Modeling HIV/AIDS and Visceral Leishmaniasis Treatment Outcomes in Northwest Ethiopia

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List of Publications

This dissertation is based on the following papers

- **Ayele, TA.,** Worku, A., Kebede, Y., Alemu, K., Kasim, A., and Shkedy, Z.,(2017). Choice of Initial Antiretroviral Drugs and Treatment Outcomes among HIV-Infected Patients in sub-Saharan Africa: Systematic Review and Meta-analysis of Observational Studies. *BMC Systematic Review 6:173.*

- **Awoke, T.,** Worku, A., Kebede, Y., Kasim, A., Belay, B., Braekers, R., Zuma, K., and Shkedy, Z., (2016). Modeling Outcomes of First-Line Antiretroviral Therapy and Rate of CD4 Counts Change among a Cohort of HIV/AIDS Patients in Ethiopia: A Retrospective Cohort Study. *PloS one, 11(11).*

- **Ayele, TA.,** Worku, A., Kebede, Y., Kasim, A., Zuma, K., and Shkedy, Z.,(2017). Model-Based prediction of CD4 cells counts in HIV-Infected Adults on Antiretroviral Therapy in Northwest Ethiopia: A Flexible Mixed Effects Approach. *BMC Infectious Diseases* [Under revision].

- **Ayele, TA.,** Worku, A., Kebede, Y., Alemu, K., Kasim, A., and Shkedy, Z., (2017). Predicting long-term ART outcomes in HIV-infected adults using longitudinal biomarkers in Northwest Ethiopia: Application of Joint Modeling *[Working paper]* .

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- Birlie, B., Braekers, R., **Ayele, TA.**, Kasim, A., and Shkedy, Z., (2017). Multi-state models for the analysis of time-to-treatment modification among HIV patients under highly active antiretroviral therapy in Southwest Ethiopia. *BMC Infectious Diseases, 17: 2017.*

- Takele, Y., Adem, E., Getahun, M., Tajebe, F., Kiflie, A., Hailu, A., Raynes, J., Mengesha, B., **Ayele T.A.,**, Shkedy, Z., et al. (2017). Malnutrition in Healthy Individuals Results in Increased Mixed Cytokine Profiles, Altered Neutrophil Subsets and Function. *PLOS ONE, 11(8).*

- Tajebe, F., Getahun, M., Adem, E., Hailu, A., Lemma, M., Kiflie, A., Raynes, J., Tamiru, A., Mengesha, B., Diro, E., Toulza, F., Shkedy, Z., **Ayele T.**, et al. (2016). Disease severity in patients with visceral leishmaniasis is not altered by co-infection with intestinal parasites. *PLOS Neglected Tropical Diseases, 11(2).*

- **Ayele, TA.,** Worku, A., Kebede, Y., Alemu, K., Kasim, A., and Shkedy, Z., (2017). Interactive web application using Shiny to predict treatment outcomes in Ethiopian hospitals *[Working paper].*

Contents

List of Abbreviations

3TC	lamivudine
ABC	Abacavir
AIC	Akaike Information Criteria
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
AUC	Area Under ROC curve
AZT	Zidovudine
BMI	Body Mass Index
CD4	Cluster of Differentiation 4
d4t	Stavudine
DDI	Dynamic discrimination Index
EFV	Efavirenz
FP2	Fractional Polynomial Second Order
GTP	Growth and Transformation Plan
Hgb	Haemoglobin
HIV	Human Immunodeficiency Virus
IQR	Inter Quartile Range

IRB Institutional Review Board

MDG Millennium Development Goal

MeSH Medical Subject Heading

NNRTIs Non-Nucleoside Reverse Transcriptase Inhibitors

NRTI Nucleotide Reverse Transcriptase Inhibitor

NVP Neverapine

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PSP Pentamidine as Secondary Prophylaxis

RNA Ribonucleic Acid

ROC Receiver Operating Characteristic

SDGs Sustainable Development Goals

TDF Tenofovir

Th T-helper

UN United Nation

UNAIDS Joint United Nations Programme on HIV/AIDS

VL Visceral Leishmaniasis

WBC White Blood Cell

WHO World Health Organization

Chapter 1

Introduction

This dissertation consists of three main parts; The first part is dedicated for modeling Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) patients under Antiretroviral Therapy (ART) in Northwest Ethiopia. The second part is focused on modeling Visceral Leishmaniasis (VL) and HIV co-infection in Northwest Ethiopia, and the third part presents publicly available software developed for the analysis of standardized ART databases.

1.1 HIV/AIDS in Low Income Countries

It has been almost 35 years since the world have been introduced to the term HIV, the virus that can lead to AIDS (Richard and Tim, 2014). Transmission of HIV can occur when HIV-infected blood, semen or vaginal secretions enter to the body (Richard and Tim, 2014). The virus is known to affect the immune system and progressively weaken the body's ability to fight infections (Blattner et al., 1988). The virus reduced cluster of differentiation 4 (CD4) cell counts, increased plasma HIV-Ribonucleic Acid (RNA) levels and, in the most severe phase of HIV-infection, can lead to an AIDS-defining complication (Kestens, 2013).

It is estimated that, since the start of the epidemic, more than 78 million people have been infected and about 35 million people have died of AIDS (WHO, 2015). In 2016, a total of 36.7 million people are living with HIV from which 95% are living in lower and middle income countries (UNAIDS, 2017a). Sub-Saharan Africa carried the highest burden of the diseases (Murray et al., 2014) having 25.5 million infected individuals (70% of the total number of people living with HIV in 2016 (UNAIDS,

2016a)). Ten countries in Africa carry 80% of the HIV burden: Ethiopia, Kenya, Malawi, Mozambique, Nigeria, South Africa, Uganda, the United Republic of Tanzania, Zambia and Zimbabwe (Roche, 2017). The first case of HIV in Ethiopia was identified in 1986 (Hladik et al., 2006) and since then 19.35 millions people were reported to be infected by HIV (UNAIDS, 2016b) at the end of 2016. It is estimated that currently 0.73 million people in Ethiopia are living with HIV and it has been one of the top three diseases which cause morbidity and mortality in the country (EPHI, 2017). Not all individuals infected with the virus know their status. It is estimated that only 54% of people living with HIV know that they have the virus.

Despite the drawbacks in the development of effective cure for HIV, the development of effective Antiretroviral Therapy (ART) drugs combinations has increased survival and reduced HIV-associated morbidities and mortality in HIV-infected individuals (De La Hoz et al., 2014; Quinn, 2008). In addition, studies revealed that the drug combination significantly reduce the transmission of the virus to uninfected sexual partner (Cohen et al., 2011; Group et al., 2015a,b). Nearly 76% of those being treated achieve viral suppression. Cohen et al. (2011) has confirmed that if an HIV-positive person enrols and adheres to an effective ART regimen, the risk of virus' transmission to their uninfected sexual partner can be reduced by 96%. Furthermore, life expectancy of HIV patients under ART treatment increase and a study in the US and Canada revealed that, a person in his/her 20s who contracts HIV can expect to live into the 70s if initiated ART early after being infected (Samji et al., 2013).

The long-term outcomes of treatment is monitored by different characteristics of HIV-infected individual who initiated ART. Keiser et al. (2011) and Roberts et al. (2012) have shown that viral load measurement is the best marker to (1) monitor the health in the HIV-infection, (2) guide treatment choices, and (3) monitor how the treatment is affected an HIV-infected individuals over time. Although measuring viral load is a standard practice in high-income countries, it is not the case in resource poor setting due to high costs and technical constraints. Instead, CD4 cell count has been used as a main marker of treatment response for HIV-infected individuals. Understanding the rate at which CD4 cell counts increases in response to ART in HIV patients is an important challenge. It is not fully explicated whether patients who receive ART can maintain continued CD4 cell count increases. Although several studies have quantified the gains in CD4 cell counts over the first 2 years after ART initiation at the population level (Mpondo et al., 2015; Rajasekaran et al., 2009), the long-term CD4 cell count response to ART remains largely uncharacterised at the individual level. As such there is a need to develop statistical models for the change of CD4 cell count over time under ART treatment. Currently, the guidelines

recommended the routine measurement of viral load for resource poor countries as well (Calmy et al., 2007).

For the last three decades, enormous number of researches involving HIV/AIDS related problems has been conducted with different approach in order to answer respective research questions. Being a life long diseases, data for HIV patients are accumulated from the date of test for an HIV-infected individual (Young, 2015). In Ethiopia, free national ART program was started in 2005 and government health organizations involved in ART provision since then. During the same year the service was limited to government hospitals, but currently health centers provide the services as well (Assefa et al., 2017). Initially, the information of the patient had been recorded on the chart (paper based). However, starting from 2009 both chart and electronic recording system was implemented. As a result, large amount of data are accumulated routinely in all hospitals and treatment centers in Ethiopia.

1.1.1 Modeling Treatment Progress for HIV-Infected Patients

Often data collected about HIV/AIDS are event-history and longitudinal outcomes (Sudell et al., 2016). Failure to take appropriate statistical models that can take into account this phenomenon can lead to biased estimation of model parameters (Sweeting and Thompson, 2011). In the first part of this dissertation, we focus on two type of endpoints that represent the response of a HIV patient under ART to the treatment: the time to composite outcome and longitudinal CD4 cell counts. Time to composite outcome is a time to event endpoint and it is defined as the occurrence of either NNRTI substitution, discontinuation, lost to follow up or death (Sarfo et al., 2013). The data analysed in the first part of the dissertation, was obtained from Gondar University Hospital database of HIV patients under ART. Our aim is to model the change in CD4 cell counts in order to asses and monitor the progress of HIV patients in the treatment center and to provide a subject-specific predictive models for treatment failures. These sets of models will be discussed in the first part of this dissertation. The general structure of the dissertation is shown in Figure 1.1.

Chapter 2 gives brief introduction about HIV/AIDS treatment in Ethiopia and presents a systematic review and meta-analysis done to determine the effect of choice of initial ART regimen on the long-term treatment outcomes of HIV-infected individuals in sub-Saharan Africa. Patients who initiated HIV treatment are expected to stay on the first regimen as long as possible as it is the treatment with different options which can combat morbidity, progression, and early mortality. However due to reasons related to drug toxicity, co-morbidity, pregnancy or treatment failure, orig-

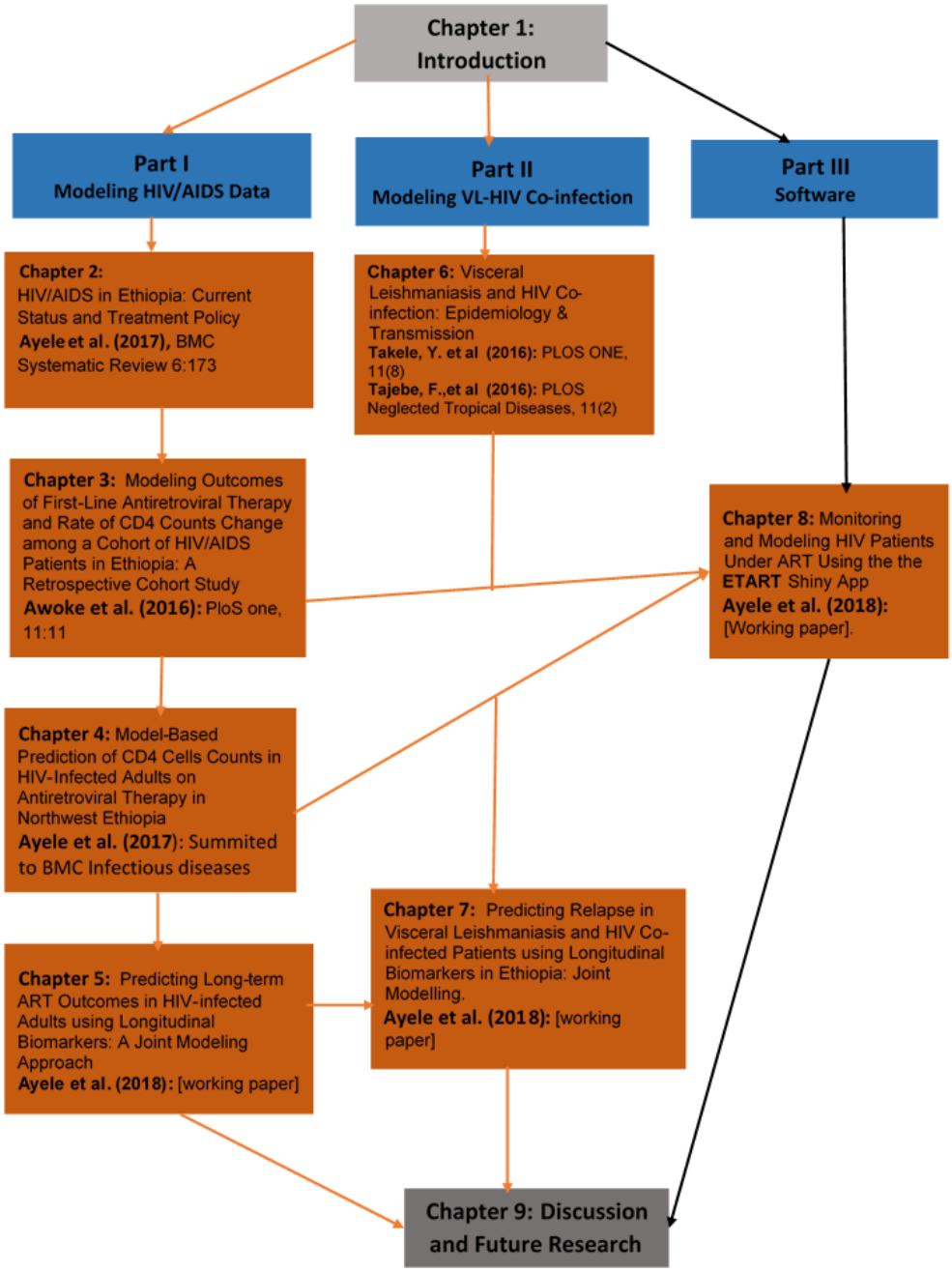


Figure 1.1. Dissertation structure and publication strategy.

inal treatment need to be modified to other alternatives. Knowing the most effective treatment combination at initiation can help the clinicians to consider the combination during modification which has optimal benefit for the patient. In **Chapter 3**, we present models for the outcome of first line ART and the rate of CD4 cell count change among a cohort of HIV/AIDS patients in Gondar University Hospital, Ethiopia. Two main statistical models are used to answer two research questions: (1) a survival analysis model for the time to first treatment change and (2) a semi-parametric random effect model for the change of CD4 cell counts over time. The first derivative of the semi-parametric model is used to describe the rate of change in CD4 cell count over time. In **Chapter 4**, using a flexible parametric modeling approach, we conducted a model based prediction to estimate the time it takes to reach a pre-specified CD4 threshold and to predict a subject specific probability of an individual to have CD4 cell count above a threshold. Since the model is developed for prediction, data are divided into two parts: an estimation period in which the unknown parameters of the model are estimated and a prediction period in which a model based prediction is made. The modeling procedure is illustrated in Figure 1.2. In **Chapter 5**, a joint model for longitudinal CD4 cell counts and time to event process, i.e., time to first treatment change, death, lost to follow up is used to determine the effect of longitudinal process of CD4 cell count on time to composite outcomes and to obtain a subject specific prediction for the probability of composite outcome and death.

1.1.2 VL and HIV Co-infection in Ethiopia

The second part of the dissertation is devoted to modeling of VL and HIV co-infection in Northwest Ethiopia. VL is an endemic and potentially life-threatening disease in the tropics, subtropics, and Mediterranean basin (Pavlia and Maltezoub, 2010). More than 90% of global burden of VL cases occur in just six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia (Bern et al., 2008; Georgiadou et al., 2015). Transmission occurs during a blood meal of female sand-fly of the genus Phlebotomus (Bates, 2007). One third of all HIV patients worldwide live in regions where leishmaniasis is endemic. Consequently co-infection with HIV and VL is very common. Globally, Ethiopia carried the highest burden of VL-HIV co-infection (Hurissa et al., 2010; Mengistu and Ayele, 2007). The north-west lowland areas bordering Sudan account for 60% of the VL burden in the country (Argaw et al., 2013). As a consequence of VL-HIV co-infection, a high rate of treatment failure, and frequent relapses were reported (Burza et al., 2014; Diro et al., 2014; Rachel et al., 2008). In

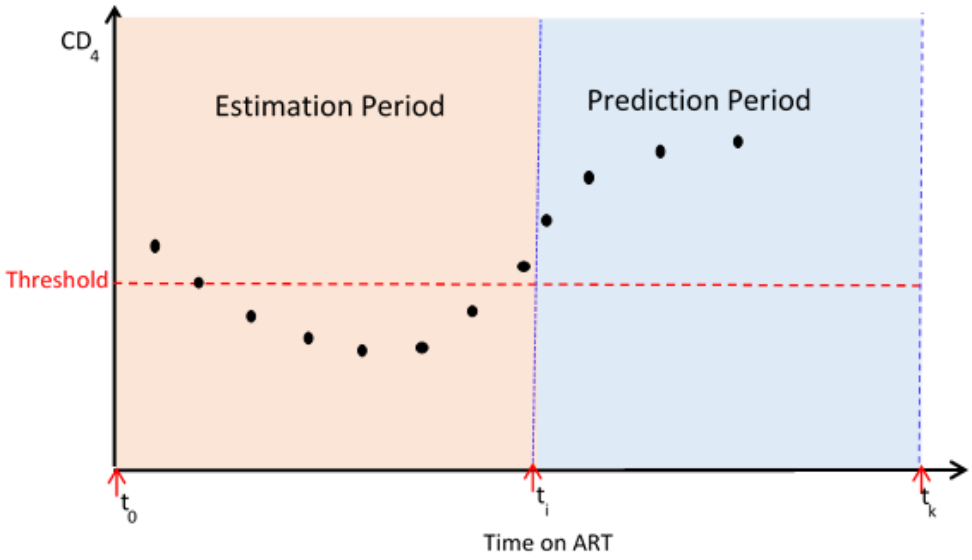


Figure 1.2. Estimation and prediction periods for early prediction diseases progression of HIV patients under ART based on CD4 cell counts. The longitudinal measurements are divided into two parts. The first part of the longitudinal sequence is used for estimation of the unknown model parameters (the estimation period) while the second part of the data, the prediction period, is used for prediction of the probability to be above a pre-specified CD4 threshold.

Chapter 6, we briefly introduce VL and HIV Co-infection in Ethiopia and present a summary of two research projects about VL conducted in Gondar University hospital and surroundings. In **Chapter 7**, we present a joint model for longitudinal laboratory biomarkers and time to relapse in order to predict relapse in VL-HIV co-infected patients.

1.1.3 Software Tools for the Analysis of HIV/ART Databases

Modern data analysis requires accessibility to software to conduct the analysis. In the third part of the dissertation, we present a new, R based, software tool, for a routine analysis of hospitals HIV patients databases in Ethiopia. Due to an increase in technological capacity, Ethiopian hospitals are accumulating data on their HIV patients in treatment centers rapidly and health professionals require data analysis tools in order to obtain information for better decision about the patients treatment and to monitoring the performance of patients in the treatment center. We present

a new R shiny package, the `ETART` shiny App, which can be used, either on-line or off-line, to conduct a basic analysis of HIV patients using a standardized database and to visualize the current situation in the treatment center with respect to effectiveness of different treatment. The `ETART` Shiny App is presented in **Chapter 8**.

1.2 Objectives of the Dissertation

The main objective of the research conducted within the PhD project reported in this dissertation is to develop flexible statistical models for long-term treatment outcomes in HIV-infected and VL co-infected patients, Northwest Ethiopia. The specific objectives are

1. To determine the effect of the choice of initial regimen on long-term outcomes in HIV-infected patients in sub-Saharan Africa.

2. To determine the long-term response of first line antiretroviral therapy and rate of CD4 cell count change in HIV-infected individuals in Ethiopia.

3. To model the evolution of CD4 cell counts over time and use this model for an early prediction of subject-specific time to cross a pre-specified CD4 threshold

4. To assess the association of long-term treatment outcomes and CD4 cell count evolution among a cohort of HIV/AIDS patients on ART in Ethiopia.

5. To identify the best biomarkers which predicts relapse in VL-HIV co-infected patients who have been taking pentamidine for the purpose of preventing relapse in Northwest Ethiopia.

6. To close the gap in local data analysis capacity by providing, a free, on-line based, user friendly, data analysis tool for the analysis of a standardised ART database.

1.3 Case Studies

Two datasets are used to illustrate the methods, models and applications presented in the dissertation.

1.3.1 HIV Database in Gondar University Hospital

Data for the first part of the dissertation were obtained from an ART clinic database which had been collected from HIV patients who initiated ART from January 2009 to December 2013 in Gondar University Hospital. It is a 570-bed university hospital, which acts as the referral center for four district hospitals in the area. It has a range of specialities including paediatrics, surgery, gynaecology, psychiatry, HIV care and treatment center. The HIV care and treatment center has voluntary counselling and testing clinics where both self-referred individuals and physician referred patients are tested for HIV and received care and support. In this hospital, free ART service was started in 2005. The most common criteria the clinician use to assign the patients to the specific regimen are laboratory results, history of drug allergy, adherence history for previous treatment and mental status. For the data analysed in this dissertation, the following inclusion criteria were applied; having at least two visits and one CD4 cell count measurements, initiated ART within the study period, initiated with either NNRTI (NVP or EFV) treatment groups, and initiated with either of the three (AZT, d4t or TDF) NRTIs backbones.

Two data sources were used in order to ensure the data quality: (1) the electronic database was accessed and data were extracted from the database according to the inclusion criteria and (2) charts of the patients' were accessed and extracted. Extraction tool was pretested and revision was made based on the feedback from the pretest on the final extraction tool. Masters of public health students who have been working in HIV care and treatment centers were recruited and trained on the procedures and tools. The extracted data were checked on daily bases by the supervisors. The data extracted from the charts were entered in to the template developed by EPI-Info software. The final dataset was obtained by amending the two datasets. This was done in order to minimize the number of the missing values which would be observed if only one of the two sources was used. A total of 2550 HIV/AIDS patients met the inclusion criteria for this study. Information on baseline and follow-up variables were recorded in the patients chart by health professional at enrolment and at each visit. The variable include HIV test date, ART start date, age, sex, original regimen, WHO stage, functional status, and weight as baseline variables. Follow up variables were recorded at each visit and include, the visits date, CD4 cell counts, WHO stage and regimen. Figure 1.3 shows the individual and average profile for log(CD4) cell counts during the study period.

Patients' follow up period ranges from 0 to 68 months. Figure 1.4 shows the Kaplan-Meier curves for the time of composite outcome. Overall, 24.9% of the patients

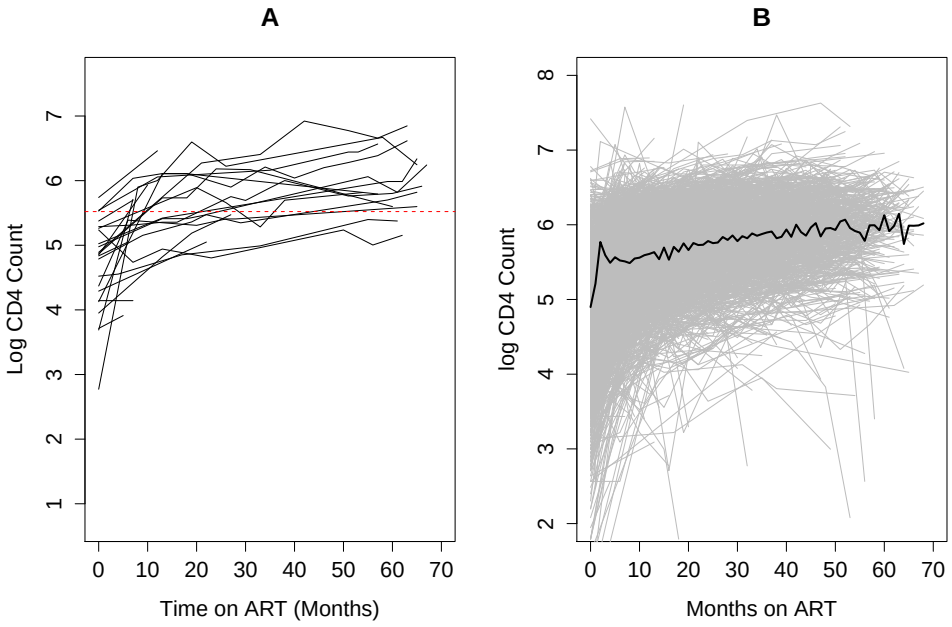


Figure 1.3. Log(CD4) cell counts over time. Panel a: Individual profiles of CD4 cell counts on log scale for selected patients. Panel B: all patients and (panel B). The dark black line in panel B shows the observed average profile.

experience the event (with median time of follow up equals to 24.9 months). An elaborate description of the data is given in Section 3.2.

Ethical Clearance

A human subject research approval for this study was received from Institutional Review Board (IRB) of the University of Gondar. As the study was retrospective, the IRB waived that the research could be done based on record review without contacting the patients. Support letter was obtained from the medical director office of the hospital for retrieving retrospective data from the database and records. All the information was kept confidential, and no individual identifiers were collected.

1.3.2 PSP Clinical Trial Data

The data we analysed in the second part of the dissertation are outcome of a clinical trial conducted in two Leishmaniasis Research and Treatment Centers (LRTC, Gondar and Abderafi) located in Ethiopia . The centers provide free VL treatment

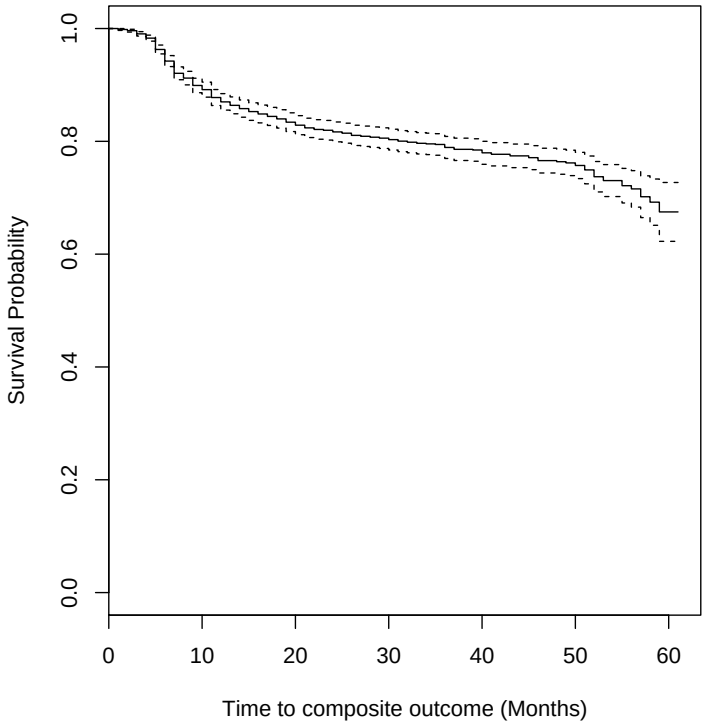


Figure 1.4. Plot of Kaplan-Meier survival curve for time to composite outcome.

and care to all patients with leishmaniasis. Patients present to the center either spontaneously or are referred from other health institutes in the catchment area. The Pentamidine as Secondary Prophylaxis (PSP) clinical trial was conducted to investigate the effectiveness, safety and feasibility of monthly PSP in VL-HIV co-infected patients that have documented parasite clearance after VL treatment when used for prevention of VL relapse. The study was started in November 2011 and recruitment of the participant taken place until August 2013. A total of 74 VL-HIV co-infected patients were enrolled. The primary end point was time to relapse or death within 12 months of PSP initiation. As can be seen in Figure 1.5, at the end of the follow up period, 20.27% of the patients relapsed (the primary endpoint of the trial). .

Pentavalent antimonials and liposomal forms of amphotericin B, and more recently paromomycin, are the main drugs to treat VL. The combination regimen sodium

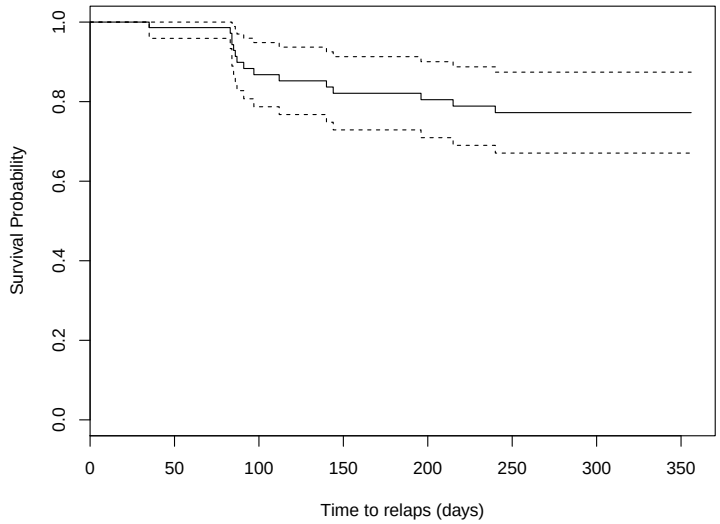


Figure 1.5. The PSP study. Kaplan-Meier survival curve for time to relapse.

stibogluconate (SSG) and paromomycin was applied as first line therapy for non-HIV/VL cases at the treatment site since September 2012. The monthly infusion of pentamidine isethionate was continued until the primary endpoints. In the second part of the dissertation, our aim is to identify a lab biomarkers that can be use for early prediction of release of VL-HIV co-infected patients. In addition to time to relapse monthly full blood count, Haemoglobin (Hgb), white blood cell (WBC), creatinine, liver function tests and blood glucose, CD4 cell count (every six months) were monitored. Figure 1.6 shows the individual profile of patients for log(WBC) (left panel) and Hgb (right panel). The red lines are corresponding for patients who experienced relapse, whereas the black line represents for patients who did not experience relapse. An elaborate discussion on the PSP data is given in Section 7.2.

Ethics Statement and Regulatory Aspects

The protocol of the study was approved by the Ethiopian Regulatory Authority (Food, Medicine, Health Care Administration and Control Authority (FMHACA)), the National Research Ethics Review Committee (NRERC) and the Institutional Review Board of University of Gondar (UoG) in Ethiopia. Additionally, it was also approved by the Ethics Review Board of Medecins Sans Frontires, and the Ethics Committee of

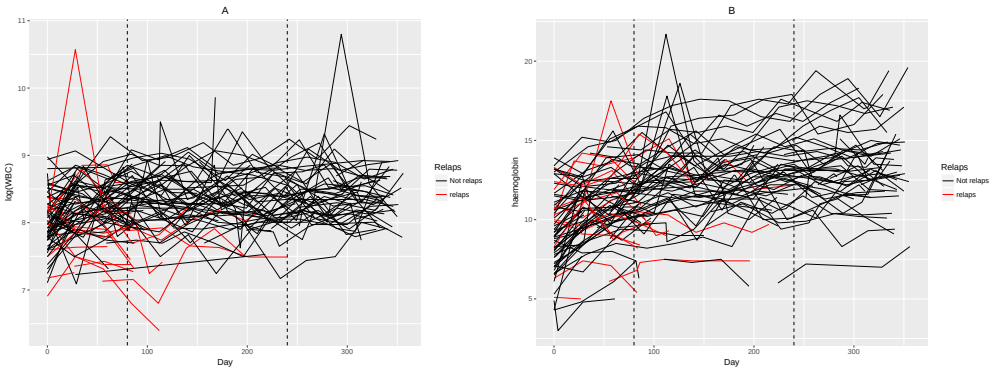


Figure 1.6. The PSP Study. Individual profile for WBC (left panel) and Hgb (right panel). The red and black lines represent patients who experienced the event and for those did not, respectively.

the Antwerp University Hospital, Belgium. All subjects were included in to the study after a written informed consent was signed. Free treatment was provided. Patients were compensated for transport and food during their visits to the study sites. All study documents were kept confidential and were accessible for the study team, monitors and inspectors. Trained clinical trial monitors carried out two pre-study visits, one initiation visit and 6 monitoring visits according to the WHO and good clinical practices standards. Regulatory inspection was carried out by FMHACA at both study sites during the study period. The independent Data and Safety Monitoring Board met five times during the study and assessed the progress of the study when every quarter of total sample recruitment was achieved. The protocol was registered in ClinTrials.gov (code NCT01360762).

Part I

Modeling HIV Data in Ethiopia

Chapter 2

HIV/AIDS in Ethiopia: Current Status and Treatment Policy

One of the most important problems after initiation of ART is a change in first line treatment. This chapter is devoted to a systematic review and meta-analysis of published studies in order to investigate if first line treatment failure and NNRTI substitution are different between initial regimen of NVP and EFV. In Section 2.1 we briefly review the current situation of HIV/ADIS in Sub-Saharan Africa and Ethiopia, while in Section 2.2 we discussed treatment strategies and ART implementation policies in low income countries. In section 2.3 we describe the systematic review and meta-analysis conducted by Ayele et al. (2017) to compare between NVP and EFV in first line ART regimens. The results are presented in Section 2.4 and discussed in Section 2.5.

2.1 HIV/AIDS in Sub-Saharan Africa and Ethiopia

Despite recent declines, HIV/AIDS still ranks in the top five global causes of disability-adjusted life years (Ortblad et al., 2013). The vast majority of infected individuals live in low and middle income countries. Sub-Saharan Africa, accounted for about 12% of the worlds population, carried the highest burden of the diseases which accounted 70% of the global total (Murray et al., 2014). Swaziland, Botswana, and Lesotho, located in the Sub-Saharan region, are the top three countries where 27.73%, 25.16%, and 23.39% of the population lives with HIV, respectively (UNICEF, 2017). Western pacific region has the lowest burden of the epidemic as can be seen from Figure 2.1.

Ethiopia is Africa's second most populous country, with 98 million people (Geck,

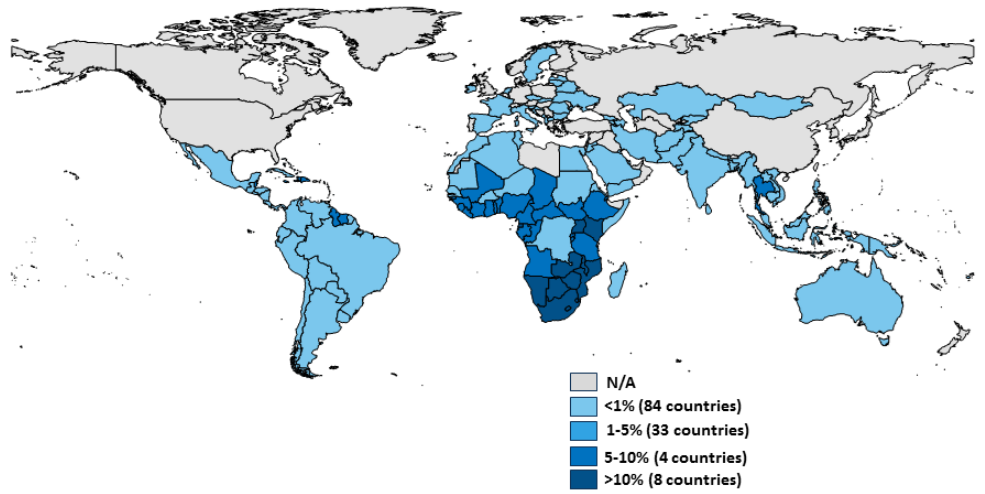


Figure 2.1. Adult HIV prevalence in 2016. Source: Kaiser Family foundation based on UNAIDS and AIDSinfo, Accessed October 2017. The figure is taken from www.kff.org/global-health-policy/fact-sheet/the-global-hivaids-epidemic.

2016). Currently, with an estimated 0.73 million people living with HIV, the country has one of the largest HIV-infected population in the world (UNAIDS, 2016b). The adult prevalence rate is estimated to be 1.2% which is one of the lowest in Sub-Saharan Africa. However, according to the globally accepted consensus, if the total number of HIV infected people in a given country exceeds 1% of the population, that country is considered to be under category of outbreak of the virus. Amhara region has high HIV/AIDS prevalence in Ethiopia with an average adult prevalence of 1.36% (CSA, 2012).

