Zambia.

Study Design Overview

This retrospective cohort study will use routine program data collected by health care providers from LGH's Pediatric Center of Excellence outpatient-clinic. We will describe the **incidence of mortality** and **loss to follow-up** and associated risk factors for HIV-infected children on cART from January 2003 to June 2015.

Clinical data for all patients are entered on a structured paper based medical record by clinicians at the time of attending to the patient and then abstracted by data entry clerks into on the electronic data base called Smartcare. The Smartcare forms are compiled together to create the patients' medical follow-up file. Based on our previous experience the electronic database is not as complete as the paper-based records. We will therefore abstract our data from the paper-based records. We will create a database of patient level variables into a Microsoft Access database on a computer that has a secure password.

Retrospective cohort design is the most efficient design for this study because it allows us to use routinely collected program data. We will be able to analyze how this program has performed in the past.

Prospective cohort study design may produce better results, but it requires a long wait time to collect the necessary information. Since the guidelines for pediatric HIV care and treatment have changed very frequently over the past 10 years, a retrospective evaluation is beneficial in assessing the impact of these changes on patient survival.

A clinical trial that would compare survival distributions with age at cART initiation may be an optimal design, but is not feasible at this time. It is not ethically justifiable because previous

research and experience from other countries have demonstrated the benefits of cART for HIV infected children.

Study Setting

We implemented this retrospective cohort study of HIV-infected infants and children who were receiving cART at the Pediatric Center of Excellence clinic (PCOE) at Livingstone Central Hospital (LCH) in Southern Province, Zambia. Livingstone Central Hospital offers primary, secondary and tertiary levels of care and serves nearly 1.2 million people in the Southern Province and parts of the Western Province of Zambia. Livingstone district has a high HIV prevalence rate and at the time that a pediatric cART program was started in 2004, it was as high as 30% among pregnant women [2]. Through a collaborative agreement between the Ministry of Health in Zambia and the Centers for Diseases Control and prevention (CDC), Livingstone Central Hospital started offering pediatric cART in 2004 in a department known as the Pediatric Center of Excellence ((PCOE). Prior to 2004, a small number of children had received treatment through the hospital's inpatient department. By June 2015, 1041 children had received treatment at the PCOE and 591 children were actively on cART. The patients who receive care at LCH are enrolled from the inpatient and maternity wards of the hospital or referred from the primary health clinics in the surrounding districts. The LCH molecular laboratory which became operational in 2008 has capacity to carry out viral loads and HIV-DNA PCR tests for HIV exposed children. The PCOE clinic offers other services for children including a child sexual abuse clinic, a family support unit, a nutritional support clinic, and outpatient services.

Study Population

Data were abstracted from the medical records of HIV infected infants and children enrolled for HIV treatment and care at the PCOE. The study sample included all infants, children and adolescents who had received cART from January 2003 to June 2015. We included children from birth and adolescents aged up to 20 years at the time of cART initiation in this study. Infants who received one antiretroviral drug for PMTCT were excluded from the study because we considered cART to be a combination of at least three antiretroviral drugs.

Pediatric HIV Treatment Guidelines

The pediatric and HIV treatment guidelines in Zambia went through significant changes giving rise to four distinct eras during the study period as follows: 1) pre-ART era (before 2004) antiretroviral drugs were not easily available at government hospitals, 2) ART era (2004-2013), In 2004 antiretroviral drugs became available at health facilities in Zambia at no charge to the patients. All children infected with HIV who had CD4+ count less than 200 cells/mm^3 or CD4% less than 20% and WHO stage 3 or 4 were started on cART. In 2008, new evidence emerged and the guidelines were updated to treat all HIV infected children aged less than 5 years old, irrespective of CD4 count, 3) Option B+ era (2013). Option B+ was implemented in Zambia as the WHO recommendation to offer antiretroviral drugs to all HIV infected pregnant and breastfeeding women for their own health and for the health of their baby for the rest of their life despite their CD4 count [3]. The guidelines were revised and recommended cART for all children aged less than 5 years old with confirmed HIV infection. 4) Test and treat era; in 2016, the WHO recommended universal cART for all people diagnosed with HIV. First-line regimens consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) which included Zidovudine (ZDV), Abacavir (ABC), Tenofovir (TDF) and Lamivudine (3TC) or Emitricibine (FTC), and one non-

nucleoside reverse transcriptase inhibitor (NNRTi), including EFV or NVP with the alternative of a Protease Inhibitor, LPV/r for children whose mothers took cART for PMTCT. Second line regimens include Raltegravir (RAL) and Atazanavir (ATV) [4].

Schedule of clinical visits (summarized in Table 3.1)

The routine procedure during patients clinical visits and the forms used in the medical records were described in another study from Zambia [5]. Briefly, caregivers of HIV infected children were counselled by trained counsellors on the need to commence cART promptly. Once the caregiver agreed, the child was enrolled into care and a thorough assessment for opportunistic infections including tuberculosis and malnutrition was carried out. Baseline samples were collected for CD4 count, viral load, hematology and chemistry. Antiretroviral therapy was commenced and a follow-up visit was scheduled after 2 weeks of treatment at which time the treatment was reviewed, adherence counseling re-enforced and targeted clinical examination and laboratory tests done based on the baseline findings. The protocols recommended repeating the CD4 count, hematology and blood chemistry every 3 months and the viral load every 6 months. The patients were given prescriptions for antiretroviral drugs every 3 months and the pharmacists with the help of the adherence counsellors created a drug pick up plan for the patients. All the nurses and clinicians at the PCOE were trained in pediatric HIV through a special workshop on management of HIV and opportunistic infections in children offered by the Ministry of Health. The clinical visit schedules were outlined in the National ART treatment guidelines [4].

Outcomes

The main exposure was initiation of cART. Since this was a retrospective cohort study, the decision to start cART was made by the attending clinicians. We abstracted the date of start of cART and the initial drug regimen. Baseline laboratory measurements including, hemoglobin and

CD4+ count or percentage, baseline WHO clinical stage, anthropometric measurements and preexisting medical conditions were abstracted from the patients records and considered baseline if they were done within 2 weeks of cART initiation.

Table 3-1: Schedule of ART visits for HIV-infected children

Visits	**Standards of care**
HIV diagnosis made today	Commence CART, collect blood for CD4 count, CBC and blood chemistry. Perform TB screening. Baseline Viral load (at LGH site)
2 weeks	Adherence counseling, assessment for adverse event and Screening for TB and opportunistic infections
Monthly visits for all children <12 months, Children aged >12 months are seen every 3 months	Growth and neuro-developing screening, adherence counseling, viral load after 6 months, nutritional counseling and physical assessments
Every six months	CD4 count, CBC, blood chemistry
Once a year	Viral load

1. Mortality and Ascertainment of Death

The main outcome was death. We ascertained death from the medical records. Some medical records of patients who had died, had copies of death certificates attached, and the rest had information on the date of death estimated by the clinic staff either from hospital records or verbal reports from the child's family.

Other patient outcomes on the medical records were 1) Lost to follow-up, 2) Transferred to another facility.

2. Loss to Follow-up

Lost to follow-up was defined as no effective clinical contact for more than 6 months in the clinic. There were patients in this clinic whose schedule was to be seen once a year and we had to make a distinction between these patients and those lost to follow-up. We did this by studying the

patient records for evidence of home visits and involvement of the outreach team. Patients who were lost to follow-up were followed up by the outreach team led by one of the clinic nurses who made efforts to trace the patients and documented in their file over a period of 6-12 months. Once outreach efforts were exhausted, the patient files were closed. All the children who were lost to follow-up or transferred were censored on the day of their last visit to the clinic. Children who survived to the end of the study were all censored on June 30th 2015, which was the day we closed the database.

Exposures

The main exposure is the age at which an HIV infected child began taking cART. This is a continuous variable that is calculated from date of birth to date of cART initiation.

Study Definitions

1. HIV positive status

HIV status is defined in conformity with the Zambia National HIV testing guidelines. A child, aged under 18 months, who has a positive DNA PCR test. A child, aged above 18 months, who has a positive HIV antibody test.

The algorithm for testing HIV exposed babies is shown in figure 5, while the algorithm for HIV antibody testing is shown in figure 6. It is routine in this population for mothers to breastfeed their babies from birth until at least 18 months of age. Moreover, breastfeeding is a crucial cornerstone for child survival because replacement food is not readily available. However, as long as a child is breastfeeding, they are exposed to HIV and are at risk of acquiring the virus from their mother.

Therefore, HIV testing for children has to be done at intervals throughout the duration of breastfeeding and at least 2 months after cessation of breastfeeding. Children older than 18 months and adults are tested using antibody tests. Serial antibody testing is the testing approach that is used in Zambia. The first test is the screening test, which has high sensitivity for HIV antibodies. If the screening test is reactive, we proceed to carry out the confirmatory HIV test, which is also the HIV differentiation test, i.e. HIV-1 vs HIV-2. The flow chart (Fig 3.2) summarizes how an HIV test result is confirmed. A third tiebreaker test is run if the first 2 test results are discordant. If a tiebreaker is not available, the client is advised to come back for a retest after at least 3 months.

Ascertainment of HIV Infection Status

The HIV treatment guidelines by the Zambian Ministry of Health recommended opt out HIV testing for all pregnant women followed by universal cART for all HIV-infected pregnant and breastfeeding women (Option B+) [4]. HIV exposed infants were tested with DNA-PCR at birth, 6 weeks and 6 months of age. HIV antibody tests were repeated for babies who tested negative at 9 and 12 months and at 18 months of age or 3 months after cessation of breastfeeding. Children older than 2 years with no history of breastfeeding within the last 3 months were tested for HIV using rapid tests (Determine, Abbot Laboratories, USA) and all positive results were confirmed using Unigold rapid test (TM HIV, Trinity Biotech, Ireland).

2. Combined Antiretroviral Therapy (cART)

Combination Antiretroviral therapy (cART) which is a combination of three drugs from different drug classes. The standard first-line cART for children is two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRT)I. In the case of infants, the choice of cART depends on whether or not the mother took ARVs during pregnancy. Infants, whose mothers are ARV naïve, can be commenced on the standard first line.

Infants whose mothers took ARVs are commenced on two NRTI's and one Protease inhibitor because of resistance mutations that may have been transmitted from the mother.

Covariates and Confounders

Potential confounders and potential interaction

The term confounding refers to an epidemiologic phenomenon in which a non-causal association between a given exposure and an outcome is observed because of the influence of a third variable or (a group of variables) called the confounder. This means that the observed association between an exposure and a given outcome is induced, strengthened, weakened or eliminated by a third variable.[6] The nature of the confounding variable is stated as follows:

1) The confounding variable is casually associated with the outcome and

2) The confounding variable is non-casually or casually associated with the exposure but

3) Is not an intermediate variable in the causal pathway between exposure and outcome?

Figure 3.3 is an illustration of the Directed Acyclic Graph constructed to explore the covariates and potential confounders of the relationship between death and age at cART initiation. The effective strategy is prevention of HIV infection. In the case of women who are HIV infected or at risk, the strategy is to avoid unintended pregnancies. HIV infected women take cART to reduce MTCT and for their own health. This is important because depending on the timing of cART and medication adherence, the risk of in utero HIV transmission reduces to below 5%. Prevention of in utero HIV infection is the priority goal. Prevention of in utero HIV infection closes the rest of the biasing path; therefore, this is an important confounder.

Timing of HIV diagnosis is an important step for an HIV-exposed infant. DNA-PCR is the standard diagnostic test, however, it is not a point of care test and is expensive to run and maintain.

Most facilities do not have easy access. Accessibility, long turnaround time and other challenges result in delayed diagnosis of HIV infection in infants. Timing of HIV diagnosis is a confounder and controlling for it in the DAG closed an important biasing pathway.

We will test for potential confounders and interaction for adding the variables to the Cox proportional hazard model one at a time and comparing the changes in the Hazard ratios.

Covariates

A covariate is a variable that is possibly predictive of the outcome under study. A covariate may be of direct interest or it may be a confounding or interaction variable. Covariate in this study refers to factors that are possibly predictive of the outcome.

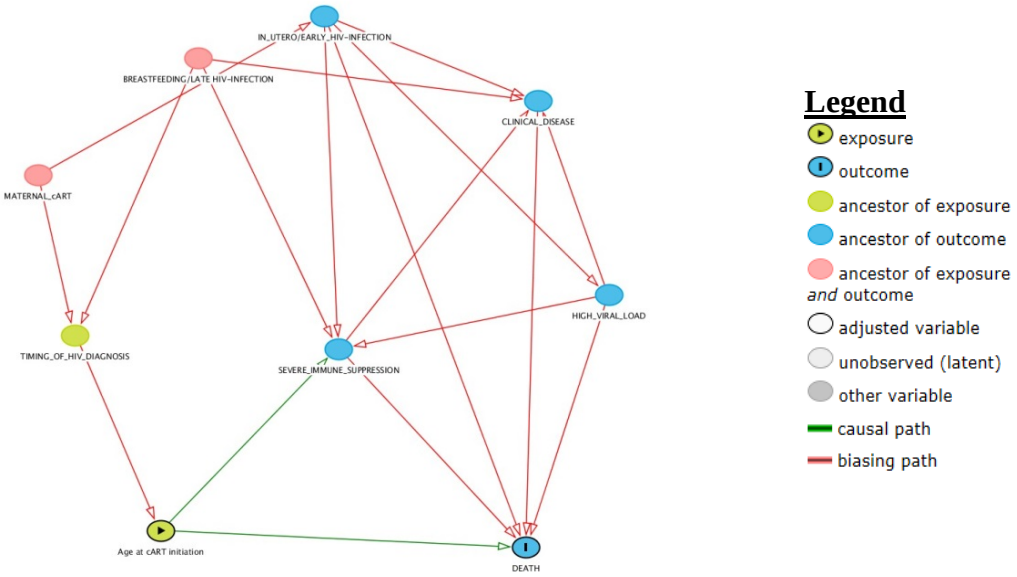
1. HIV-infected infants often have very high loads. This is because their immune system is still immature at time of infection. Therefore, the virus replicates very rapidly. There is risk of rapid disease progression in infants as a result [7]. High viral load also affects the choice of ARVs because some ARVs have a very low genetic barrier. Therefore, the virus rapidly develops resistance resulting in treatment failure [8].
2. Very low CD4 count is an indicator of severe immune suppression. Children with very low CD4 count are therefore at high risk of disease progression and development of opportunistic infections. This is accompanied by failure to thrive given the fact that low CD4 count results in immune activation, which leads to catabolic metabolism. Low Hemoglobin (anemia) at baseline has been documented in several studies to be associated with poor immune recovery. In fact, mortality has been documented to be higher in patients with low hemoglobin at cART initiation. Protein energy malnutrition is very prevalent in this population, and it is associated with severe anemia [9].

3. Weight for height z-score measures growth failure and is a consequence of severe immune suppression in children.
4. Duration of time on first-line medication: patients who do well on the first line regimen are expected to have better outcomes because the first line regimens are less toxic. As a result, the patient is more likely to be adherent to medication. However, in cases where the patient is failing on first line and they are not switched to second line, this leads to treatment failure.
5. Women who are adherent to medication and treatment during pregnancy are more likely to be adherent to the treatment regimen for their new born infants including exclusive breastfeeding up to 6 months of age which is the recommended approach in LMICs.

Measurements

The outcome variables death and loss to follow-up will be taken as recorded on the database and the patient database. Then we will verify the outcomes from the paper records. The patient status forms include the date the patient died, or in the case of loss to follow-up, the status form will include the efforts that were made to contact the patient by the social worker. We will work with the social workers in the hospital to verify the patient's status in a case where it remains unclear. It will not be possible to use the death certificates to verify the status because we cannot get access unless we know where the patient died.

The HIV status and laboratory parameters as recorded on the patient forms will be analyzed. For this part of the study, we will be using routinely collected data because we will not have the opportunity to verify much of the data. We aim to verify using laboratory records or pharmacy records and the paper files, in cases where the data is completely out of range.



After controlling for timing of HIV diagnosis, we eliminate all the biasing pathways. The major confounder of the relationship between age at cART initiation and death is timing of HIV diagnosis. All the other covariates are predictive of the outcome.

Figure 3-1: DAG showing the adjusted variables

Data Management

Data Collection

We abstracted data from the patient medical records at the clinic and created a MS access database that contained the variables of interest. The medical records were comprised of several forms called Smartcare forms which had checklists of the clinical features to look for at each visit, such as anthropometric measures, vital signs, symptoms to elicit and clinical examinations findings. We collected medical records from 2003 and abstracted the data starting with initial patient enrollment forms which included demographics such as date of birth, gender, and variables like, date of first HIV test, mode of delivery, date when they started to take ART, etc. We included all the patients whose medical files were found in the clinic registry. We collected data from 1041

files and closed our dataset on June 30th, 2015. Data abstraction was done independently by two data entry clerks and their entries were compared. Quality control was carried out by verifying the entries from every 10th medical record.

Smartcare

Smartcare is a paper and electronic medical records system that is used in antiretroviral clinics in Zambia. The entire patient visit including the laboratory, pharmacy and clinical visit are entered into the database. Patients have unique identifier numbers embedded onto a chip that they carried on a Smartcare card, which they brought with them to clinic for their visit. Before the system was fully computer based, data clerks abstracted patient information from the Smartcare forms completed by clinicians during their interaction with patients [10]. At the time of our study, we abstracted data from the medical records onto an MS access database. Table 3.3 outlines the Smartcare forms and when they are completed.

Table 3-2: Summary of Smartcare Forms

Data collection Form	**When used**
Pediatric Initial history and physical examination form	Initial enrollment visit
Patient locator	Initial enrollment and checked/updated at every visit
Adherence form	Every visit
HIV care summary sheet	Every visit summarizes laboratory results, mediations, and complications
Weight for height chart	Every visit
Pediatric follow-up visit form	Every visit
Pediatric pharmacy form	Every visit
Pediatric ARV eligibility	Initial visit and whenever changing to a new regimen
Pediatric referral form	When patient is referred to another facility
Pediatric discontinuation form	When patient dies, is lost to follow-up or defaults

Statistical Issues

Specific aim 2: To describe treatment outcomes, measure mortality rates and assess predictors of mortality among children receiving cART over a 10-year period at Livingstone Central Hospital in Zambia.

Specific aim 3: To estimate the annual incidence of loss to follow-up and identify risk factors for loss to follow-up among children on long term treatment with antiretroviral therapy at Livingstone Central Hospital in Zambia.

Analytic strategy

The Directed Acyclic Graph (Fig 3.3) shows the conceptual model of the association between age at cART initiation and death. What is clear from the literature is that HIV infected children who commence cART early have better outcomes than those who commence cART later [11, 12]. There are several factors that affect a child's likelihood to commence cART early.

Most studies showed that children whose mothers know that they are HIV infected and take cART get diagnosed early and commence cART early [13]. Breastfeeding increases the risk of transmission, but if a breastfeeding mother is on cART the transmission rate in markedly decreased [14]. Severe immune suppression, high viral load and presence of clinical disease either in the form of growth failure or opportunistic infections are factors that can increase an infant's chance of transmission [15]. Timely diagnosis of HIV in a child however keeps the child health and all the biasing pathways cease.

