DECLARATION

This proposal is my original work and has not been presented for a degree award in any other University.

Signature: . . . . . Date: . . . . . .

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SC384-C001-3997/13

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DEDICATION

To my siblings, beloved late parents and best friends for your immeasurable love, tolerance, and care.

ACKNOWLEDGMENT

First, I thank God the Almighty who has provided me the breath of life to enable the successful completion of this work and for His unconditional love. This laborious work would not have been a success without moral and financial support and guidance from various persons. I would like to express my sincere and my heart-felt appreciation to my late parents who have provided me all that is required to allow me reaching this academic level, their parental guidance and moral support have been a cornerstone to my academic achievement. Special thanks go to my mother whose affection and inspiring me towards hard work helped me up to this moment. I owe gratitude to my brother, sisters and my family in general for the unconditional love and moral support rendered to me towards the success of this study and to you all my relatives that support me in one way or another. My deepest appreciation goes to my supervisor Dr. Joseph K. Mung'atu and Dr. Jairu Ndiga for their commitment, supervision of this work and from their guidance. I gained tremendous knowledge as regards research skills. I wish to acknowledge with gratitude to my friends especially Herman Joseph and Lydia, my mentor Jean Providence and classmates especially Lucy, as it is not possible to acknowledge by names all those who directly or indirectly contributed to making this work a success, thus many thanks go to whoever assisted me in one way or another towards the accomplishment of this work. May God bless all of them abundantly.

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ACRONYMS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral drug
CD4	Cluster differentiation 4
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
KM	Kaplan-Meier
MOH	Ministry Of Health
RBC	Rwanda Biomedical Center
UNAIDS	United nations joint program on HIV/AIDS
UNICEF	United Nations Children's Fund
WHO	World Health Organization

DEFINITION OF TERMS

Accelerated Failure time: is a parametric model that provides an alternative to the commonly used proportional hazards models. Whereas a proportional hazards model assumes that the effect of a covariate is to multiply the hazard by some constant, an AFT model assumes that the effect of a covariate is to accelerate or decelerate the life course of a disease by some constant (Source: Kalbfleisch and Prentice, 2002).

ART: Antiretroviral treatment; consists of the combination of antiretroviral drugs to maximally suppress the HIV virus and stop the progression of the disease so that a person with HIV can live longer without the onset of AIDS-related diseases and occurrence of opportunistic infections (Source: WHO, 2013).

CD4 cells: Cluster differentiation 4 cells; are types of white blood cells that play a major role in protecting human body from infection. Along with other tests, the CD4 count informs on the strength of one's immune system, the stage of HIV disease progression, guide treatment options, and predictions (Source: WHO, 2013).

Censored data: when some of the subjects may not experience the event or death before the end of the study, these subjects will not be ignored, because they provide some information about survival. A look into these subjects will allow finding out whether they survived beyond a certain point, but the exact date of death will remain unknown. Those subjects are considered as censored data (Source: Kalbfleisch & Prentice, 2002).

Children living with HIV: refers to all boys and girls aged below 15 years at the time of study interest, diagnosed with HIV infection using standard clinical tests and declared HIV positive as per national HIV management guidelines (Source: WHO, 2013).

Failure time (event time, survival time or lifetime): the length of time until the occurrence of an event of interest (Source: Hosmer, Lemeshow & May, 2008).

Hazard function: is the rate at which a subject is likely to experience the event of interest in the next time interval given that the subject has not experienced the event up until that point (Source: Hosmer, Lemeshow & May, 2008).

Kaplan Meier: is an estimator for estimating the survival function from lifetime data. KM is one of the best options to be used to measure the fraction of subjects living for a certain amount of time after treatment (Source: Hosmer, Lemeshow & May, 2008).

Mortality: is a measure of deaths occurrence in a particular population, scaled to the size of that population, per unit of time (Source: WHO, 2013).

Proportional hazard model: is a well-recognized statistical technique for exploring the relationship between the survival of a patient and several explanatory variables. A proportional hazard model provides an estimate of the treatment effect on survival after adjustment for other explanatory variables. It allows us to estimate the hazard (or risk) of death, or other event of interest, for individuals, given their prognostic variables (Source: Kalbfleisch & Prentice, 2002).

Survival analysis: Survival analysis models factors that influence the time to an event (Source: Hosmer, Lemeshow & May, 2008).

Survival function: It captures the probability that the system will survive beyond a specified time t (Source: Kalbfleisch & Prentice, 2002).

ABSTRACT

The World Health Organization alerted that by the end of 2013, approximately 35 million people worldwide were living with HIV including 3.2 million who were children aged below 15 years old. The sub-Saharan region is the most affected with 24.7 million [23.5–26.1 million] people living with HIV, representing 71% of the global HIV load. The HIV infection among children bears a delicate particularity mostly associated with an exceptional high risk of poor outcomes. In the absence of clinical intervention, up to 52% of children infected with HIV die before the age of two years. The aim of this study is to analyze survival time among HIV positive children receiving ART and to model the effect of tuberculosis as a factor associated with mortality by building a proportional hazard regression model. It will be conducted among children aged 0-15 years, enrolled for HIV care and treatment in HIV clinic of pediatric excellence centers of Kigali and Butare, Rwanda. Data will be abstracted from The Electronic Medical Record database and will be analyzed by building of proportional hazards regression model for the mortality outcomes and construct Kaplan-Meier curves to assess predictors and to estimate the excess mortality attributable to HIV. Study findings are presented by demographical characteristics and categorized by study objectives. Mortality among children recruited in this study, death was likely to occur at the rate of 2.4 out of 1000 children years of follow up among 261 children who initiated ART while they were aged below 5 years. The greatest number and most proportion of terminal events occur within the first year of ART. This would suggest that patients need not only to initiate ART, but also healthcare providers should be more precautious during first months of treatment. A systematic and close monitoring system needs to be implemented in order to ensure good adherence and retention to care and treatment.

CHAPTER ONE: INTRODUCTION

1.1 Background

Over the recent decades, the world multiplied efforts in the fight against HIV infection-AIDS, focusing on research, preventing new infections and treating those who are infected. The disease remains one of the world's most significant public health concerns, particularly in low and middle-income countries. The World Health Organization alerted that by the end of 2013, approximately 35 million people worldwide were living with HIV including 3.2 million who were children aged below 15 years old. The sub-Saharan region is the most affected with 24.7 million [23.5–26.1 million] people living with HIV, representing 71% of the global HIV load (WHO, 2014).

Several organizations as well as governments joined their efforts to address the epidemic, especially in increasing access to prevention and treatment services. Thus, the number of people living with HIV receiving treatment in resource-limited countries has dramatically increased in the past decade. At the end of 2013, 12.9 million people living with HIV worldwide were receiving antiretroviral therapy (ART), of which 11.7 million were receiving ART in low- and middle-income countries. About 740,000 of those were children (UNAIDS, 2014).

Despite such tremendous achievements, HIV is the world’s leading infectious killer. An estimated 39 million people have died since the first cases were reported in 1981 and 1.5 million people died of AIDS-related causes in 2013, as reported by the WHO. Deaths are preceded by relatively long periods of morbidity, mainly due to opportunistic infections taking advantage of weakening immune system as the HIV infection takes on subject’s immune system. They may vary from bacterial, parasitic, fungal and viral infections to some

types of malignancies, as stated by the Panel on Opportunistic Infections in HIV-Exposed and HIV-Infected Children (UNAIDS, 2013).

The UNAIDS reported Rwanda among countries which have been able to achieve universal HIV treatment coverage, resulting in stabilizing the HIV prevalence at 3% throughout the last decade, dramatically reducing the number of new HIV infections by 53% and the number of HIV related deaths by 68% (UNAIDS, 2012).According to the Ministry of health in Rwanda reported an uninterrupted increase in number of people living with HIV on ART from 870 patients in 2002 when the program scale up was at its initial phases, to 123,499persons to the end of June 2013 (RBC, 2014).

1.2 Problem Statement

The HIV infection among children bears a delicate particularity mostly associated with an exceptional high risk of poor outcomes. In the absence of clinical intervention, up to 52% of children infected with HIV die before the age of two years (Newell et al., 2004). This implies the diagnosis of HIV infection among newborns as early as possible, so that you can increase chances of saving their lives. In following years of life up to the age of five, the risk of mortality and disease progression in the absence of treatment falls to rates similar to those of young adults but children are still more vulnerable compared to adults (Dunn D et al., 2008).Further to morbidity and mortality, HIV infection affects children at almost each of development stage. Children living with HIV are more likely to be stunted, and go through puberty later than their peers (Wamalwa, et al., 2008). Neurocognitive delays associated with behavioral troubles, as well as higher prevalence of mental illness were observed in children living with HIV (Abubakar, et al., 2008).

Evidences showed that better infection outcomes are strongly associated with early detection of infection, early ART initiation and treatment retention (WHO, 2013). These key aspects are challenging particularly among children due to the role of the client in the success of treatment for HIV infection.

In a recent meta-analysis conducted on outcomes in pediatric subjects living with HIV, the CD4 count was acknowledged among predictors of outcomes in children receiving ART (Peacock-Villada, 2011). However, there is limited knowledge on causes of mortality and the survival to event estimates among HIV infected patients with differentCD4 counts level.

This study analyzes the effect of CD4 count level at ART initiation on the survival time and the survival time to tuberculosis as a common opportunistic infection among HIV positive children on ART.

1.3 Objectives of the study

1.3.1 General Objective

The general objective is to model the effect of the CD4 level at initiation of ART on the survival time among HIV positive children receiving ART in pediatric excellence centers of Kigali and Butare, Rwanda.

1.3.2 Specific Objectives

1. To model the probability of survival to HIV associated outcome per CD4 count level at ART initiation among HIV positive children in pediatric excellence centers of Kigali and Butare, Rwanda .

2. To model the effect of tuberculosis as a factor associated with mortality among HIV positive children on ART in pediatric excellence centers of Kigali and Butare, Rwanda.
3. To model the effect of risk factors associated with mortality among HIV positive children on ART in pediatric excellence centers of Kigali and Butare, Rwanda.

1.4 Research questions

1. What is the association between the level of CD4 count and survival outcome among HIV positive children on ART in pediatric excellence centers of Kigali and Butare, Rwanda?
2. What is the association between tuberculosis and survival outcome among HIV positive children on ART in pediatric excellence centers of Kigali and Butare, Rwanda?
3. What are the factors associated with mortality in HIV positive children initiating ART at various levels of CD4among HIV positive children in pediatric excellence centers of Kigali and Butare, Rwanda?

1.5 Importance and Justification of the study

Several authors agree on the fact that there is limited existing data on the disease progression of HIV-infected children before access to ART, data are fragmented and most often obtained from selected children who have survived early opportunistic pathologies and who are therefore selected for these reasons (Dabis et al, 2005; Harambat et al., 2008). Detailed and accurate data on clinical evolution of HIV-infected children on ART are essential in order to evaluate the effect of HIV care and treatment now being provided. They also inform on

ART effectiveness and provide data on several other factors deserving consideration while caring for people living with HIV as they may hinder the expected outcome of the treatment on.