2.2 HIV Treatment

The introduction of ART changes HIV/AIDS from a diseases with a high mortality rate to manageable chronic diseases by decreasing the progression of AIDS and reducing HIV related illness and deaths (Broder, 2010). It has improved steadily since the advent of potent combination therapy in 1996. The scale-up of ART in the last three decades has been one of the world's greatest public health success stories (WHO, 2015). Researches revealed that an increase accessibility to ART lead to decline in HIV related morbidity and mortality (Braitstein et al., 2006; Ortblad et al., 2013; Peterson et al., 2011; Stover et al., 2008). It is estimated that nearly 7.8 million deaths averted globally during 2000-2014 due to the implementation of ART

programmes (WHO, 2015). The goal of ART is to attain maximal and durable suppression of the viral replication and prolong diseases free survival (AIDSinfo, 2014). Globally, a total of 19.5 million people living with HIV enrol to ART for which global coverage reached 46% at the end of 2016. The number of HIV patients under ART increases steadily from less than a million in 2000 to 7.5 million in 2010 and 15.8 million in 2015 (UNAIDS, 2017b). Consequently the global coverage of ART reached 54% during the same year. In eastern and southern Africa, the most affected region in the world, coverage increased from 24% in 2010 to 54% in 2016, reaching a total of 10.3 million people (WHO, 2016). South Africa alone has nearly 3.4 million HIV patients under treatment with a coverage rate of 56% (UNAIDS, 2016a). In Ethiopia an estimated 386,123 adults living with HIV are receiving ART which make the coverage to be 54% in 2015 (Wang et al., 2016).

Over the early periods ART has been administered mainly based on a patients CD4 cell counts (WHO, 2006). A non-infected individual can have a CD4 cell counts between 500 and 1900 $cells/mm^3$ (Akinbo et al., 2015). Evidence suggested that an HIV-infected individual with high CD4 cell count is healthy enough not to start ART (Siegfried et al., 2010). However, the immune system for HIV infected individuals with lower level of CD4 cell counts is severely weakened and ART is necessary. Early guideline of World Health Organization (WHO) recommend to start the ART when the CD4 cell counts are below 2000 (WHO, 2006). In 2010, the CD4 cell counts threshold for initiation of ART was increased in the WHO guidelines to 350 (WHO, 2017). Currently, following the revision of available evidence in 2013, WHO issued a new set of ART guidelines that recommended earlier initiation of ART at CD4 cell counts of 500 $cells/mm^3$ or less for all adults and children above five years (Maman et al., 2012). A patient with advanced symptoms receives treatment regardless of CD4 cell count.

First-line ART consists combinations of drugs:

1. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), either Efavirenz (EFV) or Neverapine (NVP).

2. Two Nucleotide Reverse Transcriptase Inhibitor (NRTI), one of which is either Stavudine (d4t), Zidovudine (AZT), or Tenofovir (TDF).

The later are call the backbone (WHO, 2006). The revision of WHO guidelines in 2010 brought several changes to the management of HIV-infected patients (WHO, 2010). Since 2006, WHO has recommended to reduce the usage of the drug d4t due to its long-term irreversible side effects, and instead to use either TDF or AZT as

NRTI backbone. The European Medicines Agency recommended that, in view of its long-term toxicities, d4t be used for as short time as possible and only when no appropriate alternatives exist (EMA, 2011).

Recently, randomized controlled trials (Group et al., 2015a,b) have shown modest benefit for HIV-infected persons at an individual level and for reducing HIV transmission following early ART initiation. Consequently, WHO has recently launched a recommendation that ART should be started in all HIV infected individuals, regardless of WHO clinical stage or CD4 cell count (WHO, 2015). However, despite the revision of recommendations at different times, getting access of the drug remained as a challenge for low-income countries.

2.2.1 Guidelines in Averting HIV/AIDS

There has been different policies and strategies designed and implemented to combat HIV/AIDS since the epidemic started. These include Millennium Development Goal (MDG) 6 (McArthur, 2014), the 3 by 5 initiative WHO (2011), Joint United Nations Programme on HIV/AIDS (UNAIDS) strategy for 2011-2015, and sustainable development goals (SDGs) (Osborn et al., 2015). In 2000 the United Nation (UN) set eight MDGs one of which was to combat HIV/AIDS, malaria and other diseases by 2015 (UN, 2015). In 2003, UNAIDS and WHO launched the 3 by 5 initiative was a global target to provide ART for 3 million people living with HIV in low and middle-income countries by the end of 2005 (UNAIDS, 2003). In 2010 UNAIDS set a target of getting to zero strategy in 2011-2015 (UNAIDS, 2010) with the aim to get zero new HIV infections, zero AIDS-related deaths, and zero discrimination. In 2015, the number of new HIV infection was estimated to be equal to 2.1 million and the number of AIDS related deaths was estimated to be about a million (UNAIDS, 2016a)

Post-2015 development agenda set 17 sustainable development goals (SDGs) and 169 targets stated in Osborn et al. (2015) in which target 3.3 aims to end the AIDS epidemic by 2030. UNAIDS fast track strategy was launched in 2014 aiming to step up the HIV response in low-income countries to end the epidemic by 2030. The strategy sets targets for prevention, treatment, and human rights and known as the 90-90-90 targets (Poku, 2016): 90% of all people living with HIV will know their HIV status, 90% will receive ART, and 90% of all receiving ART will have viral suppression by 2020. It refer to the pathway by which a person is tested, linked and retained in HIV care, and initiates and adheres to ART. Ultimately it is the commitment which can help to achieve one of the sustainable development goal of

ending AIDS by 2030 (UNAIDS, 2015). Ethiopia has started implementing SDGs as part of its Growth and Transformation Plan (GTP) II in 2016 (Gizaw et al., 2017)

2.3 Choice of Initial Antiretroviral Drugs and Treatment Outcomes among HIV-Infected Patients in sub-Saharan Africa: A Systematic Review and Meta-analysis of Observational Studies

2.3.1 Effectiveness of NVP and EFV: An Introduction

Staying on an initial regimen medication that successfully suppresses viral replication is essential as it slows disease progression and preserves options for future treatment (Ortblad et al., 2013). However, patients' regimen is modified or changed due to different reasons. Toxicity is the most frequently reported reason for modifying or switch the first combined ART regimens (Sarfo et al., 2013). Once a drug combination is modified, it can no longer be given to the same patient again. It also causes significant morbidity, poor quality of life and also can be an important barrier to adherence, ultimately resulting in treatment failure and viral resistance (Kiguba et al., 2007). Treatment failure due to different reasons is the challenge faced by the current ART scale up program specially in resource limited countries (Adeyinka and Ogunniyi, 2012; Hassan et al., 2014).

In resource limited countries the available evidences are not consistent on the effectiveness of NNRTI choice. In Cameroon, hematologic related adverse drug reaction was high among those who started ART which leads to treatment modification (Luma et al., 2012). According to a Ghanaian study the effectiveness of first line ART (i.e., the proportion of patients who stay on the initial regimen) was 83.3% depending on virologic failure (Barry et al., 2013). Documented virologic failure suggest that access to viral load measurements may actually reduce the rate of switching to a second line regimen (Sanne et al., 2009). The substitution due to toxicity of NVP was higher compared to EFV and according to Boulle et al. (2007), 8% and 2% substitute their initial regimen when initiated with NVP and EFV, respectively. Woldemedhin and Wabe (2012) presented a study conducted in southern Ethiopia and showed that most treatment modifications had occurred during the first 6 months of treatment.

Studies in resource-rich settings revealed that EFV regimen has better treatment outcomes than NVP regimen (Pillay et al., 2013; Shubber et al., 2013). In India, a randomized clinical trial (Swaminathan et al., 2011) showed that regimen containing NVP was inferior and was associated with more frequent virologic failure and

death compare to EFV. Similar patterns were reported in Swaziland, Zambia and Botswana (Takuva et al., 2013; van Dijk et al., 2013). However, studies in Ghana and Ethiopia indicated that there is comparable effect between EFV and NVP (Barry et al., 2013; Tirfe et al., 2013).

The choice of treatment combinations for HIV-infected patients to initiate ART depends on cost and efficacy (Pandhi and Ailawadi, 2014). Identifying the long-term treatment outcomes of these drugs is very decisive for clinical decisions. Clinical decision-making requires ongoing reconciliation of studies that provide different answers to the same question.

The above example indicate contradicting results in terms of the effectiveness of NVP and EVF in first line ART regimen. The analysis presented in this section consists of a systematic review and meta analysis conducted to investigate the difference in NNRTI substitution between NVP and EFV regimens.

2.3.2 Literature Search Strategies

The literature search, presented in Ayele et al. (2017) was as follows. MEDLINE through PubMed, google scholar, and HINARI were used to search for the relevant papers. The research question was "Does the choice of NNRTI drug affect the effectiveness of first line treatment"? The search strategy included Medical Subject Heading (MeSH) terms and a range of relevant keywords. Combinations of key words: ((((((((((((HIV) OR AIDS*) AND antiretroviral*) OR HAART*) OR ART*) OR ARV*) AND NNRTI*) AND outcomes*) OR treatment failure) OR switch) OR substitution) OR Discontinuation) AND Africa) OR sub-Saharan Africa OR developing countries OR low income countries OR resource poor settings. The authors were contacted and requested full articles by email when the article was not accessed from these sources.

Inclusion Criteria

The study eligibility was determined using the following criteria:

- Type of studies:- epidemiological study designs done in sub-Saharan Africa, including cohort, case-control, and retrospective follow up, and comparative cohort were included.

- Intervention:- studies that evaluated EFV compared to NVP regimens in a combination of three antiretroviral drugs. If a publication report on other drugs in

combination with EFV or NVP, or two NRTIs and a protease inhibitor, then only data for combination ART of two NRTIs with NVP or EFV were extracted.

- Types of outcome measures:- studies that included treatment failure or NNRTI substitution as an outcome measures were considered.
- Studies published between 2007 and 2016 in English language were included. Studies which were conducted among children (age < 15 years), published other than English language, and initiated ART other than NNRTI (NVP or EFV) drugs were excluded from the review.

Study Selection

The selection of studies from electronic databases was conducted in two stages: First decision was made based on titles and, where available, abstracts. Secondly, for studies that met the inclusion criteria, or in cases when a definite decision could not be made based on the title and/or abstract alone, the full paper was obtained for detailed assessment against the inclusion criteria. Two independent reviewers assessed study quality. The Kappa statistics was used to measure agreement between the reviewers. The paper was given to third reviewer for consensus in case of a discrepancy in decision process.

2.3.3 Quality Assessment Tools

Quality assessment of the studies included in the review was conducted using the Joanna Briggs Institute Meta-Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI) (Munn et al., 2014) and Newcastle-Ottawa quality assessment scale (Wells et al., 2013) using two independent reviewers. The first assessment tool consists of nine questions. The later consists of eight multiple-choice questions that addressed subject selection and comparability (of cases and controls in case-control studies, of cohorts in cohort studies) and the assessment of the outcome (in case-control studies) or exposure (in cohort studies). The number of possible answers per question ranged from two to five. Questions related to the assessment tools are listed in Table A.1 Appendix A

2.3.4 Data Extraction Process

A standardized data collection form (Li et al., 2015) was used to extract necessary data from the publications. For each study included in the review the following

information was collected: the title of the study, first authors last name, country where the study was conducted, study design, year of recruitment and follow up, year of publication, sample size, study population, diagnosis and identification of treatment modification, average duration of follow up (for cohort study), potential confounders that were adjusted for, main findings and quality assessment tools. Any data discrepancy was resolved by referring back to the original study. The selection process and data collection tool was pretested based on the inclusion criteria on five articles. It was aimed to check reliability of interpretation and classification of the studies appropriately and to ensure that all the relevant information were captured.

Outcomes Measure

The outcomes of interest were treatment failure and NNRTI substitution. Treatment failure is defined as either virologic, clinical or immunological failure as per the definition of WHO ART guideline (WHO, 2010). Several studies used a composite outcome as their event of primary interest. A composite outcome is defined when the a patient experienced either treatment failure, substitution or lost from the follow up. For these studies, treatment failure is a part of the composite outcome. NNRTI substitution was defined as either NNRTI modification, regimen change, NNRTI resistance, or NNRTI discontinuation.

2.3.5 Data Synthesis and Statistical Analysis

Heterogeneity among studies was examined using the I^2 statistic (Chen and Peace, 2013) given by

$$I^2 = \left(\frac{Q - (K-1)}{Q} \right) 100\%.$$

Where $Q = \sum_{i=1}^{k} W_i(\eta_i - \mu)^2$, K is the number of studies, W_i is the weight of the ith study, η_i is effect size of the ith study, and μ is the overall effect size.

According to Higgins and Green (2011), I^2 values greater than 50% was considered as indicative of moderate to high levels of heterogeneity. Adjusted point estimates were extracted from individual studies and combined together to calculate the pooled estimates. The DerSimonian-Laird random effects method was used to incorporate an additional between study component to the estimate of variability (DerSimonian and Kacker, 2007; Jackson et al., 2010). Let η_i is the observed effect size for the ith study, $i = 1, 2, \ldots, K$. The model assumes that η_i is sampled from a distribution with

true effect θ_i and variance τ^2, that is

$$\eta_i = \theta_i + \epsilon_i,$$

Here, $\epsilon_i \sim \mathcal{N}(0, \sigma^2)$. The true effect θ_i is assumed to have a mean μ and a study-specific effect ξ_i. Here, the model can be redefined as

$$\eta_i = \mu + \xi_i + \epsilon_i.$$

Subgroup analyses were conducted to explore differences in outcomes according to study outcomes. The qualitative and quantitative methods were used to present the data extracted from each study. Funnel plot is used to detect publication bias graphical in meta-analysis. It is a scatter plot of estimated treatment effects from individual studies (horizontal axis) against a measure of study size (vertical axis). Funnel plot, and Egger's test were used to check the presence of publication bias (Egger et al., 1997; Jin et al., 2014). The symmetry of funnel plots was assessed both by using visual inspection, and using Eggers test in order to test if the effect decreased with increasing sample size. A regression asymmetry test was used to detect the presence of publication bias. Let ES_i and se_i are the standardized effect size and standard error for study i. Then the regression model is defined as

$$ES_i = \beta_0 + \beta_1 se_i + \epsilon_i.$$

Under the null hypothesis $H_0; \beta_0 = 0$, the Funnel plot is symmetric. Hence, a rejection of the null hypothesis implies a publication bias. Additional heterogeneity can be modelled using a meta-regression model (Chen and Peace, 2013) in which study-level variables are included to account for the extra heterogeneity on the true effect. Length of follow up (followup), baseline CD4 cell count (bcd4), age and proportion of female (femaleprop) were considered as study level variables. The mixed-effect meta-regression model can be formulated as

$$\eta_i = \alpha + \beta_1 * followup + \beta_2 * bcd4 + \beta_3 * bage + \beta_4 * femaleprop + u_i.$$

Where u_i is random intercept.

2.4 Results

Figure 2.2 present the screening procedure, based on Systematic Reviews and Meta-Analyses (PRISMA) described in Moher et al. (2009), that was implemented for the literature review presented in this section. The inclusion criterion of this review required that studies provide a comparison between the risk of long-term treatment failure of NVP and EFV. A total of 6394 articles were identified in English-language and human domain restrictions, of which 5779 were rejected based on the publication's title that was outside the research objectives of the review. The remaining 615 articles were further screened and subsequently, 368 were considered irrelevant or duplicates. The abstracts of 247 articles were then evaluated independently. Of these, 231 records were excluded because of no comparison groups of the outcomes of interest, missing comparison of EFV versus NVP drugs.

A study that was focused on the comparison between NVP and lopinavir-ritonavir (Clumeck et al., 2014) was excluded as it was not the focus of this review. Other six papers were excluded as the studies were conducted among children (Lowenthal et al., 2013) or conducted outside of sub-Saharan Africa (Boettiger et al., 2016; de Castilla et al., 2008; Patel et al., 2006; Sinha et al., 2013). The systematic reviews and meta-analysis articles by Pillay et al. (2013) were excluded as well. Subsequently of 79 full record articles, a total of 36 were eligible studies. Further, the full text of 36 articles were reviewed in detail and 20 of them were excluded due to lack of sufficient information on sample size, design and analysis. Study by van Zyl et al. (2011) had used cross-sectional study design and the assessment tools might not evaluate the quality appropriately. Therefore, 16 studies were included in the quantitative synthesis out of 17 studies for which outcome measures were identified for meta-analysis.

2.4.1 Quality Assessment

Two independent reviewers assessed the articles prior to inclusion to maintain methodological validity using Joanna Briggs Institute Meta-Analysis of Statistics Assessment and Review Instrument (JBI-MAStARI) (Munn et al., 2014). The Kappa statistics is estimated to be equal to 0.86 indicates the presence of good agreement between the reviewers. The scores ranged from 5/9 (55.6%) to 8/9 (88.9%). In addition, the Newcastle-Ottawa quality assessment score ranges from 4 to 7 stars (Out of 9 stars).

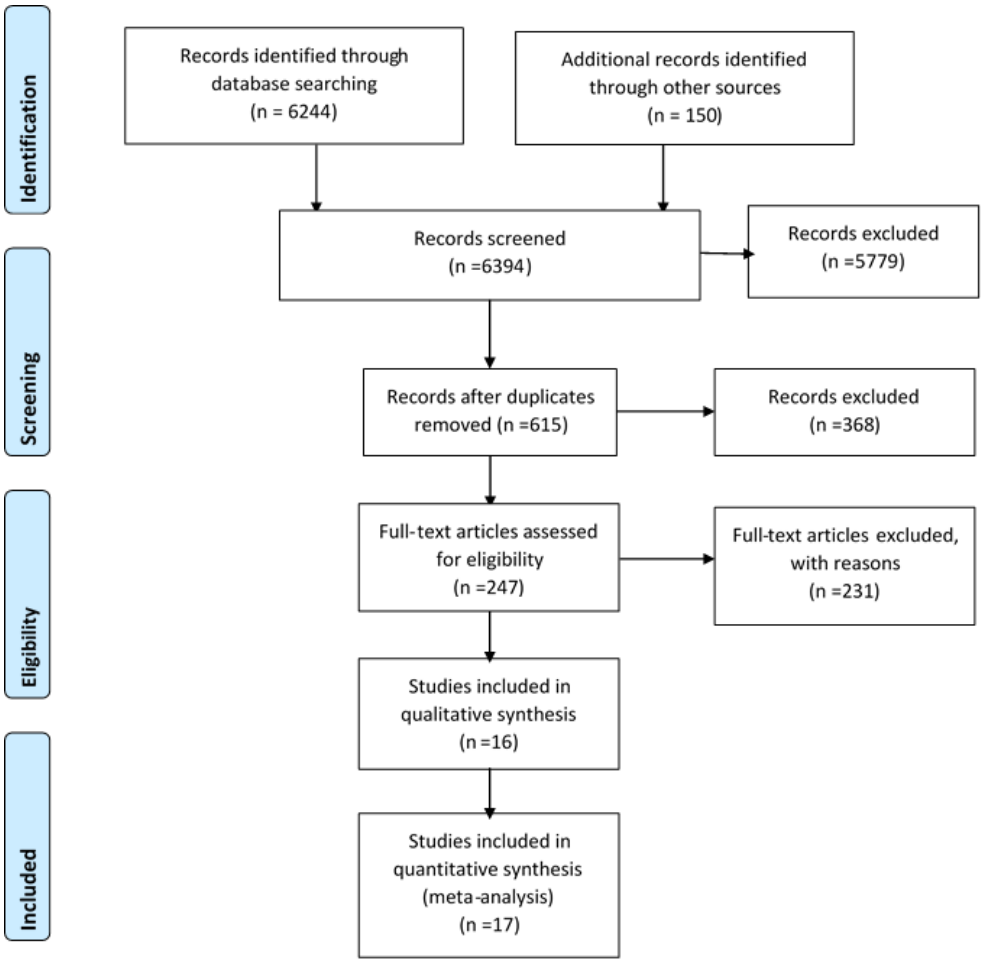
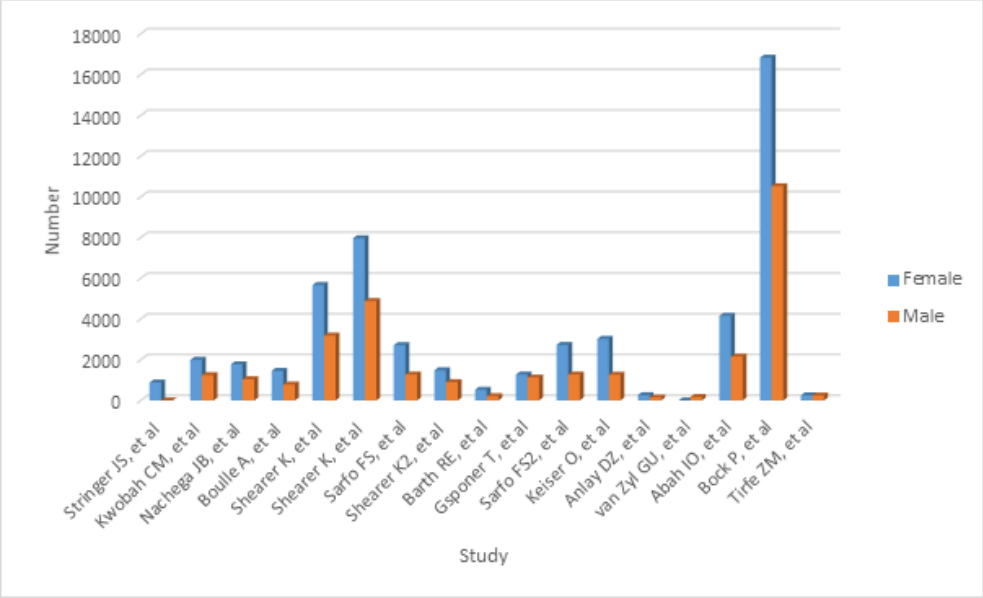


Figure 2.2. The PRISMA flow diagram of identification and selection of studies for the systematic review and meta-analysis.

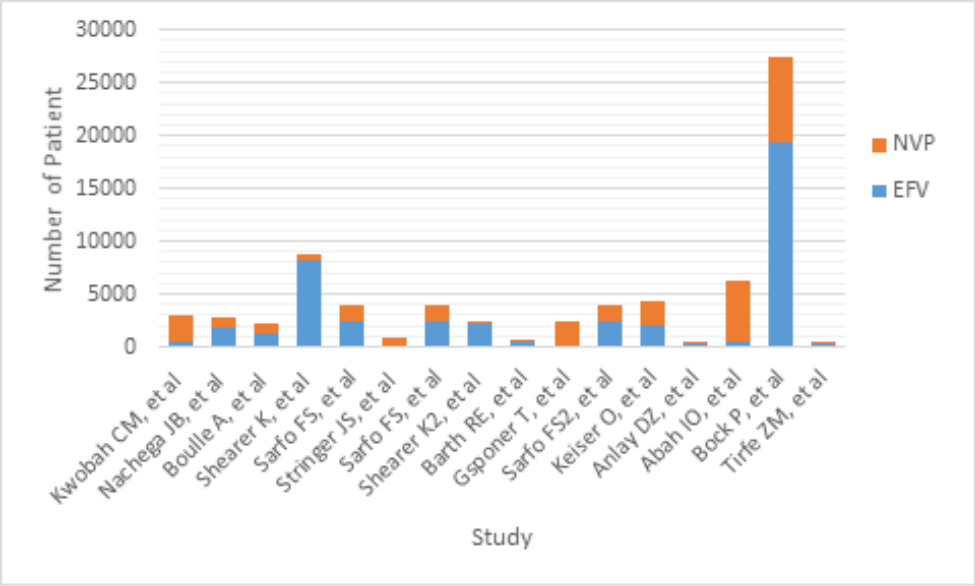
2.4.2 Characteristics of Included Studies

Table 2.1 lists the 16 studies included in the meta analysis. The studies were conducted between 2007 and 2016. As shown in the table, sample size ranges from 167 (van Zyl et al., 2011) to 27350 (Bock et al., 2013) with a total sample size of 70537. In total, 45010 (63.8%) were females (range from 51% to 72% (see Figure 2.3a). The majority of patients, 42039 (59.6%) initiated with EFV regimen. The proportion of female initiated with NVP by study is shown in Figure 2.3b. The median follow up time is 4 years (Inter Quartile Range (IQR): 3-7). Nachega et al. (2008) had the longest follow up time (10 years), while studies (Shearer et al., 2013) have the shortest follow up periods of 1 year. Stringer et al. (2010) is a multicenter study (in Kenya and in Zambia). Based on the inclusion criteria, a total of 509 and 152 patients were included in Zambia and Kenya, respectively. Study design, by publication are reported in Table 2.1. Table 2.1 also shows the median CD4 cell counts by study (ranges from 67, IQR: 21-161, to 192, IQR: 112-324) . Note that the median CD4 cell count was smaller for patients who initiated with EFV regimen.

This might be due to the occurrence of different opportunistic infection among this group of patients and EFV regimen had no organ damage like hepatotoxicity and preferred for this group at large to maintain adherence (WHO, 2010). Two studies, Tirfe et al. (2013) and van Zyl et al. (2011), did not report the median CD4 cell count at initiation. Only two studies, Nachega et al. (2008) and Keiser et al. (2010) reported the log transformed median viral load. Stavudine (d4T)/3TC was used as backbone by 13 studies while 3 studies did not use this NRTI backbone at all. AZT/3TC was used in 14 studies and 2 studies did not use this backbone at all, whilst TDF/3TC was used in 7 studies. Eleven of sixteen studies used Cox-PH model for the analysis and reported adjusted hazard ratio. Two studies used stratified and random effect Cox-PH models (Table 2.1).



(a)



(b)

Figure 2.3. Panel a: distribution of female by study. Panel b: distribution of NNRTI drugs by study.

Table 2.1. Baseline characteristics of patients on NVP and EFV by study.

Author	Country	Design	Sample size	Follow up Period	Female	Age(Median±IQR)	CD4 (Median±IQR)	Viral load(Median±IQR)	BMI(Median±IQR)
Stringer JS,et al	Zambia/Kenya	Prospective Cohort	661	4 years	661	32(2836)	148(88211)	5.0(4.45.4)	19.7(18.321.9)
Kwobah CM,et al	Kenya	Case-control	3,233	5 years	1,992	36.3(30.6-43.2)	80 (32 - 177)	NA	NA
Nachega JB,et al	South Africa	Cohort	1,817	10 years	1,771	36(8.0)	136(58-216)	5.2(4.7-5.7)	NA
Boulle A, et al	South Africa	Cohort	2,679	4 years	1,896	32(28-38)	83(34-140)	5.5(4.6-5.5)	NA
Shearer K, et al	South Africa	Cohort	1,2840	8 years	7,962	37.2(31.9-43.9)	98(36-169)	NA	21.7 (19.2-24.9)
Sarfo FS, et al	Ghana	Retrospective	3,990	7 years	2,717	40 (3547)	127 (45213)	NA	19.6 (17.422.3)
Shearer K,eta al	South Africa	Cohort	2,385	1 year	1,485	37.8 (31.844.3)	132 (58194)	NA	22.5 (19.725.9)
Barth RE, et al	South Africa	Retrospective	735	2-4 years	526	mean=35.3	68 (20140)	mean=4.9	NA
Gsponer T, et al	sub-Saharan	Collaborative	2,404	6 years	1,276	29 (25-33)	77 (41-133)	NA	NA
Sarfo FS, et al	Ghana	Retrospective	3,999	7 years	2,727	38 (3245)	132 (50217)	NA	19.8(17.522.7)
Keiser O, et al	sub-Saharan	Case control	4,281	5 years	3,022	35 (30-41)	73 (23-133)	5.0 (4.4-5.6)	NA
Anlay DZ, et al	Ethiopia	Retrospective	410	5 years	265	33.3 8.7	162.5(90.5235.5)	NA	NA
van Zyl GU, et al	South Africa	Retrospective	167	4 years	NA	NA	NA	NA	NA
Abah IO, et al	Nigeria	Cohort	6,309	3 years	4,156	35 (30-42)	154 (76-259)	NA	NA
Bock P, et al	South Africa	Multicentre	27,350	4 years	16,828	35.8 (30.742.6)	107 (51160)	NA	NA
Tirfe ZM, et al	Ethiopia	Retrospective	492	3 years	289	35(10)	NA	NA	NA

2.4.3 Systematic Review: Treatment Outcomes

In this review, treatment failure, the primary outcome of interest, was measured using clinical, virological and immunological criteria. The studies by Barth et al. (2011); Gsponer et al. (2012); Keiser et al. (2010); Kwobah et al. (2012); Nachega et al. (2008); Sarfo et al. (2013); Shearer et al. (2014, 2013); Stringer et al. (2010); Tirfe et al. (2013) defined treatment failure as their primary outcome. A total of 30,069 patients were included in the 10 studies of which 19,584 (65%) were females. The majority, 17,950 (60%) were initiated with EFV regimen. A total of 4,842 patients experience treatment failure for both EFV and NVP drugs (2,077 EFV and 2,765 NVP). The study by Nachega et al. (2008) defined treatment failure using two separate (consecutive or non-consecutive) measurements of viral load $\geq$ 400 copies/ml, or switch to another NNRTI or protease inhibitor after at least one such measurement. In this study, about 1,822 (64.7%) patients were started on EFV regimen. The two groups did not differ in viral load measurement, however patients started on EFV had a significantly shorter time to virologic suppression. Subsequently, patients started on NVP were more likely to experience virologic failure. The outcome reported in (Stringer et al., 2010) was assessed at 48 weeks after initiating ART. Participant was considered as having failed at 48 week if she/he died prior to that time, or had a plasma viral load $\geq$ 400 copies/ml (confirmed with repeat testing) at either the 24 or 48 week study visits. The difference in failure rates between the NVP-exposed and unexposed groups was 6.9%. Kwobah et al. (2012) presented a case-control study in which a case defined as adult at least one viral load measurement > 5000 copies/ml or meet the WHO 2006 immunological or clinical failure criteria (WHO, 2006). Controls were those on non-failing first-line ART with a CD4 cell count > 400/ml within the last 12 months, at the time of case incidence. Patients who were either pregnant or co-infected with tuberculosis at the time of ART initiation were excluded. A total of 1,084 cases were included with median time to ART failure of 37 months. Sarfo et al. (2013) defined the outcome measure of treatment failure as a composite of death, clinical progression or discontinuation of NNRTI for any reason. A total of 3,999 patients were included in the study from whom 2,369 (59%) initiated with EFV regimen and 633 (26.7%) experienced at least one event.

The second outcome of interest was NNRTI substitution. The studies reported in Abah et al. (2015); Anlay et al. (2016); Bock et al. (2013); Boulle et al. (2007); Sarfo et al. (2013) defined NNRTI skin rash, NNRTI discontinuation, Regimen change, NNRTI substitution, and regimen change as the outcome measure, respectively. The study presented in Tirfe et al. (2013) defined immunologic response as secondary out-

come measure and study reported in Barth et al. (2011) considered patients retention as the outcome measure. In all studies, the initial NNRTI drug was substituted by another drug in the same regimen and hence defined NNRTI substitution as the outcome measure. The studies of Bock et al. (2013); Sarfo et al. (2013); Shearer et al. (2014, 2013) defined outcome measure of death. In Sarfo et al. (2013), higher number of deaths were observed for in patients who initiated with EFV, 208 (8.8%) than NVP, 110 (6.8%). A total of 27,350 patients were included of whom 19,441 (71.1%) started EFV and 7,909 (28.9%) started NVP treatment. At the end of the study period, 1,593 (5.8%) patients were died. In the study reported in Shearer et al. (2014) 12,840 patients were included of whom 1061 died (8.3%) within the first 12 months on ART (Table A.2 in Appendix A).

Meta-Analysis Results

Figure 2.4 shows the forest plot for the relative risk of composite outcome. The pooled relative risk of composite outcome is equal to 0.72 (95% C.I 0.59-0.87) indicate that there is lower risk of developing composite outcome for patients who initiated with EFV as compared to NVP regimen. The relative risk estimates of eight individual studies were significantly different from one.

Figure 2.5 shows the subgroup analysis based on the two outcomes of interest (treatment failure and NNRTI substitution). Ten studies were included for treatment failure subgroup. The weights of the studies were reported from random effect model which ranged from 0.31% to a maximum of 28.28%. The pooled estimate of risk ratio for treatment failure is 0.85 (RR=0.85; 95%CI: 0.75-0.88). The I^2 value for treatment failure subgroup was found to be 81.0% (p-value<0.0001) indicating the presence of heterogeneity between studies.

The pooled relative risk for NNRTI substitution estimates is 0.57 (RR=0.57; 95%CI: 0.37-0.89). Similarly, the risk of NNRTI substitution is lower in patients who initiated with EFV as compared to NVP. The weight of the studies ranges from 0.37% to 38.09%.

2.4.4 Evaluation for Publication Bias

Researches reported on statistically significant results is more likely to be published than researches reported non-significant results. This could introduce bias during systematic review and meta-analysis. Figure 2.6 shows the funnel plot of the study with 95% confidence interval around the summary relative risk. The solid vertical line represents the estimate of the relative risk. Figure 2.6 show the expected distribution

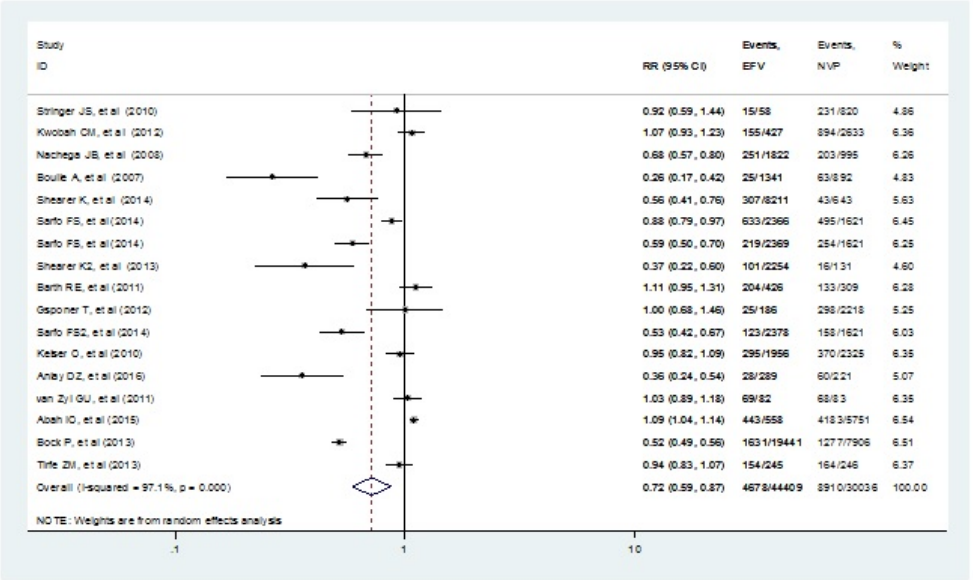


Figure 2.4. Relative risk of composite outcome associated with the choice of NNRTI drugs regiment during ART initiation. The definition of composite outcome is given in Section 2.3.4.

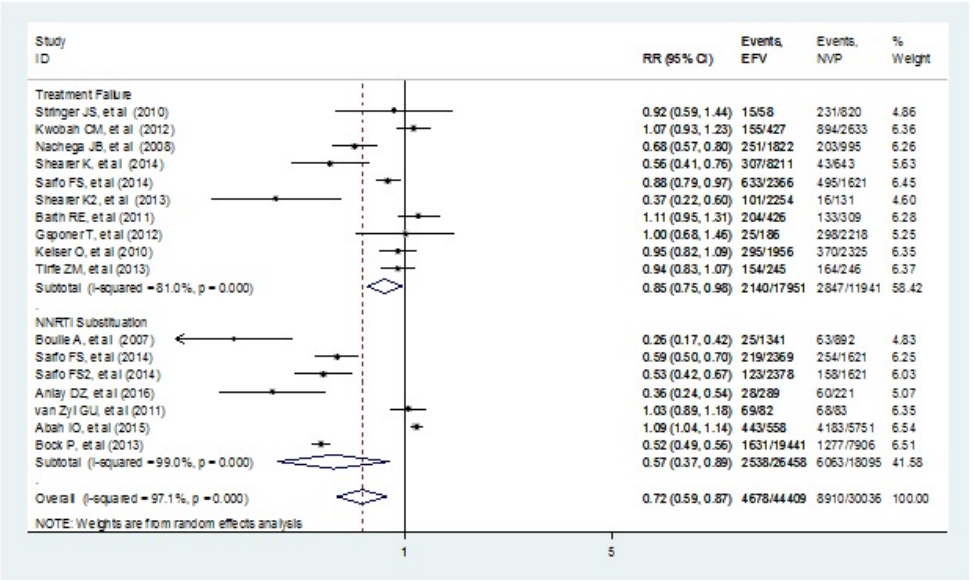


Figure 2.5. Relative risk of treatment failure (EFV/NVP) and NNRTI substitution associated with the choice of NNRTI drugs regiment during ART initiation.

of the studies' relative risk in the absence of heterogeneity or selection biases. Eggers test revealed that there was no significant bias for either of the outcome (Overall test: Intercept= -2.217, 95%CI: -5.562; 1.128), p-value=0.178).