Data analysis steps

i. **Exploratory data analysis:**

Once the database is cleaned up, we will perform item analysis of each variable. We will assess the completeness and distribution of the data using appropriate plots. We will study

the pattern of missing variables and out-of-range variables, variability and outliers for each variable.

ii. This will be followed by **correlation assessment** between the variables excluding the outcome variable. For continuous data, we use Pearson correlation coefficients and for ranked data we will use Spearman correlation. This will help us to make decisions on whether to exclude variables from the analysis, or combine variables such as weight and height to calculate a z-score. We will exclude variables for the following reasons: 1) high correlation coefficients 2) lack of variability.

iii. **Univariate analysis** will then be done. We analyze each of the variables and then cross tabulate each variable against the outcome variables. Chi-square tests will be used to test categorical variables, and student's t-test will be used to test continuous variables.

iv. Stratified analysis/Kaplan Meier

For the stratified analysis, we will plot Kaplan Meier survival distributions. The survival distributions will be stratified by age at cART initiation. We will then estimate survival distributions at 1 year and for every year of follow-up thereafter. We will use Log-rank tests to compare the survival distributions.

v. Univariate cox proportional hazards regression

Cox proportional hazards models will be used to estimate the baseline hazard of risk factors for mortality and loss to follow-up. The Cox models will be tested using graphical methods to check for violation of the Cox proportional hazards assumption. The equation for the cox model is as follows:

$$h(t, \boldsymbol{X}) = h_0\, te^{\sum_{i=1}^{n} \beta_1 x_1}$$

Where h=Hazard at time t

X= ;(X_1, X_2.....X_P) are the predictor variables

Covariates such as CD4 count, viral load and weight/height are time-dependent and this will be taken into account during the analysis.

vi. Multivariate model building

Table 3-3: Table showing outcome, exposure, covariates, and confounders to be included in the Cox model

Exposure	**Outcome**	**Covariates**	**Potential confounders**
Age at CART initiation	1. Death 2. Loss to follow-up	1. Baseline Viral load 2. Baseline CD4 count 3. Breastfeeding 4. NVP/CTX prophylaxis 5. Baseline Weight for height z-score 6. W.H.O stage 7. Adherence to medication and treatment	1. Timing of HIV diagnosis, i.e. access to DNA PCR 2. Early detection infants infected in-utero and at time of delivery.

Factors that will be found to be statistically significant ($p<0.05$) on the univariate Cox model will be entered into a multivariate model one at a time.

Death is modeled by treatment initiation and this can be written in equation form as follows:

$$h(t, \boldsymbol{X}) = h_0\, te^{\sum_{i=1}^{n} \beta x_1}$$

Where h=hazard at time t

X= ;(X_1, X_2.....X_P) are the predictor variables

The baseline hazard of death is given by

$$h(t, \boldsymbol{X}) = h_0\, t$$

The first step of the model building process will be to test the proportional hazards assumption and assess if the PH is the best way to model these data. The PH assumes that the hazard of is constant over time.

I will use three approaches to assess the PH assumption:

1) Graphical: I will compare log-log survival curves for the children who initiated cART early compared to late. We will additionally plot the observed versus expected survival time.
2) Goodness-of-fit: This approach is appealing because it provides a test-statistic (p-value) which will allow us to make clear cut decisions.
3) Time dependent variables: We will evaluate the model one at a time and also evaluate the predictors several at a time.[16]

The first step of the model building process will be to put the exposure variable, in this case, age at cART initiation in a model together with the outcome variable and assess how this compares with the other variables.

$$h(t, \boldsymbol{X}) = h_0\, te^{\sum_{i=1}^{p} \beta ixi}$$

This gives the unadjusted estimate of how the exposure affects mortality.

Where x1 is the exposure;

The second step is to add the covariates to the model one at a time

$$h(t, \boldsymbol{X}) = h_0\, te^{\sum_{i=1}^{p} \beta ixi}$$

The hazard ratio HR is given by $\frac{h_0\, te^{\sum_{i=1}^{p} \beta ixi}}{h_0\, te^{\sum_{i=1}^{p} \beta ixi}} = \exp[\sum_{i=1}^{p} \beta (X_i^* - X_i)] = e^{\beta 1}$

we will use the AIC to decide which model fits best.

After this we will then add the exposure and one covariate at a time. We will use the AIC to decide which model fits best.

$$h(t, \boldsymbol{X}) = h_0\, te^{\sum_{i=1}^{n} \beta_1 x_1 + \beta_2 x_2}$$

We will then add the confounding variables to the model one at time and assess for the change in hazard ratio before and after addition of the potential confounder. If there is difference between the crude hazard ration and the hazard ratio after addition of the potential confounder the variables is truly a confounder. We will assess for effect modification.

The final equation will look like this:

$$h(t, \boldsymbol{X}) = h_0\, te^{\sum_{i=1}^{n} \beta_1 x_1 + \beta_2 x_2 + \beta_3 x3 + \beta_4 x_4}$$

where x1=exposure

x2=covariates

x3=confounders

x4=interaction terms[16]

1) Sensitivity analysis

This will be done in order to test various assumptions that we encountered during the model building process. Sensitivity analysis tests the robustness of the results to violations of the assumptions.

Competing Risks Analysis

In the study on loss to follow-up, we hypothesized that some of the children who were lost to follow-up may be dead. When Loss to follow-up occurs before the child died, such a child is recorded as lost to follow-up and their true outcome which is death is not known. This results in a competing risks situation when we analyze lost to follow-up as an outcome. The naïve Kaplan

Meier takes competing risks as censored observations and this makes it a biased estimate of the cumulative incidence function.

Limitations and alternative approaches:

The major Limitation is that the data we plan to use is routinely collected program information that was not collected for research and is therefore not very complete, with potentially a lot of missing data. However, with good analytic methods, we will get the best results. The fact that we have to abstract data from paper records is a major limitation. If the Smartcare database had good clean data, we may not have had to use the paper records**.**

Prospective Cohort Study

To address specific aim 1, we implemented a prospective cohort study by compiling routinely collected program data for children who came to LCH for early infant testing of HIV within the first two months of life at later. This enabled us to calculate the perinatal HIV infection by calendar year and to study the uptake of HIV testing at different ages and to assess how the National HIV testing and treating guidelines have been implemented at this health facility.

Specific aim 1: To quantify the proportions of early infant diagnosis, including birth testing among HIV exposed infants at Livingstone Central Hospital (LCH), from January 2009 to December 2016.

The methods and analysis for specific aim 1 are described in chapter 4.

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CHAPTER 4 :TRACKING PROGRESS TOWARDS ELIMINATION OF MOTHER TO CHILD TRANSMISSION OF HIV IN ZAMBIA: FINDINGS FROM THE EARLY INFANT DIAGNOSIS OF HIV PROGRAM[1]

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Abstract

Introduction: In 2014, only 37% of HIV exposed infants in Zambia had an HIV DNA-PCR test for HIV diagnosis within the first two months of life. We sought to quantify the proportions of early infant diagnosis, including birth testing among HIV exposed infants at Livingstone Central Hospital (LCH), from January 2009 to December 2016.

Methods: Using a retrospective cohort study design, we abstracted data from patient registers for HIV exposed infants who had received dry blood spots for HIV-DNA PCR tests at LCH. We carried out descriptive analysis and estimated the temporal trends in perinatal HIV transmission.

Results: 3,301 dry blood spot tests were collected for HIV DNA-PCR between 2009 and 2016. Of these, 793 had demographic data recorded in the patient records but only the data of 329 children was complete data. Of the 329 children, 31(9%) were confirmed to have HIV infection. Of the 298(91%) HIV uninfected infants, only 80 (27%) came back for a second HIV test and just 44 (15%) came back for a third test. 17% of the infants tested during the first week of life (birth testing) were HIV infected while 14% of those tested between 1 to 4 weeks of age and 18% of those between 4 to 8 weeks of age were HIV infected. Overall, we observed a decline in the perinatal HIV transmission rate from 19% in 2009 to 1.4% in 2016.

Conclusion: Our finding that perinatal HIV transmission declined remarkably following implementation of universal combined antiretroviral therapy for all HIV infected pregnant women (Option B+) in Zambia suggests significant strides towards elimination of mother to child HIV transmission. Testing HIV-exposed infants during the first week of life diagnosed 17% of them with HIV infection. This is evidence that implementation of birth testing of all HIV-exposed

infants is a high yield strategy for timely diagnosis of HIV-infection and for optimizing the HIV-care cascade. There is need to improve documentation of patient information and outcomes for monitoring and evaluation purposes.

Keywords: HIV, Pediatrics, infants, early diagnosis,

Introduction

Early Infant diagnosis, defined as receipt of virologic testing for HIV exposed infants by two months of age is a key prevention of mother to child transmission of HIV (PMTCT) recommendation by the World Health Organization (WHO) [1]. HIV diagnosis in infants is complicated in resource limited settings because maternal anti-HIV antibodies are detectable in HIV exposed infant's serum, rendering HIV antibody tests ineffective for early infant diagnosis of HIV[1-3]. Nucleic acid tests with molecular assays such as DNA-PCR give the most reliable results are effective at identifying infants infected in utero who have the highest risk of disease progression and mortality in settings where they are implemented at or near birth [1, 4, 5]. However, nucleic acid tests are expensive and challenging to implement in resource limited settings [6, 7]. The Zambia Ministry of Health recommends birth testing with DNA-PCR for HIV exposed infants as of the 2016 guidelines [8].

In 2015, it was estimated that 79% of all pregnant women in Zambia were tested for HIV and 95% of those that were diagnosed with HIV commenced combined antiretroviral therapy (cART) [9]. However, only 37% of HIV exposed infants had a nucleic acid test within the first 2 months of life and only 46% of infants and children diagnosed with HIV had accessed cART [9, 10]. Early initiation of cART for HIV-infected infants is critical because it reduces the risk of

mortality by as much as 76% [11]. This gap in treatment coverage among children is the result of low early infant diagnosis.

We sought to quantify early infant diagnosis among HIV exposed infants at Livingstone Central Hospital, from January 2009 to December 2016. We present the successes and challenges of this program and generate program relevant information to inform institutional policy and/or investment in early infant diagnostic testing for HIV-exposed infants as a crucial tool for ensuring AIDS free survival in the era of continued progress towards elimination of mother to child transmission of HIV (EMTCT) in Zambia.

Methods

Study design, site and population

We conducted a retrospective cohort study of HIV exposed infants and children at the Pediatric Center of Excellence clinic (PCOE) at Livingstone Central Hospital (LCH) in Livingstone, Zambia. This hospital serves both an urban and rural population with estimated HIV prevalence of 13.4% in 2016 [9, 12]. This facility houses the only molecular laboratory for early infant HIV diagnosis in the province. Early infant testing was implemented at the PCOE in 2008. We enrolled HIV exposed infants and children who had dry blood spots tests done for HIV DNA-PCR at the LCH from January 2009 to December 2016. Universal cART for all HIV infected pregnant women (Option B+) was introduced in 2013 and the early infant diagnosis registers and patient records were updated to include the mother's demographic information and timing of HIV diagnosis/cART initiation. We therefore found that only infants tested from 2014 had any demographic information and could be linked to their mother's medical record. We abstracted data for 3,301 children who had dry blood spots collected during the study period. Prior to Option B+

implementation from 2009 to 2013, 2,508 were tested while 793 were tested during the Option B+ era from 2014 to 2016 (Figure 1). In addition, the option B+ records had information about the HIV-infected child's follow-up care including when the child was commenced on cART and the name of the facility where they received care if not at PCOE. We found detailed information about infants whose caregivers refused treatment including their reasons for refusal and follow-up plans to ensure that counseling continued. Only caregivers of 3 infants refused treatment.

Ethical considerations

This study was approved by the Zambia National Health Research Authority and the institutional review boards at Macha Research Trust and the University of Georgia. We conducted secondary analysis of anonymized routinely collected program data. Hence, informed consent was waived.

Ascertainment of HIV exposure status

HIV Testing for Mothers

Pregnant women admitted to the delivery wards with unknown HIV status were screened for HIV using a rapid test (Determine, Abbot Laboratories, USA) and all positive results were confirmed using Unigold rapid test (TM HIV, Trinity Biotech, Ireland). Women who had HIV negative test results during antenatal visits were retested for HIV as recommended by the Zambian Ministry of Health if the last test had been more than six weeks before delivery [8].

HIV testing for Infants

All the infants of HIV-infected women were commenced on Nevirapine prophylaxis right after delivery by the attending Midwife. Dry blood spots were taken at earliest contact with a nurse counselor before discharge from the delivery ward. The nurse counsellors were trained to take the

dry blood spots and link the mother-baby pair to HIV care and treatment at the hospital or at the primary health care facilities. For the dry blood spots, whole blood from the infant was collected using heel or big toe prick and the blood was spotted onto 5 circles on filter paper. The filter paper was dried overnight at room temperature and sealed in humidity free bags and later sent to the molecular laboratory. One spot from each filter paper was tested by COBAS Ampliprep/Taqman HIV-1 Version 2.0 real time PCR assay (ROCHE Diagnostics, Indianpolis IN) according to manufacturer instructions. All positive tests were repeated with a second sample from a second spot on the filter paper.

Mother-baby pair follow-up

HIV DNA-PCR results were expected back from the laboratory within 7 days. The caregivers were advised to come back to collect the results and additionally contacted by phone once the result was received at the clinic. However, because some of the patients came from long distances, the results were sometimes sent to their nearest health facility using the National SMS service that the laboratories in Zambia use to send text messages of results to health facilities [13, 14]. Clients whose results were sent in such a manner were followed up by the social worker to ensure that they received their results and they knew where to go for the 6 weeks visit to get their 6-week dry blood spots. Patients with positive tests were additionally followed up to ensure that they came back for further evaluation and commencement of antiretroviral therapy or to receive treatment at a facility near them.

Between 12 months of age and 3 months after cessation of breastfeeding, children were tested with routine HIV rapid diagnostic tests followed by DNA-PCR to confirm any positive tests.

HIV-infected infants and children were immediately commenced on cART as recommended by the Ministry of Health [8].

Data collection

We abstracted the following variables from the patient records prior to Option B+ implementation (2009-2013): file number, date of birth, gender, address, date of collection of the dry blood spots, and the result of the HIV DNA-PCR test. From 2014 (Option B+ era), we found additional information in the patient records and included the following variables: date of collection of first dry blood spot test, result of first dry blood spot test, date of second dry blood spot collection, result of second dry blood spot test, date of third test and the respective result. In addition, we abstracted the mother's details as follows: when did the mother know her HIV status, when was the mother commenced on cART, what regimen of antiretroviral drugs did she take, did the baby take antiretroviral drugs for prophylaxis, and what was the infant feeding choice.

Statistical analysis

We performed descriptive analysis of the mother-baby pairs baseline data by estimating medians, interquartile ranges, frequencies, and percentages by HIV infection status as confirmed by the DNA-PCR test results in the patient records. We plotted bar charts and boxplots to evaluate the trends in HIV testing among children. We present descriptive statistics for categorical variables and medians with interquartile range (IQR) for continuous variables. To analyze the trends in proportions of HIV positive test results, we performed linear regression of the proportion of HIV positive test results and the year. Data analysis was performed using SAS software (SAS version 9.4 (SAS Institute Inc, Cary, NC) and MS excel.

Results

A total of 3,301 children had dry blood spots for HIV DNA-PCR done at LCH between 2009 and 2016. 2,508 were done before 2013 and were not included in the analysis for baseline characteristics because they did not have baseline demographics. Of these 2,508, we excluded 1,192 tests, 176 had missing results, 700 were done after 12 months of age and 316 were repeat tests. Of the remaining 1,316 tests, 300 (23%) were confirmed to be HIV-infected, and 1,016 (77%) were HIV uninfected. Among the 793 tests done between 2014 and 2016, we excluded 14 with missing results, 78(10%) were HIV infected and 701(90%) were HIV uninfected (Figure 4-1).

Among the 793 children enrolled from 2014, we found that only 329 had complete baseline data and we therefore used this data to understand the baseline demographic characteristics of the HIV exposed children whose samples were tested for HIV with DNA-PCR during the study period. Of the 329 children, only 31(9%) were confirmed to have HIV infection and 298 (91%) were HIV uninfected. The median age at which the tests were done was 97 days (IQR: 14-214) for the HIV infected children and 4 days (IQR: 1-41) days for the HIV uninfected children (Table 4-1). Of the 298 HIV uninfected children, only 80 (27%) of the children came back for a subsequent test at median age of 6 months (IQR; 6-7 months) and just 1 of these 80 children was confirmed to have HIV infection at the time of the second test. The HIV infected child was uninfected on the first DNA-PCR test that was done within the first 2 hours after delivery. A total of 44 (15%) children had a third test at median age of 12 months (11-14 months). None of these 44 children were diagnosed with HIV (Table 4-2).

HIV-infected children were older (median age=3 months, IQR: 2 weeks to 6 months) than HIV uninfected children (median age=4 days, IQR: 1 day - to 2 months). 167 (51%) of the children

were male. Of the 31 HIV infected children, 19 (61%) were female. Only 196 (56%) of the mothers knew their HIV status before pregnancy. In total 197(60%) of the mothers took cART during the pregnancy and 167(51%) of the babies took antiretroviral prophylaxis before 6 weeks of age. In total, 22 (71%) of the HIV infected children were born to women who knew their HIV status before pregnancy (Table 4-1).

Linkage to care

Of the 31 HIV-infected children, 28 infants were successfully linked to HIV care and treatment. Of the 3 infants who didn't commence antiretroviral therapy, one died during the second week of life due to neonatal complications, one infant's caregiver refused to have the baby commenced on antiretroviral therapy and one child was lost to follow-up.

Age at the time HIV DNA-PCR test

The median age at which the HIV DNA PCR test was done declined from 8 weeks in 2009 to 2 weeks in 2014. There was more variability in age at which the test was done between 2014 and 2015 compared to the other years.

Overall, we observed a steady decline in the number of DNA PCR tests done for early infant diagnosis during the study period with the largest decline between 2014 and 2015, followed by a marked increase in 2016. In 2009 approximately 20% of the test results confirmed HIV infection and in 2015, 19% of the test results confirmed HIV infection while in 2016, only 1.4% of the tests confirmed HIV infection (Figure 4-2). Linear regression confirmed that the proportion of positive results declined every year (R squared=0.622, p=0.02).

When we stratified the DNA-PCR results by age at which the test was done, we found that 860 (26%) out of 3,301 tests were collected within the first 2 months of life. Among the 860

infants, 264 were tested during the first week of life and 277 were tested between 1 and 4 weeks of age, while 319 were tested between 4 to 8 weeks of age. Overall, 45(17%) of infants tested during the first week of life were HIV infected, 38(14%) of those tested between 1 and 4 weeks and 57(18%) of tested between 4 to 8 weeks of age were HIV infected (Table 4-3).

In 2014 after introduction of Option B+, 36% of those tested were younger than 2 months of age (Figure 4-3).

Discussion

In this study, we evaluated the early infant diagnosis program at Livingstone Central Hospital in Zambia and observed a remarkable decline in the perinatal HIV transmission rate from 19% during the period between 2009 and 2015 to only 1.4% in 2016. We found that 98% (28 out of 31) of the HIV infected infants were successfully linked to care, but only 27% (80) of the HIV uninfected infants who were tested before 2 months of age came back for subsequent tests. We observed that 17% of the infants tested during the first week of life (birth testing) were HIV infected while 14% of those tested between 1 to 4 weeks of age and 18% of those between 4 to 8 weeks of age were HIV infected.

These findings are essential for achieving the first of the WHO 90-90-90 targets to end the HIV epidemic. The first target is to ensure that 90% of all people living with HIV are diagnosed. Among infants and children this is urgent because in 2015, only 40% of HIV exposed infants globally were tested for HIV within the first 2 months of life [15]. Early infant diagnosis in resource limited settings relies on the PMTCT programs including mothers returning for postnatal care. We demonstrated in this program that early infant testing was successfully implemented in a delivery unit through coordination with the pediatric unit. The pediatric unit

through the PCOE clinic tracked the exposed infants through the patient records and registers and were responsible for ensuring that the clients got their results and were linked to care for subsequent HIV tests.