On its completion, this study will provide updated data suitable to help all counterparts involved in health policy to set up, or refine existing strategies being implemented in the care for children living with HIV. The data will be also valuable for clinicians caring on daily basis for children living with HIV, either on ART or before its initiation.

1.6. Scope of the study

The study will be conducted in the two pediatric centers of excellence in Rwanda, located respectively in Kigali University Teaching Hospital and Butare University Teaching Hospital. The two pediatric centers of excellence serve as referral centers from all children living with HIV from district hospitals and their satellite health centers all over the country.

While both centers receive and treat children with other types of diseases, each of them has an HIV clinic. The study will target only children living with HIV enrolled for care and treatment in these clinics. Considering the study objectives, only children living with HIV who already initiated ART will be included in the study.

1.7 Study limitations

Our study also accounted from some limitations. As a retrospective analysis, missing data were inevitable. Some explanatory data of potential factors were not available in the electronic data set available. This was the same for potential side effects due to rigors of ART treatment, prevalence of other opportunistic infections, as well as other individual reasons attributable to death.

CHAPTER TWO: LITTERATURE REVIEW

2.1. Introduction

This chapter details the review of the available literature related to this research study. It also attempts to explain need for the proposed work to appraise the short comings and informational gaps in earlier studies. This goes beyond scrutinizing the conclusions of the past studies, and includes the analysis of the accuracy and the credibility of these data. It also explains the conceptual framework for the current study.

2.2 Conceptual Framework

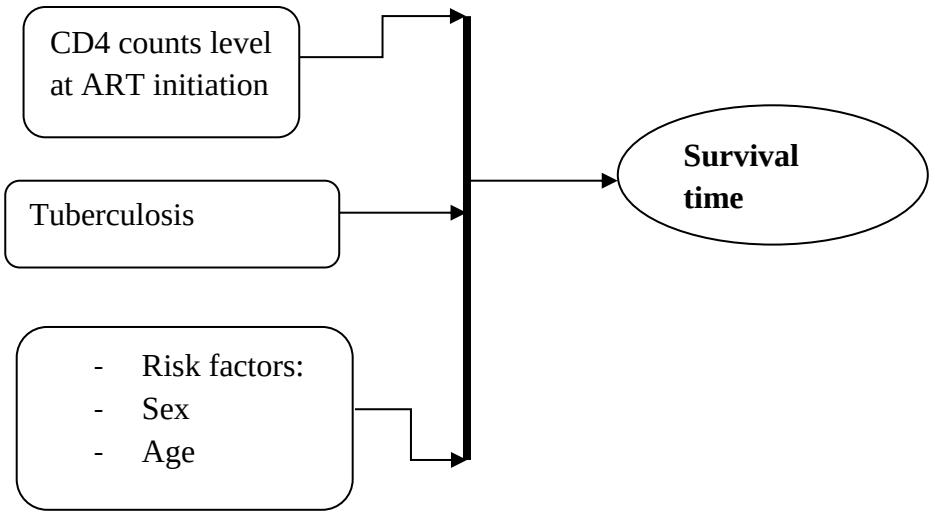

Figure 2.1 ***Conceptual framework of factors influencing survival time in HIV infected children on ART***

2.3 Review variables

Dependent variable: The response variable in this research is the "survival time" defined as the number of months from the date of enrollment of a patient in the HIV-care till one of the events "death", "lost to follow up", "stopped", "transferred out to other health centers or hospitals" occurred.

Independent variable: Age, sex, risk factors associated to mortality as TB, CD4 count

WHO (2013) reiterates that the helper cells are a type of human body cells playing an important role in the immune system, particularly in the adaptive immune system. They help the activity of other immune cells by releasing cell cytokines. These cells help suppress or regulate immune responses. Mature T cells express the surface protein CD4 and are referred to as CD4 cells. CD4 cells are generally treated as having a pre-defined role as helper cells within the immune system. The importance of helper T cells can be seen from HIV, a virus that infects $CD4^+$ cells.

Towards the end of an HIV infection, once the viral load has increased enough, the number of functional $CD4^+$ T cells falls, which leads to the symptomatic stage of infection known as the acquired immunodeficiency syndrome (AIDS); if the HIV virus is detected in bodily fluids, such as the blood, early enough, continuous therapy, if it is warranted, can delay the time at which this happens and better manage the course of AIDS if and when it occurs. There are also some rare disorders that result in the absence or dysfunction of $CD4^+$ T cells. These disorders produce similar symptoms, and many of these are fatal (UNAIDS, 2012).

UNAIDS and UNICEF (2008) alerted that children, especially those living wth HIV, are particulary vulnerable to tuberculosis. Tuberculosis is a chronic infection caused by bacteria.

It usually infects the lungs, although other organs such as the kidneys, spine, or brain are sometimes involved. It is primarily spread through droplets breathed or coughed into the air.

There are 3 important ways to describe the stages of tuberculosis:

Exposure: This occurs when a person has been in contact with, or exposed to, another person who is thought to have or does have tuberculosis. The exposed person will have a negative skin test, normal chest radiography, and no symptoms of the disease.

Latent tuberculosis infection: This occurs when a person has tuberculosis bacteria in his or her body, but does not have symptoms of the disease. The infected person's immune system walls off the TB organisms and they remain dormant throughout life in most people who are infected. This person would have a positive skin test but normal chest radiography.

Tuberculosis disease: This describes the person who has signs and symptoms of an active infection. This person would have a positive skin test and positive chest radiography (RBC, 2012).

Moderate variables: stigma

HIV-related stigma and discrimination refers to prejudice, negative attitudes and abuse directed at people living with HIV infection. The consequences of stigma and discrimination are wide-ranging. Some people are shunned by family, peers and the wider community, while others face poor treatment in healthcare and education settings, erosion of their human rights as well as psychological damage (Egger, 2006).

Control variables: CD4 counts level at ART initiation

2.4 Critique of the existing literature relevant to the study and to the variables in conceptual framework

Considering that the outcome of ART treatment depends heavily on how early the HIV infection was diagnosed and how early the treatment was initiated, several authors dedicated their work on outcomes of treatment in various contexts. The clinical evolution of HIV-infected children who have not yet initiated antiretroviral treatment (ART) is poorly explored. In most cases, it is dominated by recurrent morbidity episodes that may vary in severity as well as mortality of untreated HIV-infected children (Desmonde et al, 2011).

After ART initiation, the evolution of HIV infection is confronted not only with the treatment, but also the progression of the disease, immune suppression and potential occurrence of opportunistic infection. All of these elements are added to the ordinary requirements of the child organism mostly related to growth and physical development.

In the study conducted by Egger (2010), Cox regression was used to model the effect that initial treatment regimen and other prognostic factors have on disease progression. It was found that the ART achieves the primarily expected outcome, which is the suppression of the virus, but noted also that the choice of drugs used in the initial regimen was associated with the probability of viral suppression and with the sustained use of this regimen after starting the treatment. Among outcomes of the treatment, the study observed few differences in the rates of mortality between regimens, and these rates were much lower than were those in the pre- treatment era.

The immune system response of the child, assessed by the level of CD4, was subject of work for different authors engaged in the fight against HIV among children. In a meta-

analysis of 68 studies conducted among children from both developed countries and those with limited resources, the HIV infection outcomes varied by the level of CD4 at the time of ART initiation. The survival rate as well as resistance to opportunistic infections was higher among children who initiated the treatment with higher CD4 count, as stated by Peacock-Villada et al. (2011). These findings agree with the study conducted earlier among adult subjects on incidence and determinants of mortality and morbidity following early antiretroviral therapy initiation, as reported by Moh et al. (2007).

In their study that used Kaplan Meier survival and Cox proportional hazard model to identify independent predictors of children's mortality on ART, Gebremedhin et al. (2013) found that the level of CD4 at treatment initiation was among key aspects to consider in regard of treatment outcomes. These findings are similar to those reported by a study **on** long-term survival of HIV-infected children receiving antiretroviral therapy, whereby children with low CD4 levels were found less likely to survive (Collins et al., 2010).

Using Kaplan-Meier survival analysis, Radhakrishna et al. (2013) in a study conducted among children living with HIV, found that CD4 counts and opportunistic infections play an important role in influencing the survival chances of HIV infected children.

Additional aspects to explore include the duration and the regularity of treatment intake.

Fox and Rosen (2010) worked on patient retention in antiretroviral therapy programs on treatment in sub-Saharan Africa. From the plotted crude retention rates from each study describing observational cohorts from sub-Saharan Africa reporting on adult HIV 1- infected patients initiating ART up to five time points: 6, 12, 18, 24 and 36 months.

For each study simple retention proportions (%RT) at each time point t was calculated as:

$$\textbf{\% RTi= 100\%-[\% TAT}_i\textbf{*(1-\%RT1)]}$$

Where I_0 is all patients initiated on ART at the site; T_t is all patients transferred out of care by time t; D_t is all patients who died by time t; and $LTFU_t$ is all patients lost to follow-up by time t. This is the proportion of all patients initiated who did not transfer out of care who are still alive and in care at the end of the follow-up period.

Using linear regression the findings were: median starting CD4 count <100 (−5.8%; 95% CI: −8.9% to −2.7%), median age <36 (5.7%; 95% CI: −9.2% to −2.2%), and having <60% females (−9.4%; 95% CI: −13.9% to −4.9%) were predictive of lower retention rates at 6 months when also adjusting for median follow-up and year of initiating cohort and approximate of deaths at 41%.

Regarding to (Tegiste & Eshetu, 2010) work on research undertaken to estimate mortality rate and identify predictors(factors) that have significant impact on the survival status of a sample of patients who received ART and care in Addis Ababa, Ethiopia. As statistical method the Kaplan-Meier Method was employed to estimate mortality and the Cox Proportional Hazards Regression Method was used to identify determinants of mortality. This study used observations on 1000 HIV-positive people that were followed during 2005 to 2008. Of those, about 90% were right-censored and the remaining uncensored. HIV positive patients lived for an average of 5.65 years (CI: 3.69-7.61 years); the median survival age was found to be 3.98 years (CI: 2.98-4.97 years). Female HIV-positive patients had shorter survival on average compared to males. It was observed that the survival time of patients under ART varied along differences in functional status. People living with HIV/AIDS under ART had better survival time on average than patients who were eligible

for ART, but did not take the treatment. CD4 cells counts showed a strong influence on the survival status; patients with counts of more than 200/mm3 had higher survival experience than those with counts below 200/mm3.

It is highly regretted that due to paucity of data in this area, the researcher did not find comparable studies conducted previously in Rwanda.

2.5 Research gaps

The use of antiretroviral therapy as a good effect on the reducing disease progression and death among patients infected by HIV, but the optimal time to initiate therapy is not always achieved. Current guidelines recommend that the sooner is better, but its achievement is challenged by delays in diagnosing HIV infection. Except for children born to known mothers living with HIV, the infection in children is suspected to be late mostly after severe immunodeficiency.