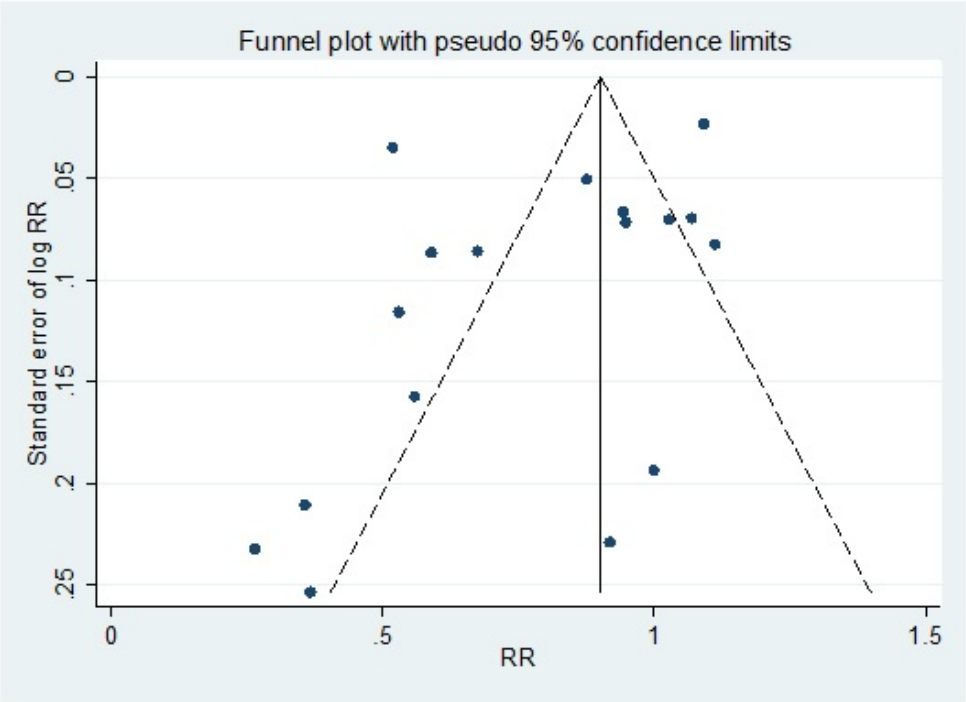


Figure 2.6. Funnel plot of estimates versus standard error of log estimate.

A meta-regression analysis was conducted to determine whether there is association between independent variables and composite outcome. Covariates included in the model are, length of follow up, median CD4 cell counts, median age, and year of publication and proportion of female. No significant relationship was found between any of the covariates and composite outcome. This indicates that these covariates may not be source of observed variability. The complete meta-regression analysis is presented in Table A.3 in Appendix A.

2.5 Discussion

The systematic review and meta-analysis presented in the chapter attempted to assess the individual and pooled estimate of the choice of NNRTI drugs on treatment failure,

and NNRTI substitution in resource poor settings. A total of 16 observational studies were found which compares EFV versus NVP, out of which 17 outcomes measures were identified in two groups. We have shown that, in resource limited settings, that initiation of ART regimen with EFV is associated with a reduced risk of treatment failure (RR=0.85, 95%CI: 0.75-0.98) compared with NVP regimen. This finding was consistent across 4 of the 10 individual studies. This is in line with previous meta-analysis by Pillay et al. (2013) conducted from 10 RCTs and 24 observational studies which concluded that EFV-based first line ART regimen is significantly less likely to lead to virologic failure compared to NVP-based ART regimen. This might be due to the hepatotoxic nature of NVP which may lead to poor adherence which might further resulted in treatment failure.

Leth et al. (2004), did not find any evidence that EFV is superior to NVP twice daily in terms of treatment failure. A Cochrane review of seven randomized clinical trials (Mbuagbaw et al., 2010) demonstrated that the two drugs provided comparable levels of viral suppression in patients infected with HIV when combined with two NRTIs. Patel et al. (2006) reported a non randomized longitudinal cohort study conducted in India, equivalent immunological response was observed among NVP and EFV based ART. The risk ratio of NNRTI switch reduced by 0.57 (95% CI: 0.37-0.89) times for patients who initiated with EFV than NVP. This finding is consistent with a multicenter randomized non-inferiority trial (Bonnet et al., 2013) in which the switching rate was found to be higher among patients who initiated with NVP than EFV. This finding is also consistent with previous meta-analyses reported in Shubber et al. (2013) which revealed that adults on NVP were two times more likely to discontinue treatment due to any adverse event compared to patients on EFV. A meta-analysis on five randomized clinical trials and four retrospective clinical trials (Jiang et al., 2014) revealed that the discontinuation rate was high among those who initiated with NVP than EFV which is consistent with the review presented in this Chapter. Similar finding was reported in (Kryst et al., 2015) in which the discontinuation rate was found to be lower among those who initiated with EFV compared to ART regimen with NVP.

The meta-regression model revealed that the log relative risk of treatment failure is not associated with the studys covariates. This might be due to the small number of studies included in the meta-analysis. Sensitivity analysis revealed that there is no single study which influences the pooled estimate. The results presented in this chapter should be interpreted with caution due to the following limitations. Although a lot of efforts has been made to find more studies, only few studies satisfied the inclusion criteria. The analysis was limited to articles published in English language;

the evidence may not be sufficiently robust to determine the comparative effectiveness of EFV and NVP due to the size of included studies. In addition, the analysis include articles with different definitions of treatment failure and different lengths of follow-up. The reviewed articles have also differences in study design, the type of statistical methods, and the variables included in the analysis. These variations may have resulted in selection bias or low statistical power. Most of our analyses detected heterogeneity between effect estimates obtained across studies. DerSimonian and Laird random effect model was used to determine the pooled effect size (DerSimonian and Kacker, 2007). However, the source of variation might not be real heterogeneity rather within study differences which may introduce bias on the pooled effect size.

In conclusion, the review presented in this chapter indicates that initiation of ART regimen with EFV leads to a reduction of the risk of treatment failure compared to ART regimen with NVP. In addition, patients who initiated with EFV are less likely to switch treatment than patients who initiated with NVP. Therefore, even though EFV is expensive to afford for resource poor settings, initiating the patient with EFV regimen could be a better option in terms of reduction of risk for treatment failure and switch.

Chapter 3

Modeling Outcomes of First-Line Antiretroviral Therapy and Rate of CD4 Cell Counts Change among a Cohort of HIV/AIDS Patients in Northwest Ethiopia: A Retrospective Cohort Study

3.1 Introduction

The effectiveness of ART can be assessed by clinical observations, CD4 cell counts and determination of plasma viral load (EMH, 2010). Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are drugs choices for initial ART for HIV infection. Studies in resource-rich settings revealed that EFV based regimen has better treatment outcomes than NVP based regimen (Pillay et al., 2013; Shubber et al., 2013). The meta analysis presented in Chapter 2 indicated the same pattern in sub-Saharan Africa. Furthermore, (Swaminathan et al., 2011) presented a randomized clinical trial from India and showed that NVP based regimen was inferior and was associated with more frequent virologic failure and death. However, there exist evidence in resource-poor settings that shows as there was no difference between EFV and NVP in the long-run (Barry et al., 2013; Collaboration et al., 2012; Tirfe et al., 2013).

In the national treatment guideline of Ethiopia 2010, the first-line ART contains four NRTIs backbone (Stavudine (d4t), zidovudine (AZT), Abacavir (ABC) and Tenofovir (TDF)) plus lamivudine (3TC) and two NNRTI drugs (EFV and NVP) (EMH, 2010). The combination regimens which have been used most frequently in Ethiopia are d4t-3TC-EFV, d4t-3TC-NVP, AZT-3TC-EFV, AZT-3TC-NVP, TDF-3TC-EFV,

or TDF-3TC-NVP. When the patient is unable to tolerate the side-effect due to toxicity, the offending drug can be substitute with another drug that does not have the same side-effect. Whereas, patients switch to second-line regimen when the first-line regimen failed due to different reasons. A failure in treatment is measured according to the WHO definition, if at least one of the three conditions happened: (1) clinical-when new or recurrent WHO stage 4 condition, (2) immunological-when persistent CD4 level below 100 or 50% fall from on-treatment peak value and (3) virological when plasma viral load above 10,000 copies/ml in duplicates after six months on ART (EMH, 2010).

As mentioned in Chapter 2, the choice of treatment combinations for HIV/AIDS patients to initiate ART depends on cost and efficacy. Thus, knowing the long-term treatment outcomes of more costly drugs is very decisive for decision making in resource limited nations. In the analysis presented in this chapter, we aim to determine the long-term outcomes of first-line ART drugs and rate of change in log(CD4) cell counts in response to ART. Furthermore, the effect of treatment choices at the initiation on the CD4 evolution was compared and tested as well.

This chapter is organized as follows. The data are presented in Section 1.3. The statistical methodology used for the data analysis is presented in Section 3.2 and the results in Section 3.4. The findings are discussed in Section 3.5.

3.2 Data and Methods

3.2.1 Study Population

Gondar University Hospital ART clinic started treating HIV/AIDS patients as part of the National AIDS control program since 2005. At the same time ART was started to be provided for free in the selected hospitals in the country, Gondar University Hospital is one of these hospitals. As a result, patients were referred to Gondar University Hospital from many areas in Northwest Ethiopia.

The study included ART nave patients aged $\geq$ 15 years-old who initiated ART containing TDF, d4t, or AZT as NRTI backbone with NVP and EFV as NNRTI drugs between 2009 and 2013. In total, following the inclusion criteria, 2386 patients included in this study. Data on patients were recorded in patients chart and entered into access database which was designed for this purpose. Baseline characteristics such as sex, age, weight, WHO staging and functional status were collected when the patient enrolled in the clinic. Whilst clinical variables such as CD4 cell counts, and regimen were collected every 6 months subsequently depending on the progress

of the patient. In some cases, patient's visit might be taken at irregular time due to different reasons such as diseases progression, toxicity or opportunistic infections. The criteria for initiating ART in Ethiopia followed WHO guideline (WHO, 2010), with an adjustment of CD4 threshold from 200 to 350 $cell/mm^3$ in 2010.

3.2.2 Data and Study Variables

Data for the study were accessed from ART clinic database and presented in Section 1.3. The information collected by the health professional from the patient were sent to the data manager who entered the data into the computer. Information on treatment substitution, treatment discontinuations, death, lost to follow up and transferred out were obtained from hospital records.

For the analysis presented in this Chapter, the following definitions were used:

1. NNRTI substitution: modifying NNRTI drugs of the original regimen for any reason.

2. Treatment discontinuation: a patient that changed his/her first-line regimen to second-line regimen.

3. Lost to follow up: defined as missing a clinic appointment for more than three months without further attendance at clinic.

4. Transferred out: transfer of patients to other ART clinic with all the history/records.

5. Death: confirmed deaths from medical records or verbal confirmation of death by relatives or friends.

6. Composite outcome: the occurrence of either NNRTI substitution, discontinuation, lost to follow up or death.

Patients who had at least two CD4 cell counts (two visits for those who experienced the event) were included in the analysis. Information on baseline characteristics were obtained from registry in the database. Whereas, follow-up variables were accessed from ART refill. The data were closed for analysis on December 31, 2013.

3.2.3 Data Quality Control

The ART clinic of Gondar University Hospital has been using database to enter information of the patients starting from the first visit. It was developed by information

technologist and well tested before used for data entry in the hospital. Well trained data entry clerks employed by the hospital perform the data entry. The entry process is supervised by the data manager for completeness and consistency in daily bases. The data for this study was accessed from this database and from patients' chart in order to increase the quality.

3.3 Data Analysis Methods

3.3.1 Data Analysis for Time to Event Outcomes

We compared patient characteristics at ART initiation by initial NNRTI treatment groups (EFV or NVP) using chi-square test for categorical covariates and Wilcoxon rank sum test for continuous covariates. There were different responses to ART treatment. A composite endpoint which represents different responses to ART treatment was defined and analyzed as time to event endpoint. These include drug substitution, lost to follow up, treatment discontinuation, and death. Three types of survival analysis were considered; primary analysis (NNRTI substitution and lost to follow up were treated as censored), two sensitivity analyses (NNRTI substitution and/or lost to follow up were treated as event).

For the primary outcome, time to the first occurrence of any of the outcome measures was calculated by subtracting the date of the event from the date of initiation of ART. Patients were censored if death was not observed until the time of the last visit for patients who were lost and December 31, 2013 for patients who were alive. Note that we assessed NNRTI substitution and discontinuation as an event (Sarfo et al., 2013). Discontinuation of NNRTI was defined as discontinuation of either NVP or EFV due to toxicity, or patient or physician preference.

For each patient in the study we observe either the time to failure or censoring. For the censored individuals we know only the time to failure is greater than the censoring time. Denote T be a random variable representing failure time. The probability of the failure time occurring at exactly time t (out of the whole range of possible t's) can be formulated as

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

where $f(t)$ is probability density function, Δt refers small change of time t. The survival function $S(t)$ can be defined as

$$S(t) = P(T \geq t) = \int_t^{\infty} f(u)du.$$

Similarly, the hazard function can be defined as

$$\lambda(t) = \lim_{\Delta t \to 0} \frac{P(t \leq T < t + \Delta t | T \geq t)}{\Delta t}.$$

Comparison of Kaplan-Meier survival curves between different groups was done using log-rank test which is used to test the null hypothesis that the probability of an event occurring at any time point is the same for each group of the covariate. The test statistic is calculated as follows

$$\chi^2(log - rank) = \frac{\sum_{i=1}^{k} (O_i - E_i)^2}{E_i}.$$

Where O_i's are the total numbers of observed events in group i, and E_i's are the total numbers of expected events in group i.

Similarly, Cox-PH regression model (Cox, 1972) was used to check the effect of NNRTI drug, NRTI backbone and other covariates at baseline on event time. The model can be formulated as

$$h_i(t|\mathbf{X_i}) = h_0(t)exp(\mathbf{X_i}\beta). \tag{3.1}$$

Where β is a $p \times 1$ vector of unknown parameters, $\mathbf{X_i}$ is the design matrix of baseline covariates such as gender, age, WHO stage, etc, and $h_0(t)$ is an unknown function giving the hazard function for the standard set of conditions $\mathbf{X_i} = 0$.

Baseline covariates such as sex, age (≥ 40 versus <40 years), WHO clinical stage (IV or III versus I or II), CD4 cell counts (<200 versus ≥200), calendar year (before 2010 versus since 2010), NRTI backbones (d4t plus 3TC, AZT plus 3TC or TDF plus 3TC) and NNRTI drugs (EFV versus NVP) were considered. The categorization of numeric variables was done based on other previous studies by Benjamin et al. (2011); Sarfo et al. (2013) for comparison purpose. Similarly, Cox-PH regression analysis was used to compare the baseline covariates for their risk of composite outcome. The hazard ratio with 95% confidence interval was used to test statistical significant association.

3.3.2 Data Analysis for Immunological Outcomes

Treatment effects on the CD4 cell counts evolution varies over time and it is expected that repeated measurements taken on the same subject to be correlated. Liner mixed effects models (Verbeke and Molenberghs, 2000) are often used for analyzing continuous correlated data (Rizopoulos, 2012b). The liner mixed effects model can be formulated as

$$\mathbf{Y}_i(t_i) = \mathbf{X}_i\beta + \mathbf{Z}_i\mathbf{b_i} + \varepsilon_{it_i}. \tag{3.2}$$

Here $\mathbf{Y}_i(t_i), i = 1, \ldots, n_i$, is n_i-dimensional response vector of log transformed CD4 cell counts for individual i at time t_i, $\mathbf{X}_i$ and $\mathbf{Z}_i$ are $n_i \times p$ and $n_i \times q$ dimensional fixed and random effects model matrices, respectively. The parameters vector β is a p-dimensional vector of fixed effects and b_i is a q-dimensional subject specific vector of random effects.

However, many biomedical experiments generate non-linear data and imposing parametric function for the mean evolution over time might yield unsatisfactory results (Bowman and Azzalini, 1997). In the context of HIV/AIDS data, the individual profiles are non-linear and parametric models may be too restrictive. Therefore, we propose a data-driven approach based on semi-parametric regression models proposed by Wood (2003). In this model, the patient-specific random intercept is used to capture correlation of the CD4 cell count measurement over time within the patient. We assumed patient-specific random parameters for both the linear and quadratic time effects to capture different evolution between the patients of log(CD4) cell count over time. It allows smoothing with respect to *time*. The Semi-parametric mixed effects model, with patient-specific random effects can be expressed as

$$\mathbf{Y}_i(t_i) = S(t_i) + b_{0i} + b_{1i}t_i + b_{2i}t_i^2 + \varepsilon_{it_i}. \tag{3.3}$$

Here $S(t_i)$ is the non-parametric component of the model. The patient-specific random effects assumed to follow a multivariate normal distribution, $[b_{0i}, b_{1i}, b_{2i}]^T \sim MVN(\mathbf{0}, \mathbf{\Sigma}_b)$, where $\mathbf{\Sigma}_b$ denote the variance covariance matrix of patient-specific random effects. The residuals ε_{it_i} are assumed to be normally distributed with mean zero and variance σ_ϵ^2.

The non-parametric component of the model, $S(t)$, is the smoother to the log(CD4) evolution given by

$$S(t_i) = \beta_0 + \sum_{\iota=1}^{\nu} \beta_\iota f_\iota(t_i),$$

where $f_\iota(t_i)$s are a set of thin plate spline basis functions.

3.3.3 Pointwise Confidence Intervals

The penalized thin plate spline model can be expressed as a mixed model of the form

$$\mathbf{Y_i} = \underbrace{\mathbf{X}_i\beta_i}_{S(t)} + \mathbf{Z}_i\mathbf{b}_i + \varepsilon_i. \tag{3.4}$$

Here $\beta_\mathbf{i}$ be a parameters vector contains all fixed and random effects for the smooth terms, $\mathbf{X}_i$ is the corresponding covariates matrix, $\mathbf{Z}_i$ is the design matrix for the random effects, and $\mathbf{\Sigma}_{b_i}$ is the covariance matrix for the random effects.

For the given values of the parameters associated with the random effect and error, application of maximum likelihood and best linear unbiased prediction (BLUP) estimate for S is given by

$$\hat{S} = \mathbf{X}\hat{\beta}. \tag{3.5}$$

A point-wise confidence interval for the average fitted problem can be obtained by Ruppert et al. (2003):

$$\hat{S}(t) \pm t_{1-\alpha/2} s.d(\hat{S}(t)). \tag{3.6}$$

Where $s.d(\hat{S}(t))$ is the square root of the diagonal of the variance covariance matrix $\mathbf{X}\hat{V_\beta}\mathbf{X}^t$, with $\hat{V_\beta} = (\mathbf{X}^t\hat{\mathbf{V}}^{-1}\mathbf{X} + \mathbf{W})^{-1}$. Here, $\hat{V}$ is the variance and covariance matrix of the coefficients of the basis functions and $\mathbf{W}$ is the wiggliness penalty matrix (Wood, 2003).

3.3.4 Pairwise Comparison of Treatment Groups

The linear mixed model formulated in (3.4) allows us to compare between the treatment groups in order to investigate whether there is difference between groups (comparing their average profiles). The model can be rewritten as

$$Y_{ig}(t_i) = \underbrace{\beta_{0g} + \beta_1 treat_g + \sum_{\iota=1}^{\nu} \beta_{g\iota} f_\iota(t_i)}_{S_g(t)} + b_{0i} + b_{1i}t_i + b_{2i}t_i^2 + \varepsilon_{it_i}. \tag{3.7}$$

where $Y_{ig}(t_i)$ is the response for the ith subject in the gth treatment group at time point t_i, $S_g(t)$ is a group specific smoother, and $f_\iota(t_i)$'s are a set of thin plate

spline basis functions, $\beta_{\iota g}$ are the coefficients of the basis function.

We estimated different spline coefficient variances for each treatment groups g, and we used penalized thin plate regression splines with a roughness penalty on the third-order derivative $k = 3$ to obtain a smooth first order derivative.
Our aim is to test if the CD4 evolution and the change in CD4 over time (i.e. the first derivative) is the same for EFV and NVP. Let $S_g(t) = \beta_g^t F(t_i)$ be a group specific smooth curve with $F_t = [f_0(t), \ldots f_\nu(t)]^t$, $\beta_g = [\beta_{0g}, \ldots \beta_{\nu g}]^t$, and $g = 1, \ldots, G$ is the group indicator.

The difference between groups were estimated using the first derivative of the smoother, that is

$$\frac{dS_g(t_i)}{dt} + b_{1i} + 2b_{2i} \times t. \tag{3.8}$$

Let $S_g^{'}(t_i) = \beta_g^t F^{'}(t_i)$ be the first order-derivatives of penalized thin-plate spline model for the gth group. The difference can be estimated by $S_\ell^{`}(t) - S_k^{`}(t)$. We can construct the point-wise confidence interval for $S_g^{'}(t_i)$ in the same way as discussed above.

3.4 Results

3.4.1 Baseline Description

A total of 2386 HIV-infected individual who initiated ART were included. Majority of the patients, 1462 (61.27%) were initiated with NVP containing NNRTI; of whom 1023 (70.0%) used AZT as NRTI backbones. Patients who were initiated with treatment containing NVP were predominantly female, 927 (63.41%), and were younger than 40 years were 1132 (77.43%). Among patients who were initiated with EFV containing treatment, 140 (15.2%) were ambulatory as compared to 124 (8.5%) who initiated with NVP containing treatment. At initiation most patients, 1149 (48.2%) were at clinical stage III, of which 483 (52.3%) and 666 (45.5%) were initiated with EFV and NVP containing treatments, respectively. The median CD4 cell count was higher for those who were initiated with NVP as compared to EFV (152 versus 141); however significant difference was not found with regard to age and weight among the two treatment groups (see Table 3.1). There was no significant difference between EFV and NVP with regard to baseline CD4 cell counts, but association was observed with other baseline covariates.

Table 3.1. Cohort characteristics at initiation of ART by Non-nucleotide Reverse Transcriptase Inhibitor (NNRTI) of HIV/AIDS patients in Gondar University Hospital, Northwest Ethiopia, 2013.

Characteristic	Efavirenz, n=924	Nevirapine, n=1462	P-value
Sex, n(%)			
Female	497(53.8)	927(63.4)	<0.0001
Male	427(46.2)	535(36.6)	
NRTI backbone, n(%)			
Zidovudine + lamivudine	215(23.3)	1,023(70.0)	<0.0001
Tenofovir + lamivudine	657(71.1)	331(22.6)	
Stavudine + lamivudine	52(5.6)	108(7.4)	
Functional Status, n(%)			
Ambulatory	140(15.2)	124(8.5)	<0.0001
Bedridden	62(4.2)	24(1.6)	
Working	722(78.1)	1,314(89.9)	
WHO stage, n(%)			
I	119(12.9)	390(26.7)	<0.0001
II	101(10.9)	285(19.5)	
III	483(52.3)	666(45.5)	
IV	221(23.9)	121(8.3)	
ART Start Year, n(%)			
2009	247(26.7)	437(29.9)	<0.0001
2010	190(20.6)	321(22.0)	
2011	154(16.7)	285(19.5)	
2012	128(13.8)	253(17.3)	
2013	205(22.2)	166(11.3)	
Age, median (IQR)	33(27-40)	31(27-38)	0.004
CD4 counts, median(IQR)	141(66-231)	152(84-210)	0.196
Weight (kg), median (IQR)	49(43-55)	50(45-58)	0.00017

3.4.2 Description of Composite Treatment Outcomes

The composite outcome, defined in Section 3.2.2, was observed among 595(24.9%) patients with rate per 100 person years of 12 (95%CI: 11.1-13.0). Amongst those who were initiated with NVP 370(25.3%) patients experienced the events. Of these death, lost to follow up, NNRTI substitution, and discontinuation accounted for 99(26.8%), 122(33.0%), 137(37.0%), and 12(3.2%), respectively. Whiles among those who were initiated with EFV 225(24.4%) experienced the event. Of these death, lost to follow up, NNRTI substitution, and discontinuation accounted for 71(7.7%), 108(11.07%), 37(4.0%) and 9(0.97%), respectively. A total of 818 (55.6%) and 515(55.7%) patients stayed in their original regimen when initiated with NVP and EFV treatments, respec-

tively. One-in-four patients who were initiated with NVP as NNRTI drug experienced the event during the follow up period (see Figure 3.1).

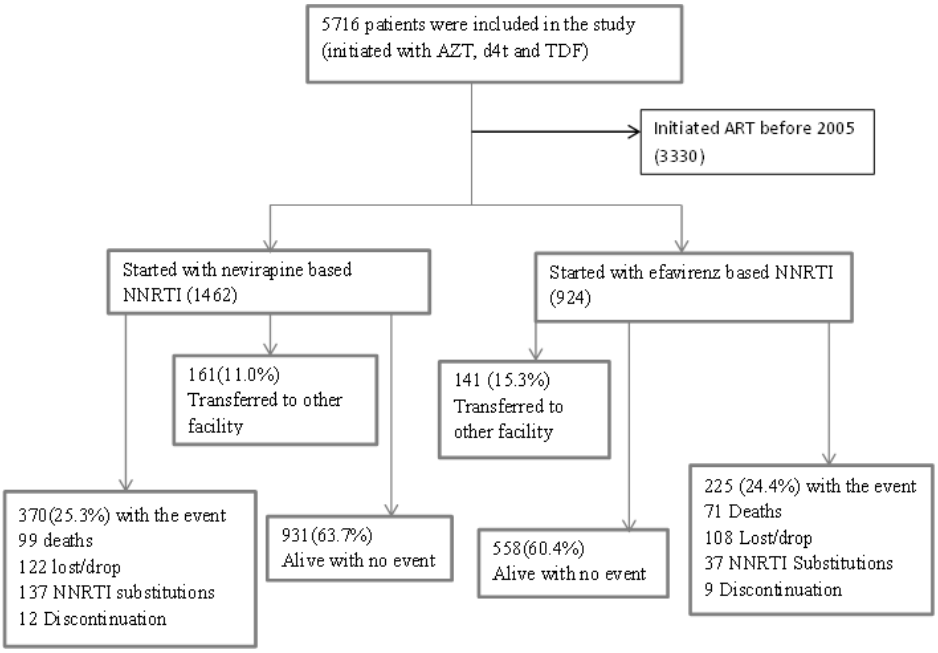


Figure 3.1. Schematic presentation showing study participants with their treatment outcomes assessed from January 2009 to December 2013.

3.4.3 Analysis of Time to Composite Treatment Outcomes

The cohort was followed for a maximum of 61 months. The cumulative probability of staying 59 months was 82.7% and contributed a total of 4958.75 person-years of the data with mean follow-up time of 25 (sd=17.8) months. The rate of composite outcome was high during the first 10 months after ART initiation. Figure 3.2 shows that the Kaplan-Meier survival curve for composite outcome decreased sharply after 40 months. Whereas the curve for death shows steady decrease.

Log-rank test was used to test the difference between the categories of baseline covariates with the probability of death. This test revealed the presence of significant difference among the categories of baseline NNRTI, and NRTI drugs (Figure 3.3). The plots of other baseline covariates are presented in Figure B.1 and B.2 in Appendix B.

Table 3.2 presents the results obtained from a Cox-PH model that was fitted in order to test the effect of baseline covariates on time to death and time to composite

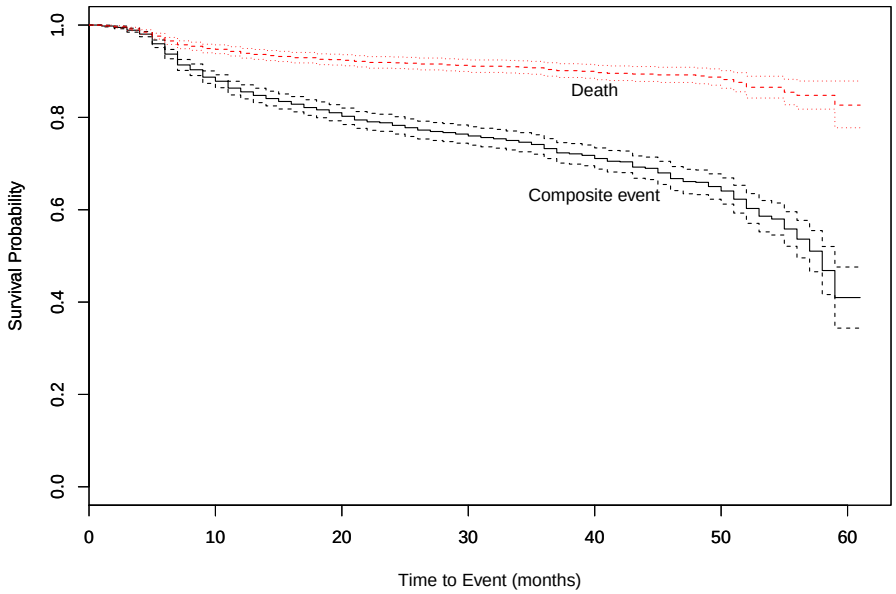


Figure 3.2. Kaplan-Meier survival curves for death and composite outcome among HIV/AIDS patients at Gondar University Hospital, 2013.

outcome. The patients could experience more than one event during follow-up, and time to the first event was used for analysis. In the primary analysis, only death was considered as an event of interest. The risk of death was not different among patients on NVP compared with EFV (AHR=1.02 (95%CI: 0.81-1.58)) which was also observed in the log-rank test. Other baseline covariates considered in the Cox-PH regression analysis were sex, age, NRTI backbone, WHO staging, baseline CD4 cell counts, functional status and ART start years. In the adjusted analysis, patients who were initiated with ART at age greater than 40 years (HR=1.65, 95%CI: 1.21-2.31), TDF backbone as compared to AZT (HR=1.90, 95%CI: 1.35-2.67) and WHO stage IV or III as compared to stage II or I (HR=1.77, 95%CI: 1.22-2.56) had higher risk of death. Whilst CD4 cell counts higher than 200 $cell/mm^3$ (HR=0.40, 95%CI: 0.25-0.64) and functional status of working (HR=0.51, 95%CI=0.37-0.71) had reduced risk of death as compared to ambulatory or bedridden.

At baseline, patients who were lost to follow up had similar CD4 cell counts, age, WHO stage and functional status as patients who died during the follow up period. Following (Brinkhof et al., 2009) who showed that lost to follow up patients

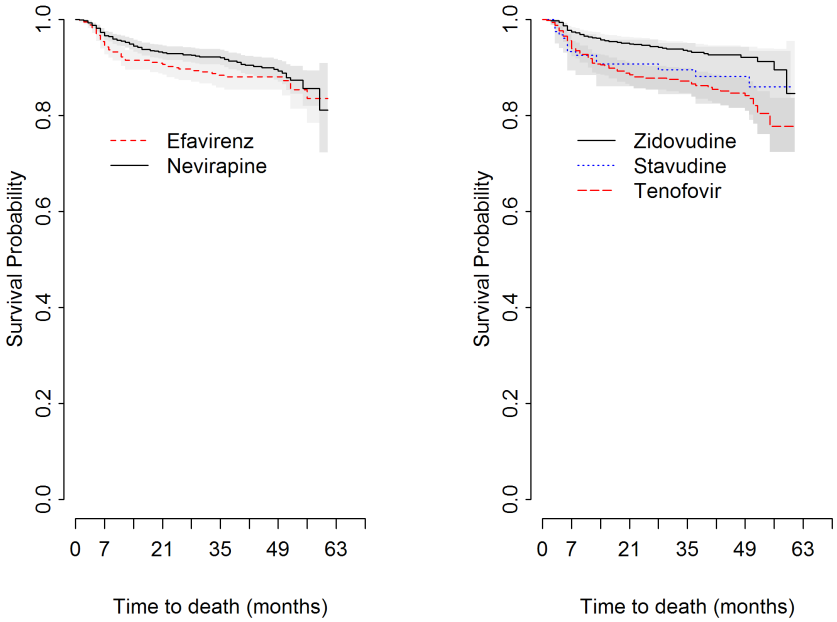


Figure 3.3. Kaplan-Meier survival curve for HIV/AIDS patients at Gondar University Hospital, 2013. Left panel: NNRTI. right panel: NRTI.

were often found to be dead when tracked a sensitivity analysis was conducted by considering lost to follow up as an event. The results are presented in Table 3.2. Despite few changes, the effects of these covariates were similar with the primary analysis. However, sex and d4t backbone were significant in the sensitivity analysis, but not in the primary analysis. The risk of composite outcome was 1.30 (95%CI: 1.07-1.58) times higher among males patients than the female patients. Whilst the hazard of composite outcome was 1.73 (95%CI: 1.22-2.46) and 1.89 (95%CI: 1.50-2.38) times higher for patients who initiated with d4t and TDF compared with AZT, respectively.

Characteristics at baseline might be different for patients initiated with NVP or EFV. A secondary analysis was performed using a propensity score weighting of the model. Covariates NRTI, age, gender, baseline functional status, WHO stage, and CD4 cell counts were included in the propensity model. The continuous measure of propensity score was used as an additional covariate in the final model. The results revealed that the risk of death or composite outcome are not different among the

Table 3.2. Cox-regression analysis of factors associated with the composite outcome of treatment failure on first-line ART in Northwest Ethiopia, 2013.

	Primary analysis (lost as censored)			Sensitivity analysis(lost as event)		
Covariate	UHR(95%CI)	AHR(95%CI)	p-value	UHR(95%CI)	AHR(95%CI)	p-value
Sex, n(%)						
Female	1	1		1	1	
Male	1.18(0.88-1.57)	0.97(0.72-1.31)	0.85	1.39(1.25-1.68)	1.30(1.07-1.58)	0.007
Age						
< 40 years	1	1		1		
≥ 40 years	1.66(1.23-2.24)	1.65(1.21-1.31)	0.001	1.12(0.90-1.39)	1.02(0.81-1.27)	0.88
NNRTI						
Efavirenz	1	1		1	1	
Nevirapine	0.75(0.56-0.99)	1.19(0.85-1.65)	0.29	0.65(0.54-0.79)	1.02(0.81-1.58)	0.88
NRTI backbone						
Zidovudine	1	1		1	1	
Stavudine	1.71(0.99-2.95)	1.20(0.68-2.12)	0.52	2.21(1.58-3.10)	1.73(1.22-2.46)	0.002
Tenofovir	2.20(1.62-2.98)	1.90(1.35-2.67)	.0001	2.19(1.78-2.69)	1.89(1.50-2.38)	<.001
WHO stage						
I and II	1	1		1	1	
III and IV	2.30(1.62-3.27)	1.77(1.22-2.56)	.0002	1.81(1.45-2.26)	1.37(1.08-1.74)	0.008
Base CD4 cells						
<200 cells/mm3	1	1		1	1	
≥ 200 cells/mm3	0.33(0.21-0.52)	0.40(0.25-0.64)	.0001	0.67(0.52-0.85)	0.79(0.62-1.01)	0.05
Functional status						
Ambulatory/Bedridden	1	1		1	1	
Working	0.35(0.26-0.48)	0.51(0.37-0.71)	<.01	0.40(0.32-0.49)	0.53(0.42-0.66)	<.001
ART start Year						
Before 2010	1	1		1	1	
Since 2010	0.67(0.50-0.91)	0.88(0.65-1.21)	0.44	0.81(0.65-0.98)	1.01(0.82-1.25)	0.93

NNRTI drugs which is consistent with multivariate Cox-PH regression model.

Comparison of Efavirenz versus Nevirapine on NRTI Backbone

In order to compare the risk of composite outcome on the two NNRTI drugs a Cox-PH model was fitted adjusting for NRTI backbones. On a backbone of d4t and AZT, there were no significant differences in the risk of composite outcome between NVP and EFV after adjusting for other baseline covariates (sex, WHO stage, functional status and ART start year). In a similar analysis for TDF, the risk of composite outcome was 1.51 (95%CI: 1.01-2.28) times higher on NVP as compared to EFV.

Comparison of Zidovudine, Stavudine and Tenofovir on NNRTI Drugs

Similarly, NRTI backbones were compared using a Cox-PH model adjusting for NNRTI drugs. In EFV of an NNRTI option, the risk of composite outcomes on TDF and D4T were 1.83 (95%CI: 1.36-2.47), and 1.70 (95%CI: 1.11-2.61), respectively. Likewise, patients who initiated with NVP, the hazard of composite outcomes on TDF and d4t

were 2.09 (95%CI: 1.38-3.18), and 0.97 (95%CI: 0.46-2.04), respectively. Thus, there were significant differences in the risk of composite outcomes on TDF as compared to AZT in both NNRTI drugs. Two way interaction of treatment with sex was tested and the terms was not statistically significant.