Our observed decline in perinatal HIV transmission must be cautiously interpreted. Firstly, because this was only for 1 year, 2016 and secondly, these results are from one hospital setting with an optimized early infant diagnosis program, thirdly, retention in care was very low (27%) and most of the children who tested negative on the first test did not come back for a subsequent test. Lastly, we did not explore the reasons for this decline. However, studies conducted in other settings associated decline in perinatal HIV transmission to optimization of universal cART for all HIV infected pregnant women[16, 17]. A study done in France found that among 2,651 infants born to HIV-1 infected women who received cART before conception and had plasma viral load less than 50 copies/ml at delivery, there was no perinatal HIV-1 transmission[18]. This decline is consistent with a recent report from the Centers for Disease control which reported perinatal HIV-1 transmission rate of 2% in Zambia [19]. In our study, we observed that 71% (n=22) of the HIV infected infants in the Option B+ era register were born from mothers who knew their HIV status and took cART during the pregnancy. Although we knew the cART status of these mothers, we did not have adequate clinical data and follow up data such as viral load to assess the level of adherence and viral suppression which is critical in reducing the chances of transmission.

Retention in care was very low in this study (27%). This was because Livingstone Central Hospital was a referral center and most of the clients may have decided to go back to their primary health care facility after delivery as recommended by the Ministry of Health guidelines.

We have no way to verify that these clients were linked or seen at the primary health care level of their choice, but a study done in a similar setting (South Africa) demonstrated low retention in care especially among HIV exposed infants who tested negative the first time. This program in South Africa documented high coverage of birth PCR testing, out of 7,085 HIV-infected deliveries, 6,358 (89.7%) of the neonates were tested. Only 91 (1.4%) were HIV-infected and with active outreach, 96% of them were initiated on cART. Of the 6,261 HIV uninfected infants, only 3,251 (52%) came back to collect their negative results [5].

Although subsequent testing for HIV negative infants was low at LCH, early infant testing was accessible through the expanded program of immunization (EPI) at primary health care facilities. Wang et al carried out a clinical trial that demonstrated that the Zambian Ministry of Health policy to integrate early infant diagnosis in the expanded program of immunization (EPI), resulted in a 17% increase in the number of dry blood spots done among HIV exposed infants and the age at which the first dry blood spot was done decreased from 4 months to 3 months during the study period [20]. Additionally, routine provider initiated testing and counseling (PITC) of infants and children was implemented in all Zambian healthcare facilities to hospitalized children [21-23]. Despite these other avenues for infant testing, studies demonstrated that HIV diagnosis for infants must be done early in life for optimal health outcomes [24, 25]. Our study showed that early infant testing is feasible in this setting.

The Ministry of Health in Zambia recently recommended birth testing for HIV-exposed infants in line with the World Health Organization 2016 guidelines [1, 8]. These new guidelines came at a time when there was urgent need to scale up early infant diagnosis in Zambia. The challenge was that the turnaround time for getting results from the laboratory was long and

resulted in delays in treatment initiation of HIV-infected infants [26, 27]. Laboratories in Zambia sent results to health facilities via text messages and this decreased turnaround time [13, 28]. However, contacting mothers to collect the results remained a challenge possibly due to low mobile phone ownership and low mobile phone coverage in rural areas [14].

The observed temporal changes in early infant testing were mainly because of policy changes. Option B+ was implemented in 2013 and Livingstone Central Hospital was one of the first facilities with capacity to adopt the infant testing guidelines and therefore the number of tests that were done among infants aged less than 2 months increased from 108 in 2009 to 233 in 2014. In 2015 the number of infants tested declined to 137 as the capacity of the primary health care system to perform early infant diagnosis improved. In 2016, birth testing of HIV exposed infants was optimized at LCH and this resulted an increase of tests to 353 (Figure 4-2).

The low uptake of HIV diagnosis in infants in Zambia, and several other sub-Sahara African countries, can be explained by: 1) inadequate number of laboratories conducting HIV DNA-PCR, 2) stock outs of commodities required to carry out the dry blood spots at health facility level, 3) long turnaround time for results to get back to the health facility and subsequently back to the patient and 4) poor retention of mother's in postnatal care [4, 26, 29]. In rural settings like most parts of Southern Province Zambia the situation is more challenging because only 56% of women delivered in a health care facility and 58% received postnatal care in 2014 [30]. Low institutional deliveries and poor utilization of postnatal care results in low PMTCT service utilization which leads to delays in HIV diagnosis for HIV exposed infants.

Limitations

We retrospectively analyzed routinely collected data that were not collected for research purposes and had inconsistencies and missing information, but we resolved this by collecting additional data from the medical files. Our finding that the records of over 50% (464 of 793) of the tested infants were not complete is of major concern. There is urgent need to ensure that all tested infants are diagnosed and linked to care.

Despite our limitations we found that the registers were useful in helping us understand the pediatric antiretroviral treatment program, specifically the uptake of early infant testing at Livingstone Central Hospital. There were challenges in completing the entries, but this can be overcome with consistent supervision. There is need for continued research to evaluate the impact and optimal timing of early treatment and the optimal dosing of antiretroviral medications to infants.

Conclusion

Our findings that the perinatal HIV transmission rate declined remarkably following implementation of Option B+ in Zambia from 19% to 1.4% suggests significant strides towards elimination of mother to child transmission of HIV. The finding that testing HIV-exposed infants during the first week of life diagnosed 17% of them is evidence that implementation of birth testing is a high yield strategy for timely diagnosis of HIV infection and for optimizing the HIV care cascade by ensuring early treatment initiation and maximal aversion of long-term sequelae due to pediatric HIV morbidity. This provides evidence for the recent recommendation by the Ministry of Health to test infants at birth and subsequently at 6 weeks, 6 months, 9 months and beyond depending on breastfeeding status. There is however urgent need to improve retention in care for

infants who test negative at birth because they are still at risk of MTCT through breastfeeding. The turnaround time for results from the molecular laboratory remains a challenge and there is need to consider use of point of care tests to eliminate this. There is need to improve documentation of patient information and outcomes for monitoring and evaluation which is critical to inform policy.

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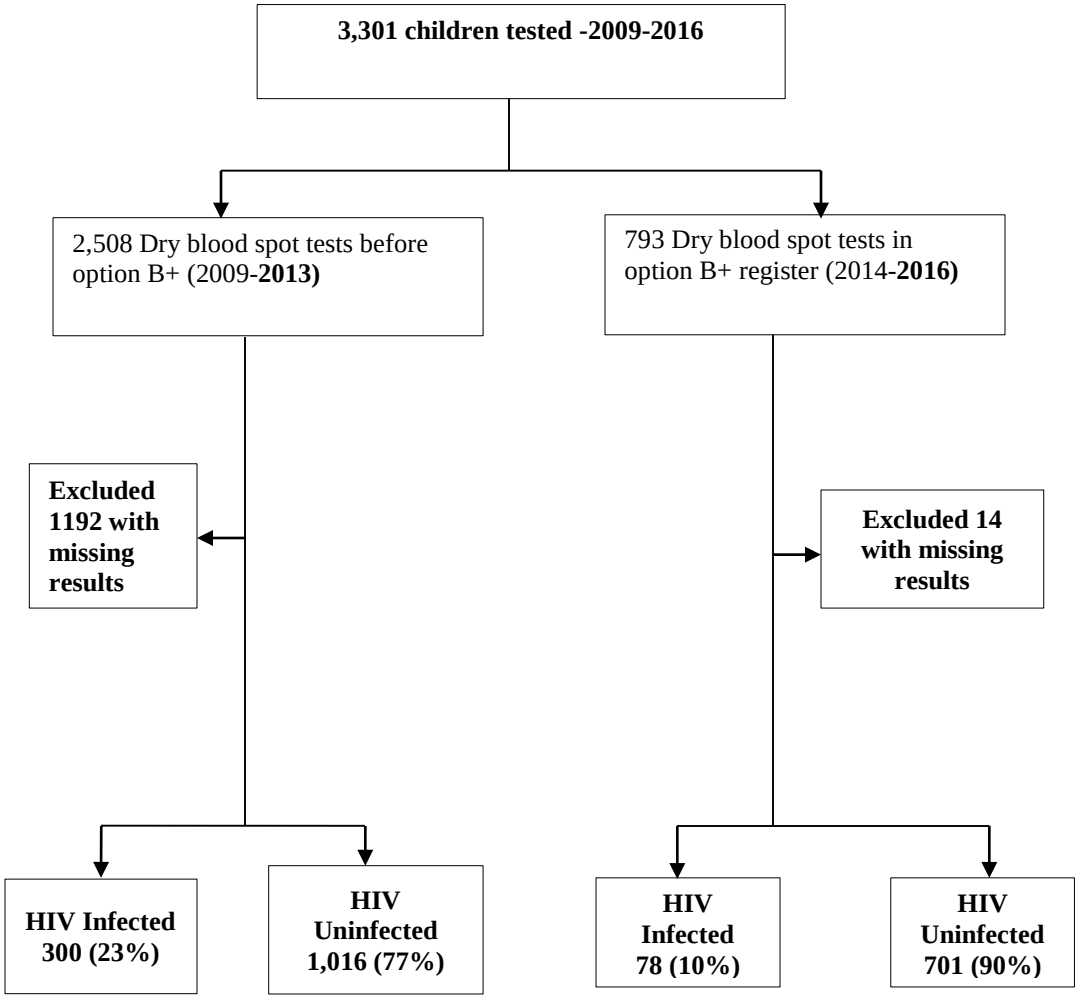


Figure 4-1.: Flow Chart of HIV DNA-PCR results in the Registers at Livingstone Central Hospital

Table 4-1: Baseline Characteristics of 329 infants had DNA-PCR for HIV diagnosis at Livingstone Central Hospital: 2014 to 2016 (Option B+ Era)

	Result of baby's first DNA-PCR test		
Characteristic	**Positive**	**Negative**	**Total**
	N=31	**N=298**	**N=329**
Age in (days) when 1st DNA-PCR was done (Median IQR)	**97(14-214)**	**4(1-41)**	
Sex			
Female	19(61%)	143(48%)	**162(49)**
Male	12(38%)	155(52%)	**167(51)**
Time of mother's first HIV diagnosis			
Before pregnancy	22(71%)	174(91%)	**196(56%)**
During pregnancy	0	13(100%)	**21(6%)**
During labor and delivery or PNC	2(6%)	4(2%)	**6(2%)**
Unknown	7(23%)	107()	**114(36%)**
Mother received ARVs for PMTCT			
NVP only	1(3%)	9(3%)	**10(3%)**
AZT during ANC	3(9.6%)	92(31%)	**95(29%)**
Full cART	23(74%)	174(58%)	**197(60%)**
none	4(13%)	23(8%)	**27(8%)**
Baby received ARVs for prophylaxis before 6 weeks of age			
Yes	18(58%)	149(50%)	**167(51%)**
No	8(25%)	43(14%)	**51(16%)**
Unknown	5(16%)	106(36%)	**111(34%)**
Year DNA-PCR was done			
2014	11(35%)	120(40%)	**131(40%)**
2015	15(48%)	76(26%)	**94(29%)**
2016	5(16%)	99(28%)	**104(32%)**

*99.7% of the infants in this population were breastfed
ANC= Prenatal clinic
cART= Combination antiretroviral therapy
NVP= Nevirapine
IQR= Interquartile range
AZT= Azido thymidine
PMTCT= Prevention of mother to child transmission of HIV
DBS= Dry blood spot

Table 4-2: Children who came back for Subsequent HIV Tests at Livingstone Central Hospital: 2014-2016

	N	**Age (months) Median(IQR)**	**Negative tests**	**Positive tests**	**Result not recorded in register**
1st Test	**661**	0.2(0-1.7)	298(45%)	31(5%)	337(51%)
2nd Test	**80**	6.0(6.0-7.0)	68(85%)	1(1%)	11(14%)
3rd Test	**44**	12(11-14)	35(80%)	0	9(20%)

*IQR=interquartile range

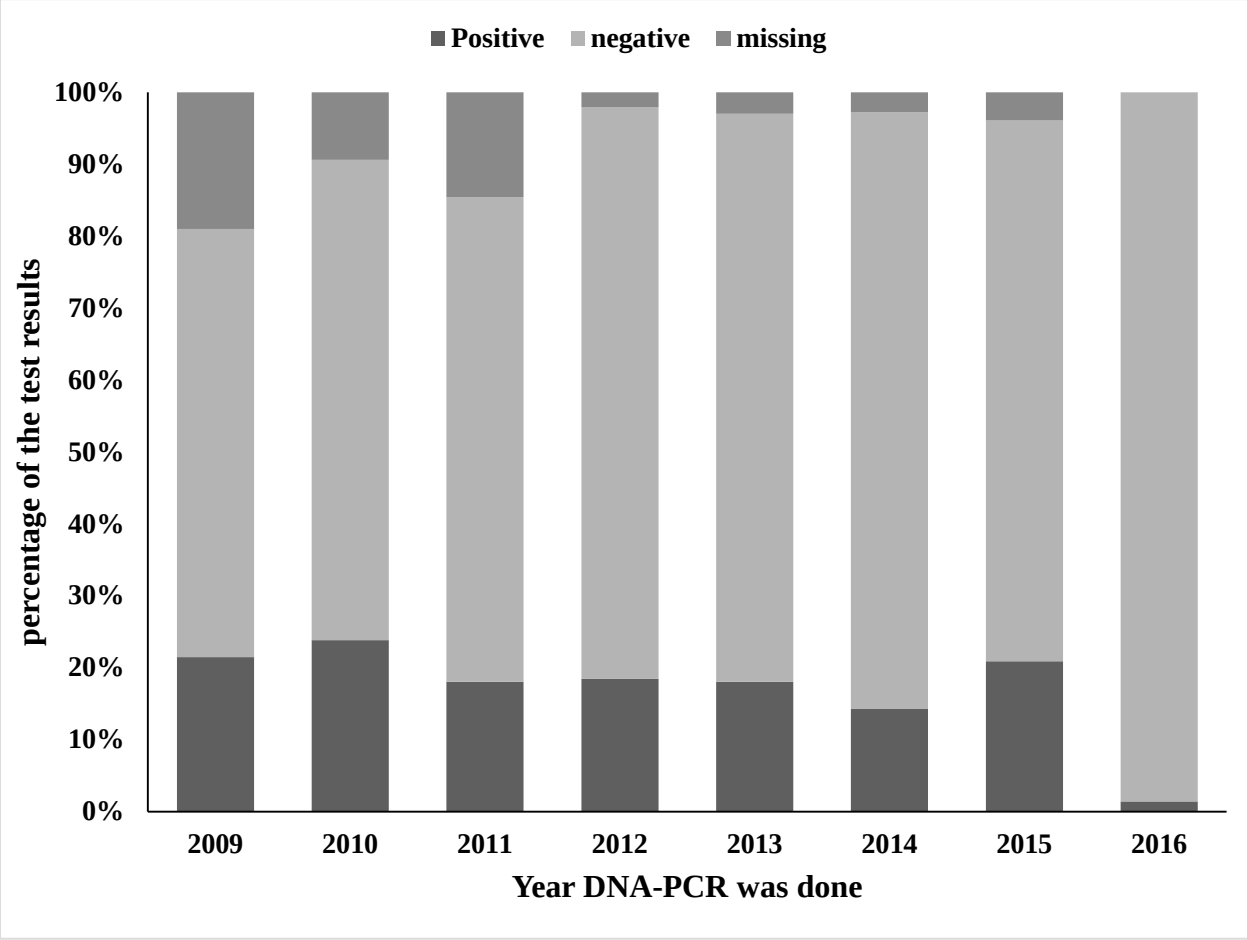
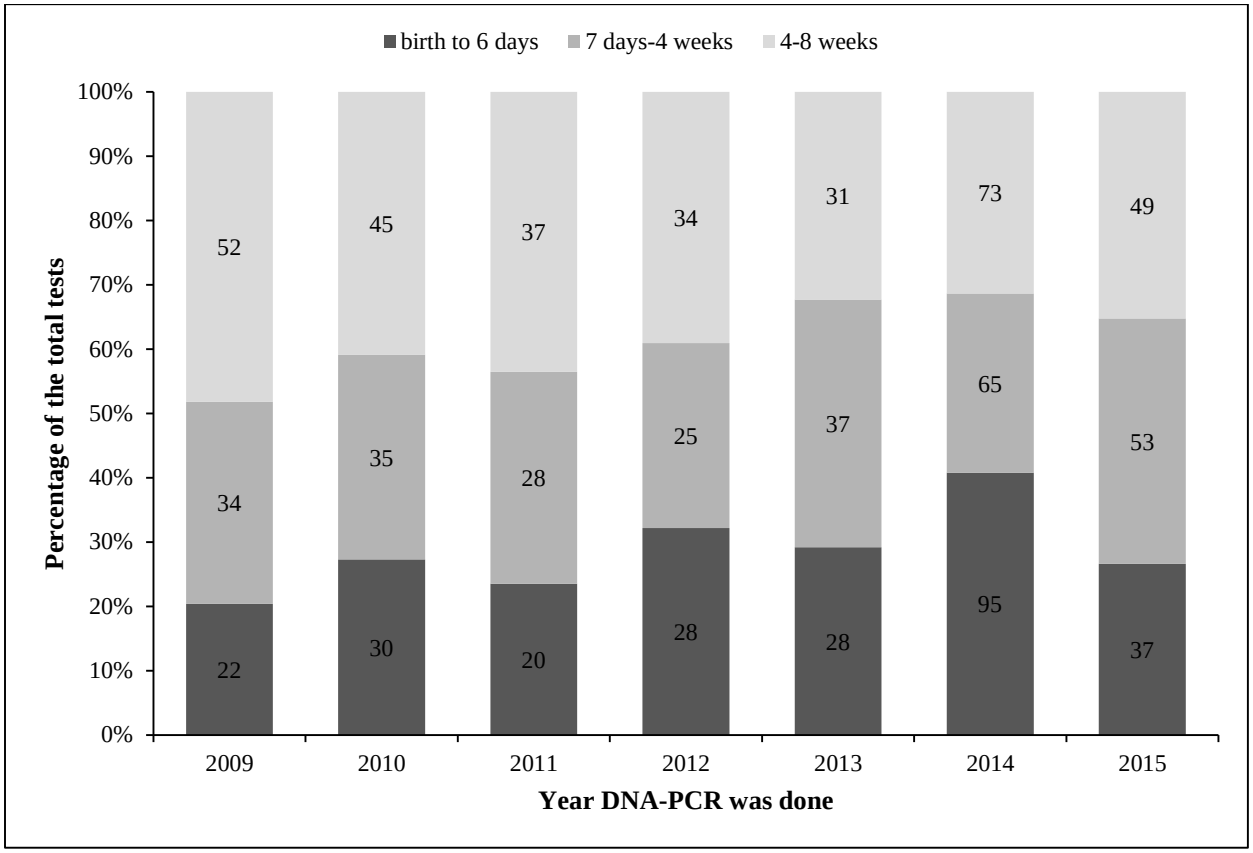


Figure 4-2: Early Infant diagnostic test results for HIV diagnosis in children at Livingstone Central Hospital: 2009- 2016

Table 4-3: Early infant testing with DNA-PCR for HIV diagnosis within the first 2 months of Life by age group at Livingstone Central Hospital: 2009-2015

Year	0 to 6 days	Total tested at birth	1 -4 weeks	Total tested at age 1-4 weeks	4-8 weeks	Total tested at age 4-8 weeks
	Positive N(%)		Positive N(%)		Positive N(%)	
2009	2(9)	22	4(12)	34	10(19)	52
2010	6(21)	29	3(9)	35	5(11)	45
2011	6(23)	26	2(7)	28	5(14)	37
2012	5(18)	28	2(8)	25	8(24)	33
2013	6(21)	28	10(27)	37	6(19)	31
2014	13(14)	95	7(11)	65	12(16)	73
2015	7(19)	36	10(19)	53	11(23)	48
Total	**45(17)**	**264**	**38(14)**	**277**	**57(18)**	**319**

*Option B+ was implemented in 2013 at Ministry of Health facilities in Zambia.
*2016 Results were excluded due to incompleteness



*Number inside the bar =n

Figure 4-3: Distribution of Age at which DNA-PCR tests were done among infants aged less than 2 months at Livingstone Central Hospital: 2009-2015

CHAPTER 5 :LONG-TERM SURVIVAL OUTCOMES OF HIV INFECTED CHILDREN RECEIVING ANTIRETROVIRAL THERAPY: A 10-YEAR OBSERVATIONAL STUDY FROM LIVINGSTONE, ZAMBIA (2003-2015)[2]

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Abstract

Background: In 2015, 61% of HIV infected Zambian children had accessed combination Antiretroviral therapy (cART). Despite this expanded cART coverage, there is paucity of information on effectiveness of the pediatric cART programs in reducing mortality. The aim of this research is to describe treatment outcomes, measure mortality rates and assess predictors of mortality among children receiving cART over a 12-year period at a hospital in Zambia.