Despite notable progress in the fight against HIV all over the world, it is widely acknowledged that serious gaps are still to be filled as far as children are concerned. These gaps include also the paucity of data on children.

Moreover, in the available few publications on children, the exploration of survival outcomes is still inadequate. In regions and countries observing higher mortality rate, there is still an information gap on the morbidities associated with the HIV related mortality as well as associated factors.

2.6 Summary of reviewed literature

The clinical evolution of HIV-infected children who have not yet initiated antiretroviral treatment is poorly explored. In most cases, it is dominated by recurrent morbidity episodes

that may vary in severity as well as mortality of untreated HIV-infected children. After ART initiation, the evolution of HIV infection is confronted not only with the treatment, but also the progression of the disease, immune suppression and potential occurrence of opportunistic infection. In the study exploring the effect that initial treatment regimen and other prognostic factors have on disease progression, it was found that the ART achieves the primarily expected outcome, which is the suppression of the virus, but noted also that the choice of drugs used in the initial regimen was associated with the probability of viral suppression and with the sustained use of this regimen after starting the treatment. Among outcomes of the treatment, the study observed few differences in the rates of mortality between regimens, and these rates were much lower than were those in the pre- treatment.

The immune system response of the child, assessed by the level of CD4, was subject of work for different authors engaged in the fight against HIV among children. In a meta-analysis of 68 studies conducted among children from both developed countries and those with limited resources, the HIV infection outcomes varied by the level of CD4 at the time of ART initiation. The survival rate as well as resistance to opportunistic infections was higher among children who initiated the treatment with higher CD4 count, as stated by Peacock-Villada et al. (2011). These findings agree with the study conducted earlier among adult subjects on incidence and determinants of mortality and morbidity following early antiretroviral therapy initiation, as reported by Moh et al. (2007).

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long-term survival of HIV-infected children receiving antiretroviral therapy, whereby children with low CD4 levels were found less likely to survive (Collins et al., 2010).

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Moreover, in the available few publications on children, the exploration of survival outcomes is still inadequate. In regions and countries observing higher mortality rate, there is still an information gap on the morbidities associated with the HIV related mortality as well as associated factors. Additional aspects to explore include the duration and the regularity of treatment intake.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

This chapter explains the design of this study, description of targeted population well as steps of its implementation. It also clarifies statistical methods used, data presentation and discussion of findings.

3.2 Study design

This is a retrospective cohort study, conducted among children enrolled for care and treatment in pediatric centers of excellence of Kigali and Butare, Rwanda. It covers the period starting from January 2004 up to December 2013. Individual follow up data kept as clinical records were handed over to researchers for analysis. They were stored in an electronic database called "Electronic Medical Records" managed by the Ministry of Health, Rwanda. Data are continuously filled in the database by technicians based in both health facilities as part of routine monitoring of clinical activities.

Data were extracted from the data base, cleaned and variables of interest were selected for analysis using survival analysis method. The principle in the dataset is that the individuals contain the start and the ends dates in the portfolio of which the lifetime is determined. Fortunately the analysis method, survival data analysis being used involves the modeling of time-to-event data, such as the time until death. In survival analysis, a data set can be exact or censored, and it may also be truncated. Censored data arises when a subject's time until the event of interest is unknown. The major advantage of survival analysis is the ability to incorporate censored and truncated data but our work is only interested in censored data.

3.3. Censored data

Censored data comes up when a subject's exact time points at which failures occur are unknown. Censoring is the missing data problem. Right censoring is the most common type of censoring and stipulates that the event is not observed within the study period.

3.3.1. Right censoring

In survival analysis, right censored data are a common missing data problem in estimating survivor and hazard function. For example, we may want to know how long individuals will survive after getting treatment if a patient drops out of a treatment in the known health facility before the event of interest has occurred or it is possible that a patient moves away before the failure time, in this case the survival time is right censored.

In biomedical applications, especially in clinical trials, these two important issues arise more often when studying "time to event" data (we will assume the event to be "death". It can be any event of interest). In these cases, some individuals are still alive at the end of the study or analysis so the event of interest, namely death, has not occurred. Therefore we have right censored data. The length of follow-up varies due to staggered entry. So we cannot observe the event for those individuals with insufficient follow-up time.

In this case we are unable to find information regarding the time to death. In right censoring we also account the subjects who are still alive after the period of interest as the exact survival time for those individuals is unknown, but we are sure that each individual's time of death will occur after some specified time point.

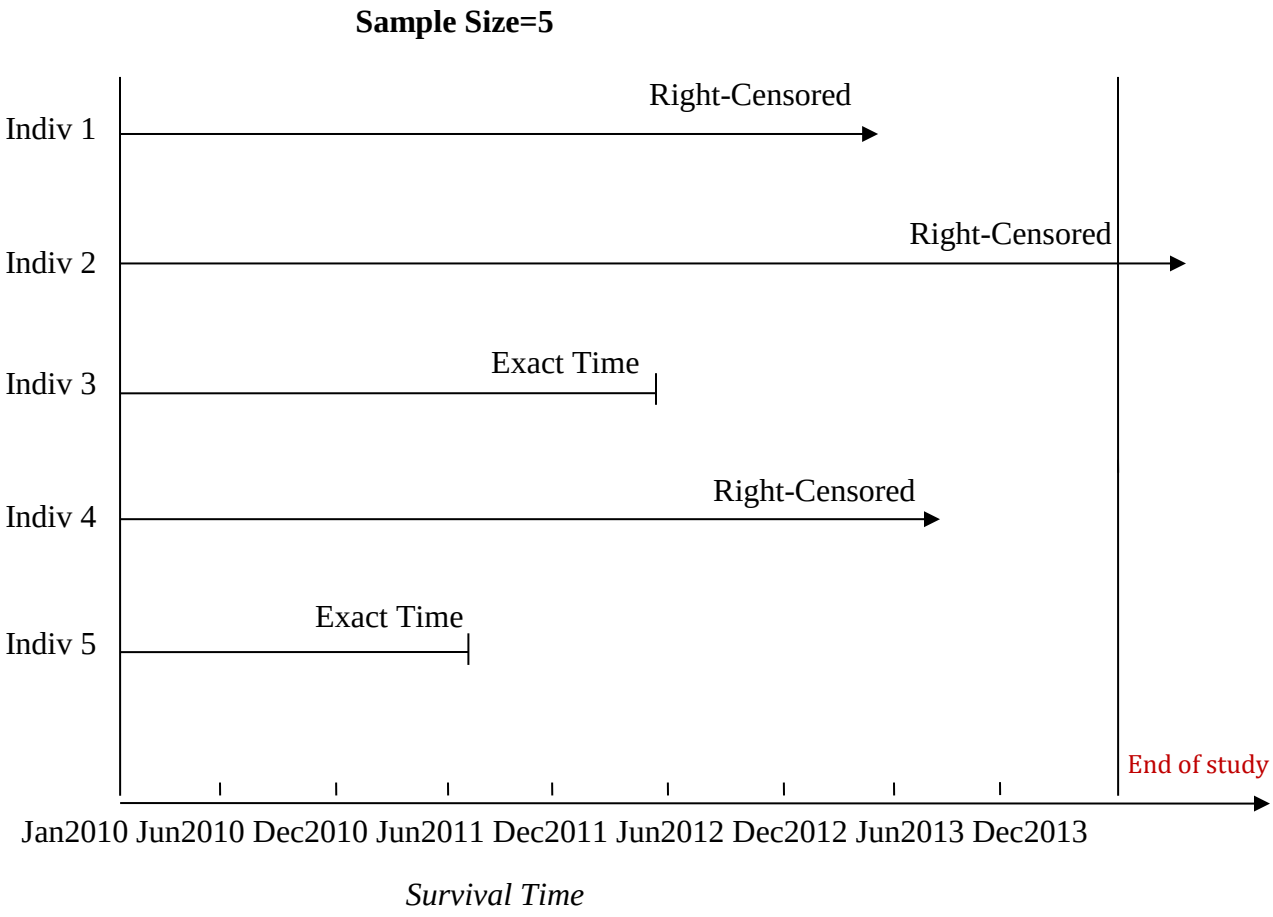

Figure 3.1: Right censoring example

Figure 3.1shows an example of graphical representation of the survival information about each of the five individuals being known. The survival information is represented by a horizontal line for each subject. An arrow at the end of an individual's line indicates a right-censored failure time. As a result, the survival time for individuals 3 and 5 is exactly known, but the failure times associated with individuals1, 2, and 4 are right-censored (unknown). Individuals 1 and 4 were lost to follow-up or transferred out in other health facility at a certain time before the end of this study. Individual 2 is still alive at 3 years, which is the end of the study period. Therefore this individual has a survival time which is right-censored at 3 years.

3.4 Target population

Actually the country is covered with 510 health facilities ranging from health centers, district hospitals and national referral hospitals. They provide the package of HIV services that may vary according to the technical capacity in each facility. They all use pediatric centers of excellence as referral setting especially for cases requiring more technical expertise. They are two: the pediatric center of excellence in Kigali University Teaching Hospital and the pediatric center of excellence in Butare University Teaching Hospital. With their geographic location, they serve the entire national territory.

The study included both children living with HIV enrolled for care and treatment in both pediatric centers of excellence. The timeframe included those who were enrolled since January 2004 through December 2013.We estimate survival function for 6, 12, 18, 24, 30 and 36 months after ART initiation including patients who died or were lost to follow-up considered as censored data but excluding transferred patients considered as truncated data.

The study has not included HIV patients who were not enrolled in ART program during the period of interest for this study and those who were the transfers in both pediatric centers of excellence center. It excludes also all patients who did not initiate ART in pediatric centers of excellence.

It also excluded the children who initiate ART regardless their CD4 count as it is recommended. Those children may be the one initiated considering only their age, and those with active TB disease and HBV co infection with severe liver disease as per national HIV management guidelines implemented in the country, all amendments considered.

3.5 Sampling Techniques and sample size

In our study, we considered the fact that all health facilities in the country refer their children with advanced treatment needs to pediatric centers of excellence. This referral service has been operational for children living with HIV since January 2004. It is common knowledge that government-funded data gathering initiatives are usually representative of the target population, thus enhancing external validity. With this in mind, the sample targeted in this study is estimated to be representative of all HIV-positive children.

Considering the study period, 720 patients initiated the antiretroviral treatment during the period from January 2004 through December 2013. Throughout this period, some of children have been transferred in other health facilities to pursue treatment for various reasons and some others have been lost to follow up and will be considered as censored observations. We decided to consider all of them as a population to get the accurate and precise findings which will be inferred to national population as the two pediatric centers of excellence serve all the country.

3.6 Data Collection

The collection of data was based on extracting data from the Electronic Medical Record database, managed by the Ministry of health. In order to protect individual privacy as well as ethical principles, there were no personal identification associated with data.