3.4.4 Longitudinal Modeling of CD4 Cell Counts Evolution

The cohort was followed for a maximum of 61 months. The median number of repeated measurements was 4 (IQR=2-5) with a maximum of 10 measurements per patient. Time from ART initiation until first regimen change was considered for analysis presented in this Chapter. The semi-parametric models discussed in Section 3.3.2 were applied to the data. Note that, for the analysis presented in this chapter, the response variable is the the logarithm of CD4 cell counts. Figure 3.4A shows an example of the CD4 cell counts for randomly selected individuals. The observed individual profile with its mean plot showed an overall increase in the level of CD4 cell counts over time as shown in Figure 3.4B. The rise was relativity quick during the first 5 months since the start of ART and became steady after month 5. The estimated overall trend of the data is shown in Figure 3.4C. In addition, as discussed in Section 3.3.2, the semi-parametric mixed effect model allows the estimation of the rate of change of CD4 cell counts (i.e. the first derivative). A derivative equal to zero implies a constant trend with respect to time. The rate of change in CD4 cell counts over time under ART treatment is shown in Figure 3.4D. Note how the derivative decreases sharply to zero in the first 10 months after the initiation of the ART treatment and thereafter remain relatively stable and closed to zero.

Non-Nucleotide Reverse Transcriptase Inhibitor (NNRTI) Drugs

The Semi-parametric mixed effects model also allows us to compare between different treatments. Figure 3.5 presents a comparison between EFV and NVP. Regardless of the type of original regimen, log(CD4) cell counts increases immediately after initiation of ART. This can be clearly seen in Figure 3.5A and Figure 3.5B which show the predicted trend and the rate of change over time, respectively. For both treatments, a sharp increase is observed in the first 10 months after initiation of ART. Further, Figure 3.5C and Figure 3.5D show the difference between the two treatments in both estimated log(CD4) trend and the rate of change in log(CD4) cell counts. A curve for which the 95% confidence band covers the value of zero indicates that the difference is not statistically significant. Figure 3.5C and Figure 3.5D reveal that the response for the two NNRTI drugs seems to be differ only in the first few months after

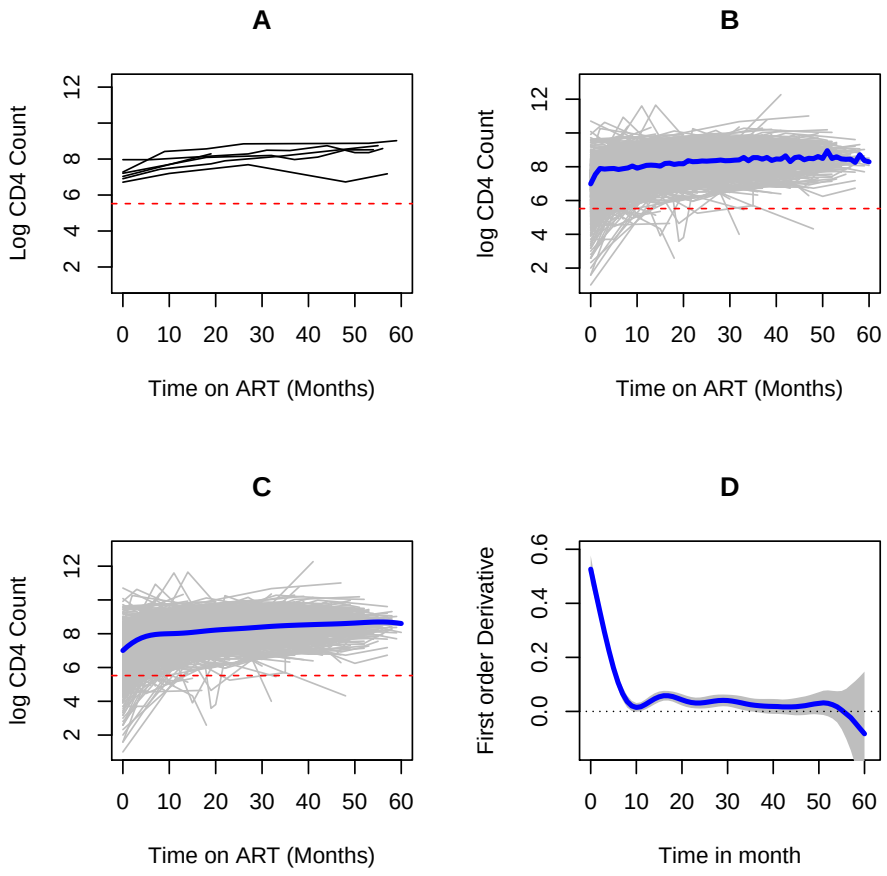


Figure 3.4. Log(CD4) cell counts over time. Panel A: individual log(CD4) cell count trajectories of 5 selected patients. Panel B: individual log(CD4) cell count trajectories with observed average plot, Panel C: individual log cd4 count trajectories with predicted CD4 cell count trend (by the semi-parametric mixed effects model) and Panel D: the estimated rate of change (the first derivative) over time.

initiation of ART and thereafter the treatments are not statistically different in both CD4 cell count levels and the rate of change since the 95% confidence bands cover the value of zero in both cases.

Nucleotide Reverse Transcriptase Inhibitor (NRTI) Backbone

The trend of log(CD4) cell counts was estimated for the three NRTI backbones (AZT, d4t and TDF). Figure 3.6A and the upper panels in Figure 3.7 show that, 10 months

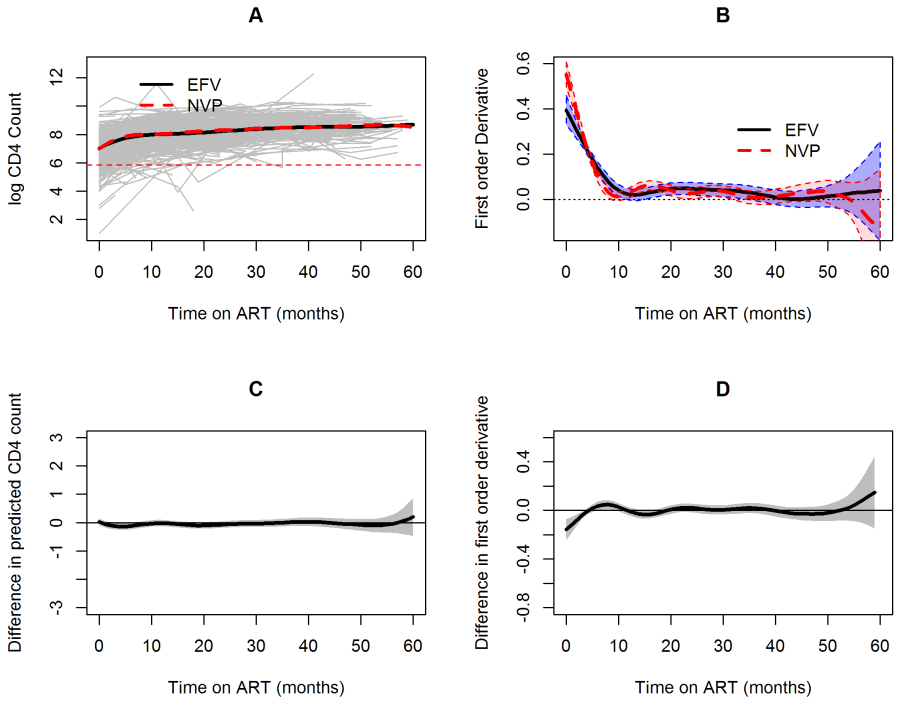


Figure 3.5. Response of log(CD4) cell counts for two NNRTI drugs (EFV and NVP). Panel A: individual and average profiles. Panel B: rate of change over time for CD4 cell counts. Panel C: estimated difference between the trend for EFV and NVP. Panel D: estimated difference between the rate of change (i.e. the first derivative) of log(CD4) cell counts, for EFV and NVP. Whenever the 95% confidence band for the curve (the gray area) covers the value of zero the difference between the two treatment is not significant.

after the initiation of treatment, patients treated with d4t reached higher levels of log(CD4) cell counts compared to the patients that were treated with AZT and TDF as the backbones. Note that from 10 months after the initiation of the treatment the rate of change in log(CD4) cell counts is the same for all backbones (see Figure 3.6B and the lower panels in Figure 3.7).

3.5 Discussion

The Scaled-up of antiretroviral therapy has shown to be effective in improving quality of life, reducing morbidity and mortality, and increase productivity in patients infected with HIV. However, there is a need to better understand the characteris-

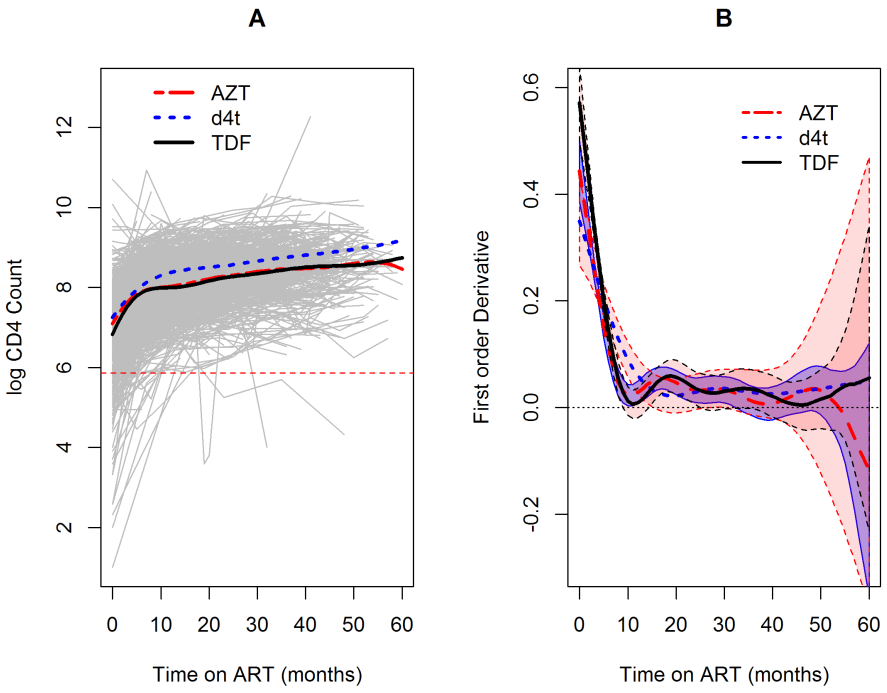


Figure 3.6. Individual and average profile plots of NRTI backbons. Panel A: fitted individual and mean plots for each backbone. Panel B: estimated rate of change for each backbone and 95% confidence band.

tics of long-term outcomes of treatment combinations. The main aim of this study was to determine and compare the long-term response of patients on NVP or EFV based first line ART regimen in Northwest Ethiopia. The analysis presented in this chapter was focused on a hospital data and the methodology presented in the chapter can be applied routinely to similar dataset from other treatment centers. We have shown that treatment responses were comparable whether NVP or EFV was chosen to initiate ART for HIV-positive patients in Gondar University Hospital, Ethiopia. Statistical significant difference was not detected in the risk of death or composite outcome among patients who initiated with the two NNRTI drugs after adjusting for baseline covariates. This is in agreement with other studies conducted in central Ethiopia (Tirfe et al., 2013), Ghana (Sarfo et al., 2013) and Botswana (Shipton et al., 2009) which indicated a non-significant difference in the long-term effectiveness of EFV and NVP based ART regimens in the population. However, it is in contrast with findings from observational study of the ART Cohort Collaboration (ART-CC)

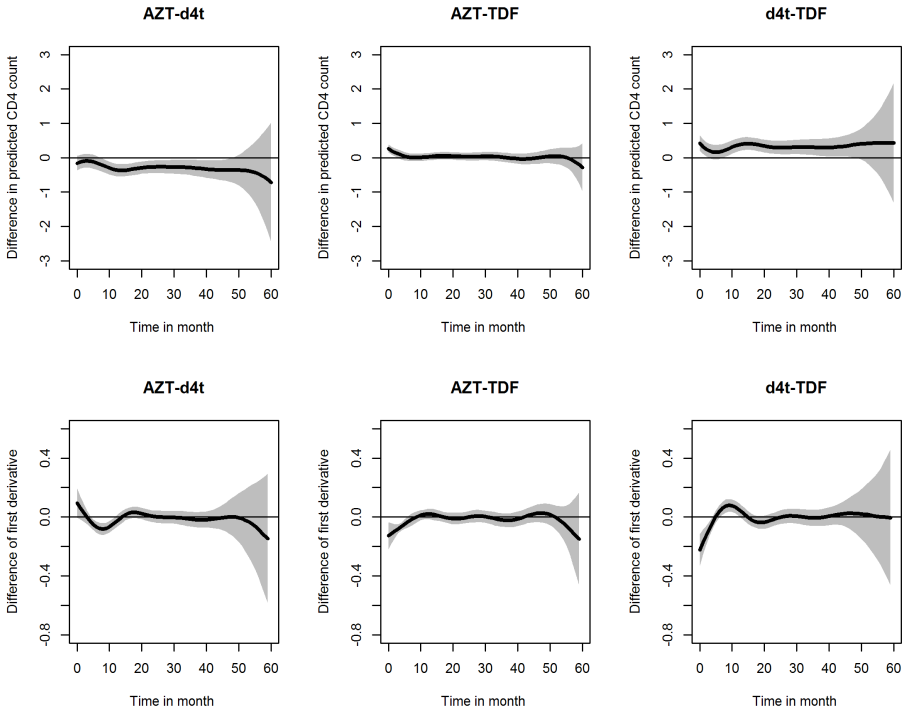


Figure 3.7. Estimated pairwise difference between the NRTI backbones. Upper panels: estimated difference between the CD4 trend over time for each pair of backbone. Lower panels: estimated difference of the rate of change of CD4 cell counts for each pair of backbones.

(Mugavero et al., 2008) and the HIV-CAUSAL collaboration reported by Cain and Hernn (2013); Collaboration et al. (2012) in which patients who initiated with NVP has an increased risk of treatment failure as compared to EFV. The systematic review reported by Pillay et al. (2013) and the meta analysis presented in Chapter 2 revealed that EFV based first line ART is a preferred NNRTI drug in first line treatment regimen for HIV treatment as it has lower risk for treatment failure.

We have shown that there is a difference in the risk of composite outcomes between patients who were initiated with TDF and those with AZT after controlling for NNRTI drugs. The risk on composite outcome for TDF when combined with NVP is two times higher as compared to AZT. This was supported by studies reported in Zambia by Benjamin et al. (2011), Nigeria by Scarsi et al. (2010) and France by Rey et al. (2009) in which TDF containing regimen was associated with higher mortality and virologic failure. In contrast with our study, studies conducted in South African reported

by Velen et al. (2013) and Botswana showed as TDF appeared to perform better than AZT with lower mortality. We have shown in the adjusted analysis the risk of composite outcome on TDF backbone has increased by 50% on NVP as compared to EFV indicating that TDF is more effective when administered with EFV than NVP. This is in line with finding reported in Thailand by Manosuthi et al. (2010) in which the frequency of TDF-associated renal impairment was significantly higher in patients receiving TDF plus NVP compared to TDF plus EFV regimen.

Our analysis reveals that there is a 73% increased risk of composite outcome for patients who were initiated with d4t containing regimen as compared to AZT containing regimen. The risk of composite outcome was 70% and 72% higher on d4t as compared to AZT for NVP and EFV, respectively. This was supported by a study conducted in Cameron reported in Laurent et al. (2008) in which patients initiated with d4t has increased risk of toxicity. However, this finding contradicts the results in a study conducted in Kenya (Kwobah et al., 2012) in which d4t leads to a decreased risk of treatment failure as compared to AZT. The risk of composite outcomes was not statistically significant for d4t with any of the NNRTI drugs when lost to follow up patients were assumed censored. This indicates that the difference in risk on composite outcome on d4t versus AZT was due to lost to follow up cases. The risk of composite outcome was higher among patients who initiated ART at clinical stage 3 or 4, low CD4 cell count and ambulatory or bedridden functional status during ART initiation which is inline with other studies (Alave et al., 2013; Mutasa-Apollo et al., 2014).

Results reported in Table B.2 in Appendix B revealed that TDF has higher risk for different event types compared to AZT which is consistent with other studies (Benjamin et al., 2011; Rey et al., 2009). Although the effect of d4t and AZT on death and NNRTI modification was the same, statistical significant difference was observed on lost to follow up. Those patients who initiated with d4t had about two fold risk of lost to follow up compared to those who initiated with AZT. This might be due to the long-term irreversible side effects of d4t (WHO, 2010). Patients who initiated with NVP had 2.5 times higher risk of modifying the NNRTI drug than those patients who initiated with EFV. Initiating with TDF of NRTI backbone has also higher risk of NNRTI modification than those who initiated with AZT. This is in contrast with finding of the study in South Africa reported in Brennan et al. (2013) in which rate of substitution was lower among those who initiated with TDF than AZT or d4t.

In Ethiopia, similar to other resource-limited countries, CD4 cell counts are used as a main surrogate marker of treatment response due to the fact that viral load

monitoring is not easily accessible. We proposed a semi-parametric mixed effects model for the longitudinal evolution of CD4 cell counts in order to investigate response to treatment based on individual and average profile plots. We have shown that there is an overall increase in CD4 cell counts over time which is consistent with other studies (Luz et al., 2014; McManus et al., 2015; Nash et al., 2008; Wright et al., 2013). Furthermore, the rate of change in CD4 cell count increase in response of treatment was high during the first 10 months and stabilized later. The analysis presented in this chapter reveals that there was no difference in the trend of CD4 cell counts in the long-run among patients who initiated with EFV or NVP. This result is in line with a study conducted in Ghana (Barry et al., 2013). Considering only the NRTI backbones, there was difference in the evolution of log(CD4) cell counts which is consistent with Bongiovanni and Tordato (2014). Even though d4t is less preferable by clinicians, it has better effect in improving CD4 cell counts than AZT and TDF which is supported in (Wainberg et al., 2007). In the long run, the improvement of CD4 level was better among patients who initiated with TDF. Even though d4t together with NVP was found to be the combination which offers better performance in increasing CD4 cells counts during the first 10 months since initiation, the rate of increase was not as good as the other combinations. All options of original regimen have similar effect between month 20 and 50. The results obtained from the semi-parametric mixed effect model can be affected by the different time to lost to follow up or death reported in this chapter. A joint model for CD4 cell counts and time to composite outcome will be discussed in Chapter 5.

The main limitation of this study was the limited number of variables that were measured in the treatment center. Since the study is based on retrospective data, many covariates were not measured. Some of the variables which were not measured includes nutritional status of the patients, adherence, opportunistic infections, viral load, side effects and reasons of regimen change. This could affect the findings of this study. Another limitation was the definition of treatment failure as composite outcome which is broad. This might overestimate the event which makes comparison with other findings difficult. Therefore, the results should be interpreted with insight of these limitations.

In conclusion, the analysis presented in this chapter revealed that the long-term treatment outcomes did not depend on NNRTI groups of the regimen. The outcomes of EFV containing regimen is comparable with NVP containing regimen. However, difference was observed for NRTI backbones chosen to initiate the treatment of HIV-infected patients in Ethiopia. The response of CD4 cell counts to treatment was high during the first 10 months and stable then after. Further clinical study is warranted

in resource limited settings to investigate the effect of EFV and NVP on long-term outcomes.

Chapter 4

Model-Based Prediction of CD4 Cells Counts in HIV-Infected Adults on Antiretroviral Therapy in Northwest Ethiopia

4.1 Introduction

In order to allow a rapid roll-out of ART, countries use the World Health Organization (WHO) public health guideline, which proposes standard first line therapy, together with treatment initiation and switch guided by clinical disease progression and, where possible, with monitoring of CD4 cell counts (Gilks et al., 2006). As mentioned in Chapter 2, the standard therapy consists of three NRTI (AZT, TDF and d4t) and one NNRTI (EFV and NVP). In resource limited settings, WHO recommends the use of two NNRTI as first line ART regimen (Blas-García et al., 2011). The number of CD4 $cells/mm^3$ of blood has been widely used as an important biomarker for progression to AIDS. Measurement of CD4 cell counts is a crucial parameter in the management of HIV patients.

In many low income countries, according to the CD4 cell count criteria, the patient would be eligible when his/her CD4 cells counts dropped below a given threshold value. The threshold value has been changed from less than 200 $cells/mm^3$ in 2006 to less than 350 $cells/mm^3$ in 2010 (WHO, 2006, 2010). The WHO 2013 guideline consolidated ART guidelines recommend that ART be initiated for all patients with CD4 cell count 500 $cells/mm^3$ or less. In 2015, WHO (2015) recommended HIV-treat all approach as presented based on two clinical trial outcomes (Group et al., 2015a,b). However, several studies are against earlier initiation of ART in patients who have

high CD4 cell counts (Collaboration et al., 2011; Sabin and Phillips, 2009). This is because early exposure to ART may precipitate early evolution of resistance and unnecessary side-effects (Wood et al., 2005).

Limited studies were focused on the trend of CD4 cell counts for patients on ART especially in Sub-Saharan Africa. A longitudinal study conducted in eastern Ethiopia (Reda et al., 2013) used a linear mixed effect model which ignores the non-linear nature of the evolution. Similarly, Lubyayi et al. (2015) analysed longitudinal study in Uganda and used cubic time effect to account the non-linear nature of CD4 cell counts. Awoke et al. (2016) proposed a semi-parametric mixed effect model to investigate CD4 cell counts response to treatments. In this Chapter, we proposed a flexible parametric modeling, the fractional polynomials framework proposed by Royston and Altman (1994), to predict a subject specific evolution of CD4 cell counts over time. We focus on two main issues: (1) early prediction of CD4 cell counts under a specific treatment and (2) estimation of the time to cross a given CD4 threshold under treatment. The later allows us to compare the efficacy between different treatments.

This chapter is structured as follows; the proposed modeling approach is formulated in Section 4.2. In Section 4.3 the proposed model is applied to the ART data of Gondar University Hospital. A discussion is provided in Section 4.4.

4.2 Methods

4.2.1 Modeling CD4 Cell Counts using Subject Specific Models

We considered a linear mixed effects model presented in Section 3.3, given by

$$\mathbf{Y}_{it_{ij}} = \mathbf{X}_i\beta + \mathbf{Z}_i\mathbf{b}_i + \varepsilon_{it_{ij}}. \tag{4.1}$$

Here, $\mathbf{Y}_{it_{ij}}(i = 1,\ldots,n_i, j = 1,\ldots,m_i)$ is n_i-dimensional response vector of log transformed CD4 cell counts for individual i at time t_{ij}, $\mathbf{X}_i$ and $\mathbf{Z}_i$ are $n_i \times p$ and $n_i \times q$ dimensional fixed and random effects model matrices, respectively. The vector $\boldsymbol{\beta}$ is a p-dimensional vector of fixed effects and $\mathbf{b}_i$ is a q-dimensional subject specific vector of random effects $\mathbf{b_i} \sim \mathcal{N}(\mathbf{0}, \mathbf{\Sigma_b})$ and $\varepsilon_{it_{ij}}$ is the random error term, $\varepsilon_{it_{ij}} \sim \mathcal{N}(0, \sigma^2 I_{n_i})$.

In what follows we discuss the usage of the mixed effects model for subject specific model based prediction of the CD4 cell counts (Section 4.2.2), and the estimation of the time to cross a CD4 cell count threshold under a given treatment (Section 4.2.3). A flexible model formulation for the mean structure using fractional polynomial random effect model is presented in Section 4.2.4

4.2.2 Model Based Prediction

The primary goal of the analysis presented in this chapter is to obtain a model based subject specific prediction under a specific treatment regimen of long term level of CD4 cell counts as early as possible. For this purpose, a two stage procedure was used. First a mixed effect model is fitted using only data between 0 to 30 months. We term this period is *the estimation period*. In the second stage, the fitted model is used to predict the CD4 cell counts in the period 31 to 68 months. We term the second period is *the prediction period*. The procedure is illustrated in Figure 4.1a, where t_0, t_i, and t_k represented the initiation time of ART treatment, estimation period and prediction period, respectively. Note that only data within the estimation period, i.e, between t_0 and t_i, is used in order to estimate the unknown parameters of the model. The observed and predicted values in both estimation and prediction period are compared and their correlation was calculated in order to determine how good the model predicts the long-term CD4 cell counts. Figure 4.2 presents profile for selected individuals.

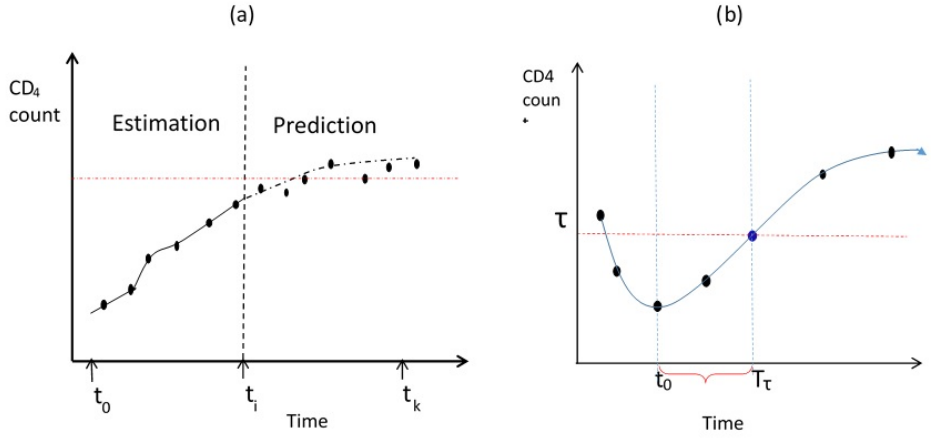


Figure 4.1. Panel a: an illustrative example for the estimation and prediction periods. Panel b: an illustrative example of a patient that cross the therhold of τ at time t_τ.

4.2.3 Model Based Prediction of Time to Cross a Pre-specified CD4 Threshold

The linear mixed effect model formulated in (4.1) can be used to predict the time that a subject will cross a pre specified threshold level of CD4 cell counts. Let τ be a thresholds value, t_0 is the time to initiate ART, and T_τ the first time in which the

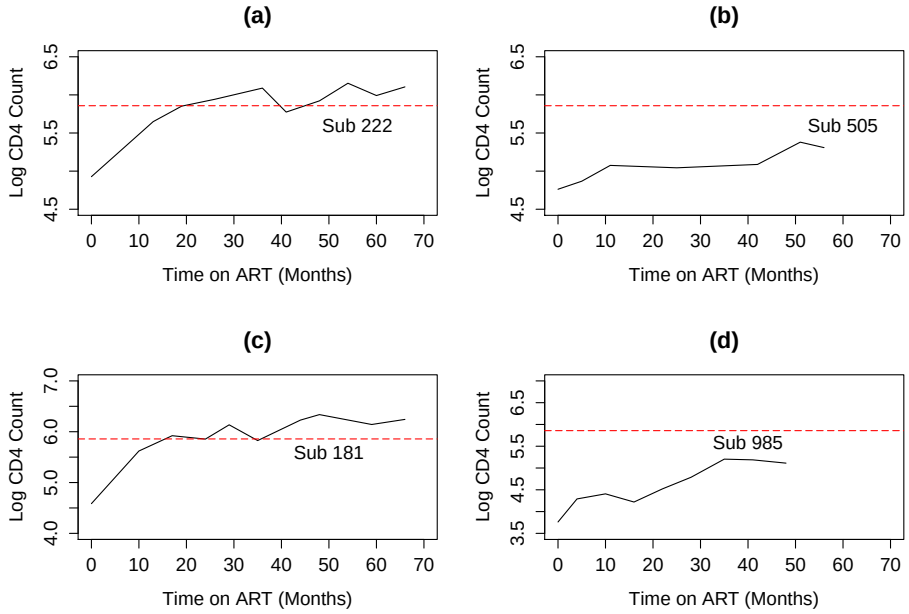


Figure 4.2. Individual profiles for selected patients who initiated ART with NVP (upper panel) and EFV (lower panel) who crossed and remain below the a threshold of 350.

subject CD4 cell counts crossed the threshold defined by

$$\top_\tau = min\{j \geq 1 : Y_{it_{ij}} \geq \tau\}, \tag{4.2}$$

Figure 4.1b illustrates schematically the trajectory of an individual who initiated ART at t_0 and cross the threshold at t_τ. Our aim is to estimate the time t_τ.
For the analysis presented in this chapter, three different threshold values of CD4 level were used for illustration: $\tau = log(200) = 5.2983$, $\tau = log(350) = 5.8579$, and $\tau = log(500) = 6.21461$ $\log(cells/mm^3)$. The threshold value of 200 $cells/mm^3$ was recommended by WHO (2006) as a criteria to initiate ART. In 2010, the criteria was modified to 350 $cells/mm^3$ by WHO (2010). In order to reduce the time between registration to treatment initiation, the 2013 guidelines recommended to initiate ART when the level of CD4 cell counts is less than 500 $cells/mm^3$. Currently, test and treat all approach is used irrespective of CD4 cell count level (WHO, 2015).

Based on the results presented in Reddy et al. (2016) the expected time for individual i to reach a CD4 cell count greater or equal to threshold τ can be express

as

$$\begin{aligned} E(\top_\tau) &= t_{i1}P(Y_{it_{i1}} \geq \tau) + t_{i2}P(Y_{it_{i1}} < \tau, Y_{it_{i2}} \geq \tau) \\ &+ t_{i3}P(Y_{it_{i1}} < \tau, Y_{it_{i2}} < \tau, Y_{it_{i3}} \geq \tau) + \dots \\ &= \textstyle\sum_{j=1}^{\infty} t_{ij} S_{ij}, \end{aligned} \tag{4.3}$$

where t_{ij} is the time corresponding to the j^{th} visit for individual i, and S_{ij} is the probability of individual i experiencing the event or stopping at t_{ij}. In practice, the infinite series will be truncated at a time point relevant to the study subjects Reddy et al. (2016). Conditioned on the random effects, the mixed model formulated in (4.1) implies that

$$Y_{ij}|\boldsymbol{b}_i \sim \mathcal{N}\left(X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i, \Sigma_i\right).$$

Hence, the joint probability that form S_{ij} reduces to the product of the individual probabilities, which can be expressed as

$$\begin{aligned} S_{ij}(X_i, Z_i, \boldsymbol{b}_i, \boldsymbol{\beta}) &= P(Y_{it_{i1}} < \tau)P(Y_{it_{i2}} < \tau)P(Y_{it_{i3}} < \tau), \dots, P(Y_{it_{ij}} \geq \tau) \\ &= [\tilde{\phi}_{i1}(\tau)][\tilde{\phi}_{i2}(\tau)], \dots, [\tilde{\phi}_{ij-1}][1 - \tilde{\phi}_{ij}(\tau)], \end{aligned} \tag{4.4}$$

where $\tilde{\phi}_{ij}(\tau)$ is a cumulative normal distribution with mean $X_i\boldsymbol{\beta} + Z_i\boldsymbol{b}_i$ and variance σ^2, that is

$$\tilde{\phi}_{ij}(\tau) = \phi\left(\tfrac{\tau - \boldsymbol{X_i\beta} - \boldsymbol{Z_i b_i}}{\sigma}\right), \tag{4.5}$$

Note that both fixed and random effects defined in the mixed model formulation in (4.1) are used to calculate $\tilde{\phi}_{ij}(\tau)$. We elaborate this point in section 4.2.3 when we discuss the mean structure of the model.

The expected time to attain CD4 cell count above the threshold in (4.3) can be computed by substituting each unknown parameter by its estimate. As shown by Reddy et al. (2016), the non-parametric bootstrap method is used to compute standard errors and 95% confidence intervals for $\hat{\top}_\tau$. Four steps procedure were applied to compute the standard errors and confidence intervals:

1. Individual i is removed from the full dataset resulting $N-1$ samples.
2. Sample $N-1$ subjects with replacement from the dataset in step 1.
3. Append the data of individual i to the bootstrap sample.
4. compute $\hat{\top}_\tau$.

This procedure is repeated 1000 times.

4.2.4 Flexible Modeling of the Mean Structure

The fractional polynomial model was proposed by Royston et al. (2006) as a flexible parametric approach to describe the dependency between a response of primary interest and continuous covariates (Royston and Sauerbrei, 2008). The responses of primary interests in the current application is the log transformed CD4 cell counts and the covariate is time under ART treatment measured in months. The mean structure of an m order fractional polynomial model can be formulated as

$$\sum_{l=0}^{m} \beta_l H_l(t) + \sum_{l=0}^{m} b_{li} H_l(t), \tag{4.6}$$

Where m is an integer, $p_1 \leq p_2 \leq \cdots \leq p_m$ is a sequence of known powers and $H_l(t)$ is a transformation function given by

$$H_l(t) = \begin{cases} t^{p_l} & \text{if } p_l \neq p_{l-1} \ , \\ H_{l-1}(t) \times \log(t) & \text{if } p_l = p_{l-1}, \end{cases} \tag{4.7}$$

with $p_0 = 0$ and $H_0(t) = 1$. Note that there are two components in the mean structure. The first consists of the fixed parameters β_l and the second the subject specific parameters b_{li}.

For the analysis presented below, both first ($m = 1$) and second ($m = 2$) order factional polynomials were considered and the model with the best goodness of fit, based on Akaike Information Criteria (AIC) (Akaike, 1973), was selected. For a second order mixed effect fractional polynomial, the mean structure is given by

$$\mathbf{X}_i\beta + \mathbf{Z}_i\mathbf{b}_i = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})t_{ij}^{p_1} + (\beta_2 + b_{2i})t_{ij}^{p_2}, \tag{4.8}$$

Here, β_0, β_1, and β_2 are fixed effect parameters, and b_{0i}, b_{1i}, and b_{2i} are subject specific parameters. To compute cumulative probability above the threshold, the unknown values of (4.5) were substituted by; $\boldsymbol{X_i\beta} = \beta_0 + \beta_1 t_{ij}^{p_1} + \beta_2 t_{ij}^{p_2}$ and $\boldsymbol{Z_i b_i} = b_{0i} + b_{1i} t_{ij}^{p_1} + b_{2i} t_{ij}^{p_2}$.

4.3 Results

The median follow up period was 27.10 months (IQR=12.1-43.1). The cohort contributed a total of 236.58 per 100 person years of follow up. The contributions were 237.11 per 100 person years and 234.28 per 100 person years by NVP and EFV regimens, respectively. There is small difference between the two treatment groups with regard to time contributed for the observation period. The median number of repeated measurements was 3 (IQR=2-6) with a maximum of 11 measurements per patient. CD4 cell counts ranges between 2 and 2057 cells/mm^3.

The evolution of CD4 cell counts over time for both treatment groups is shown in Figure 4.3 and reveal substantial variability between subjects. At baseline, 68.09% and 64.32% of the patients who initiated with EFV and NVP containing regimens had CD4 cell counts below 200 $cells/mm^3$, respectively. The percentage lower than a threshold of 350 $cells/mm^3$ increased to 94.53% and 91.97% for those who initiated with EFV and NVP, respectively.

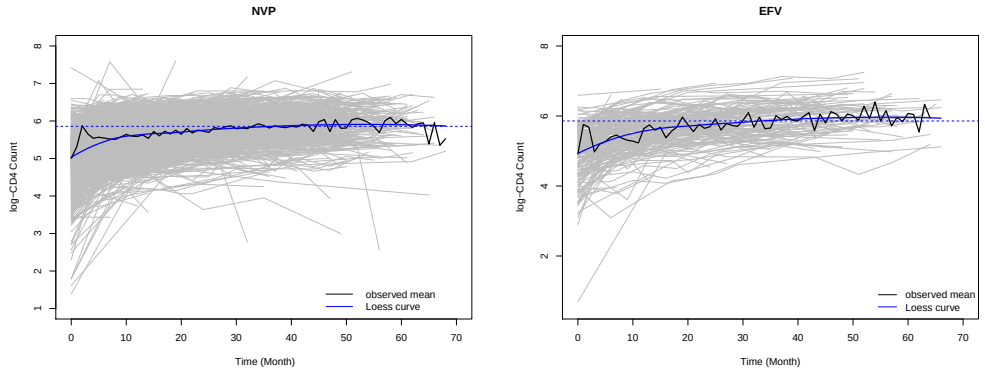


Figure 4.3. Individual and average profiles for patients who initiated ART with NVP (left panel) and EFV (right panel).

4.3.1 Model Based Prediction of CD4 cell Counts: Estimation Period 0-68 Months

The fractional polynomial mixed effect model discussed in the method section was used to estimate subject-specific evolutions for the log(CD4) cell counts within the follow-up period of the study. First and second order fractional polynomial mixed effect models were considered and compared. To select the power of the fractional polynomial mixed effect model, powers in the range $\{-2, -1.5, \ldots, 2.5, 3\}$ were considered. According to the AIC value, a second order fractional polynomial mixed effect

model (FP2) was selected. The smallest value of AIC was obtained at $(p_1 = 0, p_2 = 0.5)$ and $(p_1 = 0, p_2 = 0)$ for NVP and EFV ART regimen, respectively. The comparison of the models is presented in Figure C.1 and C.2 in Appendix C.