Methods: Using a retrospective cohort study design, we abstracted routinely collected clinical data from medical records of children enrolled in HIV care between 2003 and 2015. Our main outcome was death. We estimated the distribution of survival times for the baseline covariates using the Kaplan-Meier (KM) method. Log-rank tests were used to compare the survival curves. Cox Proportional Hazards models were used to estimate the hazard ratios for death and their 95% confidence intervals to quantify the associations.

Results: 1041 children and adolescents aged from birth to 20 years were enrolled in HIV care and there were 71 (7%) recorded deaths. Mortality was highest during the first 3 months after initiation of treatment (mortality rate of 11.7/100 person years (7.6-16.3) and survival probability of 0.97). In the multivariate Cox proportional Hazards model, the adjusted hazards of death among children aged less than 1 year were 3.1 (95% CI: 1.3-6.4) and children in WHO stage 4 had the highest hazards of mortality (HR =4.8 (95% CI, 2.3-10) ($p<.0001$). Mortality rate was 1.6/100 person years (1.4-1.8) after total follow-up time of 4450 person years.

Conclusion: We observed low attrition due to mortality among children on HIV care in Zambia. Mortality was highest during the first 3 months of treatment. Pretreatment screening and treatment

of opportunistic infections among children commencing cART needs to be strengthened to reduce early mortality.

Keywords: HIV Seropositivity, Pediatrics, therapeutics, treatment outcome, survival,

Introduction

The availability of combined antiretroviral therapy (cART) for HIV infected children and implementation of universal treatment of all HIV infected pregnant and breastfeeding women (Option B+) has been a game changer in the global fight against HIV [1]. In the absence of cART, more than half of all perinatally HIV infected children die before their second birthday [2]. However, access to cART among children remains a challenge in high HIV burden countries. In 2016, approximately 54% of adults in need accessed cART, but just 43% of children had access [3].

Zambia, a country in Southern Africa with a population of 14 million people had estimated HIV prevalence of 12.9% in 2016 [4]. There were approximately 85,000 children living with HIV and 4,700 were newly infected in 2014 [5, 6]. The pediatric HIV program in Zambia has rapidly expanded with over 61% of children in need accessing cART in 2015 [7]. However, significant gaps remain in early infant diagnosis of HIV, linkages to care and treatment for HIV exposed infants. The patterns of long term survival and associated factors including barriers to long term adherence and retention are not adequately elucidated in sub-Sahara Africa. The aim of this research is to describe treatment outcomes, measure mortality rates and assess predictors of mortality among children receiving cART over a 12-year period at a hospital in Zambia.

Methods

We conducted a retrospective cohort study of HIV-infected children who received cART at the Pediatric Center of Excellence clinic (PCOE) at Livingstone Central Hospital (LCH) in Southern Province, Zambia.

Study Setting

Livingstone Central Hospital offers preventive and treatment services to nearly 1.2 million people in the Southern and western parts of Zambia. The pediatric HIV treatment program at LCH was started in 2004 through a collaborative agreement between the Ministry of Health in Zambia and the Centers for Diseases Control and prevention (CDC) [8]. By June 2015, 1041 children had received treatment and 591 children were actively on cART.

Study population

Data were abstracted from the medical records of HIV infected children. The study sample included all children from birth and adolescents aged up to 20 years at the time of cART initiation who received treatment from January 2003 to June 2015. Infants who received one antiretroviral drug for PMTCT were excluded from the study because we considered cART to be a combination of at least three antiretroviral drugs.

Data Collection

We abstracted data from the patient medical records and created a Microsoft access database that contained the variables of interest. We abstracted the data starting with initial patient enrollment forms which included demographics such as date of birth, gender, date of first HIV test, mode of delivery, date when they started to take cART, etc. We included all the patients whose medical files were found in the clinic registry. We collected data from 1041 files and closed our

dataset on June 30th, 2015. Data abstraction was done independently by two data entry clerks and their entries were compared randomly.

Study Outcomes

The main exposure was initiation of cART. We abstracted the date of start of cART and the initial drug regimen. Baseline laboratory measurements including, hemoglobin, CD4+ count or percentage, WHO clinical stage and anthropometric measurements were abstracted from the patients records and considered to be baseline if they were done within 2 weeks of cART initiation.

The main outcome was death. We ascertained death from the medical records. We found death certificates in some medical records, the other records had information on the date of death estimated by the clinic staff either from hospital records or verbal reports from the child's family.

Other patient outcomes on the medical records were 1) Lost to follow-up, 2) Transferred to another facility. Lost to follow-up was defined as no effective clinical contact for more than 6 months in the clinic. Patients who missed appointments for more than 90 days where visited by a clinic nurse and their status established. All the children who were lost to follow-up or transferred were censored on the day of their last visit to the clinic. Children who survived to the end of the study were all censored on June 30th, 2015.

Data Analysis

Baseline demographics and clinical features were described by estimating medians and interquartile ranges for continuous variables and frequencies and proportions for categorical variables. Observed survival was defined as the duration of time in years from the date of cART initiation to the date of death or censoring. We estimated the distribution of survival times for the baseline covariates affecting survival using the Kaplan-Meier (KM) method. Log-rank tests were

used to compare the survival curves. Cox Proportional Hazards models were used to estimate the hazard ratios for death and their 95% confidence intervals to quantify the associations. We evaluated the proportional hazards assumption using log-log plots and plots of Schoenfeld's residuals and no violations of the assumption were found. To calculate the mortality rates, we estimated the actual person time that everyone contributed to the study during the first 6 months, 1 year, 2 years, 5 years and 10 years and constructed confidence intervals.

Multivariate Cox models were used to adjust for possible confounding. All p-values were two tailed. We assessed several models to assess the robustness of the final model (supplementary table). In the analysis, age was a categorical variable with 3 levels. The age categorization was based on findings from previous studies which demonstrated that infants aged less than 12 months had a different survival experience based on disease progression [2, 9]. WHO stage was a categorical variable with 3 levels in this model. Once the best model was selected, sensitivity analysis was done to assess the effect of loss to follow-up at cART initiation. We evaluated two extreme scenarios, in one scenario, we assumed that all the children lost to follow-up survived and were censored at the end of the study (June 30th, 2015) and in the second scenario, we assumed that all the children survived for 90 days after their last visit and then died. We estimated survival distributions and hazard ratios for these scenarios and compared with the main study results.

Data analysis was done using SAS version 9.4 (SAS Institute Inc, Cary, NC) and R statistical software [10]. We used the Survival package to plot the KM curves and Survminer package to visualize the KM curves [11, 12].

Ethical consideration

This study was approved by the Zambia National Health Research Authority and the institutional review boards at Macha Research Trust and the University of Georgia. We conducted analysis of anonymized routinely collected program data. Hence, informed consent was waived.

Results

1041 children aged less than 15 years commenced cART at LCH between January 2003 and June 2015. Overall, 71 (7%) were confirmed to have died after commencing treatment and 590 (57%) were alive and active in care. A total of 164 (16%) were lost to follow-up and 216 (20%) were transferred to other health care facilities during the observation period (Figure 5-1).

Baseline characteristics of children started on cART

At baseline, 520 (49%) of the children were female and 721(69%) were cared for by their biological mothers. A total of 179 (18%) of the children commenced treatment during their first year of life. At least 304 (29%) of the children were diagnosed during hospital admission and 30 (3%) were diagnosed from the delivery wards (Table 5-1).

The median age of the children at baseline was 3.6 years (IQR 1.3-8.6) and for children aged between 5 and 15 years old, the median CD4 count was 290 (IQR: 126-509). The median CD4 percentage for children under 1 year of age was 24 (IQR: 15-31), 17 (IQR: 13-23). At least 956 (97%) of the children took Cotrimoxazole at enrollment and 301(30%) had a diagnosis of Tuberculosis. 472 (46%) were WHO stage 3 and 177(17%) had advanced WHO stage 4. The first line regimen comprised of 2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and 1 Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) for 909 (87%) while 54 (5%) were

commenced on regimens that contained Protease Inhibitor (Lopinavir boosted with Ritonavir) (Table 5-2).

In 2004 the number of children on cART increased and the majority commenced between 2006 and 2009, 491(50%) (Figure 5-2).

Mortality Trends

A total of 71 deaths were documented during the study period. The largest number of deaths was among young children aged less than 12 months (n=29, 41%) (Figure 5-3). We found the death certificates for 38% (n=27) of the 71 deceased children. From the 27 death certificates, the major cause of death was diarrhea, accounting for 14% (n=10), severe pneumonia and protein energy malnutrition each accounted for 11% (n=8) and tuberculosis accounted for 7% (n=5) of the deaths (Table 5-4).

The children were followed up for a total of 4450 person years. After 10 years of follow-up, the mortality rate was 1.6/100 person years (1.4-1.8). Mortality was highest within the first 3 months of follow-up with estimated mortality rate of 11.9/100 person years (95% CI:7.6-16.3) accounting for 41% (n=29) of the deaths (Table 3). The 3-month survival probability was 0.97 (Fig 5-4).

Infants who started cART within the first year of life experienced shorter survival time compared to those who started treatment after 1 year and after 5 years of age (Fig 5-4A, log-rank test, p-value <0.0001). The survival probability for infants aged less than 1 year was 0.93 after 3 months of treatment compared to 0.96 for those aged 1 to 5 and 0.99 for those aged above 5 years old. In the multivariate Cox proportional Hazards model, the adjusted hazards of death among children aged less than 1 year were 3.1 (95% CI: 1.3-6.4, Table 5-5).

Children with severe immune-suppression (WHO stage 4) at baseline had lower survival probability of 93% compared to 97% for the WHO stage 3 and 99% for WHO stage 1 and 2, (Fig 5-4B, log-rank test, p-value <0.0001). In the multivariate Cox model, children with advanced WHO stage 4 had the highest hazard of mortality (HR =4.8 (95% CI, 2.3-10) (p<.0001), as compared to children in WHO stage 3 (HR 1.8 (0.9-3.6) p=0.1188, Fig 5-4B).

Children who took triple Nucleoside reverse transcriptase regimens had the shortest survival time (86% at 1 year) compared to children who took NRTI and NNRTI and protease inhibitor based regimen (log rank test, p<0.0001, Fig 4D). Other baseline factors associated with shorter survival were; mothers who took antiretroviral drugs during pregnancy (p=0.012), presence of anemia (Hemoglobin <8g/dl) (p=0.032) and severe wasting as estimated by WAZ score <-3SD (p=0.051). The covariates were however not statistically significant in the multivariate Cox proportional hazards model. Tuberculosis at baseline, calendar year of ART initiation and whether the infant took antiretroviral drugs for prophylaxis at enrollment did not appear to affect long-term survival (Table 5-6).

We carried out sensitivity analyses in models 2 and 3 to assess the effect of loss to follow-up. In model 2, we assumed that all defaulters completed follow-up and were censored on June 30th 2015. In model 3 we assumed that all the defaulters survived for 90 days after their last visit and then died. We therefore had 71 deaths and all the 164 defaulters as deaths in this model. The direction of the hazards ratios in these 2 hypothetical models was the same as in the main model and magnitude of the effect size was slightly less than the main model (Table 5-7). In another sensitivity analyses, we subset all the children aged 5 years and under and repeated these analyses. Our findings were consistent with what we found in the main model.

Since the results are so similar, we conclude that our main model is robust and the loss to follow-up did not affect mortality.

Discussion

In this study of HIV infected children receiving cART over a period of more than 12 years (2003-2015), attrition due to mortality was low (7%, n=71). Mortality was highest during the first 3 months after initiation of treatment (mortality rate= 11.7/100 person years (95% CI:7.6-16.3).

The mortality rate observed in this study was much lower than that observed in a study conducted in an urban setting in Zambia in which mortality was 6.6/100 person years over 3018 person years of follow-up [13] and a study done in a rural setting in Zambia (9% and 14.4% after 6 and 24 months of treatment respectively) [14]. These studies were also characterized by early mortality (17.4/100 person years within the first 3 months) and the associated risk factors were similar to what we observed (young age and advanced WHO stage) [13]. Our findings although still lower, were more similar to a study from a rural setting in Malawi where 12 months mortality was 6.6/100[5.5-7.9] and overall mortality was 3.4/100[2.9-4] after 5 years of follow-up [15]. Our observed morality rate is however higher than findings from developed countries where 10-year mortality was as low as 0.3/100 person years in the Dutch cohort [16].

The observation that hazards of death were highest within the first few months after the child starts taking cART and then declines after the first 6 months of treatment is consistent with studies done in other parts of Zambia and in similar settings [13, 17-19]. Other studies have attributed the high early mortality to late presentation to care [20]. Studies done in routine clinic settings in resource limited settings found that the cumulative incidence of mortality during the first year of treatment among older children between 5-10 years of age was less than 2% to more

than 45% among infants aged less than 12 months with severe disease [21]. Once children get past the initial 6 months, their risk of mortality declines to very low rates especially when adherence to treatment and follow-up is optimized [18, 22, 23].

Our finding that the hazard of mortality were highest among infants and children aged less than 12 months of age is consistent with predictive models that have been done in both developed and developing countries and in fact motivated the universal ART policy by WHO [24]. Results of a modelling study done in six countries in Sub-Sahara Africa suggested that mortality is higher among perinatally HIV infected children than those infected through breastfeeding [25]. Perinatally infected infants have a higher risk of mortality and disease progression [2]. Early diagnosis of HIV infection and early initiation of cART improves the outcomes of these infants [26]. Although early infant diagnosis of HIV is critical, there are still challenges in resource limited settings to diagnose children. Children who are missed by the PMTCT programs are at highest risk of mortality because they usually present to the hospital after an illness when they are already immunocompromised with high risk of mortality [27].

Children on cART in a treatment program are a highly select group and have frequent contact with healthcare providers and therefore their risk of mortality is expected to be lower than that for the general population. It was therefore of interest for us to explore the causes of mortality in this group. We strived to assess the causes of death in our study but only found death certificates for 27 children and no postmortem or thorough investigations had been done to ascertain cause of death. However, causes of death recorded on the few death certificates were similar to the causes of childhood deaths in Zambia, which are diarrhea, severe pneumonia and protein energy malnutrition [5]. This setting has a very high infant mortality rate (65/1000 in 2015) and this was

consistent with what is prevailing in the general population [28]. In the pre-ART era, AIDS related deaths led to an increase in infant mortality in high HIV burden countries. Antiretroviral therapy however improved child survival and evidence is now showing that early initiation of ART leads to better clinical outcomes [26]. In a study that pooled results of clinical trial data from Zimbabwe and Uganda, the mortality risk was attributed to pre-ART risks that persist until the ARVs reach their maximal effectiveness [18]. These early deaths have been attributed to suboptimal management of malnutrition, tuberculosis and other related medical conditions during the early treatment stage [23]. The focus may be on treating the HIV, thereby overlooking other significant causes of death.

The major strength of our study is that it was done in a routine clinic setting among children commencing cART in a high HIV burden setting. This provides a real-world effectiveness of pediatric ART treatment outcomes and provides information on important predictors of mortality. Our sample size was large and we had enough outcomes to make reasonable conclusions.

A major limitation is that there was a high proportion of defaulters (16%) and many transfers (20%). This made ascertainment of mortality very challenging because some of the children who were lost to follow-up may be dead and therefore misclassified. A study form Malawi found that at least 11% of the children who were lost to follow-up had died. The 7% that we observed might be an underestimate. Transfers are high in this clinic and this is motivated by Ministry of Health policy that encourages people to seek care at the health facility nearest to them. Studies have showed that this is an effective strategy as patients can additionally benefit from support groups and other programs that may be available at their health facility [29, 30]. However, this makes it difficult to study long-term treatment related outcomes because it is currently very difficult to track

patients once they transfer to another facility. To address this, we performed a sensitivity analysis and the results were similar in both scenarios.

Conclusion

We observed low attrition due to mortality among children on HIV care in Zambia. Mortality was highest during the first 3 months of treatment. Pretreatment screening and treatment of opportunistic infections among children commencing cART needs to be strengthened to reduce early mortality.

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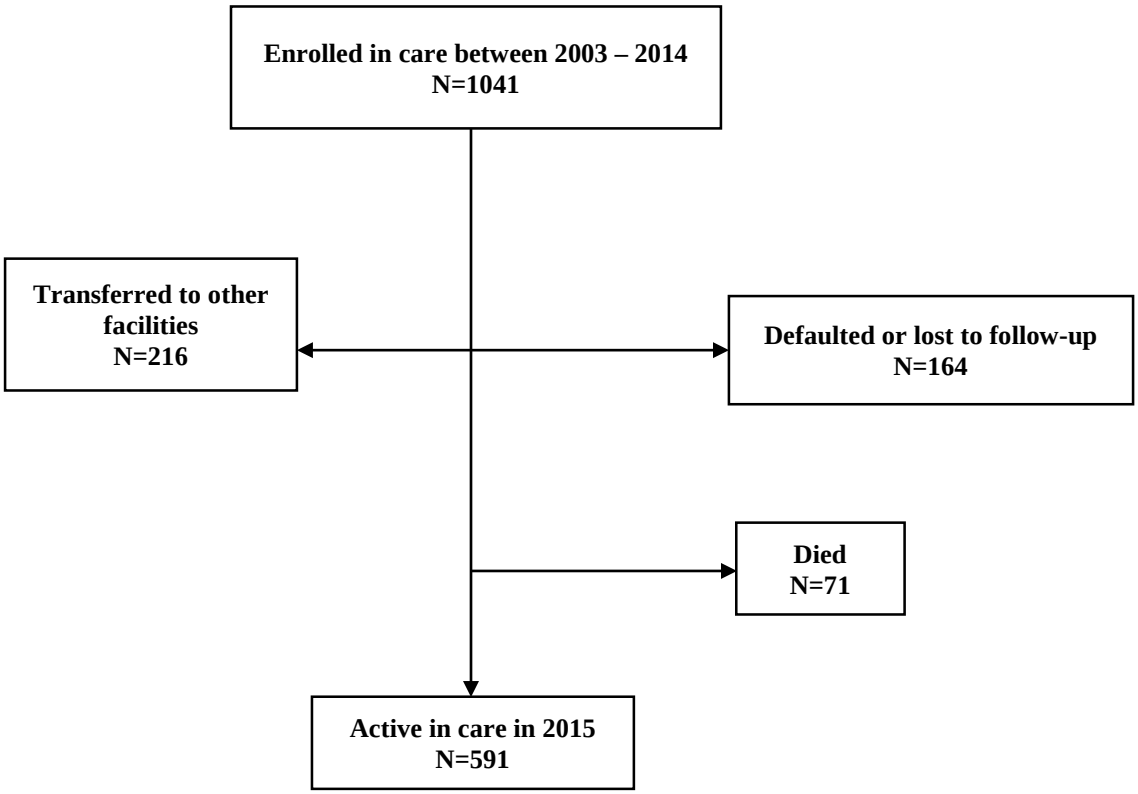

Figure 5-1: Treatment outcomes and retention in care among children on cART at LCH 2003-2014