The abstracted data was cleaned and variables of interest for this study were identified based on objectives of the current study. The data were entered into a matrix created using SPSS and after exported into STATA and R Software for further analysis.

The sample size for this study will be proportional to size of the number of patients who were being treated in both health centers within the study time. Analysis will be restricted to720HIV-positive children still on ART combination on which they were started at initiation of therapy.

3.7 Data analysis and presentation

The analysis was conducted by building of proportional hazards regression model for the mortality outcomes and construct Kaplan-Meier curves to assess predictors and to estimate the excess mortality attributable to HIV by socio-demographic factors and baseline CD4 categories. To model risk factors associated with mortality were used to build a regression proportional hazards model. We measured time from the date of initiation of therapy to the date the end points occurred. For patients free of events, follow-up was censored either on the date of the most recent follow-up visit or on the date the patient was last known to be alive. Proportional hazard model was used to model the effect that initial treatment regimen and other prognostic factors have on survival outcome.

Study results were presented by socio demographic characteristics and categorized based on study objectives. Traditional data presentation tools was used, including tables and charts, depending on type of data.

3.8 Survival analysis

Suppose that T is the length of time before a patient dies. The randomness of T can be described in three standard function ways as density, survival and hazard function (Kalbfleisch & Prentice, 2002 and Leemis, 1995).

3.9 Density Function

To predict the time to event with survival data, the best and most convenient way is to identify the probability density function *f(t)* of time to event, and then its survival cumulative function *S(t)* and hazard function *h(t)* can be easily gotten. The second way is the density function f (t). This is the probability that the event time occurs at exactly time t (out of all possible times).

For example, for continuous variable time to event (t), its density function,

$$\boldsymbol{f(t) = \lim_{\Delta t \to 0} \frac{P(t \leq T < t + \Delta t)}{\Delta t}} \quad (1)$$

The probability that an event occurs before time *t* and is given as:

$$\boldsymbol{F(t) = P(T \leq t)}\ F(t) \text{ is the } \textit{cumulative density function} \quad (2)$$

3.10 Survival Function

From distribution function, the survival function S (t), the probability the time to event (T) is larger compared to a fixed time (t), it is simply the reverse cumulative distribution function of T, can be derived as:

$$\boldsymbol{S(t) = \int_t^{\infty} f(t)dt = Pr(T \geq t) = 1 - Pr(T \leq t) = 1 - F(t)} \quad (3)$$

If there are no censored observations, the survival function is estimated as the proportion of patients surviving longer than t and is given by*:*

$$\boldsymbol{S(t) = 1 - \frac{number\ of\ patients\ with\ surviving\ time \leq t}{Total\ number\ of\ patients}} \quad (4)$$

This gives the probability of being alive just before duration t, or more generally, the probability that the event of interest has not occurred bydurationt, (Lawless, 1982).

3.11 Hazard function

And the last way is given by the hazard function. This is the probability that if a facility survives up till time t, the probability that the failure event occurs in a given interval, conditional upon the subject having survived to the beginning of that interval it will experience the event in the next instant and is given by:

$$\boldsymbol{h}\,(\boldsymbol{t}) = \boldsymbol{\lambda}(\mathbf{t}) = \lim_{\boldsymbol{\Delta t \to 0}} \left(\frac{\boldsymbol{Pr(t \le T \le t+\Delta t | T > t)}}{\boldsymbol{\Delta t}} \right) = \frac{\lim_{\boldsymbol{\Delta t \to 0}} \left(\frac{\boldsymbol{Pr(t \le T \le t+\Delta t | T > t)}}{\boldsymbol{\Delta t}} \right)}{Pr\ (T \ge t)} \quad (5)$$

$$\boldsymbol{h(t)} = \frac{\boldsymbol{f(t)}}{\mathbf{1}-\boldsymbol{F(t)}} = \frac{\boldsymbol{f(t)}}{\boldsymbol{S(t)}} \quad (6)$$

The explanation of the survival function *S (t)* is the most obvious. It is a plot with on the left axis the proportion of the population still alive and on the x axis the time. The hazard function h(t), also called incidence rate, instantaneous risk or force of mortality, is the event rate at t among those at risk at time t. The explanation is straight forward.

3.12 Types of survival models

There are three assumptions used in this analysis. *Firstly*, we assume that at any time patients who are censored have the same survival prospects as those who continue to be followed. *Secondly*, we assume that the survival probabilities are the same for subjects recruited early and late in the study. *Thirdly*, we assume that the event happens at the time specified.

3.12.1 Kaplan Meier Estimator

The KM analysis is widely used to estimate the fraction of individuals living for given length of time while considering time in many small intervals after starting get treatment and it estimates the median survival distribution function.

Suppose Y individuals experience events time in a group of individuals. Let the observed failure times be defined by $0 \leq t_{(1)} \leq t_{(2)} \leq \ldots \leq t_{(y)} \leq \infty$. Let n_i be the number of individuals at risk (uncensored and alive) just before t_i. And let d_i be the number of observed deaths at t_i. The Kaplan-Meier estimate is also called as "product limit estimate". It involves computing of probabilities of occurrence of event at a certain point of time. We multiply these successive probabilities by any earlier computed probabilities to get the final estimate. The survival probability at any particular time is calculated by the formula given below:

$$\mathbf{KM}\,(\mathbf{t}) \;=\; \hat{\mathbf{s}}\,(\mathbf{t}) \;= \prod_{x<t}\left(\mathbf{1} - \frac{\boldsymbol{d(x)}}{\boldsymbol{n(x)}}\right) \qquad \textbf{(}7)$$

Next, estimate $\Lambda(t)$ by $\widehat{\Lambda_{KM}}$ (t)= - log[KM(t)]. Their variance is estimated by:

$$\widehat{\boldsymbol{Var}}\left\{\widehat{\boldsymbol{\Lambda_{km}}}(\boldsymbol{t})\right\} = \sum_{x<t}\left[\frac{\boldsymbol{dN(x)}}{[\boldsymbol{Y(x)}-\frac{\boldsymbol{w(x)}}{\mathbf{2}}][\boldsymbol{Y(x)}-\boldsymbol{dN(x)}-\frac{\boldsymbol{w(x)}}{\mathbf{2}}]}\right] \qquad (8)$$

$$\boldsymbol{se}\left\{\widehat{\boldsymbol{\Lambda_{km}}}(\boldsymbol{t})\right\} = \sqrt{\widehat{\boldsymbol{Var}}\left\{\widehat{\boldsymbol{\Lambda_{km}}}(\boldsymbol{t})\right\}} \qquad (9)$$

The confidence interval of the KM estimator is given by:

$$\boldsymbol{CI}[\boldsymbol{S}(\boldsymbol{t})] = \boldsymbol{KM}(\boldsymbol{t}) * \boldsymbol{e}^{\pm \boldsymbol{z}\propto/\mathbf{2}*}\boldsymbol{se}\left\{\widehat{\boldsymbol{\Lambda_{km}}}(\boldsymbol{t})\right\} \qquad (10)$$

Where

- D(x) is the number of events at time x, generally either zero or one, but in case of tied survival times d(x)≥1,
- n(x) is the number of items at risk at time x,
- $\widehat{\Lambda_{km}}(t)$ is the unbiased estimator of the cumulative hazard rate at t,
- $dN(x)$ number of observed events occurring in [x, x+∆x],

- $Y(x)$ is the number of censored at time x,
- $w(x)$ is the number of censored at time x,
- $z \propto/_2$ is the (1-$\propto/_2$)th quintile of a standard normal distribution

Note that the Kaplan Meier estimator is a step function that does not change between events, nor at time censorings occur, it only changes at the time of each event.

3.12.2 Accelerated Failure time

Accelerated failure time model (AFT model) is a parametric model for estimating univariate survival and for censored-data regression problem. For non-parametric model, it estimates the survival function based on the observation only. It does not require any distribution of the data. However, the question may become much simpler if the data fits a distribution well, since there are fewer parameters should be dealt with. The AFT model means that the survival function of an individual with covariate Z at time t is the same survival function of an individual with a baseline survival function at a time $\exp(\theta^t Z)$, where θ^t is a vector of regression coefficients.

$$\mathbf{S(t|z) = S_0\ [exp(\theta^t Z)\ t]}, \text{ for } \textit{all } t \qquad (11)$$

It means, where $\exp(\theta^t Z)$ is called an acceleration factor. It means how a change in covariate alters the time scale from the baseline time scale. It implies that the median time to event with covariate Z is the baseline median time to event divided by its acceleration factor. Similarly, the hazard function is also able to be calculated. It is also related to the baseline hazard rate.

$$\boldsymbol{h(t|z) = h_0[exp(\theta^t Z)t]exp(\theta^t Z)}\ , \text{ for } \textit{all } t \qquad (12)$$

3.12.3 Proportional Hazard Model

The rule of the proportional hazards model is to link the survival time of an individual to covariates. In our case, in this study, we are seeking to find out which covariates (factors) have the most important impact on the survival time of a patient. A proportional Hazard Model is a well-known statistical technique for exploring the relationship between the survival of a patient and several explanatory variables. It allows us to estimate the hazard (or risk) of death for individuals. The hazard function is the probability that an individual will experience an event (for example, death) within a small time interval, given that the individual has survived up to the beginning of the interval. It can therefore be interpreted as the risk of dying at time t. The hazard function (denoted by h (t, X) can be estimated using the following equation:

$$\boldsymbol{h(t|X) = h_0(t)exp(X_1\beta_1 + X_2\beta_2 + \cdots + X_p\beta_p = h_0(t)exp(\beta' X)} \qquad (13)$$

1. The X is time-independent, the baseline hazard $\boldsymbol{h_0(t)}$ does not depend on X but only on t, the exponential involves the X's but not t. The effect of the predictors is the same at all times t.

2. The predictors $X_{1,\ldots,}X_p$ are assumed to act additively on $\log h(t|x)$.

3. $\log h(t|x)$Changes linearly with theβs.

Parameter estimates in the Proportional Hazard model are obtained by maximizing the partial likelihood as opposed to the likelihood. Let Yi denote the observed time (either censoring time or event time) for subject i, and let Ci be the indicator that the time corresponds to an event (i.e. if Ci = 1 the event occurred and if Ci = 0 the time is a censoring

time) and X1, ..., Xn are the covariate vectors for the n independently sampled individuals in the dataset.

The partial likelihood is given by:

$$\boldsymbol{L(\beta)} = \prod_{i:c_i=1} \frac{\exp(X_i\beta)}{\sum_{j:Y_j \geq Y_i} \exp(X_j\beta)} \quad (14)$$

The corresponding log partial likelihood is given by:

$$\boldsymbol{\ell(\beta)} = \log \boldsymbol{L(\beta)} = \sum_{i:c_i=1} \left(X_i\beta' - log \sum_{j:Y_j \geq Y_i} \exp(X_j\beta) \right) \quad (15)$$

This function can be maximized over β to produce maximum partial likelihood estimates of the model parameters (Hosmer et al 2008).