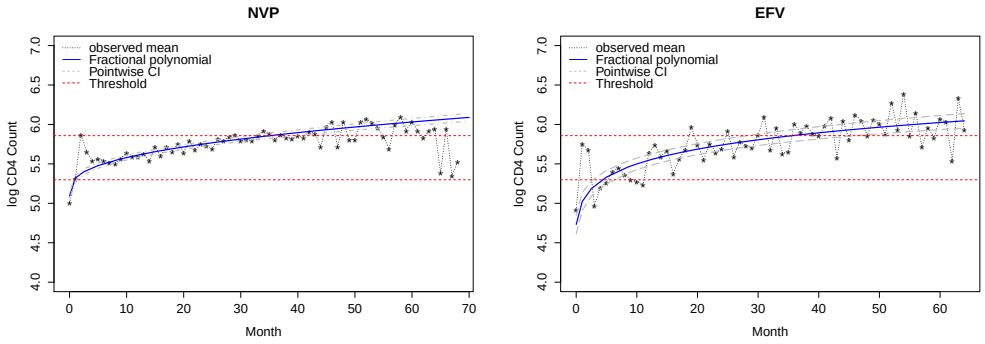


Figure 4.4. Model based predicted means for log(CD4) cell counts for NVP (left panel) and EFV (right panel) with observed average profile.

Figure 4.4 shows the estimated mean profiles for the second order FP2 models for patients who initiated with NVP (left panel) and EFV (right panel) treatment regimen. The increase in log(CD4) cell counts from baseline was maintained until the end of follow-up period. Based on estimated FP2 models we can predict a subject-specific log(CD4) cell counts for subjects under NVP and EFV, respectively, by

$$\begin{aligned} \hat{f}(t_{ij}) &= (5.22 + \hat{b}_{0i}) + (0.05 + \hat{b}_{1i})log(t_{ij}) + (0.08 + \hat{b}_{2i})t_{ij}^{0.5}, \\ \hat{f}(t_{ij}) &= (5.05 + \hat{b}_{0i}) + (0.14 + \hat{b}_{1i})log(t_{ij}) + (0.024 + \hat{b}_{2i})(log(t_{ij}))^2, \end{aligned} \tag{4.9}$$

Here, $\hat{b}_{0i}$, $\hat{b}_{1i}$ and $\hat{b}_{2i}$ are the empirical Bayes estimates for the subject specific random effects.

The density estimate for the distribution of the predicted and observed values for NVP and EFV at 6 and 12 months are shown in Figure 4.5 and indicates that the model is performing well in terms of prediction at time points within the estimation period.

Observed and predicted values at all time points are presented in Figure 4.6 which reveals a strong correlation between the predicted and observed values (0.966 and 0.977 for NVP and EFV, respectively).

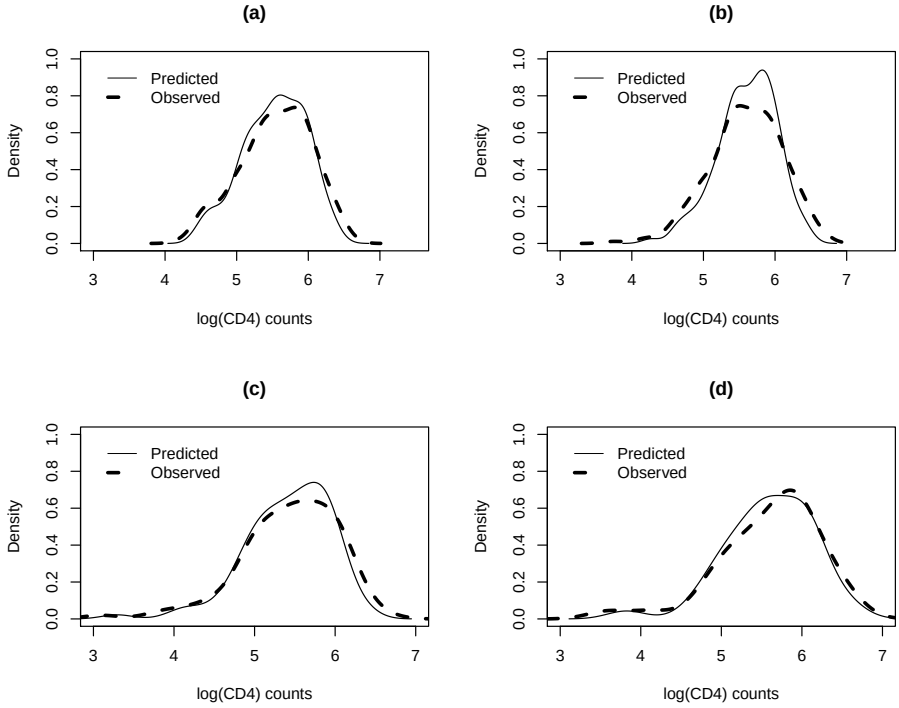


Figure 4.5. Density estimates for the distribution of the observed values (dashed line) and model-based predictions (solid line) at month 6 and 12 for NVP (panel a and b): and EFV (panel c and d).

4.3.2 Model Based Prediction of CD4 cell Counts: Estimation Period 0-30 Months

In the previous section model based prediction were obtained using FP2 models which were estimated using all data. In this section the data is divided into two periods. The first, 0 to 30 months, is used for the estimation of the model parameters while the second, 31 to 68 months, is used for prediction. Figure 4.7a and 4.7b present the observed and predicted values of log(CD4) cell counts within the estimation period and reveal, similar to the previous section, high correlation (0.976 for NVP and 0.982 for EFV). Figure 4.7c and 4.7d display the predicted versus the observed log(CD4) cell counts within the prediction period. Note that for this period the data were not used for the estimation of the model parameters. The correlations are equal to 0.805 and 0.742 for EFV and NVP, respectively.

The FP2 model estimated within the estimation period can be used to predict a subject specific last observed log(CD4) cell counts. This implies that for each subject,

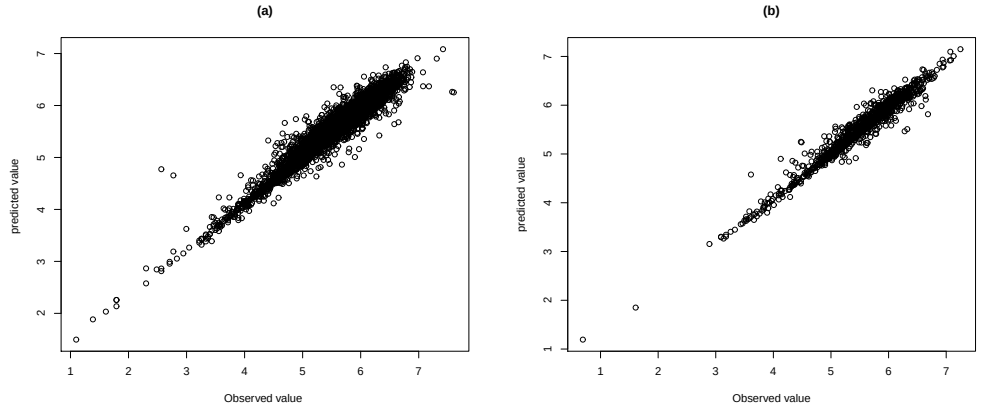


Figure 4.6. Plot of observed versus model based predicted for NVP and EFV (0 to 68 month). Note that all data are used to estimate the predictive model.

information about log(CD4) cell counts from the first 30 months of the treatment is used to predict the last observed log(CD4) cell count of the subject. Figure 4.8a and 4.8b present the observed and the predicted values. The correlations are equal to 0.764 and 0.808 for NVP and EFV, respectively.

4.3.3 Subject Specific Prediction of Time to Cross a Pre-specified CD4 Threshold

Subject Specific Prediction of Time to Cross a Threshold

The FP2 model allows us to estimate a subject specific time to cross a pre specified CD4 threshold. Three different thresholds were used (200, 350 and 500) to estimate a model-based time to cross the threshold which was consider as an event. Those patients who have time to cross the threshold longer than 70 months were censored. Note that model based predictions are obtained using a model for which the parameter were estimated using data from the estimation period (i.e. 0-30 months).

Figure 4.9 shows the observed and predicted log(CD4) cell counts for four selected patients. Panel *a* shows an example of a patient for which both the observed and predicted values cross the threshold within the estimation period. The time to cross the threshold is estimated to be 5.62 months. For the patient presented in panel *b*, both the observed and predicted values cross the threshold within the prediction period (i.e 31-68 months). Panel *c* presents a patient for which the observed log(CD4) cell counts are below the threshold of 350 and the predicted time to cross the threshold

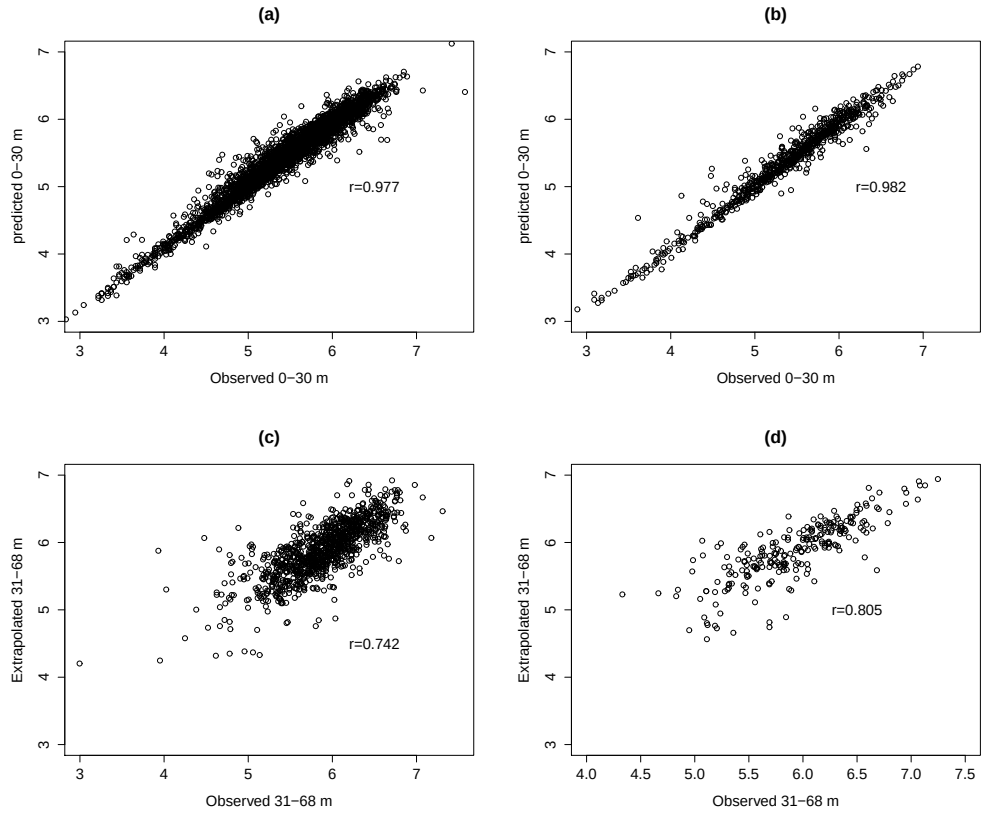


Figure 4.7. Observed and model based predicted values of log(CD4) cell counts obtained from a model that was estimated within the estimation period (0-30 months). Panel a and b: observed versus estimated values in the estimation period for NVP and EFV, respectively; Panel c and d: observed versus predicted values in the prediction period for NVP and EFV, respectively.

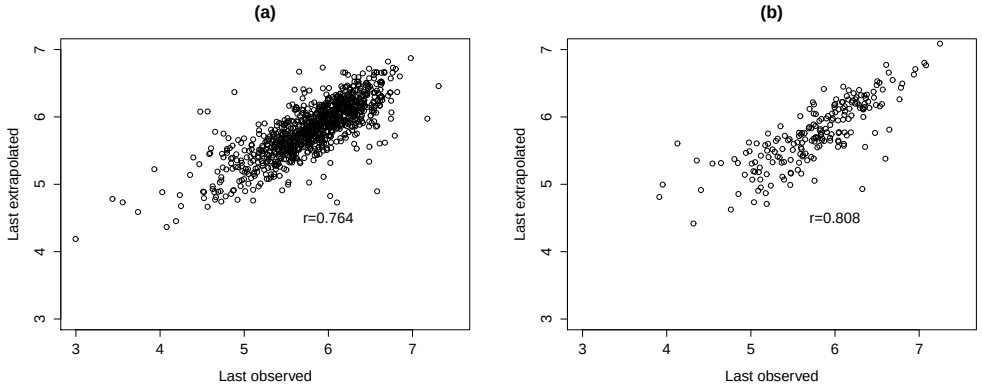


Figure 4.8. Last observed log(CD4) cell count and model based prediction based on a model which was estimated within the estimation period. Panel a: Patients who initiated with NVP containing regimen. Panel b: patients who initiated with EFV containing regimen.

is estimated to be 74.55 months. Panel *d* shows an example of patients for which both the observed and predicted log(CD4) cell counts remain below the threshold of 350 until the end of the follow up period.

Figure 4.10 presents the Kaplan-Meier curves for the estimated time to cross the threshold of 200 and 350 $cells/mm^3$. For the threshold of 200 CD4 $cells/mm^3$, there is a significant difference between EFV and NVP groups (p-values for the log rank test is 0.0422). The median time to cross the threshold is estimated to be equal to 11.6 months (95% CI: 10.8-12.4) for NVP group and 15.0 months (95% CI: 12.7-17.3) for EFV group. This implies that it took shorter time to cross the threshold of 200 $cells/mm^3$ for the NVP group. When the threshold increases to 350 (see Figure 4.10, panel *b*), the distribution of the time to cross the threshold of the groups were comparable (p-value=0.52).

For each patient, the probability to cross the threshold of 350 $cells/mm^3$, a different time points, was calculated according to Equation 4.5 and presented in Figure 4.11. The rate of increase in the probability of crossing the threshold differs for each patients irrespective of the type of regimen. The difference of the rate of increase in crossing the threshold might be partly explained by the baseline CD4 cell counts.

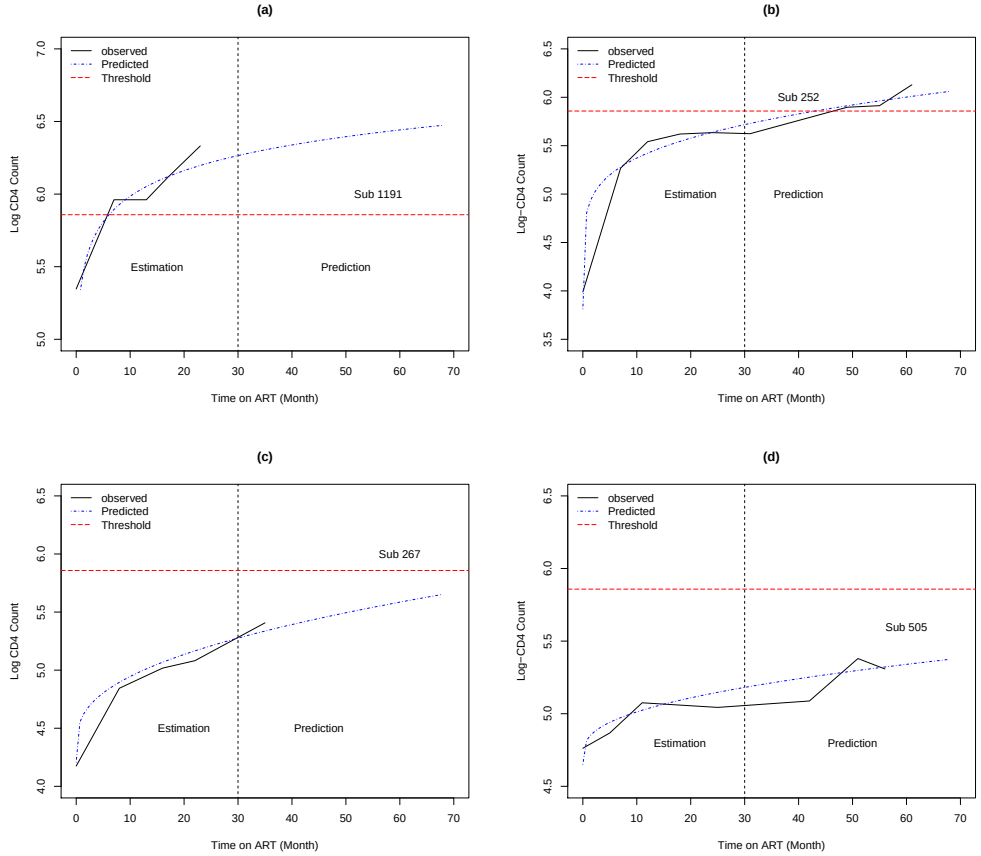


Figure 4.9. Observed and model based predicted log(CD4) cell counts for selected patients. Panel a: a patient for whom both observed and predicted values cross the threshold within the estimation period. Panel b: a patient for whom both observed and predicted values crossed the threshold after the estimation period. Panel c: a patient for whom the observed values are below the threshold and the predicted time to cross the threshold is estimated to be 74.55 months. Panel d: a patient for whom both observed and predicted values are below the threshold (350 $cells/mm^3$) during the study period.

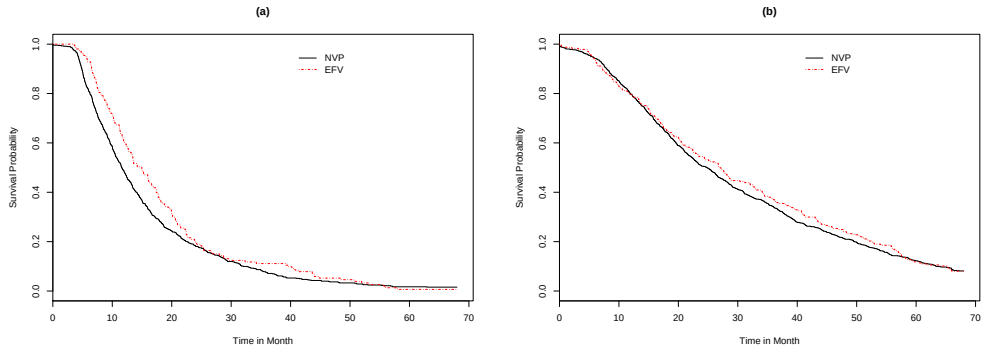


Figure 4.10. Kaplan-Meier curve for the time to cross a pre specified threshold of CD4 $cells/mm^3$ by treatment group; Panel a: Kaplan-Meier curves for threshold of 200 $cells/mm^3$ for EFV and NVP. Panel b: Kaplan-Meier courses for a threshold of 350 $cells/mm^3$ for EFV and NVP.

Predicted Probability to Cross a Pre-specified CD4 Threshold

The predicted probabilities to cross a threshold of 350 $cells/mm^3$ at 60, 90 and 120 months were calculated according to equation 4.3 and shown in Figure 4.12. For all time points, the probability to cross a threshold of 350 $cells/mm^3$ is higher for the EFV group. Although, the difference between the two treatment groups is higher for lower months. For example, at 60 months, there were 400 (36.36%) patients initiated with NVP who have CD4 cell count lower than the threshold as compared to 100 (40.48%) patients who initiated with EFV containing regimen and have CD4 cell counts lower than the threshold. The number of patients with CD4 cell counts lower than the threshold at 60% probability reduces to 200 for those who initiated in NVP containing regimen. Figure 4.12 *d* shows sorted predicted probabilities calculated using two models: (1) a model that was fitted within the estimation period and (2) a model which was fitted for all available data. We noticed that the two models lead to comparable probabilities in the estimation period and all observed dataset.

The procedures described in section 4.2.3 allows us to use (4.3) to estimate both the time and the probability to be above a given threshold. For illustration we use 8 subjects. Patients 2077, 1191, 67 and 783 are initiated with EFV, while patients 44, 240, 747, and 252 are initiated with NVP. Table 4.1 shows the estimated time to cross the threshold and the corresponding 95% confidence intervals for the selected patients.

Patients 783 and 252 started ART when their CD4 cell counts dropped below 100

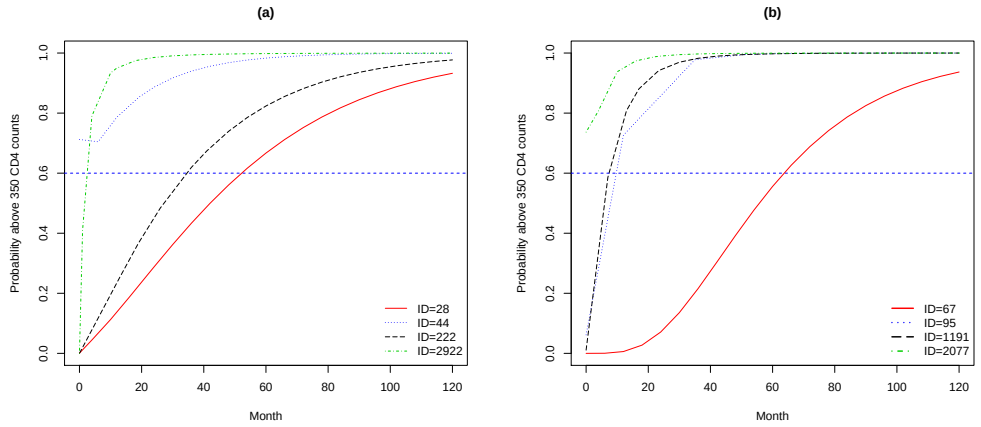


Figure 4.11. Probabilities to cross a threshold of 350 $cells/mm^3$ for selected subjects. Panel a: patients who initiated with NVP containing regimen. Panel b: patients who initiated with EFV.

$cells/mm^3$. The estimated time to cross 200 $cells/mm^3$ threshold for these patients are 20.282 (95%CI: 19.189-21.052) and 10.728 (95%CI:10.473-11.027), respectively. Patients 67 and 747 were initiated ART at CD4 cell counts between 100 and 200 $cells/mm^3$. These patients are expected to reach 200 $cells/mm^3$ at 13.667 (95%CI: 12.737-14.495) and 6.711 (95%CI: 6.765, 7.096) months, respectively. For both scenarios it takes shorter time to reach a threshold for patients who initiated with NVP.

Table 4.1. Estimated time to cross the threshold and 95% confidence interval for selected individuals (time in months).

		$\geq 200 cells/mm^3$		$\geq 350 cells/mm^3$		$\geq 500 cells/mm^3$	
PatientID	Baseline CD4	$\hat{T}_\tau$	95%CI	$\hat{T}_\tau$	95%CI	$\hat{T}_\tau$	95%CI
EFV							
2077	425	$2.8x10^{-3}$	($3.7x10^{-4}$-1.1510^{-2})	1.376	(1.045-1.763)	10.312	(9.897-10.890)
1191	210	2.789	(2.392-2.981)	9.752	(9.456-10.081)	19.786	(19.336-20.486)
67	126	13.667	(12.737-14.495)	42.918	(37.654-52.951)	79.928	(55.524-82.962)
783	67	20.282	(19.189-21.052)	51.361	(48.968-54.427)	82.1640	(71.872-85.256)
NVP							
44	490	$2.5x10^{-3}$	($5.4X10^{-4}$, $9.0x10^{-3}$)	1.601	(1.177-2.142)	16.717	(16.023-17.624)
240	271	1.406	(0.975-1.501)	12.102	(11.954-12.259)	21.481	(21.001-22.085)
747	138	6.711	(6.765-7.096)	28.107	(27.259-29.968)	69.545	62.335-72.333
252	54	10.728	(10.473-11.027)	39.161	(38.496-40.721)	69.422	(67.492-71.566)

At the start of their ART regimen, patients 1191 and 240 had CD4 cell count greater than 200 $cells/mm^3$. The expected time to cross threshold 350 $cells/mm^3$ are estimated to be equal to 9.752 (95%CI: 9.456-10.081) and 12.102 (95%CI: 11.954-

12.259), respectively. Patients 2077 and 44 were initiated ART with CD4 cell counts greater than 350 $cells/mm^3$. The estimated times to reach the threshold 500 $cells/mm^3$ are estimated to be equal to 10.312 (95%CI: 9.897-10.890) and 16.717 (95%CI: 16.023-17.624), respectively.

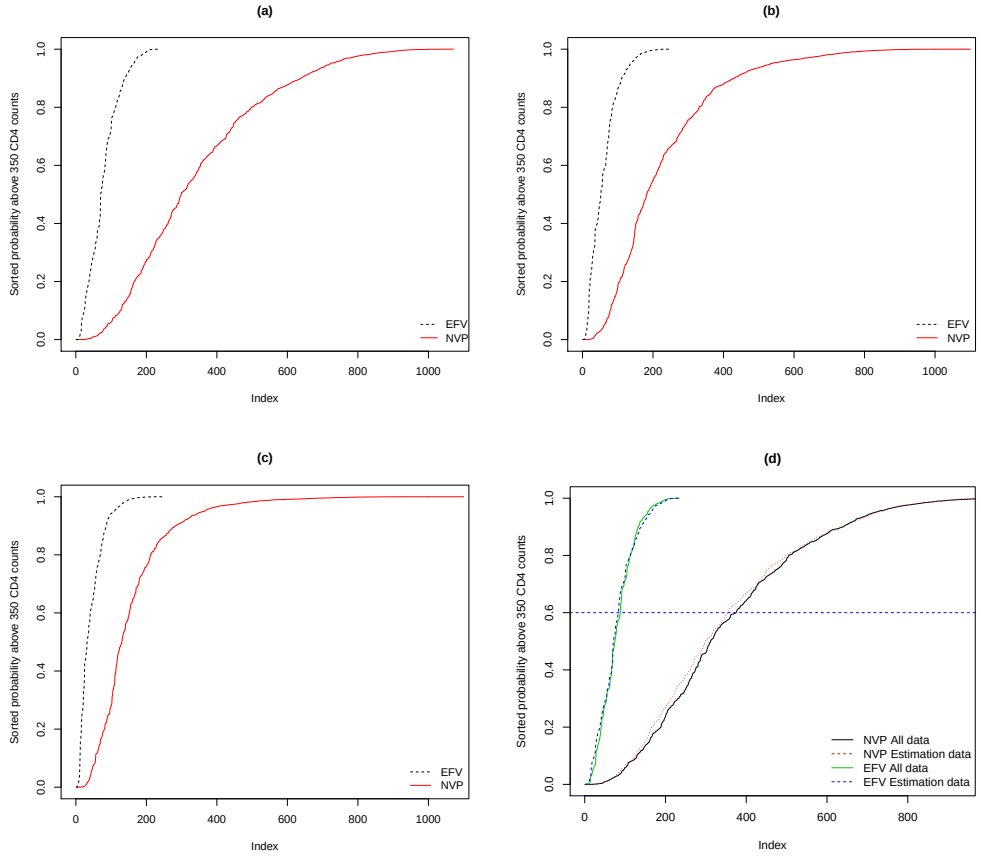


Figure 4.12. Sorted probabilities to cross a threshold of 350 CD4 cell counts. Panel a: sorted probability to cross the threshold at 60 months. Panel b: sorted probability to cross the threshold at 90 months. Panel c: sorted probability to cross the threshold at 120 months. Panel d: Sorted probability at 60 months obtained for models which were estimated within the estimation period and when using all data.

4.4 Discussion

The primary goal of ART is to reduce HIV-related morbidity and mortality, prolong survival, improve the quality of life, restore and preserve immunologic function and prevent HIV-transmission (Günthard et al., 2014). The level of CD4 cell counts is routinely used to monitoring response to ART in HIV-infected patients. It is used as a measure of the risk of development of opportunistic infections (Crampin et al., 2011). Patients with CD4 cells counts less than 200 $cells/mm^3$ are at higher risk of opportunistic infections (Stephan et al., 2012). If CD4 cell counts are close to or less than the threshold of 200 $cells/mm^3$, continued monitoring for CD4 cell counts can help to identify those needing prophylaxis for opportunistic infections (Kaplan et al., 2009). Through time, the threshold at which ART is initiated has been changed by the WHO from 200 to 350, 500 and currently ART should be initiated regardless of CD4 cell counts (WHO, 2015). Though ART treat-all approach is the strategy, however CD4 cell count remained very important indicator of immunosuppression (Ford et al., 2017).

In this chapter, we proposed a flexible method to model the relationship between CD4 cells counts and follow-up time in response to treatment. We applied a cross-validation to evaluate the performance of the prediction accuracy of a fractional polynomial mixed effect model. We have shown that model based prediction are highly correlated with the observed values within the estimation period (0.977 and 0.982 for NVP and EFV, respectively). The correlation between observed and model based prediction within the prediction period was found to be relatively high (0.805 and 0.742 for EFV and NVP, respectively). This provide evidences that our model can be used for long-term prediction of unobserved CD4 cell counts. The density plot for the distribution of observed and predicted values supported these relations. Other studies used similar approach for long term prediction based on longitudinal data (Aregay et al., 2013; Lawton et al., 2015).

The mixed effects FP2 model allow us to estimate the distribution of the time to cross a pre specified CD4 cell count threshold of interest and to use this distribution to compare between treatments. We have shown that more than half (52.87%) of the patients who initiated ART at CD4 cell counts less than 200 $cells/mm^3$ cross the threshold in six months period after initiation. When the threshold is 350 $cells/mm^3$, the proportion who crossed the threshold at 6, 24, 36 and 48 months were 13.88%, 40.14%, 52.79% and 61.65%, respectively.

Those patients who initiated at higher CD4 cell count get more pronounced CD4 cell count rise quickly than those who initiated at lower CD4 cells counts. Different

studies witnessed that the baseline CD4 cell count influences the rate of immune reconstitution (Jacobson et al., 2004; Kadima et al., 2014; Notermans et al., 1999; Smith et al., 2004). These studies indicated that markers of HIV disease stage at the time of ART initiation are critical determinants of the progression while under ART. This might be due to the damage on the immune system leads to the increase to the risk of illness.

We have shown that when ART is initiated to patients with low level of CD4 cell counts it took shorter time to reach the threshold for patients who were initiated with NVP. This pattern is reverse when ART is initiated to patients with high level of CD4 cell counts. Patients who were initiated with EFV cross the threshold of 350. The later difference between the two treatment groups persists for higher baseline CD4 cell counts which is also supported by other studies (Ford et al., 2017; Teeranaipong et al., 2016). This might be due to the high potency nature of EFV containing regiment.

The Kaplaan-Meier survival curve also shows that the median time to cross the thresholds 200 CD4 $cells/mm^3$ was shorter for patients who had been initiated with NVP as compared to EFV. Similar trend was reported by other studies (Teeranaipong et al., 2016; Van Leth et al., 2005). The possible reason is NVP has been used for patients with low CD4 level to reduce the side effect of EFV.

In conclusion, fractional polynomial mixed effect model enables the prediction of long-term treatment effects on CD4 cell counts. In addition, we used the model to estimate the predicted probability of an individual to have CD4 cell count above a pre-specified threshold. By predicting the long-term outcomes of CD4 cell count of the patient one can advise patients about the potential ART benefits that accrue in the long term. EFV regimen improves CD4 cells counts of the patient quicker than NVP regimen for higher baseline CD4 cell counts. Therefore, to our opinion, patients who have higher baseline CD4 cells counts can be initiated with EFV regimen to achieve immunological success at a faster rate. However, more strong clinical studies are needed to confirm the effect of the drugs based on CD4 label.

Chapter 5

Predicting Long-term ART Outcomes in HIV-infected Adults using Longitudinal Biomarkers: A Joint Modeling Approach.

5.1 Introduction

HIV-infected individuals in Ethiopia are followed since the date of their first test for HIV and during the time of pre and ART periods. As a result, there are two types of outcomes of interest; evolution of CD4 cell counts (longitudinal process) and, time to event outcomes (time to event process, (Rizopoulos, 2010)). Both of the outcomes pose difficulties if we attempt to model them independently. This is due to the fact that subjects with sharper rates of CD4 decline may have a higher risk to adverse events and have fewer CD4 cell count measurements (Little and Rubin, 2014).

The main focus of the analysis presented in this chapter is to determine the time to composite outcomes accounting for longitudinal outcomes. In HIV/AIDS data, CD4 cell counts have been used as a potential marker for treatment outcomes because of its observed correlation with clinical outcomes. Often only baseline values of the biomarker is used, despite the existence of repeated measurements for the biomarker (Awoke et al., 2016; McManus et al., 2012). As done in Chapter 3, one option is to fit two independent models; Cox-PH model with time-dependent covariate for the time-to-event outcomes, and mixed effect models for the longitudinal measurements. However, Cox-PH model does not account the biological variation and measurement errors of CD4 cell counts measured periodically. Prentice (1982) showed that regression coefficients on the partial likelihood are asymptotically biased when

it accommodates covariates measured with error. Failure to take appropriate account of this phenomenon and use of ordinary survival and longitudinal data models, can lead to biased estimation of average quantities of interest (Sweeting and Thompson, 2011).

Instead, different approaches have been proposed. Self and Pawitan (1992); Tsiatis et al. (1995) proposed a two stage approach in which at the first stage the repeated measurements are fitted over time using a mixed-effects models and in the second stage the individual predictions from this mixed effect model are used as either fixed or time-dependent covariates in a Cox-PH model. Kleinbaum and Klein (2005) proposed extended Cox-PH model in which the model consists of a time varying covariate. Faucett and Thomas (1996) proposed a simultaneous modelling approach of continuous covariates over time. Here, Markov chain Monte Carlo method of Gibbs sampling is used to generate the joint posterior distribution of all unknown parameters of the comprehensive model given only the observed data. Even though two stage aims to reduce bias by using a survival model that incorporates a longitudinal covariate that has been measured with error, it did not avoid all sources of bias. In the other hand, extended Cox-PH model assumes that the covariates are external and, for that reason, not related to the failure mechanism and that time-dependent covariates are measured without error. However, the approach is not appropriate for internal time-dependent covariates because it can result in biased estimations. Resent interest has focused on joint models, introduced by Self and Pawitan (1992), where models for the event time distribution and longitudinal data include a common set of latent random effects. It allows both models to share information and can lead to better estimation of the unknown parameters of the model (Seid et al., 2014).

In this chapter, our aim is to determine the relationship between CD4 cell count and the risk of composite outcome. A joint model (Rizopoulos, 2012b) that allows a broad range of dependencies between the longitudinal responses and the survival endpoints is used. Similar to Chapter 4, a flexible mean structure, based on fractional polynomials mixed effect model, is formulated to the longitudinal process. The event processes are defined as death or composite outcome. The joint model is used to predict the probability of the occurrence of the event given the longitudinal measurements of CD4 cell counts.

This chapter is organized as follow. In section 5.2 we introduce the joint model for the longitudinal and time to composite outcome will be formulated and will be applied to the data in Section 5.3. Finally, the results are discussed in Section 5.4.

5.2 Formulation of the Joint Model

The joint model for the longitudinal and time to event was implemented using the two stage procedure proposed by Rizopoulos (2012b). Briefly, in the first stage a fractional polynomial mixed effect model is fitted to the longitudinal data (CD4 cell counts) and the parameter estimates for both fixed and random effects are used in the second stage in the partial likelihood of the Cox-PH regression model for time to event model. For the analysis presented in this chapter two time to event endpoints were analysed: time-to-death and composite outcome (defined in Chapter 3). For both endpoints, a shared random-effects method (Rizopoulos, 2012b) was used.

Let T_i^* be the true event time for the ith subject, T_i be the observed event time, defined as the minimum of censoring time C_i and T_i^*, and $\delta_i = I(T_i^* \leq C_i)$ are the event indicator. For the longitudinal outcome, let $y_i(t_{ij})$ be its observed value at time point t_j (the visit time) for the ith subject. The main interest of the analysis presented in this chapter is to estimate the probability to death or composite outcome based on CD4 cell counts. This can be done using a joint model in which two submodels; the survival model for time to death or composite outcome and the CD4 cell counts, described below, are linked together via a shared random effect.