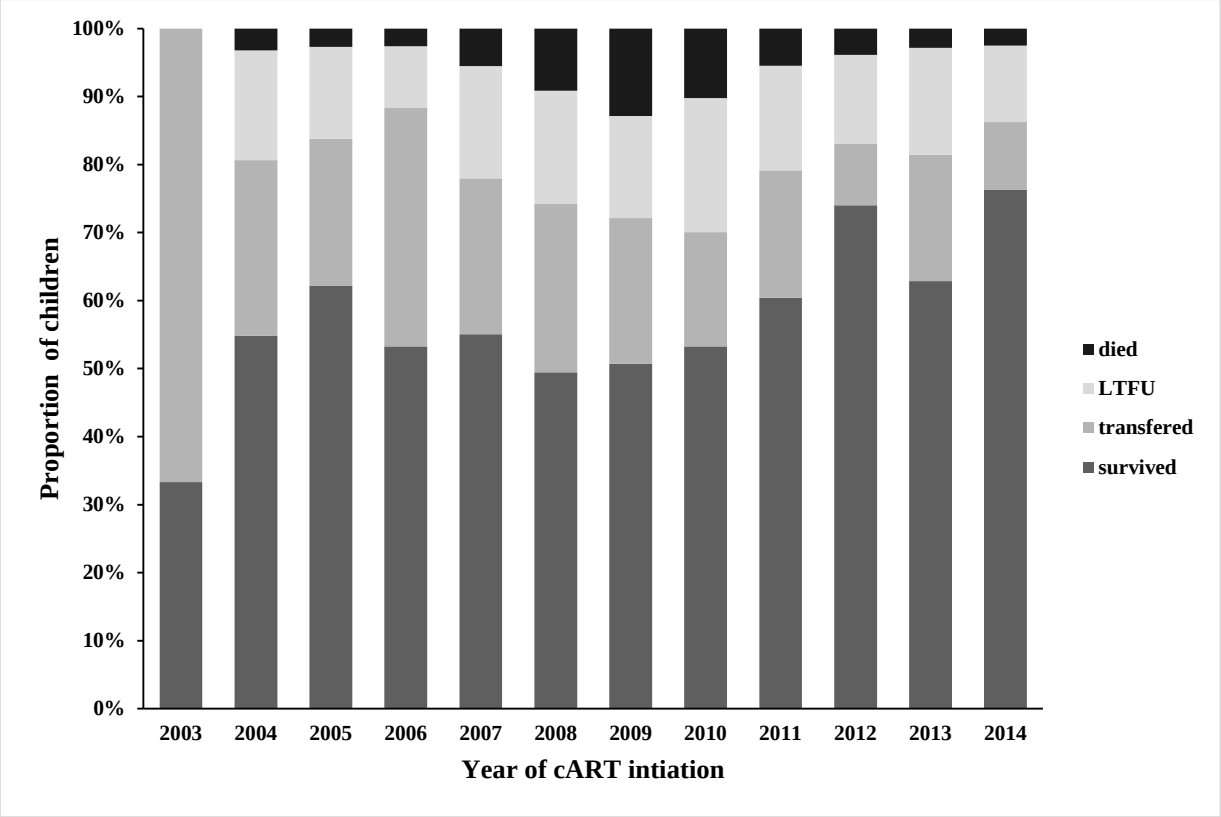

Figure 5-2: Trends in treatment outcomes among children on cART at LCH: 2003-2014

Table 5-1: Baseline Characteristics

Characteristics	**N=1045**
Gender n(%)	
Female	524 (49)
Male	521 (51)
Age(years) at enrollment Median (IQR)	3.6 (1.3-8.6)
Duration (years) on treatment Median(IQR)	3.6(1.2-6.5)
Duration of time (weeks) from diagnosis to ART initiation	6 (2-17)
Age category at enrollment N(%)	
<1 year	181(18%)
1-5 years	389(37%)
>5 years	469(44%)
Missing	*6(1%)*
Who is the child's Guardian	
Mother	722(69%)
Father	44(4%)
Grandparent	92(9%)
other relative	127(12%)
missing	*60(6)*
Mother Alive	
Yes	737(71%)
No	166(16%)
missing	*140(13%)*
Father Alive	
Yes	691(67%)
No	179(17%)
missing	*175(16%)*
Point of Entry into HIV care	
Out-patients departments	176(17%)
Inpatient Wards	304(29%)
MCH/Delivery wards	30(3%)
VCT clinic(FSU)	127(12%)
TB clinic	12 (1%)
missing	*396(38%)*
Educational level of caregiver	
None	19 (2%)
Primary or secondary school	612(59%)
Some college or university	60(6%)
missing	*354(34%)*
Occupation of caregiver	
Employed	254 (24%)
Self-employed	158(15%)
Unemployed	38(4%)
missing	*595(58%)*
Does the family have a phone	
Yes	705(68%)
Has HIV status been disclosed to the child	
Yes	191(18%)

Table 5-2: Baseline laboratory and clinical features

Characteristics	
CD4 count at enrollment Median (IQR)	**505(243-948)**
Hemoglobin at enrollment (Median IQR))	**9.6(8.3-10.9)**
CD4 percent at enrollment Median(IQR)	
<1yr	19(25-26)
1-<2yrs	17(11-21)
2-5yrs	15(9-11)
missing	*351(34%)*
Taking Cotrimoxazole at enrollment	
Yes	1002(96%)
No	43(4%)
Drug regimen at ART initiation N(%)	
3 NRTI's	78(8%)
2NRTIs +1NNRTI	913(87%)
LPV/r based	54(5%)
Year of ART start N(%)	
2003-2005	71(7%)
2006-2009	519(50%)
2010-2012	306(29%)
2013-2016	151(14%)
Mom took ARVs for PMTCT during pregnancy	
Yes	162(16%)
No	883(84%)
Child took ARV prophylaxis after birth N(%)	
Yes	123(12%)
No	861(82%)
missing	*61(6%)*
Nutritional status at enrollment	
Weight for height score<-3SD	182(17%)
weight for height score >-3 SD	863(83%)
Baseline clinical staging (WHO stage) N(%)	
WHO stage 1	219(21%)
WHO stage 2	161(15%)
WHO stage 3	472 (46%)
WHO stage 4	177 (17%)
missing	*16(1%)*
Diseases at baseline N(%)	
TB	301(30%)
pneumonia	165(16%)
Diarrhea	247(24%)
Gestation age at birth N(%)	
Premature	16(1.5%)
term	485(46.5%)
unknown	544(52%)
Birthweight Median(IQR)	**3.2 (2.8-3.5)**
Mode of delivery N(%)	
C/Section	18(1.7%)
SVD	493(47.3%)
unknown	534(51%)

Table 5-3: Cause of Death as Recorded on Death Certificate

Cause of death	Total number
Diarrhea	10
Severe Pneumonia	8
Protein Energy Malnutrition	8
Pulmonary Tuberculosis	5
TB Meningitis	2
Gastrointestinal Bleeding	2
Malaria	2
Meningitis/encephalitis	2

*We found death certificates of 27 patients. The cause of death as recorded on the death certificate is listed in the table above.
*Some patients had more than one cause of death listed on the death certificate

Table 5-4: Mortality among children receiving cART at Livingstone Central Hospital, Zambia (2005-2015)

Duration of cART	Number of children left	Deaths	Follow-up years	Mortality Rate, deaths per 100 person-years (95% CI)
3 months	927	29	243	11.9 (7.6-16.3)
6 months	868	41	469	8.7 (6.0-11.41)
1 year	813	49	894	5.5 (3.9-7.1)
2 years	702	60	1646	3.6 (2.7-4.5)
5 years	385	66	3293	2.0 (1.5-2.5)
10 years	25	71	4450	1.6 (1.4-1.8)

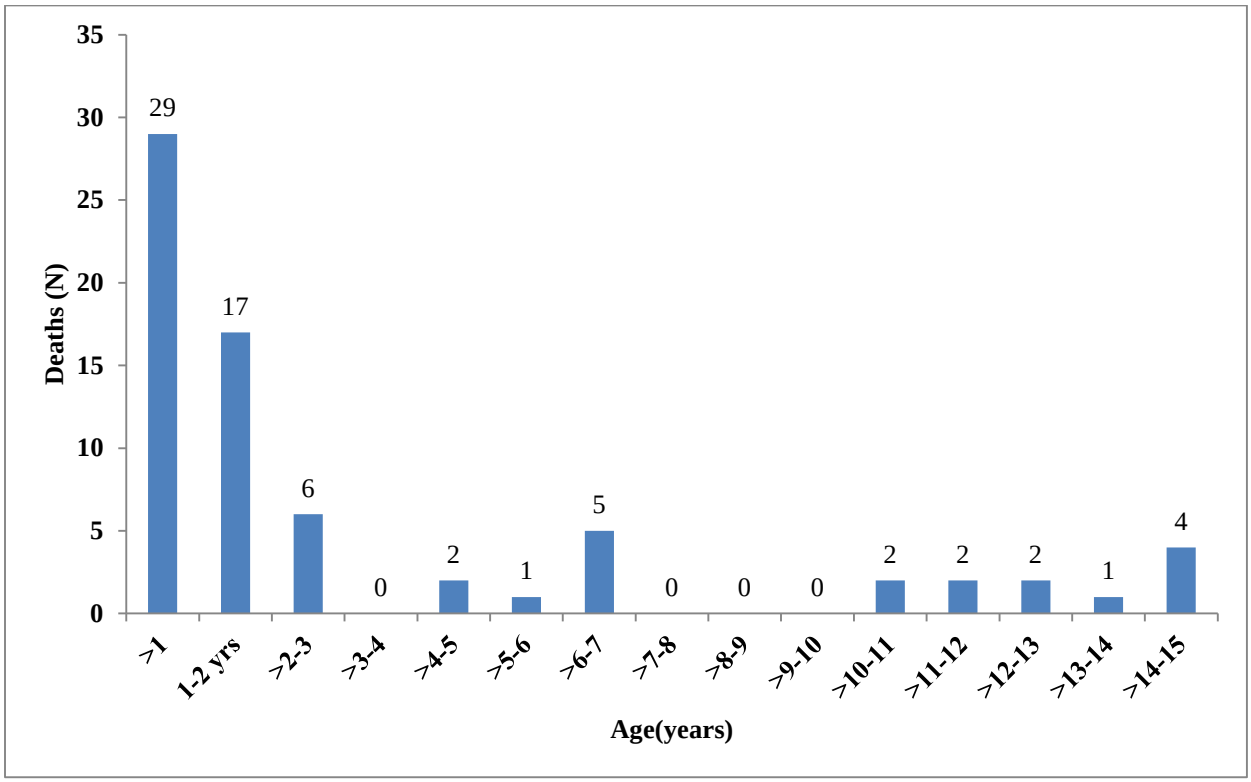

Figure 5-3: Number of deaths by age at time of cART initiation: LCH 2004-2015

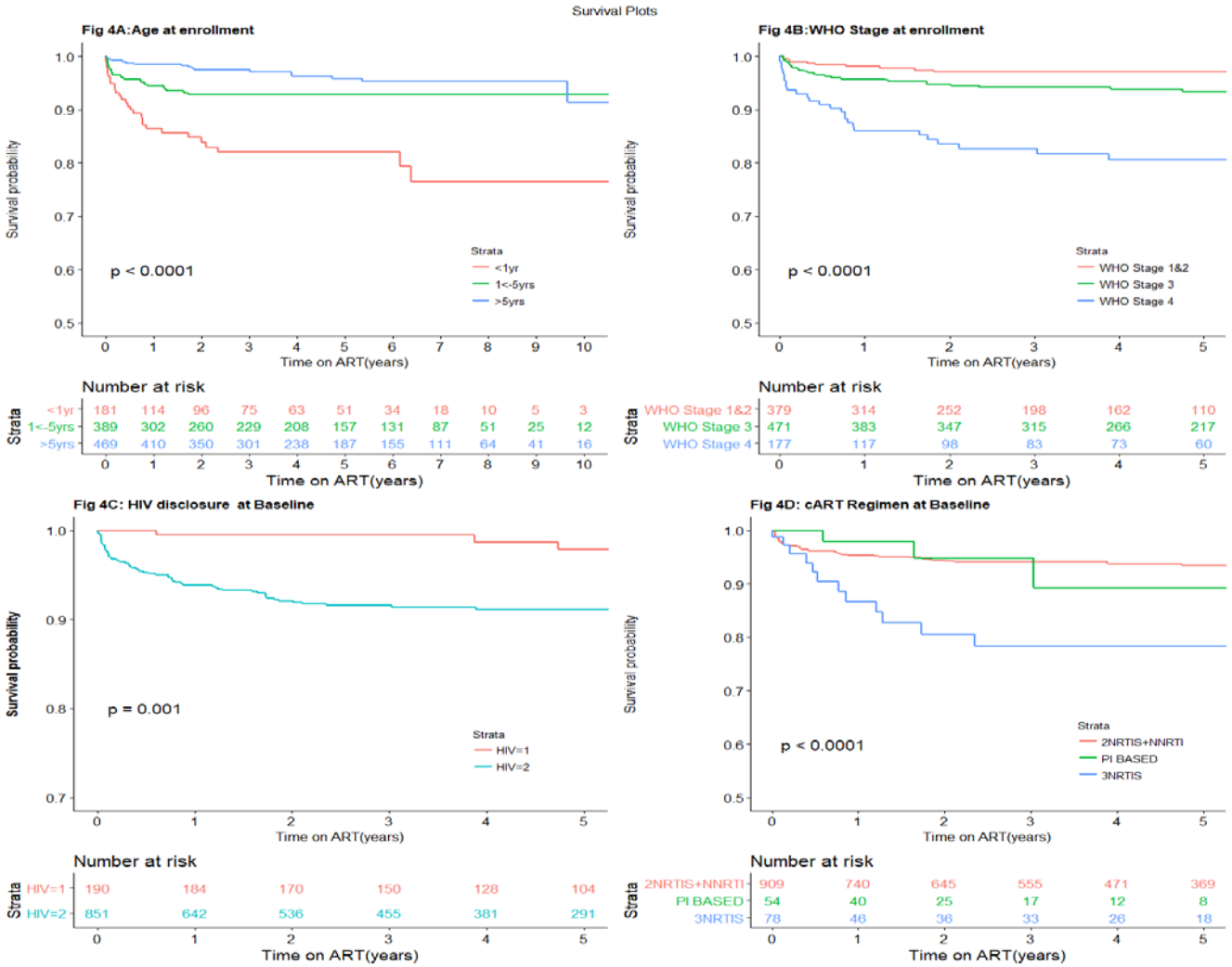

Figure 5-4: survival plots: 4A-Age at enrollment, 4B, WHO stage at enrollment, 4C, HIV disclosure at enorllment, 4D: cART regimen at Baseline

Table 5-5: Factors Associated with Mortality Among Children on cART at LCH (2004-2014)

Predictor	N	Deaths	Unadjusted Mortality HR (95% CI)	p-value
Gender (N%)				
Male	**521**	**33**	**Ref**	
Female	**524**	**38**	**1.17(0.7-1.9)**	**0.5207**
Baseline age				
<1 year	150	29	5.4 (2.9-9.9)	**<.0001**
1-5years	362	25	1.9 (1.03-3.5)	0.0423
>5years	456	17	Ref	
Nutritional status at baseline				
Wasted (WAZ <=-3SD)	165	17	Ref	
WAZ >-3SD	805	54	0.59 (0.3-1.02)	0.0564
CD4%				
<15%	139	22	3.2 (0.9-10.8)	0.0561
15-25%	285	18	2.9 (0.8-9.9)	0.08
>=25%	225	3	Ref	
missing	**351**	**28**	**3.1(0.9-10.273)**	
Baseline Hemoglobin				
<=8	665	40	1.68 (1.05-2.69)	**0.0295**
>8	305	31	Ref	
WHO stage at baseline				
stage 1 and 2	367	11	Ref	
stage 3	443	29	2.05(1.01-4.06)	0.0452
stage 4	148	29	6.41(3.19-12.85)	**<.0001**
TB at baseline				
Yes	297	21	0.82(0.54-1.49)	0.6645
No	672	50	Ref	
Regimen at baseline				
1NNRTI+2NRTi's	911	53	0.26(0.14-0.46)	**<.0001**
3 NRTI's	54	4	0.37(0.12-1.12)	0.0789
LPV/R based regimen	78	14	Ref	
Mother took ART for PMTCT				
Yes	162	17	Ref	
No	883	54	0.53(0.3-0.9)	**0.0239**
***Child know HIV status**				
Yes	191	4	0.21(0.08-0.6)	0.0029
No	854	67	Ref	

*Applies to older children aged above 10 years old,

Table 5-6: Factors Associated with Mortality Among Children on cART (Adjusted Mortality Hazard Rates): LCH 2003-2015

Predictor	**Adjusted Mortality HR (95% CI)**	**P-value**
Categorical age		
<1 year	3.1(1.3-6.4)	**0.0034**
1-5years	1.1(0.6-2.3)	0.7529
>5years	Ref	
Low-weight <-3SD		
Yes	Ref	
No	0.8(0.6-1.5)	0.5141
Baseline hemoglobin		
<=8	1.2(0.8-2.0)	0.4025
>8	Ref	
WHO stage at baseline		
stage 1 and 2	Ref	
stage 3	1.8(0.9-3.6)	0.1188
stage 4	4.8(2.3-10)	**<.0001**
Regimen at baseline		
1NNRTI+2NRTi's	Ref	
3 NRTI's	1.9(0.9-3.7)	0.0603
LPV/R based regimen	0.9(0.3-2.6)	0.806
Mother took ART for PMTCT		
Yes	Ref	
No	1.0(0.5-1.9)	0.961
***HIV status disclosed at baseline**		
Yes	Ref	
No	0.4(0.1-1.05)	0.0627

*Applies to older children aged above 10 years old, won't add to multivariate model

Table 5-7: Sensitivity Analysis for Risk factors for Mortality and Loss to follow-up

	Model 1		**Model 2**		**Model 3**	
Predictor	**HR**	**p-value**	**HR**	**p-value**	**HR**	**p-value**
Age at baseline						
<1 year	3.4(1.6-7.1)	0.0008	2.9(1.4-6.1)	**<.0032**	2.3(1.5-3.5)	**<.0001**
1-5years	1.2(0.6-2.4)	0.5781	1.1(0.5-2.3)	0.7049	0.9(0.6-1.3)	0.6464
>5years	Ref		Ref		Ref	

Table 5-8: Supplementary Table 1

	Univariate Model		**Model 1**		**Model 2**		**Model 3**	
Predictor	**HR(95% CI)**	**p-value**	**HR**	**P-value**	**HR**	**p-value**	**HR**	**p-value**
Categorical age								
<1 year	5.4(2.9-9.9)	**<.0001**	3.4(1.6-7.1)	0.0008	2.9(1.4-6.1)	**<.0032**	2.3(1.5-3.5)	**<.0001**
1-5years	1.9(1.1-3.5)	**0.0423**	1.2(0.6-2.4)	0.5781	1.1(0.5-2.3)	0.7049	0.9(0.6-1.3)	0.6464
>5years	Ref		Ref		Ref		Ref	
Low-weight <-3SD								
Yes	1.7(0.9-2.9)	**0.0564**	0.8(0.4-1.4)	0.3891	0.9(0.5-1.5)	0.5817	0.6(0.4-0.9)	0.0051
No	Ref		Ref		Ref		Ref	
Hb categorical								
<=8	**1.7(1.1-2.7)**		1.2(0.7-2.0)	0.4163	1.1(0.7-1.8)	0.699	1.4(1.0-1.9)	0.0425
>8	Ref		Ref		Ref		Ref	
WHO stage at baseline								
stage 1 and 2	Ref		Ref		Ref		Ref	
stage 3	2.05(1.0-4.1)	**0.0265**	2.6(1.1-6.3)	0.0301	1.9(0.9-3.8)	**0.0886**	0.9(0.6-1.2)	0.4335
stage 4	6.4(3.2-12.9)	**<.0001**	8.3(2.5-27.8)	0.0006	4.7(2.3-19.3)	**<.0001**	1.7(1.2-2.5)	**<.0061**
Mother took ART for PMTCT								
Yes	**Ref**		Ref		Ref		Ref	
No	0.5(0.3-0.9)	**0.0239**	1.0(0.5-1.9)	0.9389	0.9(0.5-1.7)	0.8066	0.8(0.6-1.2)	0.3779

Model 1: The most parsimonious model included categorical age with 3 levels, and WHO stage with 3 levels as independent variables in the Cox PH. The outcome is death. Loss to follow-up and transferred out are censored on the dates that they happened. Children who were alive and active in care were censored on June 30th 2015. Selection of variables to include in the final model were done from the univariate analysis ($p<0.1$). The model was built by forward, stepwise selection.