CHAPTER FOUR: RESULTS

4. Statistical analysis

Descriptive statistics such as median; mean and proportions were used to describe the general characteristics of the cohort. Person-months/years of follow up were calculated by assessing the date of enrollment for ART and death or censoring. The role of the variables on patient survival was analyzed using Kaplan-Meier survival analysis method. Log Rank test was used to test the equality of survival probabilities and compared across the different groups of covariates. The overall survival function and separate estimates for the stratum of covariates were considered as statistically significant at p-value < 0.05 in the Log- rank test. Hazard ratios (HR) with 95% confidence intervals were used as effect measures. Multivariable Cox proportional hazards regression was used to assess the effect of baseline predictors on the survival of children on ART. Variables with p <0.05 in univariate and bivariate analysis were taken to multivariate analysis to estimate hazard ratios with 95% confidence intervals for the mortality rate among children on ART for the covariates at their CD4 counts at initiation.

4.1 Cohort description

This study recruited a cohort made of 720 children living with HIV. They are enrolled for care and treatment in HIV clinic of pediatric center of excellence of Kigali and in HIV clinic of the pediatric center of excellence in Butare. These centers are located respectively in Kigali university teaching hospital (CHUK) and Butare university teaching hospital (CHUB). All subjects of this study initiated ART in both clinics at different dates from January 2004 throughout December 2013.

They were aged from 0 to 15 years when they initiated ART, and the median age was 6.7 years. Both genders are almost equally represented, with a slight higher prevalence of female (54%). The two clinics contributed nearly evenly to the sample size: 47% initiated

ART in Kigali clinic whereas the rest initiated ART in Butare clinic. After ART initiation, subjects continued care and support associated with ART in both clinics. The median time of follow up was 55 months [interquartile range (IQR) 1-119]. Different scenarios are associated with drop out of follow up in a clinic, including change of health facility, abandonment as well as death. More than half of the children (51%) have been followed for at least 50 months after ART initiation in both clinics.

4.2 Distribution of study subjects

Children enrolled in the current study are distributed in various demographic characteristics as well as HIV clinic of ART initiation. They are summarized in the table 4.1

Table 4.1 Distribution of study subjects

	HIV clinic		
	CHUK	**CHUB**	**Total**
	n(%)	**(%)**	**N(%)**
Age in years			
<5	131(38.6)	(35.2)	265(36.8)
05-10	108(31.9)	(44.9)	279(38.8)
11-15	100(29.5)	(19.9)	176(24.4)
Gender			
Male	178(52.5)	(50.7)	371(51.5)
Female	161(47.5)	(49.3)	349(48.5)
CD4 cells/ μl at baseline			
<=50	35(10.3)	(4.7)	53(7.4)
51-199	29(8.6)	(10.8)	70(9.7)
200-349	39(11.5)	(16.8)	103(14.3)
350-499	49(14.5)	(14.4)	104(14.4)
>=500	187(55.2)	(53.3)	390(54.2)
Total	339	381	720

Table 4.1 shows that a total of 720 children living with HIV were recruited for this study. Only 37% of children were younger than 5 year (range 7.8% to 42.8%). The majority of children (54.2%) initiated ART with more than 500 CD4 cells per micro liter. Only 7.4% of

children started ART with the lowest CD4 count level, below 50 CD4 cells per micro liter. In this cohort, the median CD4 count at ART initiation was 557 CD4 cells per micro liter.

4.3 Follow up time of children receiving ART

The treatment provided to children living with HIV requires a lifelong follow up, ensuring that they are coping well with rigors of treatment, detecting any opportunistic infection as well as providing necessary adjustments in the treatment plan as the child grows. It allows also witnessing and recording the occurrence of events. In this study, the event of interest is death and the follow up time is estimated in months. The follow up duration for subjects of this study in both clinics is summarized in the table 4.2.

Table 4.2 Follow up time and event occurrence.

Event	p1	p25	p50	p75	p99	N	mean
No	1	28	59	79	118	661	56.08321
Yes	0	1	3	10	76	59	10.59322

As displayed in the table 4.2, the duration of children's follow-up after ART had considerable variations. In a group of 661 children who survived, the median duration of follow up was 55 months after ART initiation. However, among 59 children who died, the median duration of follow-up on ART was only 3 months (25^{th}percentile =1 month; 75th percentile =10 months).

4.4 Censoring

Several events may occur throughout the follow up period, including those that cause the discontinuation of the client's follow up. The figure 4.1 displays the occurrence of death, as event investigated by this study, per duration of follow up. The vertical axis represents the number of subjects and the horizontal axis represents the duration of follow up after ART initiation, estimated in months.

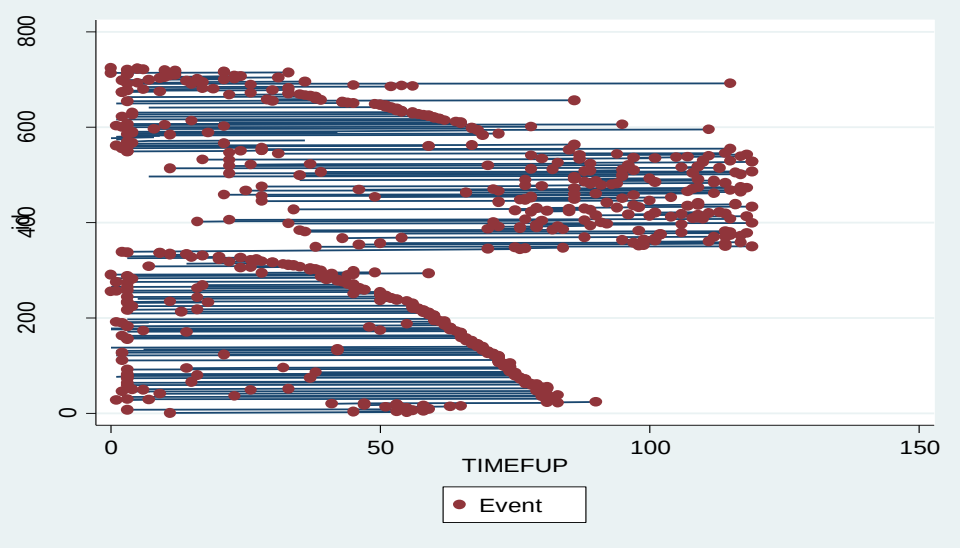

Figure 4.1 Censored data in the study

The events mostly experienced in HIV clinic that lead to the discontinuation of follow up include change of health facility due to migration, loss to follow up for unknown motives as well as death. In the figure 4.1 red dots denote intervals in which the event "death" occurred, whereas intervals without red dots signify censoring. It appears that a number of subjects dropped out of follow up after only a relatively short time subsequent ART initiation. Another group of subjects did not die by the timeframe of this study and no one can predict the event occurrence if the study timeframe was to be longer.

4.5 Correlates to mortality

The CD4 count level at ART initiation is among key elements to consider throughout the follow up of children on lifelong ART. The distribution of CD4 count among study subjects is summarized in table 4.3. The event of interest is death and the number of CD4 is estimated in number of cells per micro liter.

Table 4.3 Distribution of CD4 counts at baseline

Event	p1	p25	p50	p75	p99	mean
No	3	310	580	951	3021	698.204
Yes	2	143	356	612	2994	514.881

As stated in table 4.3, among subject who were alive, the median CD4 count at baseline was 580 cells per micro liter (1st percentile =3; 75th percentile =951, 99th percentile =3021). However, in the group of children who died during the ART follow up, the median CD4 count at baseline was 356 cells per micro liter (1st percentile =2; 75th percentile =612, 99th percentile =2994).

4.6 Incidence Death Rate

4.6.1 Incidence death rate per CD4 level at ART initiation

Deaths per 1000 child years (DPCY) were calculated by using the number of events and time of occurrence. The total number of child-years was estimated as the sum of years contributed by living patients at the time of mortality measurement and one-half of this follow-up time for deceased patients. The table 4.4 summarizes the comparison of incidence death rate per level of CD4 count at ART initiation.

Table 4.4 Incidence death rate by CD4 level at ART initiation

CD4 counts at initiation	Exposure Group		Comparison Group	P-Value
	IR[1]	n	IR[1]	
<50	4.0/1000 (PY)	48	1.1/1000 (PY)	<0.001
<200	2.2/1000 (PY)	112	1.1/1000 (PY)	=0.012
<350	1.8/1000 (PY)	210	1.0/1000 (PY)	=0.017
<500	1.7/1000 (PY)	307	0.9/1000 (PY)	=0.02
N=691				

The table 4.4 shows that death occurred at the rate of 4 out of 1000 Child years of follow up time among 48 children who initiated ART with CD4 counts below 50 cells per micro liter.

Though, in the group made of 307 children who initiated ART with CD4 count below 500 cells per micro liter, the death occurred at the rate of 1.7 out of 1000 Child years.

Children initiating ART with CD4 count below 50 cells per micro liter were significantly at higher risk of dying than those who initiated ART at CD4 count above 50 cells per micro liter with P value <0.001. The table shows also that the risk of death among children in groups made by CD4 level at initiation of ART appears significantly higher than those in groups of children who initiated ART with higher CD4 count, with respective P values.

In all groups, the average incidence of death rate by 15 out of 1000 child years of follow up died in the period of study. We found that a child initiating ART with lower CD4 counts is at a higher risk of death than ones who were initiated at higher CD4 counts.

4.6.2 Incidence death rate by age at ART initiation

Using categories of age at ART initiation, the study explored the incidence death rate and comparisons among categories were conducted. Findings are summarized in table 4.5

Table 4.5 Incidence death rate by age at ART initiation

Age in years	IR[1]	N	**P-Value**
<5	2.4/1000 (PY)	261	=0.0012
05-10	0.6/1000 (PY)	277	
11-15	1.5/1000 (PY)	173	

As showed by the table 4.5, death was likely to occur at the rate of 2.4 out of 1000 children years of follow up among 261 children who initiated ART while they were aged below 5 years. This level was the highest compared to other groups of age at ART initiation, and this difference was statistically significant with P-value of 0.0012 .

4.6.3 Incidence death rate by gender

The incidence death rate was assessed based on the gender of the children. The study did not find any difference among boys and girls. They were on the same level as death was likely to occur at the rate of 1.4 out of 1000 children years of follow up in each group of children.

4.6.4 Incidence Death Rate by TB

Tuberculosis, one of common opportunistic infections developed by children living with HIV, occurred 81 times among 720 children assessed. The incidence death rate was assessed among children who got tuberculosis during their follow up under ART and those who did not get it. Both groups of children have the same incidence death rate of 1.4 out of 1000 child years of follow up.