5.2.1 Model Formulation

Longitudinal Process

Similar to Chapter 4, a fractional polynomial mixed effect models was formulated for the mean structure of the longitudinal process. This model is a flexible parametric approach to describe the dependency between longitudinal response of primary interest and a covariates (Royston and Sauerbrei, 2008). Let $m_i(t_{ij})$ be the mean structure (include both fixed and random effects) for the fractional polynomial model given by

$$m_i(t_{ij}) = \sum_{l=0}^{k} \beta_l H_l(t_{ij}) + \sum_{l=0}^{k} b_{li} H_l(t_{ij}), \tag{5.1}$$

where k is an integer, $p_1 \leq p_2 \leq \cdots \leq p_k$ is a sequence of powers and $H_l(t_{ij})$ is a transformation function defined in (4.2.4) with $p_0 = 0$ and $H_0 = 1$. As shown in Chapter 4, for a given powers, p_1 and p_2, the second order mixed effect fractional polynomial can be written as

$$y_i(t_{ij}) = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i}) t_{ij}^{p_1} + (\beta_2 + b_{2i}) t_{ij}^{p_2} + \epsilon_i(t_{ij}).$$

In matrix notation the above mixed fractional polynomial model can be expressed

as:

$$\begin{aligned} y_i(t_{ij}) &= m_i(t_{ij}) + \epsilon_i(t_{ij}), \\ &= \mathbf{x}_i^\top(t_{ij})\beta + \mathbf{z}_i^\top(t_{ij})\mathbf{b}_i + \epsilon_i(t_{ij}), \\ &= (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})t_{ij}^{p_1} + (\beta_2 + b_{2i})t_{ij}^{p_2} + \epsilon_i(t_{ij}). \end{aligned} \tag{5.2}$$

Here, $\mathbf{b_i} = (b_{0i}, b_{1i}, b_{2i})$ is a subject specific random effect vector, $\mathbf{b_i} \sim \mathcal{N}(0, \mathbf{\Sigma_{b_i}})$ were $\mathbf{\Sigma_{b_i}}$ is the covariance matrix for the random effects and $\epsilon_i(t_{ij}) \backsim \mathcal{N}(0, \sigma^2)$

Survival Process

For the time to event, a Cox-PH model is used. The survival probability can be expressed in terms of the risk function as

$$S_i(t|\omega_i) = exp(-\int_0^t h_0(s)exp\{\gamma^\tau \omega_i\}ds), t > 0. \tag{5.3}$$

Consequently, the cox-proportional hazard model is formulated as

$$h_i(t) = h_0(t)exp\{\gamma^T \omega_i\}. \tag{5.4}$$

Here, $h_i(t)$ is the hazard function, $\omega_\mathbf{i}$ is the vector of baseline covariates, γ is the corresponding vector of regression coefficients and $h_0(t)$ is the baseline hazard. It assumes that the hazard ratio $h_i(t)/h_0(t)$ depends only on covariates, whose value is fixed during the follow-up.

A joint Model for the Longitudinal and Time to Event processes

Following the modeling approached proposed by Rizopoulos (2012b), we formulated a joint model for the time to event (time to composite outcome) and longitudinal outcome (CD4 cell count). The two endpoints described in the previous section were link together via the linear predictor of the hazard function,

$$h_i(t|\mathcal{M}_i(t_{ij}), \omega_i) = h_0(t)\exp(\gamma^\tau \omega_i + \alpha m_i(t_{ij})), t > 0. \tag{5.5}$$

Where $\mathcal{M}_i(t_{ij}) = (m_i(t_{ij}), 0 \leq s < t)$ denotes the history of the true unobserved longitudinal outcomes process up to time point t (Rizopoulos, 2012b) and $m(t_{ij})$ represents the model for the longitudinal outcome. Hence, the parameter α quantifies the effect of longitudinal outcomes on the risk for composite outcome (Njagi et al., 2013). The case with $\alpha = 0$ implies that the longitudinal outcome and the risk to composite outcome are independent. Indeed, $exp(\alpha)$ be the relative increase in the

risk of composite outcome at time t that results from one unit increase in $m_i(t_{ij})$ at the same time point t. The parameter $exp(\gamma)$ be the hazard ratio for one unit change in ω_i at any time t. A joint model in which the time to death is the survival endpoint can be formulated in the same way.

The risk of composite outcomes at time t depends on the current value, $m_i(t_{ij})$, and the change (slope), $m_i'(t_{ij})$, of CD4 cell counts, that is

$$h_i(t|\mathcal{M}_i(t_{ij}),\omega_i) = h_0(\exp(\gamma^\tau \times CD4 + \alpha_1 m_i(t_{ij}) + \alpha_2 m_i'(t_{ij}))), t > 0. \tag{5.6}$$

where

$$m_i'(t_{ij}) = \frac{d}{dt_{ij}}\left(\mathbf{x}_i^\top(t_{ij})\beta + \mathbf{z}_i^\top(t_{ij})\mathbf{b}_i\right).$$

The choice of the baseline hazard $h_0(.)$ is essential. In this chapter we used piecewise-constant approach proposed by Rizopoulos (2012b).

5.2.2 Estimation

Maximum likelihood estimation for joint models is based on the maximization of the log-likelihood corresponding to the joint distribution of the time-to-event and longitudinal outcomes $\{T_i, \delta, y_i\}$. We assumed that the longitudinal process and the survival process are conditionally independent given b_i:

$$p(T_i, \delta_i, y_i|b_i;\theta) = p(T_i, \delta_i|b_i;\theta)p(y_i|b_i;\theta),$$

with

$$p(y_i|b_i;\theta) = \prod_j p\{y_i(t_{ij}|b_i;\theta)\}.$$

Where $\theta = (\theta_t^T, \theta_y^T, \theta_b^T)$ denotes the full parametric vector for the event time outcome, the longitudinal outcome and for the random effects and covariance matrix, respectively. Under the conditional independence, the joint log-likelihood contribution for the ith subject can be formulated as;

$$log(p(T_i, \delta_i, y_i;\theta)) = log \int p(T_i, \delta_i|b_i\theta_t, \beta)[\prod_j p\{y_i(t_{ij})|b_i;\theta_y\}]p(b_i;\theta_b)db_i, \tag{5.7}$$

where the likelihood of the survival part is written as

$$p(T_i, \delta_i | b_i; \theta, \beta) = \{h_i(T_i | \mathcal{M}_i(T_i); \theta_t, \beta)\}^{\delta} S_i(T_i | \mathcal{M}_i(T_i; \theta_t), \beta). \tag{5.8}$$

Then the overall log-likelihood for all the observed data is formulated as

$$l(\theta) = \sum_i log p(Y_i, \delta_i, y_i; \theta). \tag{5.9}$$

The maximization of this function (5.9) with respect to θ requires a combination of numerical integration and optimization algorithms, because both the integral with respect to the random effects in (5.7) and in the survival function given by (5.8) do not have an analytical solution. The Expectation-Maximization (EM) algorithm has been traditionally preferred (Wulfsohn and Tsiatis, 1997). The idea behind the EM algorithm is to maximize the log-likelihood in two steps: the Expectation step, where missing data are filled, so we replace the log-likelihood of the observed data with a surrogate function, and the Maximization step, where this surrogate function is maximized. For an elaborate discussion about the estimation procedure we refer to Rizopoulos (2012a).

5.2.3 Predicted Time to Composite Outcome

Our interest is the subject-specific event within a time interval $(s, t + s]$ given the whole information available on the subject accumulated till the landmark time s. The time t denotes a fixed window of prediction whereas the varying landmark time s denotes the time at which predictions are made conditionally to the subject-specific biomarker history. Based on the fitted joint model, our aim is to predict the survival probabilities for a new subject i that has provided a set of longitudinal measurements $\mathcal{Y}_i(t_{ij}) = \{y_i(s); 0 \leq s \leq t\}$. For the subject-specific survival probabilities, $y_i(t_{ij})$ is directly related to the failure mechanism. This is due to the fact that providing longitudinal measurements up to t implies survival up to this time point. Thus, it is more relevant to focus on the conditional probability of surviving time $u > t$ given survival up to t. Here survival implies that the patient dose not experience composite outcome. For any time $u > t$ we are interested in the probability that subject i will survive at least up to u, given survived up to time t can be computed as:

$$\pi_i(u|t) = Pr(T_i^* > u | T_i^* > t, \mathcal{Y}_i(t_{ij}), \mathcal{D}_{\mathbf{n}}; \theta). \tag{5.10}$$

Where $\mathcal{D}_n = \{T_i, \delta_i, y_i; i = 1, \ldots, n\}$, being the sample on which the model was

fitted, and on which we wish to base our predictions (Rizopoulos, 2011). These predictions are dynamic in the sense that they change with increasing landmark time u and available information $(\mathcal{Y}_i(u), T_i > u)$. The time dynamic nature of $\pi_i(u|t)$ is evident from the fact that when new information is recorded for subject i at time $t > t'$, we can update these predictions to obtain $\pi_i(u|t')$, and proceed in a time dynamic process.

5.2.4 Prediction Accuracy Measures

Assume we have collected a set of longitudinal measurements $\mathcal{Y}_i(t) = \{y_i(s); 0 \leq s < t\}$ up to time point t for subject i. We are interested in events occurring in the time window of $(t, t + \Delta t)$ within which the physician can initiate or change treatment to increase the time of composite outcome of this patient. From all the longitudinal history $(\mathcal{Y}_i)$ of the patient, we aim to determine which values contribute to the specification of the prediction. A value of CD4 cell count lower than a specific threshold c is considered as predictive for composite outcome (death). The prediction rule is defined using the (estimated) probability to experience a composite outcome, $\pi_i(t + \Delta t|t)$,). The case in which $\pi_i(t + \Delta t|t) \leq c$ is termed as success (occurrence of the event), and $\pi_i(t+\Delta t|t) > c$ is a failure. The sensitivity and specificity then defined respectively as:

$$\begin{aligned} Se(c,t) &= Pr\{\pi_i(t + \Delta t|t) \leq c | T_i^* \in (t + \Delta t]\}. \\ Sp(c,t) &= Pr\{\pi_i(t + \Delta t|t) > c | T_i^* > (t + \Delta t]\}. \end{aligned} \tag{5.11}$$

The performance of the model in discriminating between patients who will experience the event of composite outcome and those who will not experience the event of composite outcome was assessed using Area Under Receiver Operating Characteristic (ROC) curve (AUC) values. An AUC equal to 1 indicates maximum discrimination, whereas AUC = 0.5 indicates random discrimination. Predictive accuracy can be assessed at certain time points and for given time windows, using the time-dependent AUC. An AUC at time t is obtained by varying c as

$$AUC(t) = Pr[\pi_i(t + \Delta t|t) < \pi_j(t + \Delta t|t) | \{T_i^* \in (t + \Delta t]\} \cap \{T_j^* > t + \Delta t\}]. \tag{5.12}$$

Here i and j represent a pair of comparable subjects (Antolini et al., 2005). Note that at each time point, and for a given time period of interest, if we consider two patients, one of whom experiences the event within the time window, and the other

who is not in the time window, the calculated conditional survival probability for the first patient should be lower. So far, we assumed that the prediction rule utilizes only the last available measurement of the marker. However, we can define another prediction rule which utilizes the last two measurements of the marker. We could define success when the pre-last marker value is c and the last one as kc, for $k \in (0,1)$, indicating that a k% decrease is strongly indicative for death or composite outcome.

The overall performance can be assessed using a summary of the AUC_s, in the form of the dynamic discrimination index (DDI), $C_{dyn}^{\Delta t}$ and is defined as Rizopoulos (2011)

$$C_{dyn}^{\Delta t} = \frac{\int AUC(t, \Delta t) Pr(T_i^* > t) dt}{\int Pr(T_i^* > t) dt}. \tag{5.13}$$

where

$$\varepsilon(t, \Delta t) = [\{T_i^* \in (t, t + \Delta t)\} \cap \{T_i^* > t + \Delta t\}].$$

Here, $Pr\{\varepsilon(t, \Delta t)\}$ is the probability that the random pair of patients is comparable at time t. The value $C_{dyn}^{\Delta t}$ depends on the time interval of interest, Δt. Technical details regarding the estimation of these quantities are discussed in Rizopoulos (2011, 2012b).

5.3 Application to the Data

The data used for the analysis presented in this section are the ART database from Gondar University Hospital that was introduced in Section 1.3. Table 5.1 presents the baseline characteristics of the patients induced in the analysis. A total of 1041 (40.8%) patients initiated with TDF containing backbone, from whom 688(66.1%) initiated with NVP containing regimen. The event of interest accounted for 115(11.05%) deaths and 289(27.76%) composite outcome. During the follow-up a total of 69(10%) and 187(27.2%) death and composite outcomes among NVP treatment groups, respectively. The cohort contribute a total of 2410.307 per 100 person months during the follow-up time. The incidence rate of death and composite outcome were 4.77 and 11.99 per 100 person months, respectively.

5.3.1 Survival Process

A Cox-model with treatment effect (EFV vs NVP) for the two events of interest (death and composite outcome) is fitted. The results revealed that the risk of death decrease by 28% (95%CI:0.539; 0.9618) when initiated by NVP containing regimen

Table 5.1. Baseline characteristics for patients who initiated by TDF containing backbone.

	NNRTI		
Covariate	NVP	EFV	Total
Death (n)			
No	307	619	926
Yes	46	69	115
Composite event (n)			
NO	251	501	752
Yes	102	187	289
Sex			
Female	245	401	646
Male	108	287	395
BWHOS			
I	76	105	181
II	66	84	150
III	163	339	502
IV	48	160	208

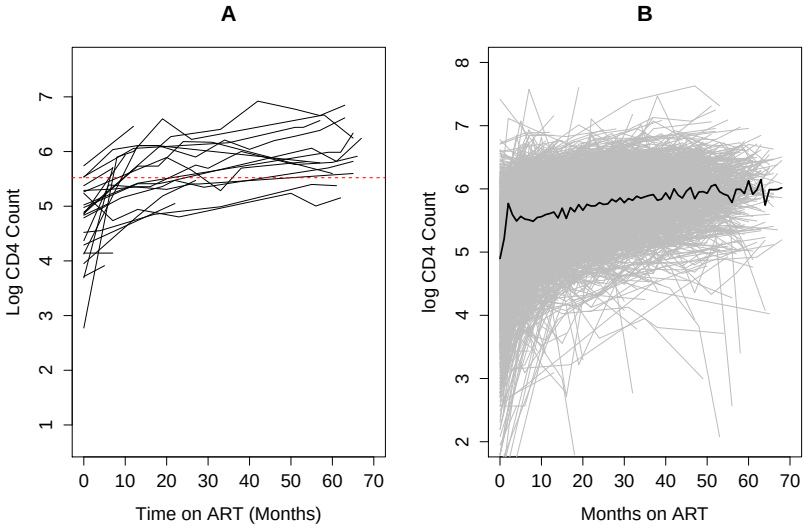


Figure 5.1. Individual profile selected patients (panel A) and individual and average profile for all patients (panel B).

as compared to EFV. However, for composite outcome, the risk of failure was not statistically significant among NNRTI treatment groups (Table D.1 in Appendix D).

Table 5.2. Model comparison for log(CD4) cell counts.

Model	Log-Likelihood	AIC	BIC
Linear Time + random int-Slope	-3049.802	6115.604	6164.859
Square time + random int-Slope	-2956.97	5935.94	6003.665
Cubic time + random int-Slope	-2867.695	5759.39	5833.268
FP(0,0)	-2672.377	5366.755	5434.48
FP(0,0.5)	-2660.899	5343.797	5411.522

5.3.2 Longitudinal Process

The fractional polynomial mixed effect models discussed above were used to estimate subject-specific evolutions for the log(CD4) cell counts within the follow-up period of the study. Different models for log(CD4) cell counts were fitted and compared using the AIC. Table 5.2 shows that the smallest value of AIC was obtained at $(p_1 = 0, p_2 = 0.5)$. Unstructured covariance structure was used for the random effects. Figure 5.2 shows the observed mean and predicted plot for the model with quadratic time, and fractional polynomials.
Estimated, subject specific, mean structures, is given by

$$\hat{m}_i(t_{ij}) = (\beta_0 + \hat{b}_{0i}) + \beta_1 \times treatment + (\beta_2 + \hat{b}_{1i}) log(t_{ij}) + (\beta_3 + \hat{b}_{2i}) t_{ij}^{0.5}. \qquad (5.14)$$

Note that the rate of change in log(CD4) cell count evolution, defined in Section 5.2.1, is given by

$$\hat{m'}(t_{ij}) = \frac{0.046 + \hat{b}_{1i}}{time} + \frac{0.097 + \hat{b}_{2i}}{2 * \sqrt{time}}.$$

5.3.3 Joint Modeling of the Longitudinal and Time to Event Process

Time to Composite Outcome

The rate of change in CD4 cell counts (first derivative) parametrization is the model with lower value of AIC (see Table D.8 in Appendix D). The mean structure for log(CD4) cell count is defined as

$$\hat{m}_i(t_{ij}) = (5.036 + \hat{b}_{0i}) + 0.102 \times treatment + (0.047 + \hat{b}_{1i}) log(t_{ij}) + (0.096 + \hat{b}_{2i}) t_{ij}^{0.5}.$$

For time to composite outcome, the model include both the current value, $m_i(t_{ij})$, and the change (slope), $m_i^{'}(t_{ij})$, of the longitudinal process of CD4 cell counts as

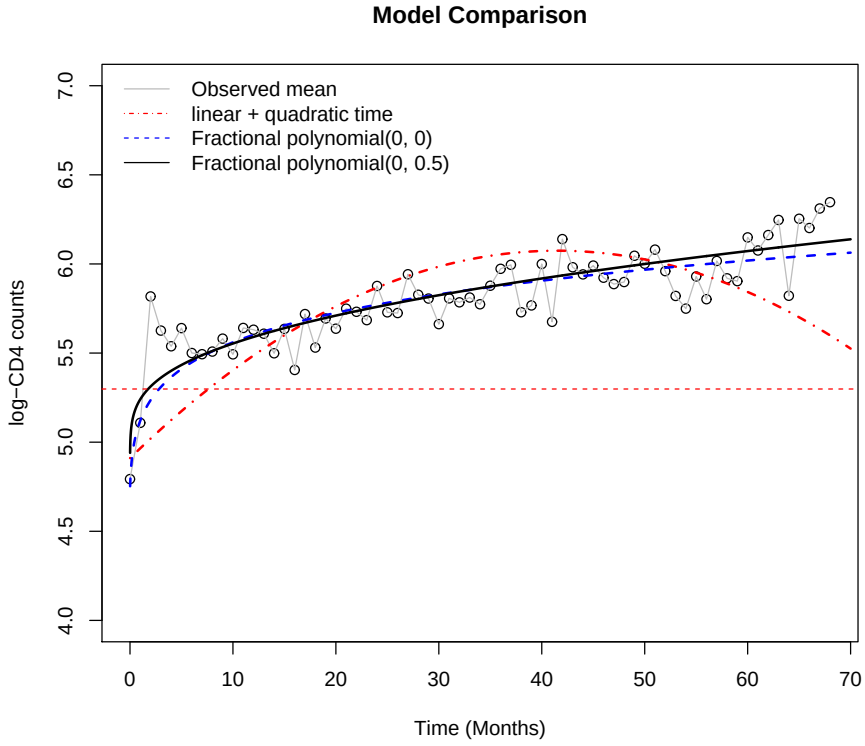


Figure 5.2. Plot of the fitted models for the longitudinal process.

predictor, that is

$$h_i(t) = h_0(\exp(\gamma^\tau \times treatment + \alpha_1 m_i(t_{ij}) + \alpha_2 m_i'(t_{ij}))), t > 0. \tag{5.15}$$

The parametrization of $m_i(t_{ij})$ is given in (5.14). Table 5.3 presents the parameter estimates for the joint model. The parameter estimate for α_1 is equal to 0.601 and found to be significant. A unit increase in the current value of log(CD4) cell counts has 60.1%(95% CI: 0.520; 0.695, P=0.0001) reduction in the risk of composite outcome. The parameter estimate for α_2 is equal to 0.009 which implies that a unit increase in the rate of change in log(CD4) cell count (i.e. a unit change in the derivative), the hazard of composite outcome reduced by 99.0%(95%CI:0.001; 0.131, P=0.0006). There was no significant difference between NNRTI groups on the risk of composite outcomes.

Time to Death

The model formulation presented in (5.15) was used for time to death. Similarly, current value, $m_i(t_{ij})$, and the rate of change, $m_i^{'}(t_{ij})$, of the longitudinal process of log(CD4) cell counts are included as predictor for time to death as well. The parameter estimate for α_1 is equal to 0.458, which indicates that a unit increase in the current value of log(CD4) cell count has 45.8%(95% CI: 0.370; 0.566, P=0.0001) reduction in the risk of death. The parameter estimate for α_2 is equal to 0.014(95%CI:0.001; 0.363, P=0.009) implies that a unit increase in the rate of change in log(CD4) cell count, the hazard of composite outcome reduced by 98.4% (see Table 5.3).

Table 5.3. The effect of current value and rate of change on the risk of death and composite outcome .

	Death				Composite event			
Covariate	Estimate	SE	95%CI	P-value	Estimate	SE	95%CI	P-value
Longitudinal process								
(Intercept)	5.034	0.035	4.965; 5.103	<0.0001	5.037	0.0351	4.968; 5.106	<0.0001
NNRTINVP	0.101	0.035	0.032; 0.170	0.0042	0.102	0.0352	0.033; 0.171	0.0038
log(time)	0.046	0.004	0.039; 0.053	<0.0001	0.0468	0.0035	0.040; 0.053	<0.0001
$time^0.5$	0.097	0.006	0.085; 0.108	<0.0001	0.0959	0.0059	0.084; 0.107	<0.0001
Random Effect								
σ_{b_0}	0.676				0.677			
σ_{b_1}	0.060				0.060			
σ_{b_2}	0.037				0.037			
σ	0.299				0.300			
Event process								
NNRTINVP	0.841	0.194	0.574; 1.231	0.3738	1.130	0.1249	0.885; 1.444	0.3269
α_1	0.458	0.109	0.370; 0.566	<0.0001	0.601	0.0743	0.520; 0.695	<0.0001
0.001; 0.363 α_2	0.014	1.655		0.0101	0.009	1.3611	0.001 0.131	0.0006
AIC	5971.252					7668.195		

5.3.4 Individual Prediction for Time to Composite Outcome

The joint model discussed in the previous section allows us to estimate the probability that a patient is free of composite outcome given the longitudinal history of CD4 cell counts. Note that the event free survival probabilities can be predicted using different number of the longitudinal measurements. For illustration purposes, we considered four patients (two from each treatment group), who provided CD4 cell counts measurements for 60 and 68 months. For each of these patients, the measurements at the observation time t is considered. Given that the patients survived up to each of these time points, we compute the predicted conditional survival probabilities at each of the remaining time points until the study ends. Figure 5.3 shows the log(CD4) cell counts measurements at the observation time for selected patients.

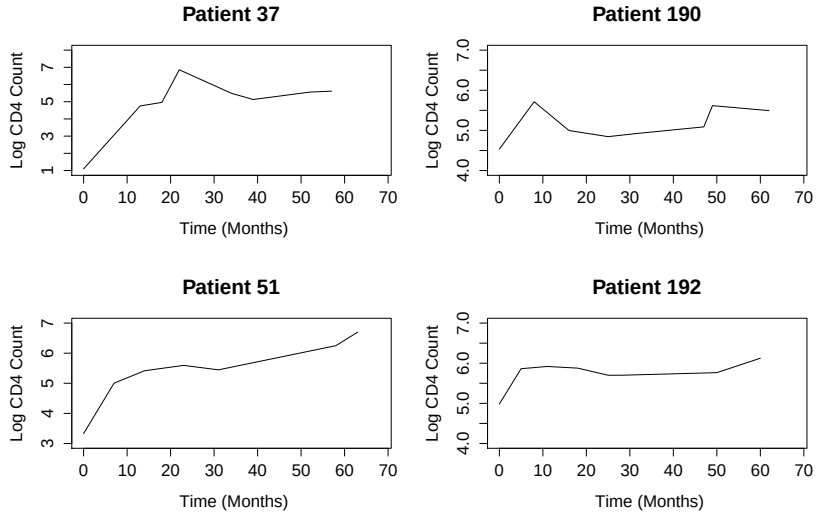


Figure 5.3. Individual profile for selected patients. Patients 37 and 51 were initiated with EFV and patients 190 and 192 were initiated with NVP.

For each scenario, 400 Monte Carlo sample were generated and their median event free survival with 95% point wise confidence intervals were computed. The median probability with 95% point wise confidence interval for patients 37 and 51, who initiate with EFV. The upper panels of Figure 5.4 shows the predicted probability of event-free survival within the observed time when only one measurements of the CD4 cell count is used for prediction. The lower panels show the event-free survival probability when additional CD4 cell counts measurements are used. The scatter points appearing before the vertical dashed lines represent a plot of log(CD4) cell counts up to that particular time point. The conditional survival probability curve (and 95% confidence intervals) are shown to the right of the vertical line. Note that subject 37 and 51 were excluded from the original data and the joint model was fitted.

The probabilities of event-free survival at month 68 were 70% and 50% for patient 37 and 51 respectively when we use CD4 cell count measurements until month 10. Consequently, the probability of event-free survival increased to 90% and 70% when CD4 cell counts measurements until time equals to 40 months were used for prediction. As expected, the length of confidence interval decreases as the number of log(CD4) cell counts measurements increases.

Figure 5.5 shows results for patients 190 and 192 who initiated with NVP. Note that the two patients were excluded from the analysis before the joint mode was fitted.

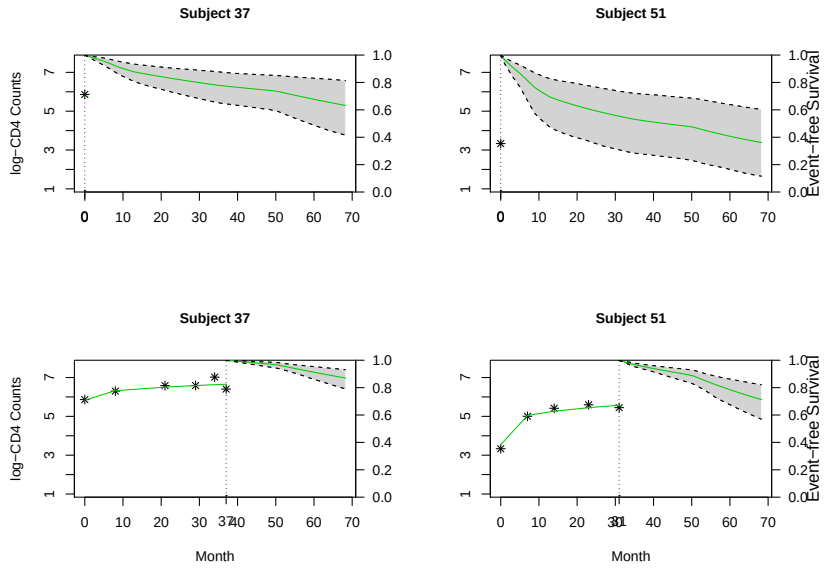


Figure 5.4. Prediction of the probability for composite outcome. Dynamic survival probabilities for Patients 37 and 51 during follow-up. The vertical dotted lines represent the time point of the last CD4 cell count measurement at time t=0 and t=40 months. Left of this vertical line, the fitted longitudinal trajectory is depicted. Right of the vertical line, the solid line represents the median estimator for $\pi_i(u|t)$, and the dashed lines the corresponding 95% point-wise confidence intervals. Upper panels: log(CD4) cell count up to 10 moths are used for prediction. Lower panels: log(CD4) cell counts until 60 months are used for prediction.

The first CD4 cell counts measurement was used to estimate the event-free survival probability at last follow up time (68 months). The event-free survival probabilities were 47.73% and 57.16% for patients 190 and 192, respectively. When CD4 cell counts until month 60 were used to predict the probability of developing composite outcomes (see Figure 5.5, lower panel) , the probabilities of event-free survival increased to 70% and 80%, respectively.

The conditional survival probabilities are updated as more measurements become available. Figure 5.6 illustrates the change in CD4 cell counts profile in the dynamic updates of the survival probabilities. This is done by comparing the estimates of $\pi_i(u|t)$ between patient 37 and 190 who were initiated with EFV and NVP regimens, respectively. A thousand Monte Carlo samples was used for estimation. The conditional probabilities were estimated along with 95% confidence interval at four time

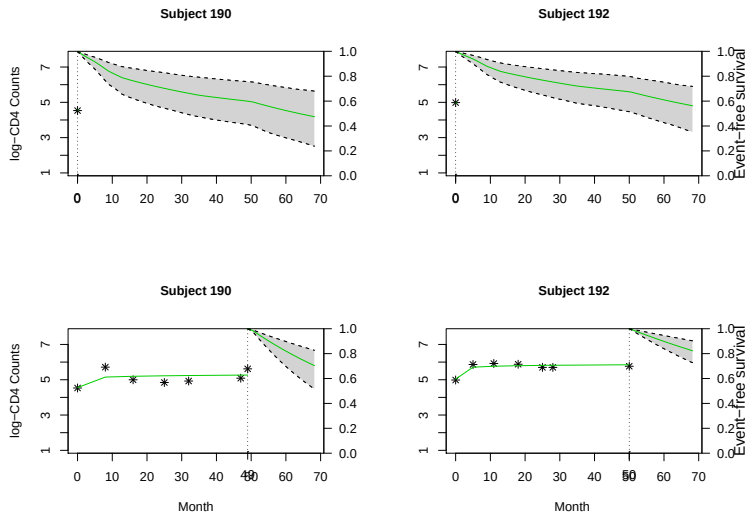


Figure 5.5. Dynamic survival probabilities for Patients 190 and 192 during the follow-up. The vertical dotted lines represent the time point of the last CD4 cell count measurement at time t=0 and t=60 months. Left of this vertical line, the fitted longitudinal trajectory is depicted. Right of the vertical line, the solid line represents the median estimator for $\pi_i(u|t)$, and the dashed lines the corresponding 95% point-wise confidence intervals.

points (t= 12, 24, 36 and 48 months), and $u = t + \Delta t$ at different values of Δt. We observed that patient 37 (top panel) showed much more stable CD4 cell counts profile, has higher survival probability of not experiencing composite outcome as compared to patient 190 (bottom panel).

Next, we investigate how well the model performs in terms of discriminating between subjects who will experience a composite outcome and those who will not experience a composite outcome. For illustration, we use patients 31 and 57. The sensitivity and specificity, defined in section 5.2.4 were calculated for all different possible prediction rules. Figure 5.7 shows that the ROC curve for Δt = 48 lies above the ROC curves for Δt = 12 and Δt = 24, indicating that at months 48 the marker can discriminate better between patients who will experience composite outcome before months 85 from patients who will not experience a composite outcome before month 85. Similar pattern was observed for patient 51.

Table 5.4 provides the time-dependent AUCs under the different options for Δt =12, 24, 36 and 48 months, and the time points of t=7, 14, 23, 31, 58 and 63 months for

patient 51. We notice varying degrees of discriminative ability for different time windows at different time points. At time point t = 63, time window Δt = 48 provides slightly better discriminating power between those who will experience the event and those who will not. This means, for instance, if interest was on predicting composite outcome within a 48 months window, and such a prediction was done at 63 months, then the probability that the model would allocate a lower conditional survival probability to a patient who was going to develop composite outcome within the next 48 months as compared to one, that was not, would be 0.6621.

Table 5.4. Dynamic Discrimination Index at different time windows for patient 51.

Time Window Δt	Time Point	AUC(t)	DDI
12	7	0.6458	0.6557
	14	0.6143	
	23	0.6218	
	31	0.6479	
	58	0.6521	
	63	0.6483	
24	7	0.6435	0.6179
	14	0.6151	
	23	0.6237	
	31	0.6510	
	58	0.6538	
	63	0.6533	
36	7	0.6401	0.6341
	14	0.6160	
	23	0.6282	
	31	0.6510	
	58	0.6556	
	63	0.6578	
48	7	0.6360	0.6176
	14	0.6196	
	23	0.6305	
	31	0.6513	
	58	0.6576	
	63	0.6621	

The DDI was calculated for the windows of interest Δt =12, 24, 36 and 48 months, to get a summarized measure of the discriminative ability over the follow-up period. It provides a weighted average of the AUC, with weights accounting for how many patients are still at risk. The indices's range from 0.6176 for a time window of 48 months to 0.6557 for a time window of 12 months.

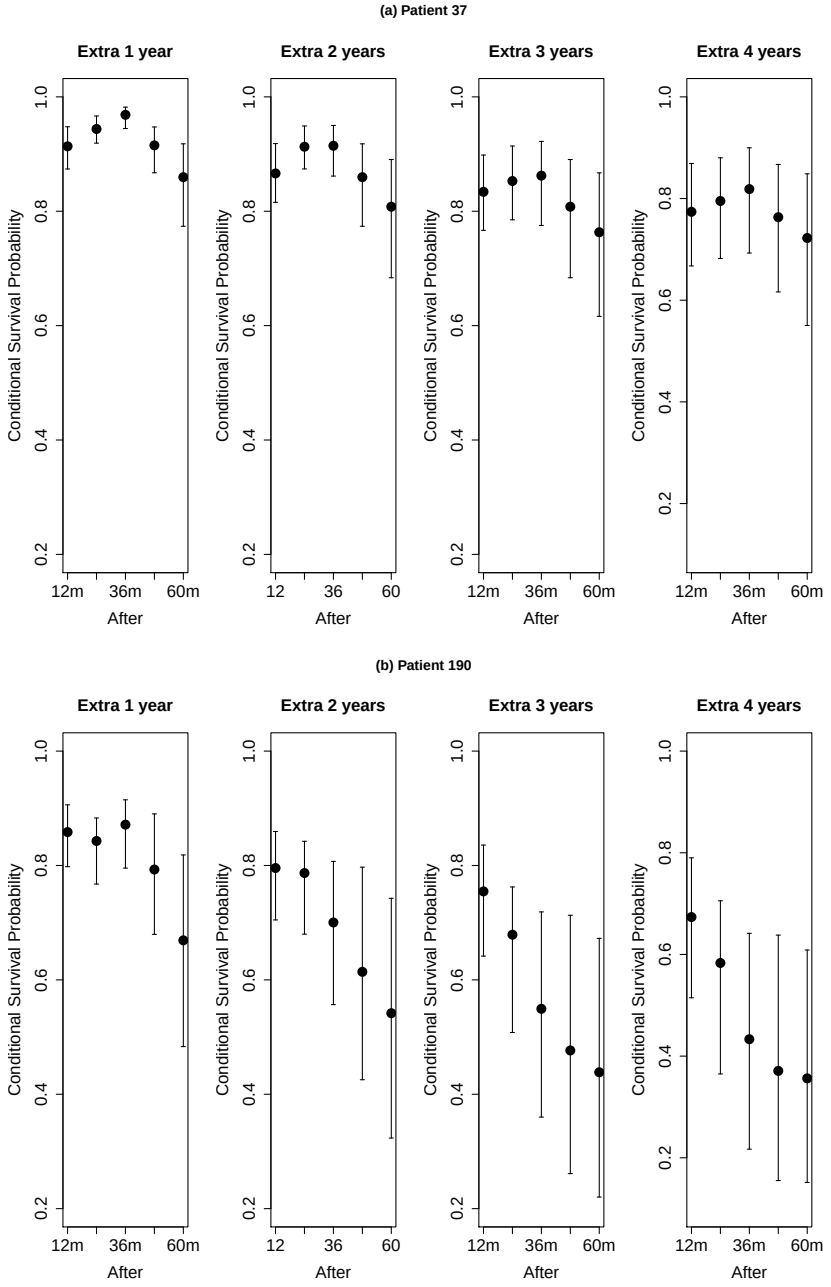


Figure 5.6. Conditional survival probabilities of surviving an extra 12, 24, 36 and 48 months, with each additional 12 months of measurement. The left panels corresponds to t = 12 months, second column corresponds to t=24, third column corresponds to t = 36 months and right panels corresponds to t=48 months. The dots represent the median conditional probability.

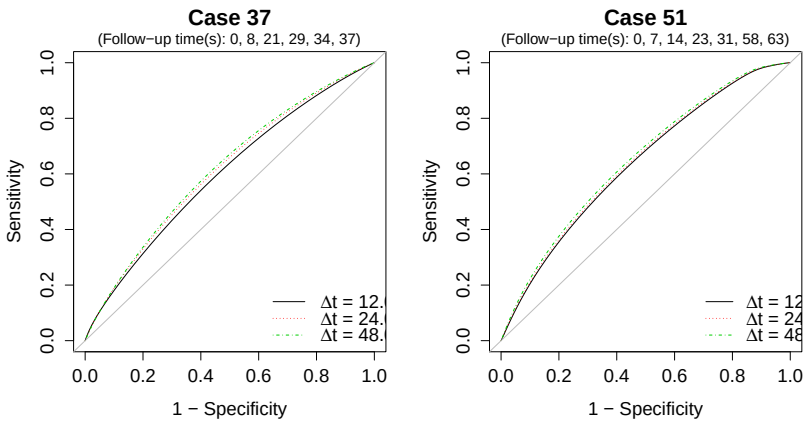


Figure 5.7. Receiver Operating Curve (ROC) at time $t = 63$ and three options for *Deltat* under the simple prediction rule for two patients (id=37 and id=51).