Model 2: is sensitivity analysis in which we assumed that all lost to follow-up children lived until the end of the study, 30th June 2015. We censored all the lost to follow-up on that day and ran the KM analysis and the CPH with this, the number of deaths was still 71. All the patients who were transferred out were censored on the day of their last visit and those who survived were censored on the day 30th June 2015.

Model 3: Sensitivity analysis in which we assumed that all the patients who were lost to follow-up survived for 90 days and then died. In this model we had a total of 71+all the lost to follow-up patients recoded as dead patients. The transfers were censored on the day of their last visit and the survived were censored on 30th June 2015. Sensitivity analysis was done by considering all defaulters as lost to follow-up.

CHAPTER 6 :PREDICTORS OF LOSS TO FOLLOW-UP AMONG CHILDREN ON LONG-TERM ANTIRETROVIRAL THERAPY[3]

[3] Mutanga. J.N, Mutembo, S, Ezeamama, A.E., Song X., Fubisha, R. C., Kapembwa-Mutesu, K., Sialondwe D., Simuchembu, B., Mucwani, M., Chinyonga, J., Thuma, P.E, Whalen. C Submitted to *the Journal of the International AIDS Society* (JIAS) 11/26/2017

Abstract

Introduction: Retention in care is crucial for people living with HIV who are taking combined antiretroviral therapy (cART). Loss to follow-up is high in treatment programs in resource limited settings. We estimated the annual incidence of loss to follow-up and identified associated risk factors among children on cART at Livingstone Central Hospital (LCH) in Zambia.

Methods: Using a retrospective cohort study design, data were abstracted from medical records of children who received cART between 2004 and 2014. Lost to follow-up was defined as no effective clinical contact for more than 90 days. We estimated the distribution of survival times for the baseline covariates using the Kaplan-Meier (KM) method and Log-rank tests to compare the survival curves. Cox Proportional hazards models estimated the hazard ratios and their 95% confidence.

Results: A total of 1041 children aged from birth to 20 years old commenced cART at LCH during the study period. 167 (16%) were lost to follow-up and we traced 151(92%). Of these, 39(26%) had died, 71(47%) had stopped cART. Calculation of the incidence of loss to follow-up by calendar year showed declining trends from 29/100 person years in 2004 to 1.6/100 person years in 2014. From the Kaplan Meier curve, loss to follow-up was high during the first 3 months after cART initiation (4%), 8% after 1 year and 30% after 10 years. The risk factors for loss to follow-up were: 1) lack of disclosure of HIV status to older children at baseline, HR=1.9(1.2-2.9), 2) Lack of access to a phone, HR=2.1(1.6-2.9), 3) starting treatment between 2012 to 2014 (Option B+ era) HR=5.6 (2.2-14.1).

Conclusion: There is increased need to strengthen engagement in care for HIV infected infants in the Universal ART era. Access to a phone and disclosure of HIV infection status can be used as strategies to improve retention in care.

Keywords: Pediatrics, Risk factors, Treatment, Adherence, Loss to follow-up

Introduction

Retention in care and viral suppression are optimal outcomes for HIV infected people who are taking antiretroviral therapy [1]. Poor medication adherence leads to lack of viral suppression which results in development of HIV viral resistance and subsequently treatment failure. Treatment failure is undesirable in HIV infected people because the treatment is lifelong and there are only very few drug choices especially in resource limited settings. To achieve optimum adherence and ultimately viral suppression, retention in care is critical for every antiretroviral treatment program.

Retention in care is particularly challenging in pediatric HIV treatment programs in Sub-Sahara Africa where the proportion of children lost to follow-up have been estimated to be around 9-14% during the first year of treatment and 28% during the second year of treatment [2]. Disruption in HIV care because of missed appointments can undermine clinical outcomes including assessment of adverse events, ongoing provision of prophylactic medications, clinical and neurodevelopment assessment and identification of treatment failure as soon as it occurs [3]. Most studies of Loss to follow-up in Sub-Sahara Africa focus on shorter periods of follow-up time and the findings vary widely across different settings [4-9]. We carried out a retrospective analysis of HIV infected children on cART at Livingstone Central Hospital in Zambia. We estimated the annual incidence of loss to follow-up and identified associated risk factors among children on cART at Livingstone Central Hospital (LCH) in Zambia.

Methods

Study setting and design

We conducted a retrospective cohort study of HIV-infected infants and children who received combined antiretroviral therapy (cART) at the Pediatric Center of Excellence clinic (PCOE) an outpatient children's clinic at Livingstone Central Hospital (LCH) in Southern Province, Zambia. Livingstone Central Hospital serves nearly 1.2 million people in the Southern Province and parts of the Western Province of Zambia.

Study population

We abstracted data from the all the patient medical records at the clinic registry and validated with the patient registers. We created a Microsoft Access database with this information. Our variables of interest included: Patient ID number, date of start of cART and date of last visit to the clinic, physical address and contact information of the caregiver. We collected clinical information including, date of birth, date of HIV diagnosis, WHO clinical staging at baseline and baseline laboratory measurements, clinical history at baseline and at the last visit. We included all the patients whose medical files were found in the clinic registry. We collected data from 1041 files and closed our dataset on June 30th, 2015. Data abstraction and entry was done independently by 2 data entry clerks. Quality control was carried out by the verifying the entries from every 10th medical record by the principal investigator.

Study Outcomes

The main outcome was loss to follow-up. Lost to follow-up was defined as no clinical and pharmacy contact for more than 90 days. There were patients in this clinic whose schedule was to be seen once a year and we had to make a distinction between these patients and those lost to follow-up. We did this by studying the patient records for evidence of home visits and involvement

of the outreach team. Patients who were lost to follow-up were followed up by the outreach team led by one of the clinic nurses who made efforts to trace the patients and documented this in the medical record over a period of 6-12 months. All the children who were lost to follow-up were censored on the day of their last visit to the clinic.

We ascertained that patients were lost to follow-up firstly together with the clinic staff by looking through the registers and documents of who they have followed up. Secondly, we pulled out all the files of patients who were seen at the PCOE and as we did our file reviews we found a few more patients who were lost to follow-up. Thirdly, we queried the electronic medical records database for patients who were flagged as late for their appointments. We found a total of 164 children who were lost to follow-up and traced 151(92%). We did not find the whereabouts of the remaining 13 (8%) (Figure 6.1).

Data Collection

The clinic outreach team comprised of a social worker and nurse counsellor who collected the files and attempted to trace the caregivers of the lost children. They contacted those who provided a phone number by calling them and if they were reachable asked them if they could go to visit them at their home. They then went to the home of those who accepted and asked those who declined if they could come to the clinic or if they can call them again at another time. As for those who did not provide a phone number, the outreach team went to the last address that had been provided on the patient file. During the home visits, the caregivers were asked questions aimed at understanding why they missed their clinic visits and to determine if they sought care elsewhere. This was done in a friendly atmosphere by a qualified social worker and psycho-social counsellor who completed the status forms in the patient's files once they determined the outcome of the follow-up.

After understanding their circumstances, the outreach team made tailored interventions for each family to ensure that they engage in care. Those who refused treatment were encouraged to contact the clinic if they change their mind later. This assessment was done as part of routine clinical care.

Data Analysis

Baseline demographics and clinical features were described by estimating medians and interquartile ranges for continuous variables and frequencies and proportions for categorical variables. To calculate the annual loss to follow-up rates, we estimated the actual person time that each individual contributed to the study during each calendar year and constructed confidence intervals.

We estimated the distribution of survival times for the baseline covariates affecting loss to follow-up using the Kaplan-Meier (KM) method and log-rank tests were to compare the survival curves. Cox Proportional Hazards models were used to estimate the hazard ratios of loss to follow-up. We evaluated the proportional hazards assumption using log-log plots and plots of Schoenfeld's residuals and no violations of the assumption were found. Multivariate Cox models were used to adjust for possible confounding. Selection of variables to include in the model was done by forward stepwise selection. All p-values were two tailed.

Because we found that 26% of the patients who were lost to follow-up had died, we compared the cumulative incidence of death, transfer out and loss of follow-up for all the children in the clinic using a cumulative incidence curve for a competing risks model with lost to follow-up, death and transfer to another facility as competing risks. This allowed us to study the risk of loss to follow-up while controlling for death and transfer out which are competing risks especially in the first one year after cART initiation [10].

Data analysis was done using SAS statistical software (SAS version 9.4 (SAS Institute Inc, Cary, NC) and R statistical software [11]. We used the Survival package to plot the KM curves and Survminer package to visualize the KM curves [12, 13].

Ethical consideration

This study was approved by the Zambia National Health Research Authority and the institutional review boards at Macha Research Trust and the University of Georgia. We conducted analysis of anonymized routinely collected program data. Hence, informed consent was waived.

Results

A total of 1041 children aged less than 15 years old commenced cART at LCH between January 2003 and June 2015. The clinic had 591(57%) children who were alive and active in care, 216(20%) had been transferred to other facilities, 71(7%) had died and 164(16%) were lost to follow-up. Of the 164 who were lost to follow-up, we traced 151(92%) and did not find 13(8%). Of the 151 children that we found, 39(26%) had died, 71(47%) had stopped cART, 20(13%) had sought cART at other clinics and 21(14%) had continued treatment but with poor adherence (Figure 6-1).

Baseline characteristics of children started on cART

At baseline, 520 (49%) of the children were female and 721(69%) were cared for by their biological mothers. A total of 179 (18%) of the children commenced treatment during their first year of life. At least 304 (29%) of the children were diagnosed during hospital admission and 30 (3%) were diagnosed from the delivery wards. Only 191(18%) of the children knew their HIV status (Table 5-1).

The median age of the children at baseline was 3.6 years (IQR 1.3-8.6). The median CD4 count at baseline was 290 (IQR: 126-509) for children between 5 and 15 years of age. The first line regimen comprised of 2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and 1 Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) for 909 (87%) while 54 (5%) were commenced on regimens that contained Protease Inhibitor (Lopinavir boosted with Ritonavir) (Table 5-2).

Lost to Follow-up

Calculation of the incidence of loss to follow-up by calendar year showed decreasing trends from 29/100 person years in 2004 to 1.6/100 person years in 2014 (Figure 6-2). From the Kaplan Meier curves, loss to follow-up was highest during the first 3 months after cART initiation (4%). The cumulative probability of a patient being lost to follow-up by the end of the first year of treatment was 8%. During the second year, losses decreased and the probability of being lost was 11% and by the end of the 6th year 20%. As patients stayed longer on treatment, their probability of loss to follow-up increased to 30% by the end of the 10th year (Figure 6-3).

When we accounted for those who died and those who transferred to other facilities using the cumulative incidence curve, that attrition was highest within the first year of treatment for all loses. The estimate for the cumulative incidence of loss to follow-up at the end of the first year of cART initiation was 6.6%, cumulative incidence for death was 4.7% and 7.1% for transfer to another facility (Figure 6-4).

Children who started treatment, between 2013 to 2015, had shorter time to event (0.88 after the 3rd year) compared to those who started treatment between 2003-2005 (1.00 after the third year) (Figure 6-5A). Those who started treatment during the period of rapid program expansion from 2006 to 2009 had 3-year time to event of 0.93 (Log Rank p-value=0.00012) (Figure 6-5A).

In the unadjusted model, the hazard ratio (HR) for children who started treatment between 2013-2015 was 5.1 (2.2-11.9), p-value=0.0002, those who started between 2010 to 2012 was 3.6(1.7-7.7), p=0.0009 (Table 6-1). In the adjusted model, the HR was 5.6(2.2-14.1), p=0.0002), and for those who started between 2010 to 2012 HR was 3.5(1.6-7.9), p-value=0.002 (Table 6-2).

In this sample, 68% of the caregivers had access to a phone. Children whose caregivers had no access to a phone had shorter time to event compared to those whose caregiver had access to a phone ((survival probability=0.95 versus 0.88 after 1 year, Log-Rank p-value<0.0001) (Figure 6-5B). In the unadjusted model, children whose caregivers did not have access to a phone had a HR of loss to follow-up of 2.1(1.6-2.9, p<0.0001). In the adjusted model, the hazard of loss to follow-up was 1.8(1.3-2.5, p=0.0002)

Only 18% (n=191) of the children knew their HIV status at baseline. HIV status was only disclosed to older children aged above 7 years old. We found that children older than 5 years old whose HIV status was disclosed at baseline had longer time to event than those who were not disclosed (survival probability =0.99 at 1-year vs 0.94 for those not disclosed, Log-Rank P-value=0.0035) (Figure 5C). In the univariate Cox model, children whose HIV status was not disclosed to them had a hazard of loss to follow-up of 1.9(1.2-2.9, p=0.004) (Table 6-1). In the multivariate model, in which we stratified by age, WHO stage and gender and controlled for the other covariates, the hazard of loss to follow-up was not statistically significant (Table 6-2).

Discussion

After 12 years of treatment 16% (n=167) of the children were lost to follow-up and their whereabouts were unknown to the clinic. The outreach team at the clinic traced 90% (n=151) of the 167 children through a combination of phone calls and home visits. Of these 151 children, 26%

(n=39) had died and 47% (n=71) stopped treatment. The annual incidence of loss to follow-up among children at this clinic declined from 29/100-person years in 2004 to 1.6/100 person years in 2014.

Our finding that children who commenced cART during the option B+ era between 2013-2015 had the highest hazard of loss to follow-up was surprising because we expect that since their mothers are on lifelong treatment and therefore already in care, it should be easier to retain their children in care. However, a study from South Africa found a similar trends in which children commenced on cART in recent years experienced poorer retention in care [14]. This could be because mothers commenced on cART in the option B+ era were healthy and may not be motivated to take the medication and give it to their infants. During the earlier years of program expansion (2003 to 2005) the hazard of loss to follow-up was lower because cART was only accessible to very sick children whose caregivers associated survival to cART and were motivated to stay on treatment.

The finding that children whose HIV infection status was disclosed at baseline had better retention in care must be carefully interpreted because disclosure of HIV infection status to a child is an incremental process that starts with partial disclosure to younger children leading to full disclosure among older children [15, 16]. Disclosure rates in Sub-Sahara Africa remain low, some studies attribute this to caregiver lack of skills to disclose to children. Training caregivers in disclosure skills has improved disclosure rates in most places [17, 18]. In this sample only 18% of the children were disclosed to at baseline and these children had better retention in care than those who were not disclosed.

Despite the decline observed in annual incidence of loss to follow-up, the Kaplan Meir curves revealed that most of the loses occurred within the first 3 months of treatment. This suggests

lack of engagement in care for the child and caregiver within the clinic. Studies done in other parts of Sub-Sahara Africa have revealed similar patterns. In a case control study from Botswana, it was found that 47.6% (n=51) of the children who were lost to follow-up failed to engage after just one clinic visit as compared to 1% (n=2) in the control group [4]. The authors suggested that engagement in care can be improved by addressing personal concerns at the initial clinic visit [4]. Other researchers proposed use of risk scores to identify patients at risk of loss to follow-up at baseline and provide individualized risk assessment [19]. Risk scores would definitely be useful in the PCOE clinic for assessing risk of short term loss to follow-up. In addition to risk scores, we suggest that engagement in facility and community treatment support groups would improve engagement in care [20].

Several approaches have been proposed for dealing with patients who are lost to follow-up including: enhancing engagement in care by addressing all patient related concerns during the initial visit[4], early tracing of patients who missed their appointments [6], dealing with stigma and child disclosure related issues early in the course of treatment. What we found in this program in addition that engagement in care can be strengthened by following up patients by contacting them through their telephones as shown by the finding that access to mobile phone improved retention in care. In Malawi, they found that access to a phone doubled success of tracing a lost patient [7]. This is supported by results of a meta-analysis of studies from Sub-Sahara in which 77% of the lost patients were successfully traced using a combination of phone calls and home visits [8]. What made patient tracing in this way possible is that at each visit, the nurse counsellors updated the contact information, and phone number and discussed with the caregivers the circumstances that would lead to them getting a phone call or home visit from the clinic outreach team, in turn, the patients were given a phone number that they could use to call the clinic and talk

to a health provider if they had concerns regarding the child's treatment. This process was critical to engagement in care for the pediatric patients and their caregivers.

One of the strengths of our study is that the data was collected over a 10-year period in a health facility that had resources to trace children with missed appointments. Most studies have been done on children who have been on treatment for a short period time and all found that tracing is best done within the first weeks of a missed appointment [5, 6, 21]. The PCOE clinic had programs to trace children who missed appointments every week and every month. This probably explains why the proportions of children lost to follow-up after 10 years is only 16%. It was however necessary to analyze the data using a competing risks model in addition to Kaplan Meier analysis because we found that 26% of the children who were lost to follow-up were actually dead. The cumulative incidence curve for the competing risks model allowed us to study the cumulative incidence of all the major outcomes at the same time. The increase in loss to follow-up that we observed from the cumulative incidence curve (21% at 10 years' vs 30% at 10 years from the KM curves) suggest that loss to follow-up and death are competing events.

A major limitation of our study is that we did not document the for reasons for loss to follow-up among the children who were traced. There is need for future studies to explore the reasons that children default on cART.

Conclusion

This study showed that annual incidence of patients who were lost to follow-up declined steadily as this program matured. Children who could be traced by the clinic outreach either by phone or home visits had higher retention in care. Older Children whose HIV status was disclosed

had better retention in care. There need to strengthen engagement in care for HIV infected infants in the universal cART.