4.7 Survivor function

The survival function has been computed for the entire cohort, throughout the period of follow up after ART initiation. Findings are summarized in the table 4.6

Table 4.6 HIV survival throughout follow up

Time	N	Events	Survivor function	Standard Error	[95% Conf. Int.]	
1 month	711	10	0.9859	0.0044	0.9740	0.9924
12 months	579	30	0.9409	0.0091	0.9203	0.9563
24 months	526	6	0.9306	0.0099	0.9084	0.9476
36 months	475	3	0.9249	0.0104	0.9017	0.9428
48 months	408	3	0.9185	0.0109	0.8942	0.9375
60 months	323	1	0.916	0.0112	0.8912	0.9354
72 months	237	0	0.916	0.0112	0.8912	0.9354
84 months	137	1	0.9114	0.0121	0.8845	0.9322
96 months	93	0	0.9114	0.0121	0.8845	0.9322
108 months	57	0	0.9114	0.0121	0.8845	0.9322
119 months	6	0	0.9114	0.0121	0.8845	0.9322

The table 4.6 shows that mortality rate was higher in the first months following ART initiation and progressively stabilizes in the following months under treatment. Thus, 6 of the 720 patients had a follow-up time till 10 years (119 months), and so a survival rate of 91.1% (95% confidence interval (CI), 88.4% to 93.2%) at 10 years was presented based on this 6 patients.

4.7.1 HIV survival per CD4 level

Children living with HIV recruited for this study have been initiating ART at different levels of CD4 count. The survival function has been computed per level of CD4, throughout the period of follow up after ART initiation. Findings are summarized in the table 4.7

Table 4.7 HIV survival per CD4 count at ART initiation

Time in months	CD4 <50			CD4 >50			CD4 <200			CD4 >200		
	N	Event	Prob	N	Event	Prob	N	Event	Prob	N	Event	Prob
12	38	7	0.849	531	26	0.9574	93	11	0.8996	475	22	0.9596
24	34	2	0.8017	482	4	0.9498	85	2	0.8794	431	4	0.9511
36	34	0	0.8017	432	3	0.9434	80	0	0.8794	386	3	0.9439
48	27	1	0.772	371	2	0.9387	65	1	0.8658	333	2	0.9387
60	22	0	0.772	294	1	0.936	54	0	0.8658	261	1	0.9356
119	1	0	0.772	5	1	0.9308	1	0	0.8658	5	1	0.9297

Time in months	CD4 <350			CD4 >350			CD4 <500			CD4 >500		
	N	Event	Prob	N	Event	Prob	N	Event	Prob	N	Event	Prob
12	177	15	0.9267	391	18	0.9599	259	21	0.93	309	12	0.9658
24	162	2	0.9158	354	4	0.9495	233	2	0.9224	283	4	0.9528
36	147	1	0.9096	318	2	0.9437	208	2	0.9139	257	1	0.9491
48	124	2	0.8957	274	1	0.9406	182	2	0.9043	216	1	0.9452
60	103	1	0.8879	212	0	0.9406	152	1	0.8989	163	0	0.9452
119	3	1	0.8759	3	0	0.9406	4	1	0.8901	2	0	0.9452

The table 4.7 shows that the overall survival probabilities were 0.986% [95% confidence interval (CI), 0.974- 0.992) at 1 month till 119 month. The group of children who initiated ART

with CD4 counts below 50 cells per micro liter experienced most of the deaths in first year following ART initiation and no child died after four years of follow up. At 60 months, 93.6% (95%CI: 0.9120, 0.9536) of the children who initiated ART at with 50 CD4 cells per micro liter were still alive whereas 77.2% (95%CI: 0.6151, 0.8713) of the children who initiated ART with CD4 counts below 50 cells/μl. Comparing children who initiated ART with less than 200 CD4 cells per micro liter and those with higher CD4 cells count, it was found that 86.6% (95%CI: 0.7829, 0.9187) of the children who initiate ART with lower CD4 counts were still alive and 93.5% (95% CI: 0.9097, 0.9543) of the children who initiate ART with higher CD4 count were still alive.

The probability of surviving with higher CD4 was high at the start but was slightly decreasing with the time (from 96% at one year to 94% at 10 years). At 5 years 94.1% (95% CI: 0.9130, 0.9596) of the children who initiated ART above 350 cells/μl CD4 were still alive compared to 88.8% (95% CI: 0.8321, 0.9260) of the children initiating ART with CD4 counts below 350 cells/μl. At 60 months 94.5% (95% CI: 0.9140, 0.9652) of the children who initiated ART above 500 cells/μl CD4 counts were still alive compared to 89.9% (95% CI: 0.8561, 0.9295) of the children initiating ART with CD4 counts below 500 cells/μl.

4.7.2 HIV survival and tuberculosis co-infection

Children who hadn't gotten TB their probability survival time was higher than those who had not gotten TB. At 48 months 92.2% (95%CI: 0.8965 0.9412) of the children who did not have TB were still alive compared to 89.6% (95%CI: 0.7919 0.9497) of the children who had TB were still alive.

4.8 Simulation Study

In survival analysis, we looked at the Kaplan-Meier curves for all the categorical predictors, to provide insight into the shape of the survival function for each variable. We also considered the tests of equality across strata to explore the predictor's likelihood to be included in the final model. For the categorical variables we used the log-rank test of equality across categories, which is a non-parametric test. For the continuous variables we used a univariate Cox proportional hazard regression which is a semi-parametric model. We considered including the predictor if the test has a p-value of 0.05 or lower. This

elimination scheme appears most suitable because all the predictors in the data set are variables that could be relevant to the model. If the predictor has a p-value greater than 0.05 in a univariate analysis it is highly unlikely that it will contribute anything to a model which includes other predictors.

4.8.1 Kaplan Meier for CD4 counts

The probability of HIV survival throughout the follow up period was assessed based on the level of CD4 count at ART initiation. Findings are summarized in the figure 4.2 whereby the probability of survival is displayed on vertical axis and the time of follow up estimated in months is found on horizontal axis.

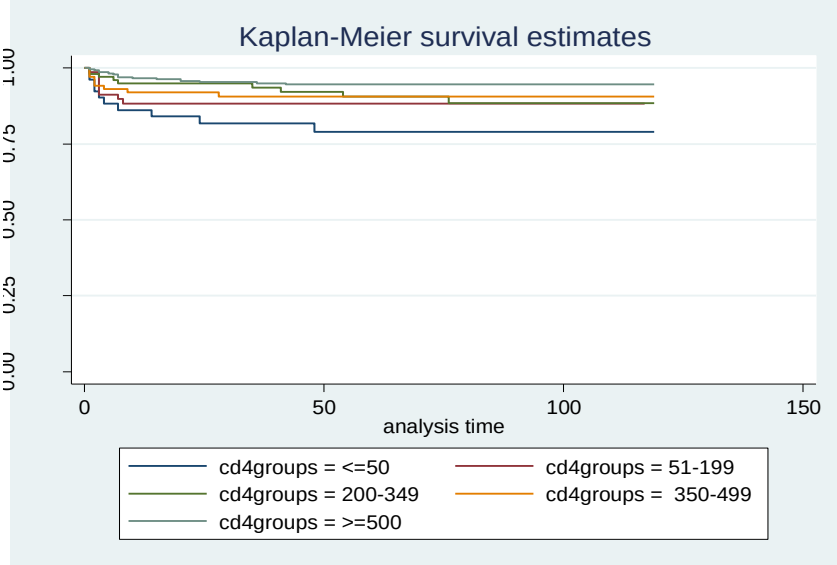

Figure 4.2 HIV Survival per CD 4 at ART

The visual assessment shows that curves are constantly parallelisms with episodes where the curves are very close. It is only at the time next to ART initiation whereby curves appear close for a number of months of follow up. Curves were tested statistically and findings are presented in the table 4.8.

Table 4. 8 Test of equality of survival distributions for the different levels of CD4 counts

	Chi-Square	Sig.
Log Rank (Mantel-Cox)	11.719	.001
Breslow (Generalized Wilcoxon)	12.205	.000
Tarone-Ware	11.986	.001

The table 4.8 provides overall tests of the equality of survival times across the groups. The log rank test calculates the chi-square (X^2) for each event time for each group and sums the results. The summed results for each group are added to derive the ultimate chi-square to compare the full curves of each group. Since the significance values of the tests are all lesser than 0.05, there is a statistically significant difference in survival time between the different CD4 groups range in which children initiated ART with.

4.8.2 Kaplan Meier for HIV and tuberculosis co-infection

The probability of HIV survival based on tuberculosis co-infection among children receiving ART was assessed. Findings are summarized in the figure 4.3 whereby the probability of survival is displayed on vertical axis and the time of follow up estimated in months is found on horizontal axis.

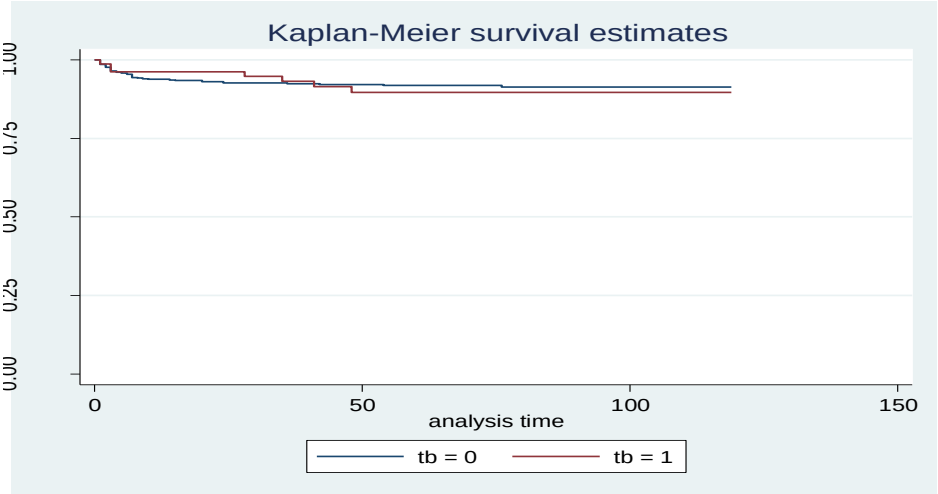

Figure 4.3 HIV Survival per HIV-tuberculosis

The figure 4.3 shows that survival curves are parallel all along the follow up period; with only at the beginning of ART treatment whereby the two curves appear very close together. Curves were then tested statistically and findings are presented in the table 4.9

Table 4.9: Test of equality of HIV survival and tuberculosis co-infection.

Test	Chi-Square	Sig.
Log Rank (Mantel-Cox)	.695	.405
Breslow (Generalized Wilcoxon)	.471	.492
Tarone-Ware	.575	.448

As showed by the table 4.6, the Log Rank test for the data had a P value equal to 0.405 suggesting no inclusion in the final model. Similarly with the curves that remain parallel all over the follow up period, the two curves show that the probability of HIV survival did not vary whether the children developed tuberculosis throughout ART treatment or otherwise.

4.8.3 Kaplan Meier of age group at ART initiation

The study explored the probability of HIV survival throughout the follow up period based on age of children at ART initiation. Findings are summarized in the figure 4.3 whereby the probability of survival is displayed on vertical axis and the time of follow up estimated in months is found on horizontal axis.