5.4 Discussion

The number of HIV/AIDS patients are increasing from time to time and so do the number of measurements resulting a wealth of data available from each treatment providing institutes. Such follow-up data produces multiple types of events and repeated measurements taken from each subject. It has been evidenced that the longitudinal evolution of biomarkers provide important additional information on the development of time to event outcomes compared to the baseline or current value of the biomarkers. The main aim of the current study was to assess the association of CD4 cell counts trajectory over time and the risk of death or composite outcome among a cohort of HIV/AIDS patients on ART in Ethiopia.

Different approaches have been proposed in the literature to take the longitudinal evolution to predict the time to event outcome. such as Cox-PH regression model used to study the relationship between CD4 cell counts as time-dependent covariate and time-to-event outcome. However, CD4 cell counts is measured periodically and with substantial measurement error and biological variation. Standard methods for estimating the parameters in the Cox-PH model by maximizing the partial likelihood are no longer appropriate. Further, two stage approach was proposed as an option (Tsiatis et al., 1995). Evidence show that using two stage approaches severely underestimate any association between the current underlying longitudinal value and the hazard of the event (Sweeting and Thompson, 2011). These provides extensive opportunities to utilize the joint modeling framework Self and Pawitan (1992).

In this chapter, the joint model proposed by Rizopoulos (2012b) is used. This general class of model allows accurate inference regarding time-to-event outcomes while adjusting for the longitudinal response. As can be seen from the individual and average profile in Figure 5.1, the relationship between CD4 cell counts and time seems nonlinear. We used fractional polynomial random effect model to capture the curvature of the relationship. Cox proportional hazards model is used for the event process (death or composite outcome). In the longitudinal process, there was no statistical significant difference in CD4 cell counts among the treatment groups over time. Whereas, logarithm and square root transformed time were significantly associated with CD4 cell count evolution.

The results of time-dependent slop parametrization revealed that the hazard of death or composite outcome depended on log(CD4) cell counts trajectory. The risk of death increased by 2.31-fold (95%CI:1.652; 3.235) for a unit decrease in the current value of log(CD4) cell counts for patients who initiated with EFV. Meanwhile, for patients who initiated with NVP, the risk of death increase by 1.942-fold for a unit

decrease in current values of log(CD4) cell counts. This finding is in-agreement with a collaboration observational HIV Epidemiological Research in Europe (COHERE et al., 2012), which shows that a higher CD4 cell count is associated with a reduced risk of death.

For composite outcome, the risk increased by 2.10-fold (95%CI:1.645; 2.653) for a unit decrease in the current value of log(CD4) cell counts in patients who initiated with EFV. The risk increased by 1.385-fold for patients who initiated with NVP containing regimen. There was no significant difference among the interaction of drug and log(CD4) cell counts on the risk of death. This is in contrast with study findings that employed separate analysis by Alemu and Sebastián (2010), Morquin et al. (2012) and Okomo et al. (2012). These studies reported that the risk of death was not related to the level of CD4 cell counts at baseline. This might be due to the fact that separate analysis did not take the longitudinal evolution of CD4 cell counts over time in to account.

Joint modelling provides a framework for performing individual predictions of the outcomes. Such prediction are dynamic in the sense that when additional information becomes available for the individual, the prediction can be updated taking in to account this new observation. The method predict the probability of an event in some future time for an individual with available baseline information and longitudinal outcomes.

The study was a retrospective cohort which limits the availability and quality of data collected. Some follow up variables were not complete in the record. All the patients are supposed to have CD4 cell counts in every six months. However, the current data structure do not keep the six monthly records for the patients.

In conclusion, joint modeling of longitudinal biomarker and time-to-event processes are more efficient than the two-stage method and extended Cox-PH regression for estimating the coefficients related to the longitudinal biomarkers. There was strong relationship between the risk of death and rate of change in CD4 cell counts. This relation remains constant between composite outcome and rate of CD4 cell counts change. We have explored how dynamic prediction can assist physicians make intervention for patients who experience composite outcome.

Part II

Modeling VL-HIV Co-infection in Ethiopia

Chapter 6

Visceral Leishmaniasis and HIV Co-infection

6.1 Introduction: Visceral Leishmaniasis Infection

6.1.1 The Epidemiology of Visceral Leishmaniasis

Visceral leishmaniasis (VL) is an endemic and potentially life-threatening disease in the Tropics, Subtropics, and Mediterranean region (Pavlia and Maltezoub, 2010). Worldwide, leishmaniasis occurs in 88 countries or territories (Desjeux, 2006). It mainly affects the poorest segment of the population, those living in rural areas, and migrants are affected in the African and Indian subcontinent (Figure 6.1) while in southern Europe the epidemic re-emergence was associated with HIV, drug abuse and immunosuppression (Alvar et al., 2008, 1997, 2006). Without treatment VL is nearly always fatal. Lack of effective, drugs resulted the suffering of people infected with VL worldwide (Narain et al., 2010).

The epidemiology of leishmaniasis depends on the characteristics of the parasite species, the local ecological characteristics of the transmission sites, current and past exposure of the human population to the parasite, and human behaviour. There is an estimated 350 million people in 88 countries at risk of leishmaniasis (Den Boer et al., 2011; Murray et al., 2015). Worldwide, the number of new cases is estimated to be 0.2-0.4 million with 20,000 to 40,000 estimated deaths occurring each year (Alvar et al., 2012). However, these numbers are widely acknowledged to be a gross under-estimation of the real burden (Bern et al., 2008). Visceral leishmaniasis has immense impact on the developing world and delay economic development, with an estimated loss of 2.3 million disability-adjusted life years (WHO, 2014). Most transmission in rural areas occurs outside village, primarily affecting farmers working in agriculture

Figure 6.1. Geographic distribution of visceral leishmaniasis: Source: Desjeux (2004).

fields in the endemic area in East Africa (Pearson and Queiroz, 1996).

6.1.2 Transmission

There are about 20 different species and subspecies of leishmania parasite which can cause leishmaniasis, from which leishmania donovani complex causes VL (Desjeux, 2004). The transmission occurs commonly during a blood meal of the female phlebotamine sandfly (Bates, 2007). A susceptible female phlebotomine sand fly can be infection when it bites an infected animal or human and vice versa, a susceptible animal or human can be infected after an infected female's bite. Less common forms of transmission include blood transfusions, contaminated needles, and from pregnant mother to her child. Symptoms typically appear two to eight months after the person has been bitten by an infected female sand fly (CDC, 2013).

6.1.3 VL-HIV Co-Infection

VL-HIV co-infection has an important clinical and epidemiological implications. There is overlap between the transmission areas of VL and HIV which results an increasing number of cases of VL-HIV co-infection spread throughout these regions (Lindoso et al., 2016). The two diseases reinforce each other and lead to profound immune deficiency. Most HIV-infected people worldwide live in regions where leishmaniasis

is endemic and they are particularly vulnerable to VL, while VL accelerates HIV replication and progression to AIDS (WHO, 2017).

A VL-HIV co-infection represents a challenging diagnosis since clinical characteristics of VL is similar to that of other disseminated opportunistic diseases (Cota et al., 2014). Both VL and HIV infections reduce cellular immunity. As a result, VL-HIV co-infection leads to accelerated progression of VL, drug toxicity, treatment failure, repeated relapse and increased mortality (Burza et al., 2014; Diro et al., 2014; Rachel et al., 2008). Up to 50% of patients fail to clear parasites from infected tissues which ultimately leading to treatment unresponsiveness and overwhelming parasite load (van Griensven et al., 2014a). As many as 35 countries throughout the world have reported cases of VL-HIV co-infection (Lindoso et al., 2016). High co-infection rates are reported from Brazil, Ethiopia and the state of Bihar in India (Diro et al., 2014). In this highly endemic area for VL, the rate of HIV co-infection among VL patients ranges between 15-30% (WHO, 2017). The highest rate of co-infection was observed in Ethiopia (van Griensven et al., 2014b) with an estimate of 30% co-infected individuals among the total number of VL infections.

First line treatment for VL-HIV co-infected patients include liposomal amphotericin B and paromomycin (Meyerhoff, 1999; Saravolatz et al., 2006; WHO, 2017). Second-line treatment options for VL in HIV-co-infected patients include miltefosine and paromomycin. Pentamidine isethionate has been used as a second-line alternative but is no longer recommended, because of toxicity that sometimes includes irreversible insulin-dependent diabetes mellitus (Kaplan et al., 2009). Using first line antileishmanial drugs as secondary prophylaxis increases the risk to develop resistance that can easily be transmitted in anthroponotic transmission regions. Thus pentamidine was chosen as an aromatic diamidine that is not used in first intention because of toxicity but that was found to be safe when used as prophylaxis at a lower dose therapeutic dosage (Calza et al., 2001; Patel and Lockwood, 2009; Perez-Molina et al., 1996).

6.2 Visceral Leishmaniasis in Ethiopia

6.2.1 Epidemiology

In Ethiopia VL is found widespread in several regions as shown in Figure 6.2. The first case was documented in 1942 in the lower Omo plains, the south-western part of the country (Leta et al., 2015). The country has the second largest number of VL cases in sub-Saharan Africa, with an estimated annual incidence of VL ranging from 3,700 to 7,400 cases (Alvar et al., 2008). The Northwest lowland areas (Metema

and Humera districts) bordering Sudan have the highest prevalence of the disease, accounting for 60% of the VL burden in the country (Argaw et al., 2013). Each year, it is estimated that 0.3 to 0.5 million young highlanders migrate into the VL endemic region for a daily labour work in the cash-crop farms (Leta et al., 2014). These non-immune migrants stay for several months to years in the farm where they can be acquire VL infection.

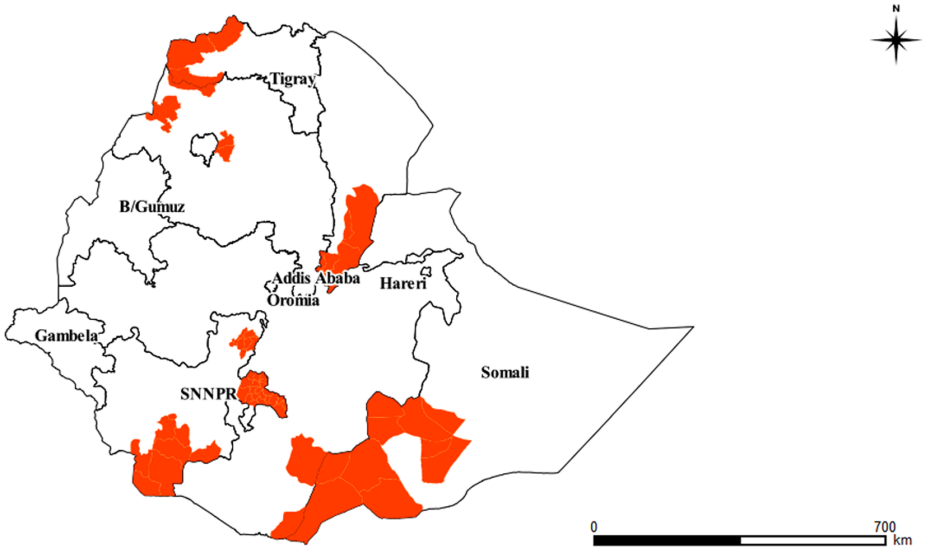


Figure 6.2. Distribution of Visceral Leishmaniasis in Ethiopia: Source: (Leta et al., 2014).

Worldwide the highest burden of VL-HIV co-infection is found in north-west Ethiopia, where up to 30% of VL patients are co-infected with HIV (Alvar et al., 2008; Hurissa et al., 2010; Mengistu and Ayele, 2007).

6.2.2 Effect of Malnutrition and Intestinal Parasites on VL

Several studies were conducted in Gondar University to investigate the possible impact of malnutrition and intestinal parasitic infections on VL severity of VL (by modulating cell-mediated immunity (Diro et al., 2015a)). Helminth infections are characterised by a strong T-helper (Th)2 response (Maizels et al., 2012) and it has been suggested that this might suppress a protective Th1 response in VL patients and therefore contribute to the strong immunosuppression characteristic of these patients (Nylén and Sacks, 2007)

Malnutrition plays a crucial role in an increased susceptibility to infection and/or disease severity by weakening the immune system, however the causal links between malnutrition and infections are not yet well established. Moreover, the majority of the work on malnutrition has been mainly done with malnourished patients suffering from infectious diseases, or other pathological conditions, and, apart from studies on patients with eating disorders such as anorexia nervosa (Marcos et al., 2003). Little is known about the impact on malnutrition on the immune system of apparently healthy adult individuals. Previous studies on immune responses of patients with VL has shown that the majority of these patients suffer from severe malnutrition (Abebe et al., 2013; Takele et al., 2013). The immune status of these patients is characterised by a profound suppression of T-cell responses, high levels of cytokine and chemokine production and strong inflammatory responses (Goto and Prianti, 2009; Nylén and Sacks, 2007).

Takele et al. (2016) compared immunological outcomes between malnourished and individuals with normal Body Mass Index (BMI). Several immunological parameters that have been shown to be impaired in patients with non-healing VL: haematological profile, cytokine profiles in the plasma, CD4 and CD8 T-cell ratio and activation status, as well as neutrophil effector functions. Takele et al. (2016) shows that several immunological parameters are altered in apparently healthy malnourished individuals: we observed significantly increased production of mixed cytokines as well as impaired neutrophil effector functions. In contrast, the haematological data were similar amongst all groups, suggesting that the malnourished individuals did not have an infection, were not anaemic, neutropenic, lymphopenic or thrombocytopenic. Furthermore, the frequency and percentage of CD4 and CD8 T-cells were similar in the malnourished healthy individuals and those with normal BMI.

In addition, intestinal parasites may also contribute to malnutrition by competing for nutrients in the gut, inducing chronic inflammation and causing malabsorption. Northwest Ethiopia, has a high prevalence of intestinal parasitic infections (Diro et al., 2015a) and malnutrition appears to be relatively common (Branca et al., 1993). However, precise information about the impact of co-infection with intestinal parasites on the severity of patients with VL patients is typically not available. Tajebe et al. (2017) present an investigation of the impact of intestinal parasite co-infections on the disease status of patients with VL, before the start of anti-leishmaniasis treatment. Clinical data were collected and haematological, inflammatory mediators and cytokines were determined. All these parameters were compared between patients presenting with VL and VL co-infected with intestinal parasites. The result indicated that co-infection of VL patients with intestinal parasites does not affect VL

disease severity, since clinical, haematological data and the treatment outcome are not altered by co-infection. This is in contrast with VL-HIV co-infection that, as mentioned above, can lead to an increases risk for treatment failure (for both VL and HIV).

Chapter 7

Predicting Relapse of Visceral Leishmaniasis in HIV Co-infected Patients using Longitudinal Biomarkers: A Cohort Study in Northwest Ethiopia

7.1 Introduction

As mentioned in Chapter 6, Ethiopia is the country with the highest VL-HIV co-infection rate which can lead to an increase risk of treatment failure. Available treatments for VL are expensive and have serious associated toxicities and may lead to the development of drug-resistant parasites (Croft et al., 2006). Recurrent epidemics of VL in East Africa (Ethiopia, Kenya, South Sudan and Sudan) have caused high morbidity and mortality in affected communities (Richard and Tim, 2014).

The evidence regarding best treatment practices for co-infected patients is limited specially for resource limited countries such as Ethiopia. The data analysed in this chapter are outcome of a clinical trials in which co-infected VL-HIV patients were treated by pentamidine. Patients in the clinical trails were fallowed for a period of 12 months and their response to treatment status (relapse /not relapse) was measured. At the end of the follow up time, patients who were not relapsed were followed for another 6 months based on their CD4 cell count ($< 200 cells/mm^3$). In addition to the response to treatment status, laboratory indicator such as WBC, and Hgb were measured regularly.

Our aim in this chapter is to use clinical or laboratory variables in order to predict probability of relapse after treatment. In particular, we model the relationship between WBC, and Hgb, with relapse, using a shared random effects joint model for

the time to relapse and laboratory indicators. The joint model used in this chapter is similar to the model presented in Chapter 5 for composite outcome and CD4 cell counts.

This chapter is organized as follow. In Section 7.2 we introduce the data, study setting and design. The joint model for the longitudinal and time to relapse is formulated in Section 7.3 and will be applied to the data in Section 7.4. Finally, the results are discussed in Section 7.5.

7.2 Data and Methods

7.2.1 The PSP Clinical Trial

As mentioned in Section 1.3.2, the Pentamidine as secondary prophylaxis (PSP) clinical trial was conducted on the use of PSP to prevent VL relapses in HIV co-infected patients in Gondar, since May 2011 for a year. Recruitment of the patients for the study proceeded in two steps. During pre-screening, age 18 or more years, parasitological diagnosis of VL, documented HIV test result and acceptable distance of residence from the trial centres for monthly follow-up were checked. Eligible patients were then approached for consent. For detailed information of the study we refer to Section 1.3.2 and Diro et al. (2015b).

Study Setting

The study was conducted in Northwest Ethiopia at two LRTC; University of Gondar Hospital (UoGH) and Abdurafi Health Centre. They are the largest VL treatment centers in the region and are supported by the non-governmental organizations Drugs for Neglected Diseases initiative (DNDi) and Mdecins sans Frontires respectively. Patients register to the center either spontaneously or are referred from other health institutes in the catchment area. Pentavalent antimonials and liposomal forms of amphotericin B, and more recently paromomycin, are the main drugs used to treat VL. While liposomal amphotericin B is recommended for VL-HIV co-infected patients, due to the inadequate supply, it is often reserved for more severe cases such as patients with organ dysfunction. Miltefosine is infrequently available. As a result, most VL-HIV co-infected patients are being treated with SSG.

Study Design and Population

The PSP clinical trial was started in March 2011 and closed in February 2016 ($http://clinicaltrials.gov/show/NCT01360762$). It was an open label, single arm trial designed to investigate the effectiveness, safety and feasibility of monthly pentamidine prophylaxis to prevent relapse for VL-HIV co-infected patients. All VL-HIV co-infected patients that were registered at LRTC were screened for enrolment to the clinical trial and their findings and initial treatment responses are documented in individual patient record files. The detail on diagnosis procedure and treatment monitoring procedure are presented in Diro et al. (2015b). At the end of the trial PSP, VL-HIV co-infected patients were enrolled and treated by SSG.

Data Collection Procedures

Pentamidine isethionate was started one month after VL cure for the current VL patients; and soon after the inclusion criteria were met for past VL cases. Patients were followed until the event of interest or over a period of 12 months. By the end of the study period, patients that were not experienced the event (relapse or death) were censored. All adverse events were documented, and all the serious adverse events were reported to the sponsor and concerned Ethics Committee via a fast track procedure.

Analysis of Baseline Characteristics

Table 7.1 present a summary values of baseline variables in the PSP trail. A total of 74 VL-HIV co-infected patients were enrolled to the PSP study, from whom 71 (95.9%) were male. The mean age of the participants was 32.6 (sd=7.01). The maximum number of repeated measurements was 13 (IQR=5-14). Nearly half (47.3%) of the patients were followed in Gondar University hospital. The mean baseline weight of the patients was 49.44 (sd=6.13). The median WBC count and Hgb were 3253 (IQR=2300-3900) and 9.365 (IQR=7.725-10.975), respectively. All patients enrolled to the study were under ART.

Treatment Outcomes

Figure 7.1 shows possible treatment outcomes. At the end of the main study period (12 months), 28 patients (37.84%) had CD4 cell counts greater than 200 $cells/mm^3$ followed by CD4 cell count less than or equal to 200 $cells/mm^3$. The cohort contribute a total of 619.6 person-months. The incidence of relapse was 2.4 per 100 person months of follow-up. Seven (9.46%) patients lost from the follow up, and 5 patients

Table 7.1. The median, and inter quartile range for selected baseline clinical and immunological variables of the patients.

Variable	Number
Sex, male	71(95.9%)
Baseline CD4, $> 200 cells/mm^3$	37(50%)
Pulse rate, median(IQR)	91.14(84.00-96.00)
Temperature, median(IQR)	36.12(35.60-36.40)
Liver span, median(IQR)	12.98(10.00-13.00)
White Blood cell(WBC), median(IQR)	3253(2300-3900)
Lymphocyte, median(IQR)	31.26(22.35-38.35)
Haemoglobin, median(IQR)	9.365(7.725-10.975)
Creatinine, median(IQR)	0.9297(0.700-1.100)
Blood glucose, median(IQR)	100.4(86.00-112.00)

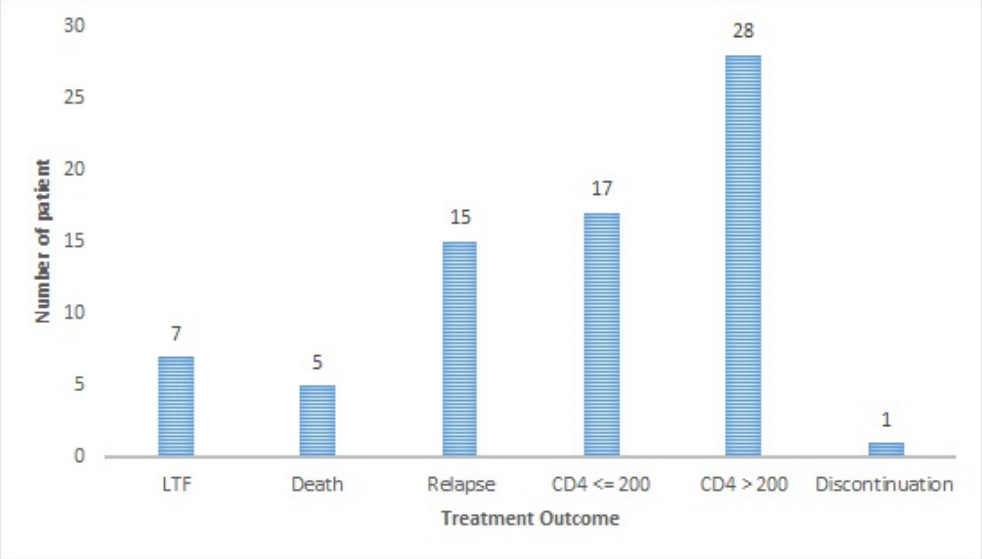


Figure 7.1. Outcomes of Treatment at 12 months since the start of treatment.

(6.76%) were died during the follow-up period. Figure 7.2 shows the Kaplan-Mair estimate for the probability of time to relapse. The probability of event free survival at 83, 140 and 240 days were 0.972 (95%CI: 0.933-1.000), 0.837 (95%CI:0.748-0.925) and 0.772 (95%CI:0.671-0.874), respectively. The first event was observed 35 days after the start of treatment and the last event was observed at 240 days. Note that the incidence of relapse was null from day 35 to day 84 Most of the relapse happen between 84 days to 100 days.

The individual profiles of log(WBC) and Hgb levels are presented in Figure 7.3.

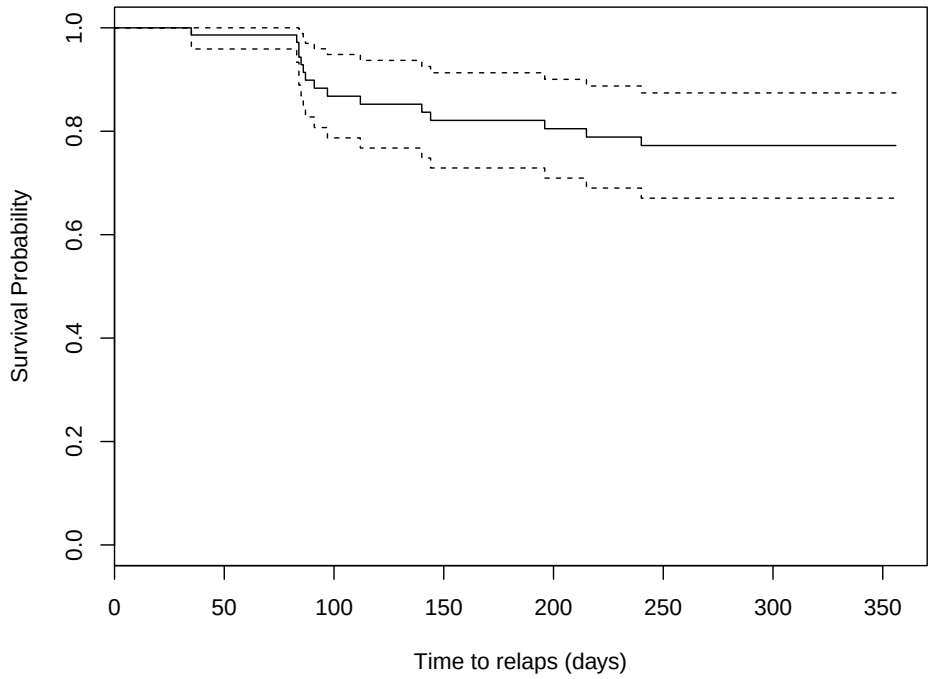


Figure 7.2. Plot of Kaplan-Meier survival curve for time to relapse.

The incidence of relapse was almost null until day 84 except the patients who experienced at day 35. Most of the relapse (8 out of 15, 53.33%) happen between 84 days to 100 days (see Panel C and D in Figure 7.3).

Table 7.2 shows that the time interval when the patients developed relapse. Only one event was observed until 80 days after the start of treatment. Note that one lapse was observed in day 35, while the rest of the relapse are observed between day 84 to day 240. The longitudinal processes until 84 days were plotted in Figure 7.4. This portion of the repeated measurements of log(WBC) and haemoglobin will be used to formulate the longitudinal process.

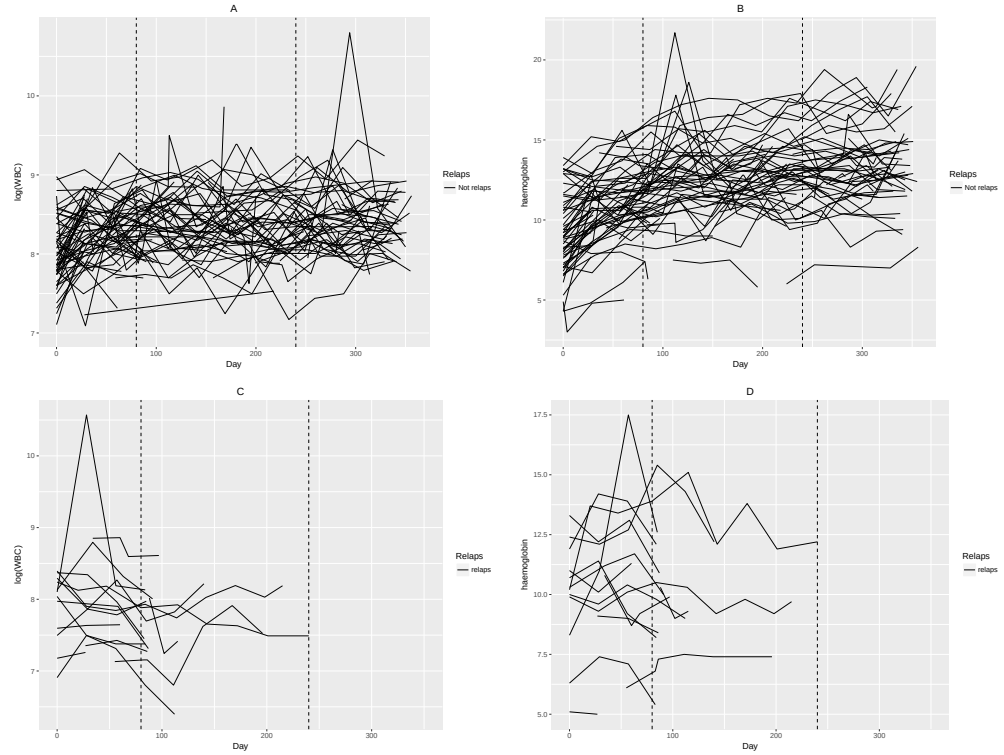


Figure 7.3. Individual profile for log(WBC) and Hgb. Panel A: log(WBC) for patients who did not develop relapse. Panel B: Hgb for patients who did not develop relapse. Panel C: log(WBC) for patients who developed relapse. Panel D: Hgb for patients who developed relapse.

Table 7.2. The number of relapse at different follow-up periods.

Time Interval	Number of Relapse	Cumulative Relapse
<80 days	1	1
80.1 to 85 days	4	5
85.1 to 90 days	2	7
90.1 to 100 days	2	9
100.1 to 150 days	3	12
150.1 to 240 days	3	15

7.3 A Joint Model for Time to Relapse and Laboratory Indicators

Our aim, in this chapter, is to predict the probability of relapse using the laboratory markers WBC and Hgb. Note that, since only one relapse was observed within the first 84 days of the study, only the longitudinal data within this period will be included

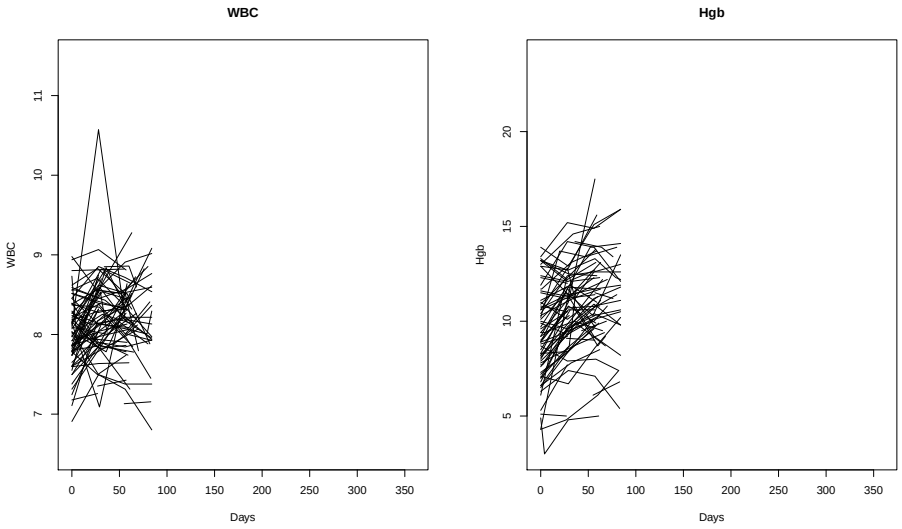


Figure 7.4. Individual profile for WBC(left panel) and Hgb (right panel) for the first 84 days after the start of treatment.

in the model. This part of the data is shown in Figure 7.4. The joint model described in Section 5.2.1 is used to jointly model the longitudinal laboratory markers and time to relapse. Each laboratory marker, WBC and Hgb is modelled separately. Similar to Chapter 5, the model for the hazard function include information about the longitudinal marker. For example, the risk of a relapse at time t depends on the current value of WBC, $m_i(t_{ij})$.

$$h_i(t|\mathcal{M}_i(t_{ij}), \omega_i) = h_0(\exp(\gamma^\tau * CD4 + \alpha_1 m_i(t_{ij}))), t > 0.$$

Here, $CD4$ represents the $CD4$ cell counts at baseline. Similarly, the risk of relapse at time t depends on the current value and the change of Hgb, that is

$$h_i(t|\mathcal{M}_i(t_{ij}), \omega_i) = h_0(\exp(\gamma^\tau \times CD4 + \alpha_1 m_i(t_{ij}) + \alpha_2 m_i'(t_{ij}))), t > 0.$$

Where,

$$m_i'(t_{ij}) = \frac{d}{dt_{ij}} \left(\mathbf{x}_i^\top(t_{ij})\beta + \mathbf{z}_i^\top(t_{ij})\mathbf{b}_i \right).$$

For the mean structure of the longitudinal outcome, we used the fractional polynomial model, described in Section 4.2.4. Note that, as explained above, only the

first 84 days are used for the estimation of $m(t_{ij})$.

7.3.1 Predicted Time to Relapse

As in Section 5.2.3, our primary interest is to predict a subject-specific relapse within a time interval $(s, t+s]$ given the whole information available on the subject accumulated until the landmark time s. The time t denotes a fixed window of prediction whereas the varying landmark time s denotes the time at which predictions are made conditionally to the subject-specific WBC or Hgb history.

7.4 Application to the Data

7.4.1 Joint Modeling

A Joint Model for Hemoglobin and Time to Relapse

As pointed out in Section 7.2.1, the longitudinal measurement of the clinical variables until 84 days were used to predict the incidence of relapse later. For Hgb, fractional polynomial mixed effects model with $p_1 = 0.5$ and $p_2 = 1$ with random intercept, random slope of time with degree p_1 and p_2 was the model with the best goodness to fit the data. Additional covariates that were included in the model are baseline CD4 cell count (categorized in to two levels, CD4 cell count < 200 $cells/mm^3$ versus CD4 cell count ≥ 200 $cells/mm^3$) and study site (Gondar versus Abderafe). The estimated mean structure for Hgb is defined as

$$\hat{m}(t_{ij}) = (8.46+\hat{b}_{0i}) + 1.78 \times site + 1.028 \times CD4 + (1.62+\hat{b}_{1i})t_{ij}^{0.5} + (-0.204+\hat{b}_{2i})t_{ij}. \quad (7.1)$$

The predicted and observed vales are presented in Figure 7.5 left panel (correlations equals to 0.983). For time to relapse, the initial model include both the current value, $m_i(t_{ij})$, and the change (the derivative), $m_i^{'}(t_{ij})$, of the longitudinal process of Hgb were included in the model as predictor.

The parametrization of $m_i(t_{ij})$ is given in (7.1). Table 7.3 presents the parameter estimates for the joint model. The parameter estimate for α_1 is equal to 0.91 and found to be not significant. The parameter estimate for α_2 is equal to 0.341 (95%CI: 0.132-0.883, P=0.0267) implies that a unit increase in the rate of change in Hgb (i.e. a unit change in the slope) reduces the probability of relapse by 65.9% (HR=0.341, 95%CI: 0.132-0.883).

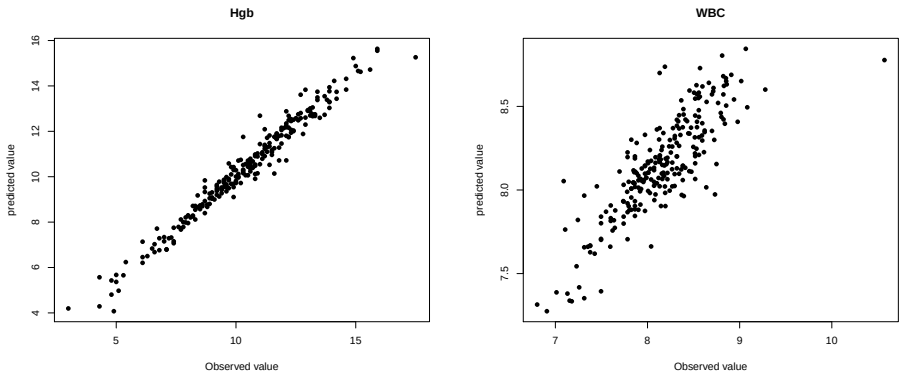


Figure 7.5. Model based predicted versus observed values for Hgb (left panel) and log(WBC) (right panel). Predicted values were obtained using a model that include Hgb (log(WBC)) measurements until 84 days.

Table 7.3. Parameter estimates of joint model for Hgb and time to relapse processes.

Covariate	Estimate	SE	95%CI	P-value
Longitudinal process				
(Intercept)	8.1673	0.3712	(7.4397-8.8948)	<0.0001
SiteG	1.6222	0.3968	(0.8444-2.3999)	<0.0001
CD4>200$cells/mm^3$	0.9164	0.4062	(0.1204-1.71248)	0.0240
$time^{1/2}$	1.2676	0.3509	(0.5799-1.9552)	0.0003
time	-0.0573	0.1958	(-0.4411-0.3265621)	0.7699
Random Effect				
σ_{b_0}	2.1330			
σ_{b_1}	1.2225			
σ_{b_2}	0.2671			
σ	0.8958			
Event process				
CD4>200$cells/mm^3$	0.280	0.6588	(0.0943-1.2482)	0.1045
α_1	0.910	0.1714	(0.6815-1.3343)	0.7815
α_2	0.341	0.4851	(0.1319-0.8833)	0.0267
log likelihood	-566.6732			
AIC	1177.346			

A Joint Model for WBC and Time to Relapse

A fractional polynomial model with $p_1 = -1$ and $p_2 = 1$ that includes random intercept and degree p_1 slope was found to be the best model (based on AIC) for log(WBC).