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Figure 6-1: Treatment outcomes and retention in care among children on cART at LCH (2003-2014)

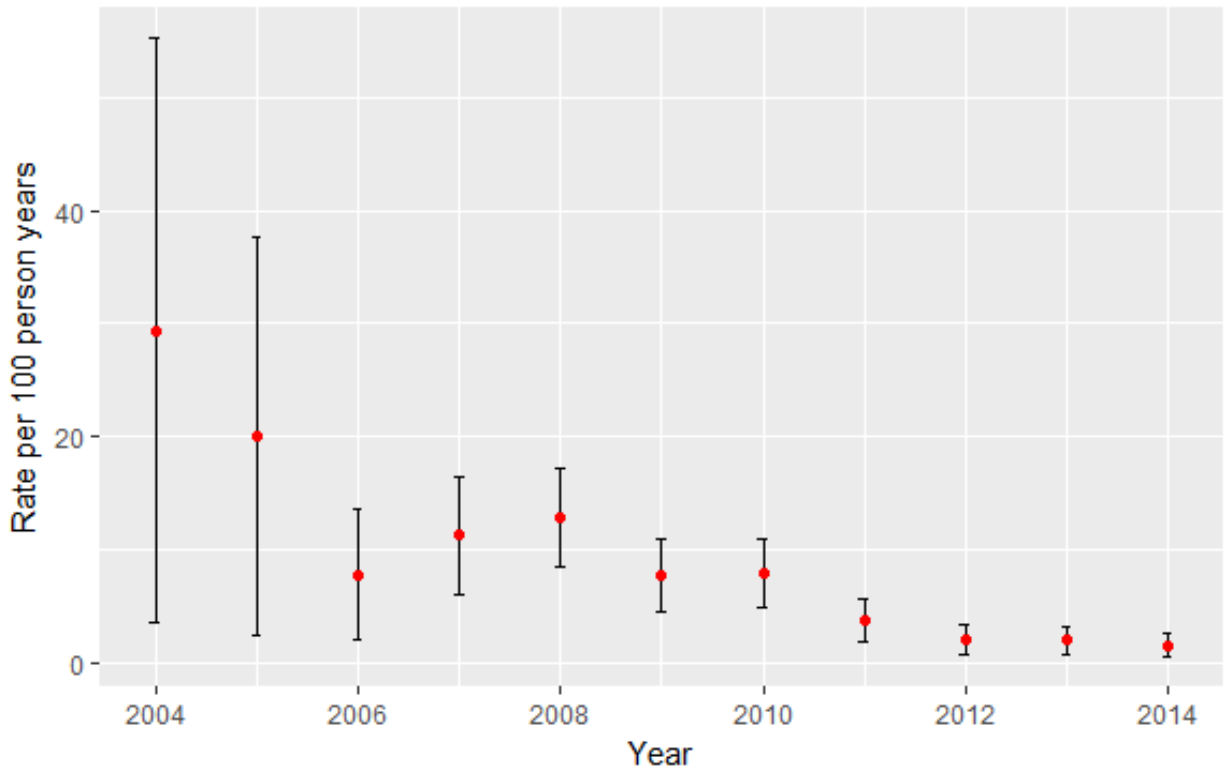

Figure 6-2: Loss to follow-up Rates by Calendar Year

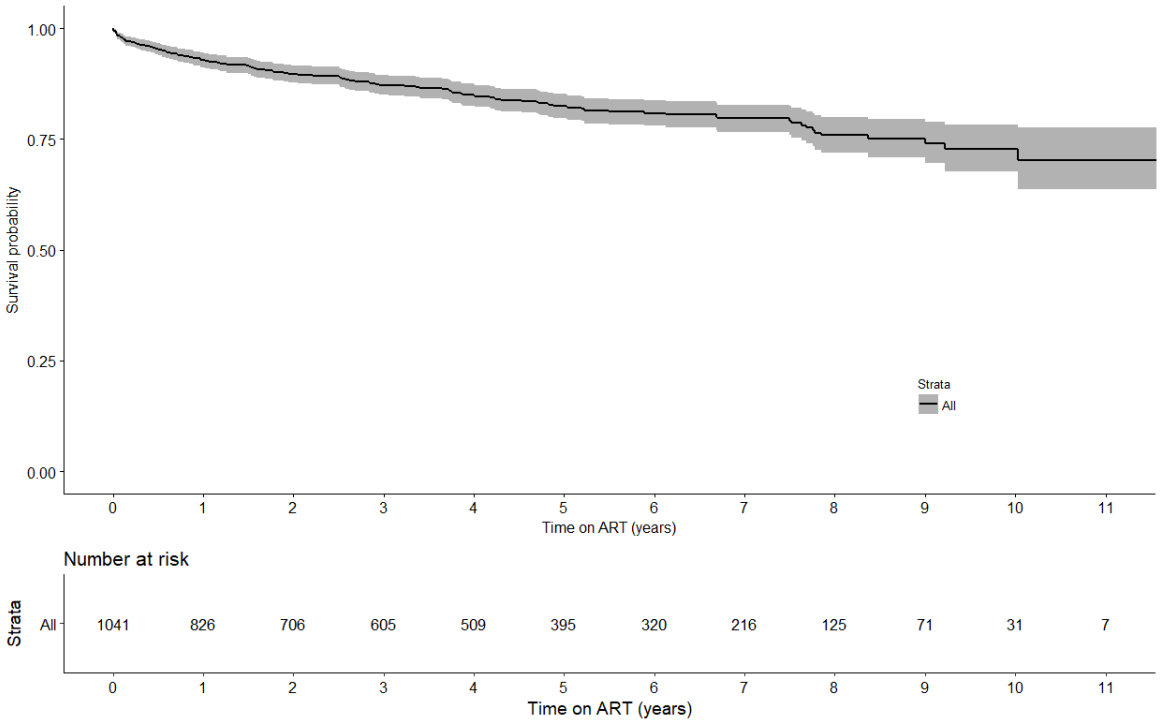

Figure 6-3: Time to event Curve

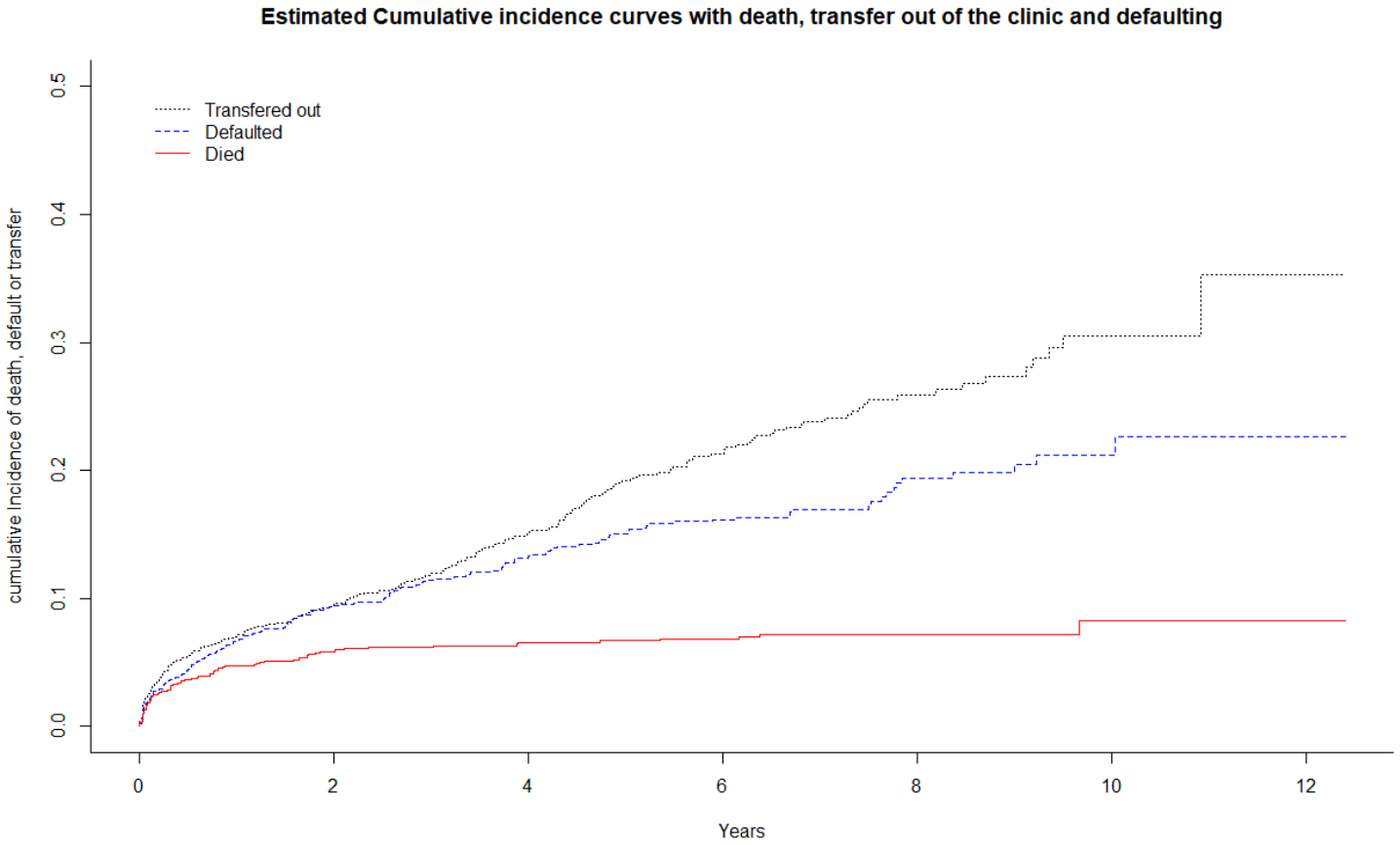

Figure 6-4: Cumulative Incidence Curve For children who died, transferred to another facility or were lost to follow-up based on a competing risks Model

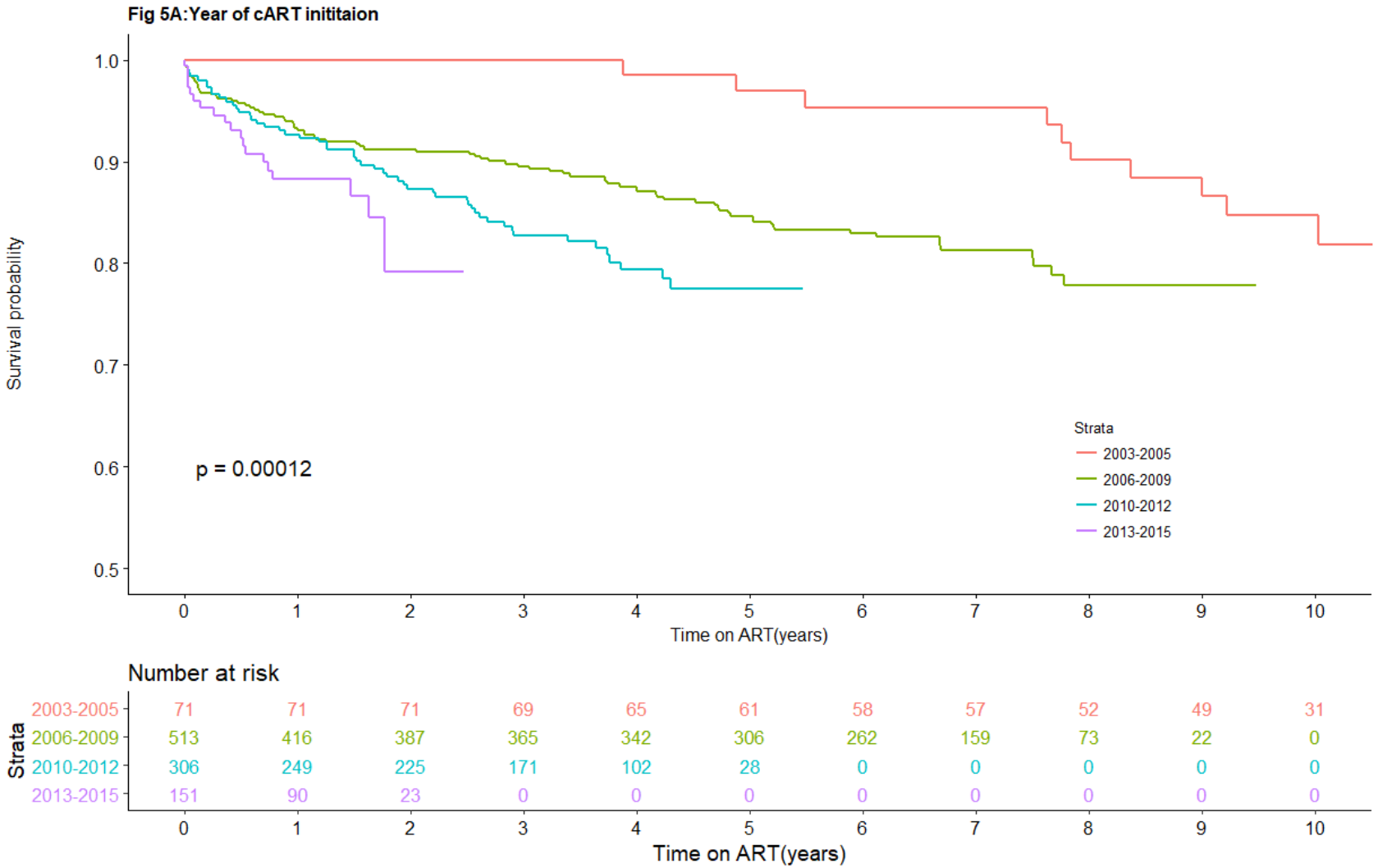

Figure 6-5: Time to event plot for children on CART who were lost to follow-up at LCH by year of treatment initiation

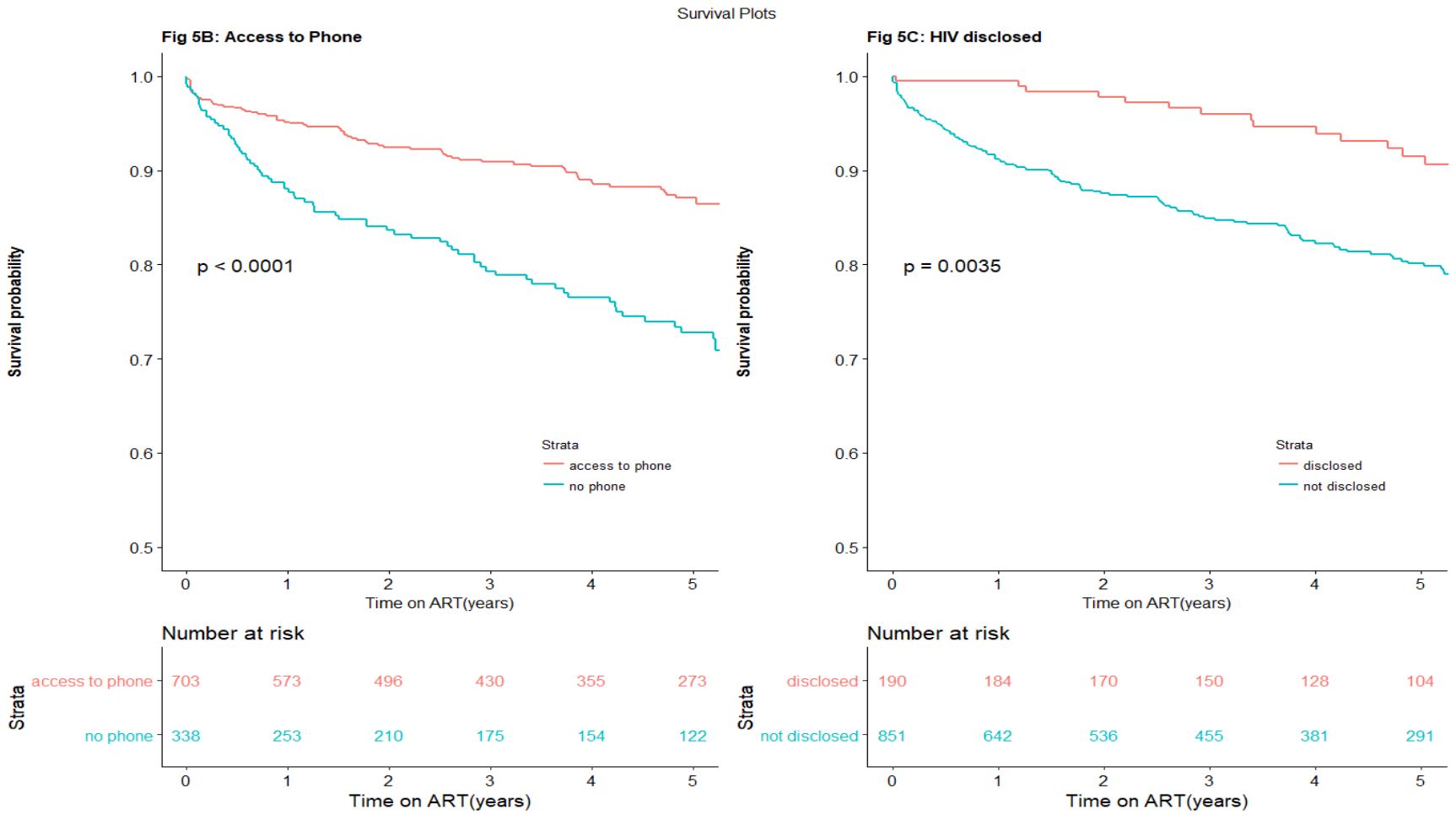

Figure 6-6: Kaplan Meier Time to Event Plots for children lost to follow-up in the Pediatric ART program at LCH (2003-2015): Fig 5A: Children whose guardians own a mobile phone Children, Fig 5C: Who know their HIV status,

Table 6-1: Univariate Analysis for Lost to follow-up

Predictor	N	LTFU	Unadjusted Loss to follow-up HR (95% C1)	p-value
Gender (N%)				
Male	521	92	1.2(0.9-1.7)	0.1929
Female	524	75	Ref	
Age continuous	1039	71	0.94(0.91-0.98)	**0.001**
Categorical age				
<1 year	181	45	2.7(1.8-3.9)	**<.0001**
1-5years	389	59	1.3(0.9-1.8)	0.1717
>5years	469	59	Ref	
Year of ART initiation				
2003-2005	71	10	Ref	
2006-2009	513	81	2.2(1.0-4.5)	**0.0293**
2010-2012	306	53	3.6(1.7-7.7)	**0.0009**
2013-2016	151	20	5.1(2.2-11.9)	**0.0002**
Low-weight <-3SD				
Yes	182	34	1.6(1.1-2.4)	**0.0117**
No	863	133	Ref	
Baseline CD4%				
<15%	307	58	1.9(1.1-3.4)	**0.03**
15-25%	261	36	1.3(0.7-2.4)	0.3675
>=25%	112	14	Ref	
missing	292	59		
Baseline Hemoglobin (continuous)	1045		0.87(0.7-0.9)	**0.0036**
Baseline Hemoglobin (categorical)				
<=8	336	67	1.4 (1.03-1.9)	0.0477
>8	709	100	Ref	
WHO stage at baseline				
Stage 1 and 2	380	57	Ref	
Stage 3	472	70	0.9(0.6-1.3)	0.5036
Stage 4	177	36	1.6(1.0-2.3)	0.0356
TB at baseline				
Yes	319	46	Ref	
No	723	118	0.8(0.5-1.0)	0.1279
Access to Phone				
Yes	705	85	Ref	

No	340	82	2.1(1.6-2.9)	<.0001
Regimen at baseline				
1NNRTI+2NRTi's	911	124	Ref	
LPV/R based regimen	54	16	2.9(1.7-4.9)	<.0001
3NRTIs	78	25	3.4(2.3-5.4)	<.0001
Mother took ART for PMTCT				
Yes	162	31	Ref	
No	883	136	0.6(0.4-0.9)	0.03
***HIV status disclosed at baseline**				
Yes	191	24	Ref	
No	854	143	1.9(1.2-2.9)	0.004

Table 6-2: Multivariate Analysis

Predictor	**Multivariate Loss to follow-up HR (95% CI)**	**p-value**
Year of ART initiation		
2003-2005	Ref	
2006-2009	2.3(1.1-4.9)	0.0316
2010-2012	3.5(1.6-7.9	**0.002**
2013-2016	5.6(2.2-14.1)	**0.0002**
***HIV status disclosed at baseline**		
Yes	Ref	
No	1.4(0.9-2.4)	0.1704
Access to Phone		
Yes	Ref	
No	1.8(1.3-2.5)	**0.0002**
Underweight		
Yes	1.14(0.76-1.7)	0.5151
No	Ref	
Hemoglobin categorical		
<=8	1.2(0.9-1.7)	0.1958
>8	Ref	

CHAPTER 7 : SYNTHESIS OF RESULTS, PUBLIC HEALTH IMPLICATIONS AND CONCLUSION

Overview

This chapter reviews and synthesizes the important findings of this project and discusses the potential public health implications. We approached this task by analyzing the key findings and key implications of each aim. Figure 7.1 shows the scope of this work within the PMTCT cascade which was explained in chapter 1. This work mainly focused on prong 4, which deals with provision of care and support for women living with HIV and their families.

The overarching goal of this project was to generate evidence on the effectiveness of pediatric antiretroviral therapy (ART) programs, specifically, the impact on morbidity and mortality, obstacles to treatment access, uptake and barriers to long-term adherence and retention in care. Focusing research efforts on generating targeted evidence that improves program implementation through a better understanding of what works for infants and children is recognized by the WHO and the rest of the global health agenda as a critical strategy to inform global health policy on how to ensure better outcomes for infants and children across the HIV cascade [1].