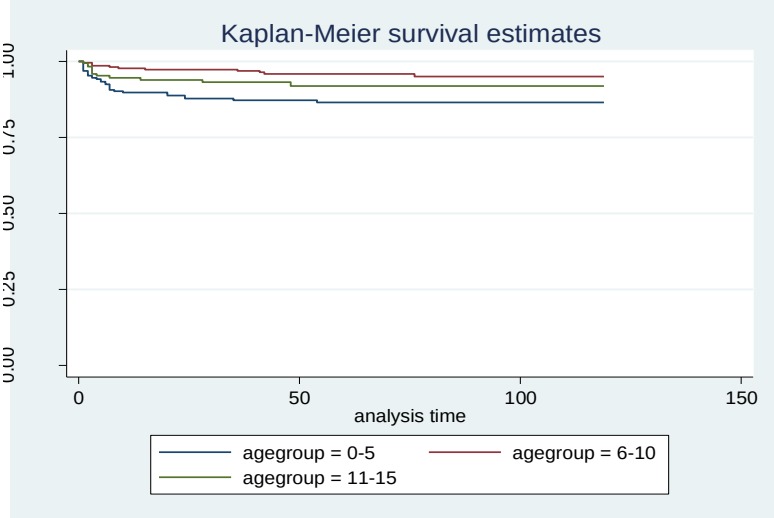

Figure 4.4 HIV Survival per age at ART

The figure 4.3 displays curves per age groups which are not consistently parallel but remaining separate except at the very beginning of ART. The statistical significance in the difference showed by the curves was tested, and findings are summarized in the table 4.10

Table 4.10 Test of equality of survival distributions for the different levels of age group

Tests	Chi-Square	Sig.
Log Rank (Mantel-Cox)	13.917	0.001
Breslow (Generalized Wilcoxon)	15.173	0.001
Tarone-Ware	14.771	0.001

As stated in the table 4.10, the log-rank test of equality across strata for the predictor age at ART initiation has a p-value of 0.001, qualifying for being included in the final model. This says that there was a statistical significance in probability of death based on the age of the child when initiating ART.

4.8.4 Kaplan Meir for clinic providing treatment

Children recruited for this study were enrolled for ART treatment in two HIV clinics located respectively in pediatric center of Kigali (CHUK) and the pediatric center of Butare (CHUB). The probability of HIV survival based on the clinic where the child is receiving ART was assessed. Findings are summarized in the figure 4.3 whereby the probability of survival is displayed on vertical axis and the time of follow up estimated in months is found on horizontal axis.

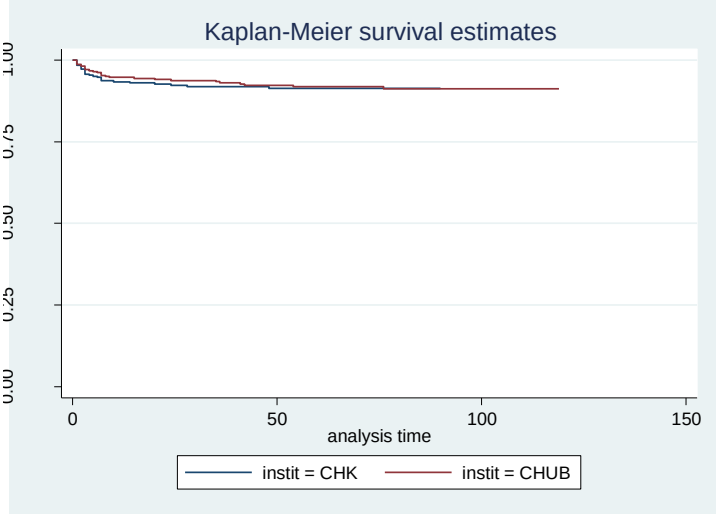

Figure 4.5 HIV Survival per enrolling clinic

The fig 4.9 shows that survival curves appear very close throughout the follow up period, and did not appear parallel at any time of follow up. Further exploration were conducted using statistical tests, and findings are presented in the table 4.11

Table 4.11 Test of equality of survival distributions for sites

Tests	Chi-Square	Sig.
Log Rank (Mantel-Cox)	.615	.433
Breslow (Generalized Wilcoxon)	1.043	.307
Tarone-Ware	.838	.360

The table 4.11 shows that the Log-Rank test of equality across strata for the predictor "clinic of enrollment" has a p-value of 0.433, thus it will not be included as a potential candidate for the final model.

4.8.5 Kaplan Meir of gender

The study explored the probability of HIV survival throughout the follow up period based on gender of children at ART initiation. Findings are summarized in the figure 4.6 whereby the probability of survival is displayed on vertical axis and the time of follow up estimated in months is found on horizontal axis.

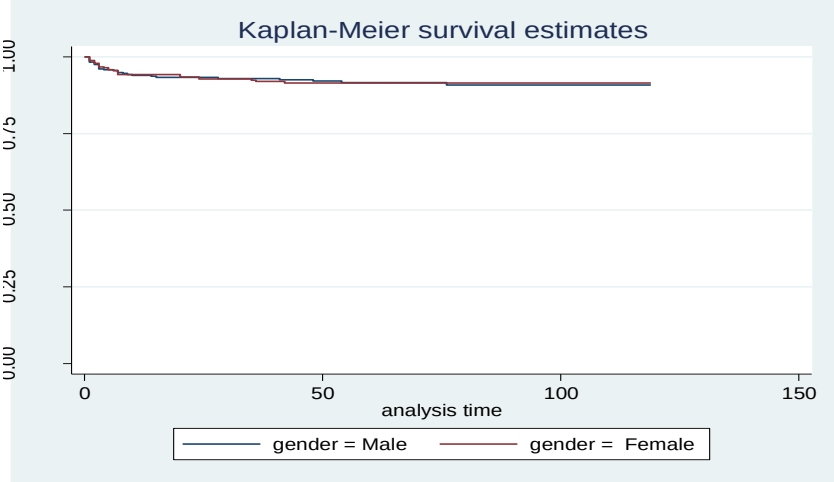

Figure 4.6 HIV Survival per enrolling

The fig 4.6 shows that survival curves appear very close throughout the follow up period, and did not appear parallel at any time of follow up. Further exploration were conducted using statistical tests, and findings are presented in the table 4.12

Table 4.12 Test of equality of survival distributions for the different levels of gender

Tests	Chi-Square	Sig.
Log Rank (Mantel-Cox)	0.194	0.66
Breslow (Generalized Wilcoxon)	0.197	0.657
Tarone-Ware	0.184	0.668

The table 4.12 shows that Log-Rank test had a P value of 0.66 implying that the probability of HIV survival did not differ among boys and girls. Thus, gender will not be eligible to be included in the final model.

4.9 Empirical Study

4.9.1 Cox proportional Hazard Model

In determining our final model with a cox proportional hazard model we used the hazard ratio, which is resulting from this model, deals of a statistical test of an estimate of relative risk of events of interest.

4.9.2 Multivariate cox proportional hazard model

For our model building, we will first consider the model which will include all the predictors that had a p-value of less than 0.5 in the univariate analyses. We adjusted for TB, gender and study sites in the model for the less than 0.05 and they were not significant. A multivariate model using a forward elimination method showed initiating ART with CD4 below 50 had 4.4 times higher after adjusting for age as a continuous variable with an effect for the children aged 6 to 10 and initiating ART with CD4 cell below 200 had 2.7 after adjusting for age as a continuous variable with an effect for the children aged 6 to 10. Finally also using a forward elimination method to get a parsimonious model by eliminating the predictors which are not significant showed initiating ART with CD4 below 500 had 2.3 times higher after adjusting for age as a continuous variable with an effect for the children aged 6 to 10.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

A total of 720 children aged below 15 years enrolled for care and treatment in HIV clinic of pediatric center of excellence of Kigali and in HIV clinic of the pediatric center of excellence in Butare were recruited for this research. The timeframe of the study goes from January 2004 throughout December 2013 corresponding to the time of ART initiation for all study subjects. The median time of follow up after ART initiation was 55 months. More than half of the children (51%) have been followed for at least 50 months after ART initiation in both clinics.

The majority of children (54.2%) initiated ART with a good level of immunity indicators, mainly at more than 500 CD4 cells per micro liter. Only 7.4% of children started ART with the lowest CD4 count level, below 50 CD4 cells per micro liter and the median CD4 count at ART initiation was 557 CD4 cells per micro liter.

5.1.2 ART initiation and cohort context

The study found that the duration of children's follow-up after ART initiation had considerable variations. In a group of 661 children who survived, the median duration of follow up was 55 months after ART initiation. However, among 59 children who died, the median duration of follow-up on ART was only 3 months (25^{th}percentile =1 month; 75th percentile =10 months). The duration of follow up is encouraging as the retention of children in HIV clinics can also be a challenge both prior and post ART initiation. It was reported to vary from 71% to 95% and 62% to 93% at 12 and 24 months, respectively, among children and adolescents in ART programs in several countries of the region (McNairy et al., 2012). This point is worth noting as Massavon et al. (2014) found in their recent study that children receiving with better retention in care clinics have a long-term HIV survival.

5.1.3 Exploration of outcomes associated with HIV survival.

Among subject who were alive, the median CD4 count at baseline was 580 cells per micro liter (1^{st} percentile =3; 75th percentile =951, 99th percentile =3021). However, in the group of children who died during the ART follow up, the median CD4 count at baseline was 356

cells per micro liter (1st percentile =2; 75th percentile =612, 99th percentile =2994). It was found also that death occurred at the rate of 4 out of 1000 Child years of follow up time among 48 children who initiated ART with CD4 counts below 50 cells per micro liter. Though, in the group made of 307 children who initiated ART with CD4 count below 500 cells per micro liter, the death occurred at the rate of 1.7 out of 1000 Child years. Children initiating ART with CD4 count below 50 cells per micro liter were significantly at higher risk of dying than those who initiated ART at CD4 count above 50 cells per micro liter with P value <0.001. The study found also that the risk of death among children in groups made by CD4 level at initiation of ART appears significantly higher than those in groups of children who initiated ART with higher CD4 count, with respective P values.

Overall, the average incidence of death rate by 15 out of 1000 child years of follow up died in the period of study. We found that a child initiating ART with lower CD4 counts is at a higher risk of death than ones who were initiated at higher CD4 counts. These findings agree with several other findings, including Banerjee et al. (2010) who found that Initiation of ART in children aged 1–5 years was associated with a significant increase of 1.66 in CD4 cells (p=0.0001) whereas initiation of ART in those aged 5 years and above was associated with a non-significant decrease of 0.16 in CD4 cells compared with children who initiated HAART before 1 year of age.

About mortality among children recruited in this study, death was likely to occur at the rate of 2.4 out of 1000 children years of follow up among 261 children who initiated ART while they were aged below 5 years. This level was the highest compared to other groups of age at ART initiation, and this difference was statistically significant with P-value of 0.0012. The incidence death rate was assessed based on the gender of the children.