We addressed our objectives by compiling routinely collected program data from Livingstone Central Hospital in Zambia and carrying out analysis of the data to culminate into three manuscripts. In the first manuscript, we quantified the proportions of early infant diagnosis, including birth testing among HIV exposed infants at Livingstone Central Hospital (LCH), from January 2009 to December 2016. In the second manuscript, we described treatment outcomes,

measured mortality rates and assessed predictors of mortality among children receiving cART over a 10-year period. In the third manuscript, we estimated the annual incidence of loss to follow-up and associated risk factors for loss to follow-up among children on long term treatment with antiretroviral therapy

Main Findings

Aim 1: Early Infant Diagnosis of HIV

Timely diagnosis HIV-infection remains a key challenge in resource limited settings. Early diagnosis of HIV followed by early initiation of antiretroviral therapy greatly reduces infant mortality and HIV progression. In the absence of antiretroviral therapy, 50% of HIV infected children died before their 2nd birthday [2].

In this study, we evaluated the early infant diagnosis program at Livingstone Central Hospital in Zambia and found that the perinatal HIV transmission rate among children seen at the hospital declined remarkably following implementation of Option B+ in Zambia from 19% to 1.4%, a significant stride towards elimination of mother to child transmission of HIV.

Our finding that 98% of the HIV infected infants were successfully linked to care is a good indicator that children were accessing treatment at this facility. Linking mother-infant pairs to treatment remains a challenge in most resource limited settings. What made this possible in this health facility is that testing and long-term treatment was offered within the same facility with adequate staff and the presence of outreach services to trace those who were lost to follow-up.

Another interesting finding under the theme of early Infant diagnosis is that 17% of the HIV infected infants were identified during the first week of life. This finding provides evidence that implementation of birth testing of all HIV exposed infants is a high yield strategy for timely

diagnosis of HIV infection and provides a platform for promptly linking children born with HIV to treatment. This finding supports the recent recommendation by the Ministry of Health to test infants at birth and subsequently at 6 weeks, 6 months, 9 months and beyond depending on breastfeeding status. These findings are essential for achieving the first target of the WHO 90-90-90 strategy. The first target is to ensure that 90% of all people living with HIV are diagnosed. Among infants and children this is urgent because in 2015, only 40% of HIV exposed infants globally were tested for HIV within the first 2 months of life [3]. Early infant diagnosis in resource limited settings relies on the PMTCT programs including mothers returning for postnatal care. We demonstrated in this program that early infant testing was successfully implemented in a delivery unit through coordination with the pediatric unit. The pediatric unit through the PCOE clinic tracked the exposed infants through the patient records and registers and were responsible for ensuring that the clients got their results and were linked to care for subsequent HIV tests

There is however urgent need to improve retention in care for infants who tested negative at birth because they are still at risk of MTCT through breastfeeding. Of the 661 infants who were tested at birth, only 27%(n=80) came back for their second test at a median age of 6 months and just 15% (n=44) came back for a third test at median age of 12 months (11-14). Retention in care is a challenge in most pediatric HIV and PMTCT programs in sub-Sahara Africa [4-6]. Several studies have been done to understand the poor retention in care and a myriad of factors have been identified including: 1) health systems factors such as poor health infrastructure to offer optimal antenatal and postnatal care to the mother-baby pair, mother not engaged in care, lack of patient education, long turnaround time for results to get back to the patient, 2) patient related factors such as forgotten appointments, lack of disclosure of HIV status to partner. Strategies to mitigate retention in care have been proposed and are being piloted in several places

such as: 1) use of Short message service using mobile phones to send results to health facilities and in some instances to remind mothers about their appointments. This has been piloted in Zambia, Kenya, Uganda, Mozambique and other countries [7-12]. In Kenya and Zambia, use of short message service to send reminders to mothers increased the proportion of infants accessing ART [8, 9]. Other suggested interventions to improve retention in care are: 1) integration of infant testing in other child health platforms like expanded program of immunization in Zambia [13]. 2) Routine provider initiated testing and counseling (PITC) of infants and children was implemented in all Zambian healthcare facilities to hospitalized children [14-16]. Despite these other avenues for infant testing, studies demonstrated that HIV diagnosis for infants must be done early in life for optimal health outcomes [17, 18]. Our study showed that early infant testing at birth is feasible in this setting.

Key challenges however remain in early infant diagnosis in Zambia, and several other sub-Sahara African countries including: 1) inadequate number of laboratories conducting HIV DNA-PCR, 2) stock outs of commodities required to carry out the dry blood spots at health facility level, 3) long turnaround time for results to get back to the health facility and subsequently back to the patient and 4) poor retention of mother's in postnatal care [19-21]. In rural settings like most parts of Southern Province Zambia the situation is more challenging because only 56% of women delivered in a health care facility and 58% received postnatal care in 2014 [22]. Low institutional deliveries and poor utilization of postnatal care results in low PMTCT service utilization which leads to delays in HIV diagnosis for HIV exposed infants.

Aim 2: Mortality among children on antiretroviral therapy

We observed low attrition due to mortality among children antiretroviral therapy. After the tenth year of the program, mortality was 7%(n=71). The observation that hazards of death were highest within the first 3 months after the child starts taking cART and then declines after the first 6 months of treatment is consistent with studies done in other parts of Zambia and in similar settings [15, 23-25]. Other studies have attributed the high early mortality to late presentation to care [26]. Studies done in routine clinic settings in resource limited settings found that the cumulative incidence of mortality during the first year of treatment among older children between 5-10 years of age was less than 2% to more than 45% among infants aged less than 12 months with severe disease [27]. Once children get past the initial 6 months, their risk of mortality declines to very low rates especially if there is adherence to treatment and follow-up is optimized. Studies from resource limited settings demonstrated good survival after the first 6 months of treatment [24, 28, 29].

Our finding that the hazard of mortality were highest among infants and children aged less than 12 months of age is consistent with predictive models that have been done in both developed and developing countries and in fact motivated the universal ART policy by WHO [30]. Results of a modelling study done in six countries in Sub-Sahara Africa suggested that mortality is higher among perinatally HIV infected children than those infected through breastfeeding [31]. Perinatally infected infants have a higher risk of mortality and disease progression [32]. Early diagnosis of HIV infection and early initiation of cART improves the outcomes of these infants [33]. Although early infant diagnosis of HIV is critical, there are still challenges in resource limited settings to diagnose children. Children who are missed by the PMTCT and early infant diagnosis

programs are at highest risk of mortality because they usually present to the hospital after an illness when they are already immunocompromised with high risk of mortality [18].

Aim 3: Loss to Follow-up Among children on antiretroviral therapy

The aim of this study was to estimate the annual incidence of loss to follow-up and identify risk factors for loss to follow-up among children on long term treatment with antiretroviral therapy at Livingstone Central Hospital in Zambia. Although this ART program had frequent monitoring of patient outcomes, after 10 years of follow-up, we found that 16%(n=167) of the children were lost to follow-up and their whereabouts were unknown to the clinic. The outreach team at the clinic traced 90% (n=151) of the 167 children who were lost to follow-up through a combination of phone calls and home visits. Of these 151 children, 26% (n=39) had died and 47% (n=71) stopped treatment. The annual incidence of loss to follow-up among children at this clinic declined from 29/100-person years in 2004 to 1.6/100 person years in 2014. The risk factors for loss to follow-up in this sample of children on treatment were 1) lack of disclosure of HIV status to older children at baseline, HR=1.9(1.2-2.9), 2) Lack of access to a phone, HR=2.1(1.6-2.9), 3) starting treatment between 2013 to 2015 (Option B+ era) HR=5.6 (2.2-14.1).

Our finding that children who commenced cART during the option B+ era between 2013-2015 had the highest hazard of loss to follow-up was surprising because we expect that their mothers are on lifelong treatment and therefore already in care, so it should be easier to retain their children in care. However, a study from South Africa found a similar trend in which children commenced on cART in recent years experienced poorer retention in care [34]. This could be explained by the fact that HIV infected infants during the option B+ era were likely born to mothers who may not have had access to medication during their pregnancy and their challenges may have

continued after their infant was diagnosed with HIV This reinforces the need for strengthened early infant diagnosis and engagement in care from the initial visit. During the earlier years of program expansion (2003 to 2005) the hazard of loss to follow-up was lower because cART was only accessible to very sick children whose caregivers associated survival to cART and were motivated to stay on treatment.

The finding that children whose HIV infection status was disclosed at baseline had better retention in care must be carefully interpreted because disclosure of HIV infection status to a child is an incremental process that starts with partial disclosure to younger children leading to full disclosure among older children [35, 36]. Disclosure rates in Sub-Sahara Africa remain low, some studies attribute this to caregiver lack of skills to disclose to children. Training caregivers in disclosure skills has improved disclosure rate in most places [37, 38]. In this sample only 18% of the children were disclosed to at baseline and these children had better retention in care than those who were not disclosed.

Despite the decline observed in annual incidence of loss to follow-up, the Kaplan Meier analysis revealed that most of the loses occurred during the first 3 months of treatment. This suggests lack of engagement in care for the child and caregiver within the clinic. Studies done in other parts of Sub-Sahara Africa have revealed similar patterns. In a case control study from Botswana, it was found that 47.6% (n=51) of the children who were lost to follow-up failed to engage after just one clinic visit as compared to 1% (n=2) in the control group [39]. The authors suggested that engagement in care can be improved by addressing personal concerns at the initial clinic visit [39]. Other researchers proposed use of risk scores to identify patients at risk of loss to follow-up at baseline and provide individualized risk assessment [40]. Risk scores would definitely

be useful in the PCOE clinic for assessing risk of short term loss to follow-up. In addition to risk scores, we suggest that engagement in facility and community treatment support groups would improve engagement in care [41].

Contribution of this study to science

1. What was known before this study

Evidence that treating HIV-infected pregnant women with antiretroviral therapy reduces mother to child HIV transmission by as much as 75% has been available for almost three decades [42, 43]. Implementation of this evidence in developed countries led to virtual elimination of mother to child transmission of HIV. In developing countries, critical barriers remain to scale-up of PMTCT and pediatric ART programs largely due to limited resources and fragile health infrastructure to deal with the complexity of the existing approaches to testing and treating children. Innovative approaches to testing, treatment and service delivery strategies are needed but there is lack of evidence on the effectiveness of current strategies.

2. What this study adds

We used epidemiologic methods to analyze routinely collected program data and described effectiveness of a pediatric ART program in Livingstone, Zambia. This study covered the fourth prong of PMTCT (Figure 7.1) and addressed the first 90 (90% of HIV infected people should know their status) and second 90 (90% of HIV infected people should be on antiretroviral therapy) of the WHO 90-90-90 approach. The fourth prong of PMTCT is targeted at provision of care for women living with HIV and their families. Timely HIV diagnosis is a prerequisite for access to ART. We studied the uptake of early infant diagnosis at LCH and this enabled us to highlight the

decline in perinatal HIV transmission in 2016 and that poor record keeping is a hindrance to program evaluation.

This is the first study to assess survival and retention in care among children on ART in Livingstone, Zambia which is critical for optimal patient outcomes. Our findings show that there is need for strengthened pretreatment screening and treatment of opportunistic infections among children commencing cART. Clinical management needs to be especially strengthened and there is need to improve diagnosis of HIV among children and ensure that they access treatment early. There is increased need to strengthen engagement in care for HIV infected infants in the Universal ART era as their mothers may not have had access to cART during pregnancy. Access to a phone and HIV disclosure to HIV infected children were effective strategies that seemed to improve retention in care. Mobile phone technology has been found to be effective as a tool to improve adherence to medication and treatment in several clinical trials from Sub-Sahara Africa [8, 9, 44, 45]. Disclosure of HIV infection status to a child remains challenging in most settings.
These findings touch on key challenges of elimination of mother to child transmission of HIV in resource limited settings and are applicable to similar settings.

Future Directions

1. Policy Makers

The Ministry of Health in Zambia recently updated the HIV treatment guidelines to include a test and treat approach for all and to strengthen Early infant diagnosis of HIV. This approach implies that the number of people on ART will increase. However, critical gaps remain health service delivery especially for key populations like children and adolescents. We recommend that the Ministry Health needs to focus on the following key area to improve pediatric ART in Zambia:

1. **Testing**

   - Efforts should also be made at National level to ensure that novel diagnostic tools are accessible by health care workers at all levels of care so that early infant diagnosis of HIV is effectively carried out.
   - Best practices on timely access of diagnosis and treatment should be actively looked for and highlighted then replicated at other facilities. Supervision of health care workers on integrated management of childhood illnesses to ensure that children are given optimal care.
   - There should be efforts at national level to guide and support operational research at facility level that can be used to inform policy about best practices, effective interventions and factors that hinder progress.

2. **Treatment and service delivery**

   - National monitoring and evaluation systems should be strengthened and research facilitated and supported so that there can be data to inform policy
   - Systems to collect program data should be strengthened and innovative approaches such as use of paperless medical records should be implemented in the urban health facilities. This will assist in monitoring and evaluation.
   - There should be deliberate efforts to include key stakeholders such as networks of people living with HIV, advocacy and civil organization in the decision making process.
   - There is need for implementation research. The ministry of health should find ways of incorporating implementation research into their activities.

3. For Researchers

There remain significant gaps in pediatric HIV research. Based on our findings, the following key areas are critical;

1. Testing
    - Research is needed for best approaches to early infant diagnosis in resources limited settings. Several researchers are currently working novel point of care diagnostic tests and there is need to assess models of care that can be implemented in different settings to ensure cost effectiveness and maximum yield while providing optimal care.
    - Breastfeeding HIV exposed uninfected children are a particularly vulnerable group with high loss to follow-up rates across high HIV burden settings. There is need for research to study strategies of keeping them engaged in care.
2. Treatment and service delivery
    - There is heightened need to study outcomes of children on ART especially mortality among those lost to follow-up or transferred to other facilities [46, 47].
    - There are gaps in knowledge on retention in care for breastfeeding infants and their mothers and for children on antiretroviral therapy.
    - As universal ART is implemented, gaps remain on how to maintain optimal adherence and retention in care for adolescents and children.
    - Optimal drug dosing especially for very young children remains an issue. There is need for research on pharmacokinetics of antiretroviral drugs in children.
    - Innovative strategies to provide tools for monitoring viral loads of children in resources limited settings.

- Strategies to collect better program data
- Studies on implementation of tools to strengthen adherence such as mobile phone technology.
- Other areas of research that were not studied in this project include the last 90, which is ensuring that 90% of people on ART are virally suppressed and related to this is assessment of adherence to medication and dosing of ARVs in children. There is need for continued research on HIV vaccine development.

4. For key stakeholders

The Global health agenda led by the W.H.O in collaboration with the International AIDS Society's Collaborative Initiative for Pediatric HIV Education and Research (CIPHER) in July released a document with recommendations for the global research agenda for pediatric HIV [1]. This documents outlines a list of top research priorities in pediatric HIV and is a helpful tool for all stakeholders in pediatric ART because the gaps are still a lot and tackling them will involve a multidisciplinary team approach. Key stakeholders include civil society organizations and community based organizations, research funders such as Bill and Melinda Gates, CIPHER, and NIH and program managers.

1. Funders

- Invest in research areas that will have the greatest impact on pediatric HIV, such as vaccine research

2. **Civil society and advocacy groups**

   - Engage with the researchers and policy makers and in meaningful activities that will have impact on pediatric HIV. are in line with policy and researchers and they are included in decision making.
   - Advocate for inclusiveness in decision making

Conclusion

In conclusion, our finding that perinatal HIV transmission declined remarkably following implementation of universal combined antiretroviral therapy for all HIV infected pregnant women (Option B+) in Zambia in 2013 suggests significant strides towards EMTCT. We observed that attrition due to mortality and loss to follow-up among children on cART in Zambia was highest during the first 3 months of treatment. Pretreatment screening and treatment of opportunistic infections among children commencing cART needs to be strengthened. There is need to improve documentation of patient information and outcomes for monitoring and evaluation purposes.

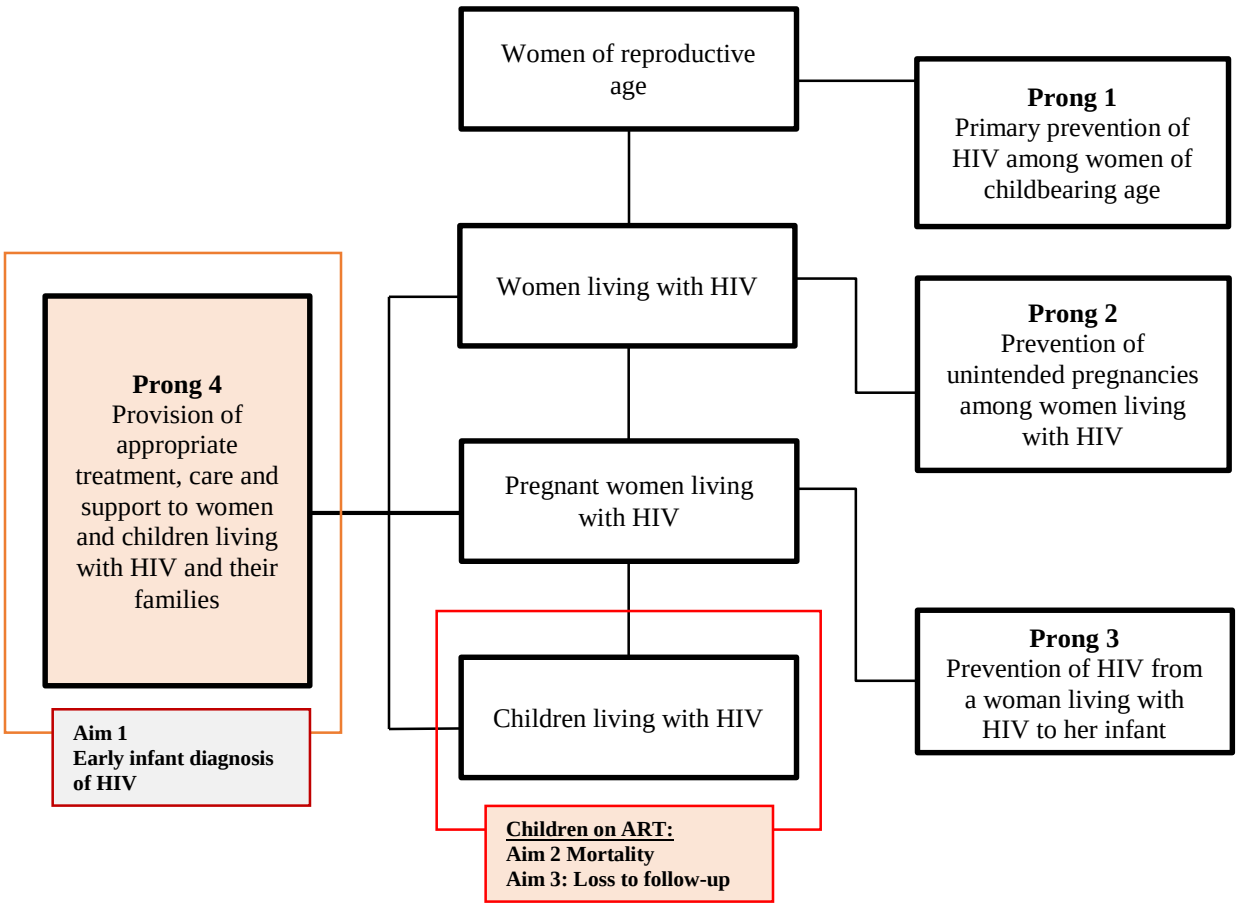

Figure 7-1: Four prong to eliminate mother-to child transmission of HIV and improve maternal health

Statement of Contribution

Jane Namangolwa Mutanga conceptualized this study in all its parts, collected and analyzed the data, interpreted the results and wrote the whole dissertation. This report is original and has not been published elsewhere. The findings and conclusions are those of Jane Mutanga and do not represent the official position of the University of Georgia or the Zambian Ministry of Health.

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