The study did not find any difference among boys and girls. They were on the same level as death was likely to occur at the rate of 1.4 out of 1000 children years of follow up in each group of children. The mortality rate was higher in the first months following ART initiation and progressively stabilizes in the following months under treatment. Thus, 6 of the 720 patients had a follow-up time till 10 years (119 months), and so a survival rate of 91.1% (95% confidence interval (CI), 88.4% to 93.2%) at 10 years was presented based on this 6 patients. These findings agree with Penazzato et al (2012) who reported comparable findings in their study on survival and effectiveness of ART among children.

5. 1.4 HIV survival and tuberculosis as opportunistic infection.

In sum, the overall survival probabilities were 0.986% [95% confidence interval (CI), 0.974-0.992) at 1 month and 119 month respectively. The group of children who initiated ART with CD4 counts below 50 cells per micro liter experienced most of the deaths in first year following ART initiation and no child died after four years of follow up. At 60 months, 93.6% (95%CI: 0.9120, 0.9536) of the children who initiated ART at with 50 CD4 cells per micro liter were still alive whereas 77.2% (95%CI: 0.6151, 0.8713) of the children who initiated ART with CD4 counts below 50 cells/μl. Comparing children who initiated ART with less than 200 CD4 cells per micro liter and those with higher CD4 cells count, it was found that 86.6% (95%CI: 0.7829, 0.9187) of the children who initiate ART with lower CD4 counts were still alive and 93.5% (95% CI: 0.9097, 0.9543) of the children who initiate ART with higher CD4 count were still alive. The probability of surviving with higher CD4 was high at the start but was slightly decreasing with the time (from 96% at one year to 94% at 10 years). At 5 years 94.1% (95% CI: 0.9130, 0.9596) of the children who initiated ART above 350 cells/μl CD4 were still alive compared to 88.8% (95% CI: 0.8321, 0.9260) of the children initiating ART with CD4 counts below 350 cells/μl. At 60 months 94.5% (95% CI: 0.9140, 0.9652) of the children who initiated ART above 500 cells/μl CD4 counts were still alive compared to 89.9% (95% CI: 0.8561, 0.9295) of the children initiating ART with CD4 counts below 500 cells/μl.

Children who hadn't got TB their probability survival time was higher than those who had not gotten TB. At 48 months 92.2% (95%CI: 0.8965 0.9412) of the children who did not have TB were still alive compared to 89.6% (95%CI: 0.7919 0.9497) of the children who had TB were still alive. Thus, there was no difference between children who got TB and those who did not on likelihood of dying.

5.2 Conclusion and recommendation

The greatest number and most proportion of terminal events occur within the first year of ART. This would suggest that patients need not only to initiate ART, but also healthcare providers should be more precautious during first months of treatment. A systematic and close monitoring system needs to be implemented in order to ensure good adherence and retention to care and treatment.

Once again, this study reiterated the fact that ART had a substantial beneficial impact on the survival of HIV-infected children in Rwanda. Along with the reinforcement and widening the coverage of HIV services, , this study presents a valuable set of knowledge, of the extent to which therapy decreases mortality in a highly relevant population of HIV infected children. Our estimate provides compelling evidence that the accelerating rollout of ART access and initiating as early as possible could save substantial numbers of lives. Pooled data from pediatric cohorts in less developed areas should be used in future research not only to generate more precise effect estimates, but also to focus on the effects of HAART within particular groups such as undernourished children, which remain incompletely understood.

5.2.1 Call for further research

A study focusing on risk factors associated with death among children living with HIV would answer some questions than were not enough covered by the current study. A physical contact with parents of children while collecting data for such studies would allow capturing further key features that the researcher may not anticipate prior study implementation.

REFERENCES

1. Abubakar, A., Van Baar, A., Van de Vijver, F.J. H, P., Newton CR.(2008) Pediatric HIV and neurodevelopment in sub-Saharan Africa: A systematic review. Tropical Medicine & International Health. 2008;13(7):880 887.
2. Banerjee T, Pensi T, Banerjee D, Grover G. (2010) Impact of HAART on survival, weight gain and resting energy expenditure in HIV-1-infected children in India. Annals of Tropical Paediatrics (2010) 30, 27–37
3. Collins IJ, Jourdain G, Hansudewechakul R, Kanjanavanit S, Hongsiriwon S, Ngampiyasakul C, (2010) Long-term survival of HIV-infected children receiving antiretroviral therapy in Thailand: A 5-year observational cohort study. Clin Infect Dis. 2010; 51(12):1449-57
4. Dabis F, Bequet L, Ekouevi DK, Viho I, Rouet F, Horo A, Sakarovitch C (2005) Field efficacy of zidovudine, lamivudine and single-dose nevirapine to prevent peripartum HIV transmission. *AIDS* 2005, **19**(3)**:**309-318.
5. Desmonde S, Coffie P, Aka E, Amani-Bosse C, Messou E, Dabis F, Alioum A, Ciaranello A, Leroy V (2011) Severe morbidity and mortality in untreated HIV-infected children in a paediatric care programme in Abidjan, Côte d'Ivoire, 2004-2009. *BMC Infectious Diseases* 2011, 11:182
6. Dunn D., Woodburn P., Duong T., Peto J., Phillips A., Gibb D., and Porter K. (2008) Current CD4 cell count and the short-term risk of AIDS and death before the availability of effective antiretroviral therapy in HIV-infected children and adults. *Journal of Infectious Diseases* 197(3):398–404.
7. Egger M (2006) Rates of Disease Progression according to Initial Highly Active Antiretroviral Therapy Regimen: A Collaborative Analysis of 12 Prospective Cohort Studies. Accessed at http://jid.oxfordjournals.org/content/194/5/612.full
8. Fassinou P., Elenga N., Rouet F., Laguide, R., Timite, M. (2004) Highly active antiretroviral therapies among HIV-1 infected children in Abidjan, Cote d'Ivoire. AIDS. 2004;18(14):1905–13.
9. Fox, M. P., and Rosen, S. (2010) Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: Systematic review.

Tropical Medicine and International Health, *15*(june), 1–15. doi:10.1111/j.1365-3156.2010.02508.x

10. Gebremedhin A., Gebremariam S., Haile F., Weldearegawi B. and Decotelli C. (2013) Predictors of mortality among HIV infected children on anti-retroviral therapy in Mekelle Hospital, Northern Ethiopia: a retrospective cohort study. *BMC Public Health* 2013, 13:1047
11. Harambat J., Fassinou P., Becquet R., Touré P., Rouet F. (2008) 18-month occurrence of severe events among early diagnosed HIV-infected children before antiretroviral therapy in Abidjan, Côte d'Ivoire: A cohort study. *BMC Public Health* 2008, 8:169 doi:10.1186/1471-2458-8-169
12. Hosmer D., Lemeshow S., and May S. (2008) Applied Survival Analysis: Regression Modeling of Time to Event Data (2nd ed.). Wiley-Interscience.
13. Kalbfleisch J. and Prentice R. (2002) The Statistical Analysis of Failure time Data. (2nd edition ed.). Hoboken, New Jersey: John Wiley Sons, Inc.
14. Lawless, J. F. (1982) Statistical Models and Methods for Lifetime Data Analysis. New York: Wiley.
15. Leemis, L. (1995) Reliability. Englewood Cliffs: Prentice-Hall.
16. Massavon W, Barlow-Mosha L, Mugenyi L, McFarland W (2014) Factors Determining Survival and Retention among HIV-Infected Children and Adolescents in a Community Home-Based Care and a Facility-Based Family-Centred Approach in Kampala, Uganda: A Cohort Study. ISRN AIDS Volume 2014, Article ID 852489.
17. McNairy ML, Lamb MR, Carter RJ, Fayorsey R, Tene G (2012) Retention of HIV-infected children on antiretroviral treatment in HIV care and treatment programs in Kenya, Mozambique, Rwanda and Tanzania. *Journal of Acquired Immune Deficiency Syndromes*, vol. 62, no. 3, pp. e70–e81, 2012.
18. Moh R, Danel C, Messou E, Ouassa T, Gabillard D (2007)Incidence and determinants of mortality and morbidity following early antiretroviral therapy initiation in HIV-infected adults in West Africa. AIDS (London, England), 2007; 21 (18) doi:10.1097/QAD.0b013e3282f09876

19. Newell ML, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F (2004) Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. Lancet 2004 Oct 2-8; 364 (9441):1236-43.
20. Panel on Opportunistic Infections in HIV-Exposed and HIV-Infected Children. Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Exposed and HIV-Infected Children. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/oi_guidelines_pediatrics.pdf. Section accessed [May 25, 2015].
21. Peacock-Villada E, Richardson BA, John-Stewart GC (2011) Post-HAART outcomes in pediatric populations: comparison of resource-limited and developed countries. Pediatrics. 2011Feb;127(2):e423-41. doi: 10.1542/peds.2009-2701. Epub 2011 Jan 24.
22. Penazzato M, Prendergast A, Tierney J, Cotton M, Gibb D (2012) Effectiveness of antiretroviral therapy in HIV-infected children under 2 years of age. Cochrane Database Syst Rev 2012: CD004772.
23. Radhakrishna M., Durga K., Kondal Rao R., Mallikarjuna Reddy D. and Kumar Kondapi A. (2013) Factors associated with conversion of long-term non-progressors to progressors: A prospective study of HIV perinatally infected paediatric survivors. Indian J Med Res 138, September 2013, pp 322-328
24. RBC (2012) National HIV annual report, Kigali, Rwanda.
25. RBC (2013) National HIV annual report, Kigali, Rwanda.
26. RBC (2014) National HIV annual report, Kigali, Rwanda.
27. Rouet, F., Fassinou, P., Inwoley, A., Anaky MF, Kouakoussui, A., Rouzioux, C. (2012)Longterm survival and immuno-virological response of HIV-1 infected children to highly active antiretroviral therapy regimens. AIDS. 2006;20(18):23152319.
28. Tegiste A. and Eshetu W. (2012) Survival analysis of patients under chronic HIV-care and antiretroviral treatment at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. 2012; 26 (1): 22-29.
29. UNAIDS (2012) Together we will end AIDS. Geneva, Switzeland.
30. UNAIDS and UNICEF (2008) Second stocktaking report: Children and AIDS. Geneva, Switzeland.

31. Wamalwa, D.C., Farquhar, C., Obimbo, E.M., Selig S, bori-Ngacha, D.A., Richardson, B.A.(2007) Early response to highly active antiretroviral therapy in HIV-1-infected Kenyan children. Journal of Acquired Immune Deficiency Syndromes. 2007;45(3):311 317.
32. WHO (2014) Global Health Observatory data. Section available and accessible online at http://www.who.int/gho/publications/world_health_statistics/en/.Section accessed [May 25, 2015].
33. WHO (2013) Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Geneva, Switzeland.