The estimated mean structure is given by

$$\hat{m}(t_{ij}) = (8.322 + \hat{b}_{0i}) - 0.11 \times site + 0.215 \times CD4 + (-0.003 + \hat{b}_{1i})t_{ij}^{-1} - 0.036 \times t_{ij}. \quad (7.2)$$

The observed and predicted vales are presented in Figure 7.5 right panel (correlations equals to 0.835). The parametrization for the survival process includes the current value of WBC, $m_i(t_{ij})$, of the longitudinal process of WBC as predictor has the best fit.

Table 7.4 presents the parameter estimates for the joint model. For the hazard function, α = 0.151 (95%; 0.026-0.874, P=0.0348) implies that a unit increase in the current value of log(WBC) reduce the probability of relapse by 84.9

Table 7.4. Parameter estimates for the joint model for the log(WBC) and time to relapse.

Covariate	Estimate	SE	95%CI	P-value
Longitudinal process				
(Intercept)	8.221	0.094	(8.038-8.406)	<0.0001
SiteG	-0.130	0.084	(-0.294-0.035)	0.1237
CD4>200$cells/mm^3$	0.218	0.082	(0.057-0.379)	0.0080
$time^{1/2}$	-0.003	0.001	(-0.004;-0.001)	0.0003
time	-0.039	0.036	(-0.11-0.032)	0.2801
Random Effect				
σ_{b_0}	0.3517			
σ_{b_1}	0.0018			
σ	0.3222			
Event process				
CD4>200$cells/mm^3$	0.397	0.653	(0.110-1.428)	0.1572
α	0.151	0.896	(0.026; 0.874)	0.0348
log likelihood	-192.7376			
AIC	419.4751			

7.4.2 Predicted Survival Probability

Hemoglobin

The joint model discussed in the previous section allows us to estimate the probability that a patient is event free (i.e. not develop relapse yet) given the longitudinal history of the biomarker. Note that the event free survival probabilities can be predicted

using different number of the longitudinal measurements. Baseline Hgb measurement of a patient was used to predict the probability of event free survival in the follow up period. In the next step, more measurements of the Hgb were used to update the predicted probability for an event free subject. For illustration, let us focus on patients 13 and 20 shown in Figure 7.6. Patient 13 relapsed at day 215 while patient 20 did not experience relapse up to 277 days (last observed time).

Figure 7.6 shows the event free predicted probability using Hgb as a biomarker. For patient 13 the predicted event free survival at 29 and 355 days using only baseline measurement of the biomarker were 0.92 (95%CI: 0.940-0.995) and 0.775 (95%CI: 0.229-0.965), respectively. For patient 20, the probabilities were 0.969 (95%CI: 0.911-0.990) and 0.738 (95%CI: 0.123-0.931), respectively. Using the Hgb measurements up to 84 days, the probabilities at 355 days were 0.796 (95%CI: 0.423-0.921) and 0.809 (95%CI: 0.398-0.941) for patients 13 and 20, respectively.

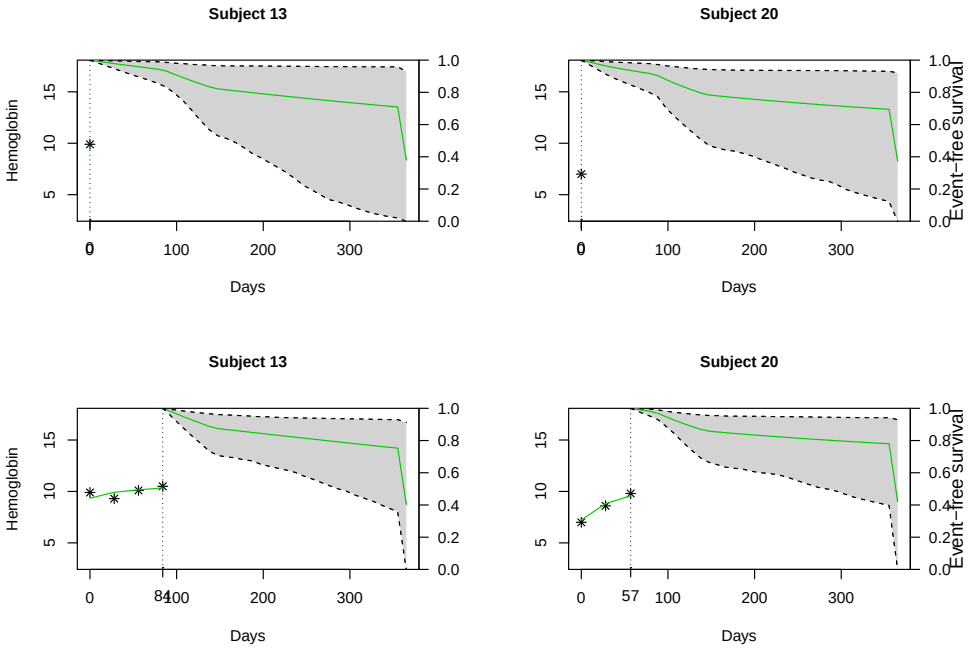


Figure 7.6. Hgb. Prediction of event free probabilities at baseline (top panel) and 84 days (bottom panel) for two selected patients.

White Blood Cell

Figure 7.7 shows the event free predicted probability for patients 13 and 20 using log(WBC) as a biomarker. When only baseline WBC measurement was used, median event-free survival probabilities for patient 13, at 29 and 355 days were 0.988 (95%CI:0.945-0.998) and 0.868 (95%CI: 0.523-0.983), respectively. For patient 20, the event-free survival probabilities were 0.990 (95%CI: 0.943-0.999) and 0.867 (95%CI: 0.489-0.985) at the two time points, respectively. When the measurements of WBC up to day 84 were included, patients 13 and 20 have median probability of event free survival 0.805 (95%CI: 0.502 0.928) and 0.880 (95%CI: 0.653-0.971) at 355 days, respectively.

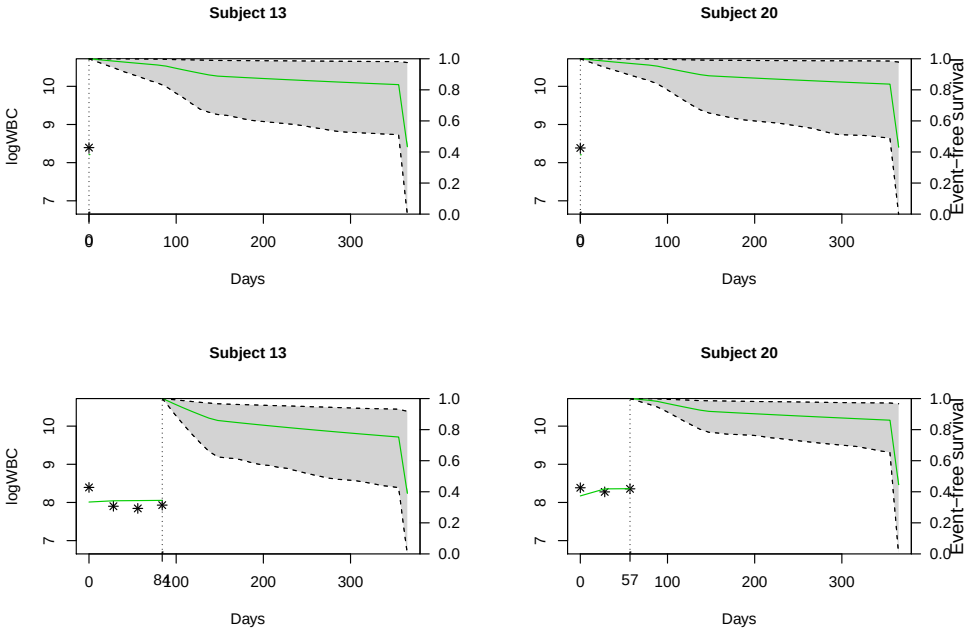


Figure 7.7. WBC. Prediction of event free probabilities at baseline (top panel) and 84 days (bottom panel) for two selected patients.

Figure 7.8 shows the predicted probability for event free (i.e. a patient not yet experience relapse) for an extra 30, 60, 90 and 120 days of measurements given a fixed number of days for which the longitudinal sequence for WBC was observed. Patient 13 (upper panels) experienced a relapse at 215 days. It is, therefore less likely to

survive an extra 120 days which implies of 300 days of observed time. Patient 20 has higher probability of event free survival for an extra 120 days. Patient 20 (lower panels) did not experience relapse until 277 days has higher probability of event free survival for an extra 120 days.

7.4.3 Prediction Accuracy

The joint model allows to investigate whether Hgb or WBC is a potentially useful marker in discriminating between patients who experience relapse within a short time window after their last assessment and patients who did not experienced relapse. In order to asses the overall discrimination power of the markers, the sensitivity (true positive rate, i.e., a patient that was relapsed was classified as relapse) and specificity (i.e., a patient that was not relapsed was classified as non relapsed patient) were calculated. Given the sensitivity and 1-specificity (false positive rate) the ROC curve can be constructed by varying the threshold of the biomarkers. Figure 7.9 shows the ROC curve for WBC and Hgb at t equals to 28, 56 and 84 days, and four options of Δt (14, 28, 42, 56). Hemoglobin has higher discriminating power of patients who will experience relapse from those who will not.

Later we changed the prediction rule in to two threshold where each of these measurements could be utilized. We considered the last two available measurements of the marker, and when there is 20% decrease from the pre-last to the last Hgb it is considered as strong indicator for relapse.

As we mentioned in Section 5.2.4, a summary of the predictive accuracy index of the marker for a possible threshold value c_s is given by AUC. The medically relevant time window, Δt, is assumed to be 14 days. To obtain the estimates for the discrimination measures, AUC and DDI, discussed in Section 5.2.4 were used. At each observed time, the specific time at which the discrimination measures were estimated was assumed to be the last available time (in addition to the baseline).

Table 7.5 presents the AUCs and DDIs values of Hgb marker for patient 13. The discrimination quality alters for different time windows at different time points, from a high of 0.878 for $\Delta_t = 14$ at baseline, to a low of 0.775 for $\Delta_t = 56$ at baseline. This implies that if the primary interest is to predict relapse within a 14 day window, and such a prediction was done at baseline, then the probability that the model would allocate a lower conditional survival probability to a patient who will experience relapse within the next 14 days compared to a patient who will not experience relapse within the 14 days window is equal to 0.878. Based on 20% relative decrease from the previous measurement of Hgb, AUCs varies between 0.890 for $\Delta_t = 14$ at 84 days,

and 0.788 forΔ_t = 56 at 56 days.

Table 7.5. Area under the ROC curve and the estimated DDI of Hgb marker (based on 1000 Monte Carlo samples for patient 13) for two prediction rules.

Parametrization		Simple value		20% Rel.decrease	
Δt	t	AUC(t)	DDI	AUC(t)	DDI
14	0	0.8779	0.7225	0.8858	0.7225
	28	0.8728		0.8730	
	56	0.8730		0.8352	
	84	0.8656		0.8904	
28	0	0.8376	0.6627	0.8531	0.6627
	28	0.8652		0.8561	
	56	0.8637		0.8222	
	84	0.8545		0.8599	
42	0	0.7989	0.6507	0.8283	0.6507
	28	0.8594		0.8446	
	56	0.8549		0.8019	
	84	0.8491		0.8413	
56	0	0.7747	0.6401	0.8088	0.6401
	28	0.8538		0.8349	
	56	0.8468		0.7876	
	84	0.8454		0.8288	

The DDIs presented in Table 7.5 for patient 13, is used as a summarized measure of the discriminative ability over the follow-up period. As shown in Section 5.2.4, it provides a weighted average of the AUCs, with weights that account for the size of the risk group within the follow-up period. The indices range from 0.6401 for a time window of 56 days to 0.7225 for a time window of 14 days. As time progresses, we see a decrease in the separation between the three values of Δ_t. Similarly, Table D.9 in Appendix D and presents the results for WBC marker. A similar analysis for patients 20 is presented in Table D.10 Appendix D.

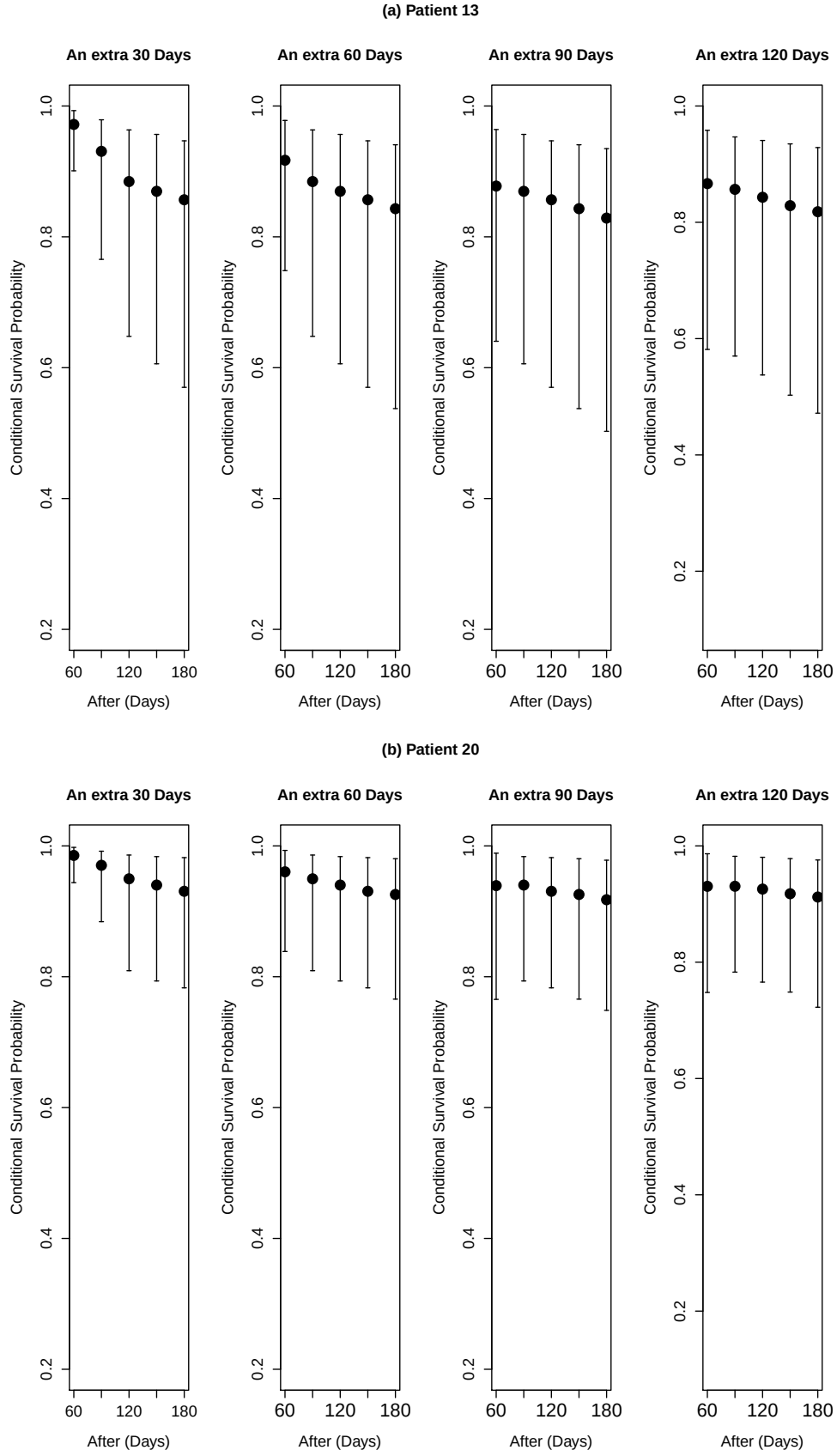


Figure 7.8. WBC. Predicting conditional survival probabilities and 95% confidence intervals of surviving an extra 30, 60, 90 and 120 days, with each additional 30 days of measurement.

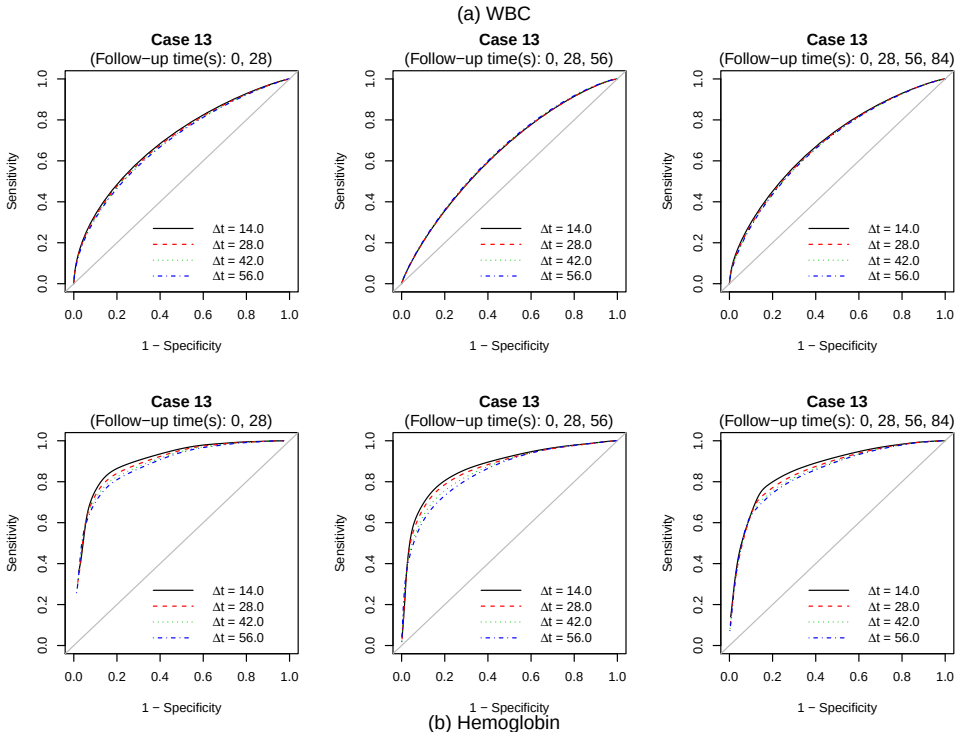


Figure 7.9. Time-dependent ROC curves for WBC (upper panel) and Hgb (lower panel) at t=28, 56 and 84 and four options of Δt=14, 28, 42 and 56 days (based on 1000 Monte Carlo samples).

7.5 Discussion

Kala-azar, also known as visceral leishmaniasis (VL), is a chronic multi systemic disease. Co-infection with VL and HIV is recognized as a major public health challenge in Africa which leads to frequent treatment failure, relapse, lost to follow up and death (Cota et al., 2014). Ethiopia is one of the sixth country where 90% of the global burden of VL originated and highest burden of VL-HIV co-infection is found (Argaw et al., 2013). Available treatments have serious associated toxicities and may lead to the development of drug-resistant parasite (Richard and Tim, 2014). Recurrent epidemics of VL have caused high morbidity and mortality in affected communities. In a cohort of VL-HIV co-infected patients, pentamidine has been used as secondary prophylaxis to prevent relapse primarily (Diro et al., 2015b; Singh et al., 2016).

The analysis presented in this chapter aimed to investigate the clinical or laboratory variables to be used as biomarker for the prediction of time to event process in VL-HIV co-infected patients who have been taking pentamidine for the purpose of preventing VL relapse. This involves testing of the different clinical, laboratory, and immunological follow-up variables if they can be used as a biomarker to predict relapse. A total of 74 VL-HIV co-infected patients were included in the study. Nearly one in five patient experience relapse. The cohort contribute a total of 619.6 person-months of follow-up. The current value of WBC and the change in Hgb were significantly associated with relapse (Table 7.3 and 7.3).

Most of the patients were male. This might be explained by the nature of the study area where most of the people in this area are migrant workers coming from different part of the country for work. This is also supported by others studies from Brazil (Cota et al., 2011; Daher et al., 2009) in which most VL-HIV co-infected patients were male. The mean age of the study participates was 33.36 ± 7.01 years, which is similar with another studies by Cota et al. (2011); Daher et al. (2009). The median Hgb level in this study is higher as compared to the study in Southern Sudan and North-east Brazil (Collin et al., 2004; Tvora et al., 2015).

The proportion of relapse in this study was 15 (20.27%). The first patients who experienced relapse was observed after 35 days of treatment initiation. A study in North-east Brazil by Tvora et al. (2015) showed that the mean time for recurrent event was 38 days, which is close to the time the first event was observed in our study. The second event was observed after 83 days. The probability of event free survival at 83, 140 and 240 days were 0.972 (95%CI: 0.933-1.000), 0.837 (95%CI:0.748 -0.925) and 0.772 (95%CI:0.671-0.874), respectively. A study in India revealed that VL-HIV co-infected patients had higher 6-month relapse rate, and less relapse-free

12-month survival (Goswami et al., 2017). The prevalence of relapse in the current study is lower than the study conducted in Spain by Molina et al. (2007), but much greater than the study conducted in India by Mahajan et al. (2015).

We have shown that, based on the joint model for Hgb and time to relapse, the rate of change in Hgb over time is associated with relapse. Different studies conducted in India showed that Hgb was not significantly associated with relapse in the adjusted analysis (Burza et al., 2014; Mahajan et al., 2015). These studies used the baseline value of Hgb as a predictor for relapse. A unit increase in the slope of Hgb level reduces the probability of relapse by 65.9% (HR=0.341, 95%CI: 0.132-0.881). Similarly, probability of relapse can be predicted by the current value of WBC. A unit increase in log WBC results in a 84.9% (HR=0.151, 95%; 0.026-0.874) reduction in the probability of relapse.

In addition, the longitudinal process of the biomarker was used to predict event free survival probability of relapse in the future for each patient. The biomarkers were updated with additional measurements up to 84 days, and the event free survival probability were predicted at any time after 84 days. The higher the number of measurements of the biomarker included in the model, the higher precision of the predicted probability. We have shown that the predictive of Hgb marker is better the predictive ability of WBC as evidenced by ROC and AUCs values for relapse. Lower values of Hgb has higher discriminating ability between patients who will experience relapse and who will not. Time window of 14 days is the optimal time to discriminate patients who will experience relapse from those who will not. For a 20% relative decrease of Hgb (9.7423 to 7.7939), the value of area under ROC curve was equal to 0.8904 for time window of 14 at 84 days.

One of the limitations of this study was small sample size that leads to a lower power. As a results, clinical and laboratory markers could turn to be not significant. In conclusion, using the joint model presented in this chapter we have shown that both WBC and Hgb can be used for biomarkers for time to replace. This implies that the patients performance in the early stage of the follow up can be used to predict the patients outcome at the end of the study.

Part III

Monitoring and Modeling HIV Patients Under ART using the ETART Shiny App

Chapter 8

The ETART Shiny App

8.1 Introduction

HIV care and treatment services are available, since the epidemic started in Ethiopia. The facilities provide counselling, testing, pre-ART services, and ART treatment where both self-referred individuals and physician referred patients receive services. It is estimated that currently, 386, 123 patients are enrolled to ART program in Ethiopia (UNAIDS, 2016a). Being a life long diseases, data for HIV patients are accumulated from the date of test for an HIV-infected individual throughout his/her treatment period (Young, 2015). Initially, the service was limited to government hospitals, but since 2006 government health centers started providing the services as well. Currently, a total of 1045 treatment centers are providing the services in Ethiopia (Assefa et al., 2017; Frehiwot et al., 2014). Since 2009 both paper based and electronic data archiving system is implemented in treatment centers and the data of a treated patient are recorded at each visit during the treatment period.

Although the databases contain treatment information and patient performance and typically available in the hospitals, they are rarely analysed due to limited capacity in data analysis skills. Health professionals and policy makers should base their decision on the current and future treatment strategy based on evidence available in the data. However, due to lack of expertise in data analysis, the information available in the treatment center database is typically not used. For example, local treatment efficiency in a health center can be evaluated using the centers database but this is usually not done.

This chapter is aimed to close the gap in local data analysis capacity by providing,

a free, on-line based, user friendly, data analysis tool for the analysis of a standard HIV patients database. We provide a Shiny R application, the ETART Shiny App, that can be used to produce a similar analyses that was presented in Chapter 3-5. Both on-line and standalone version of the ETART package are available. The latter is useful for treatment centers with poor internet infrastructure. The ETART Shiny App can be downloaded from:

```
https://hivshiny.shinyapps.io/shiny-DataAnalysis_level1/
```

The ETART Shiny App can be applied to a local patients database in any treatment canter provides that the treatment canter uses a standardized database to store the patients data. In Section 8.2, we introduce the standardized databases structure that should be constructed by a treatment canter. In Section 8.3, we overview the data analysis tools, the methods and the analysis output that can produced using the ETART Shiny App.

8.2 Local Standardized Database for ART Patients

In this section we introduce the structure of the standardized local database that can be used as an input data for the ETART Shiny App. Figure 8.1 shows the three stage procedures that should be applied to construct and analysed the ART patients' standardized database. There are two type of outcomes that can be analysed by the ETART Shiny App: time to event (i.e, time to death, time to composite outcomes, etc) and longitudinal outcome (i.e. CD4 cell counts, etc). Two databases should be constructed using the following procedure:

- Stage 1: The user should prepare the data in excel format based on the outcome of interest:
  - For time-to-event outcome, the data should include time variable (with the specified unit), censoring indicator, and possible covariates.
  - For the longitudinal outcomes, the time variable (i.e. date of each visit), response variable and other baseline covariates are required.
- Stage 2: Once the databases were created, the dataset has to be uploaded to ETART Shiny App.
- Stage 3: Based on the information required, select and execute the data analysis in order to produce the outputs.

An example of the databases created for Gondar University Hospital is discussed in the next sections.

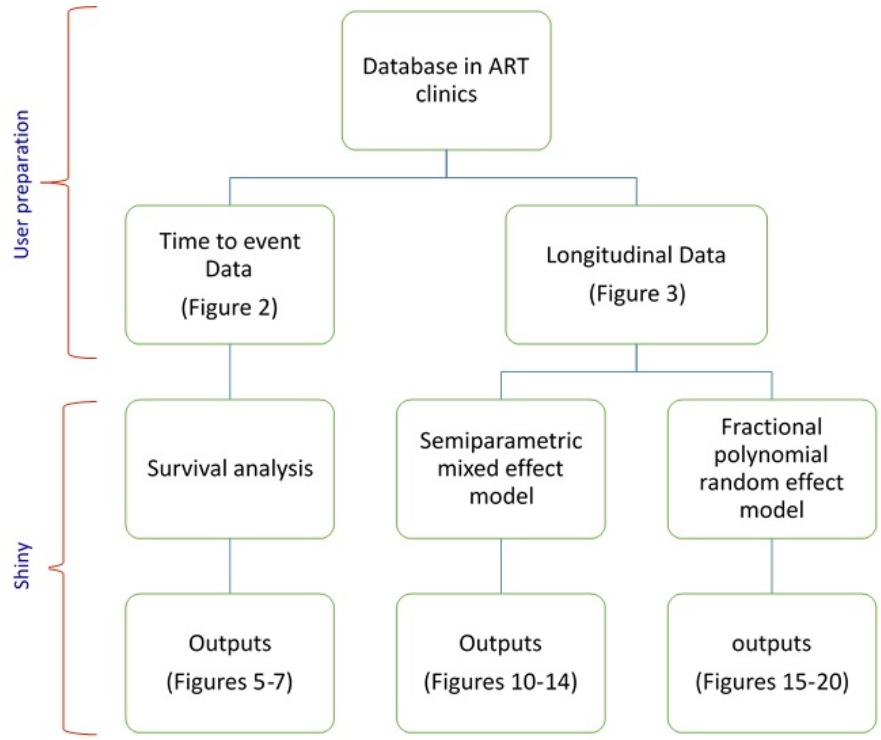


Figure 8.1. Schematic presentation of data preparation for the `ETART` Shiny App. The user should organized and prepared the data based on the research question, time to event analysis or longitudinal analysis of CD4 cell counts (second lines). The data is uploaded and executed to produce the required outputs (third and fourth lines). Note that the data analysis using fractional polynomial requires an initial modeling step.

8.2.1 ART Database at Gondar University Hospital

Gondar University Hospital grew out of the Gondar Public Health College and Training Center (PHC) established in 1954. It is a 570-beds university hospital, which acts as the referral centre for four district hospitals in the area. It serves as the main hospital for more than 5 million individuals in the region and neighbouring regions. It has a range of specialities including paediatrics, surgery, gynaecology, psychiatry, HIV care and treatment clinic. The HIV clinic provide pre-ART and ART services since 2005. A total of 14333 adult HIV-infected individuals ever enrolled and currently

5492 HIV-infected individuals are on ART care. In the clinic, baseline and follow-up variables are recorded at registration and over the treatment period, respectively. Baseline variables include gender, age, weight, WHO staging and functional status were collected when the patient registered in the clinic. Follow up variables include patient performance indicators such as CD4 cell counts, weight, functional status, adherence, opportunistic infection (OIs), TB status and regimen. The follow up variables are recorded every 6 months subsequently depending on the progress of the patient.

Two databases were created. The first dataset, `1survdata.csv`, shown in Figure 8.2, contains information about time to event data and treatment and will be used to compare treatment efficacy in terms of time to event outcome (for example, survival time under NNRTI treatment, NRTI backbone that was presented in Chapter 3). The second dataset, `3longData.csv`, shown in Figure 8.3 contains information about patients performance using CD4 cell counts and treatment and will be used to compare treatments efficacy in terms of CD4 evolution and change over time that was presented in Chapter 3 and 4.

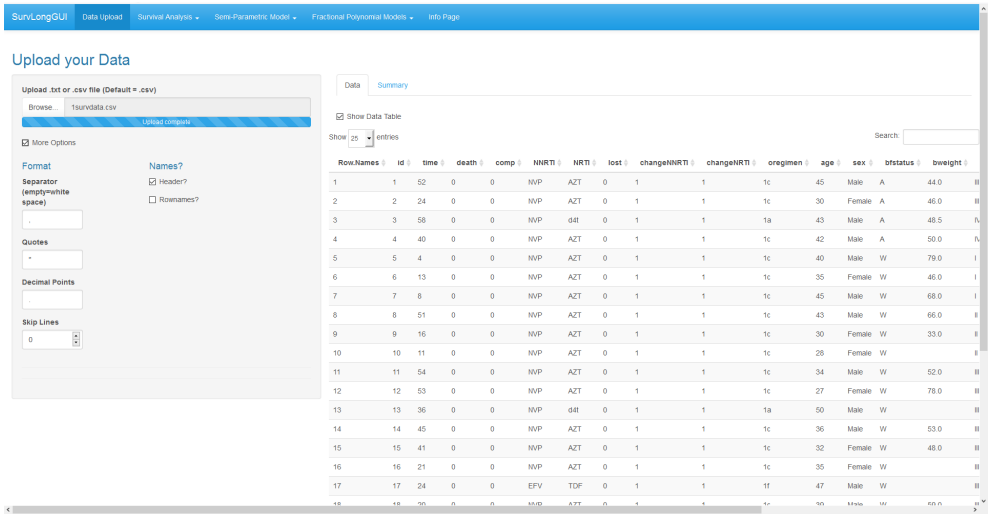


Figure 8.2. Variables for the analysis of time to event data are specified in the left panel. A short description of the data and a partial printout are shown in the right panel.

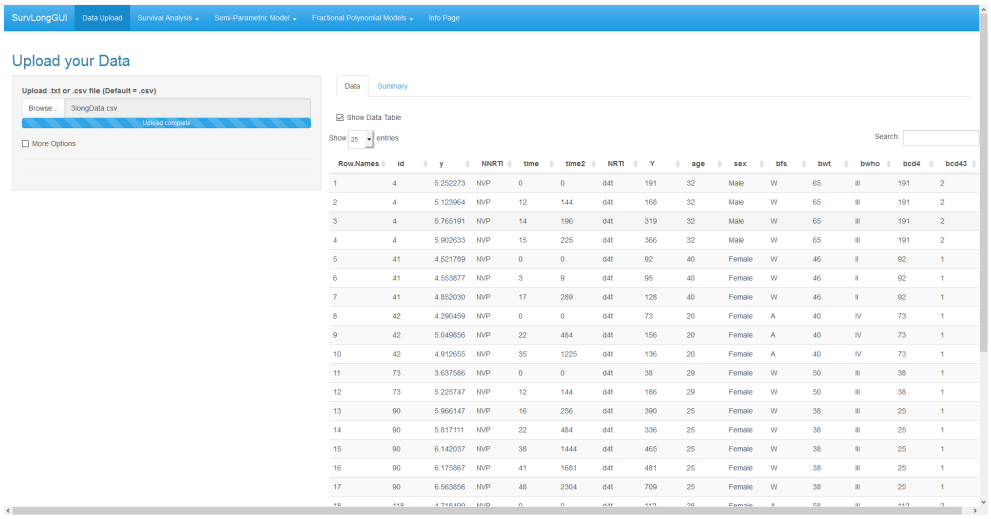


Figure 8.3. The ART `3longData.csv` analysis is specified. A short description of the data and a partial printout are shown in the right panel.

8.3 Data Analysis Tools and Methods

8.3.1 Time to Event

Distribution of Patients

Once the time to event dataset `1survdata.csv` is uploaded to the `ETART` Shiny App, several analyses can be conducted. A summary of patients distribution by backbone and treatment is shown in Figure 8.4.

Kaplan-Meier Curves and Log-rank Test

Two time to event outcomes can be analysed by the `ETART` Shiney App: time to death and time to composite outcome. For both outcomes, a comparisons between the treatment groups in the center can be conducted. Let T be a random variable representing the time to event (i.e. death or composite outcome). Then the probability of the event occurring at exactly time t can be formulated as;

$$S(t) = P(T \geq t) = \int_t^{\infty} f(u)du.$$

The Kaplan-Meier survival curve for both outcomes can be produced automatically using the middle tab in Figure 8.5. Note that although the analysis conducted using the `R` software, the `ETART` Shiny App users are not expose to the `R` code used to

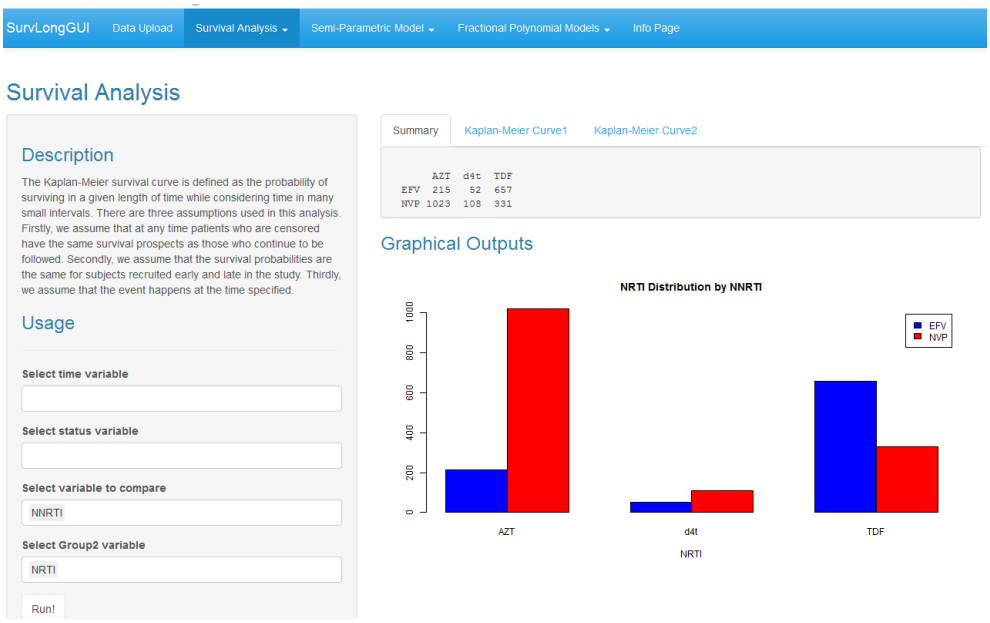


Figure 8.4. Summary table (upper panel) and bar-chart plot for the ART groups (lower panel).

produce the analysis and do not need to install `R`. The Kaplan-Meier plot, presented in Figure 8.5, can be produce using the R function `survfit`,

```
tfailure<- survfit(Surv(time, comp)~1, conf.type ="plain",
 data = dataInput())")
```

The variables `time` and `comp` are, respectively, the time to event and censoring variables in the dataset `1survdata.csv` presented in Figure 8.1.

Comparison of Kaplan-Meier survival curves between different groups can be done using log-rank test Grafféo et al. (2016).

$$\chi^2(log-rank) = \frac{\sum_{i=1}^{k}(O_i - E_i)^2}{E_i}. \tag{8.1}$$

Here, k the total number of groups, O_i is the total numbers of observed events in the ith group, and E_i is the total numbers of expected events in the ith group. Figure 8.6 presents the results for the comparison of time to composite outcome between the EFV and NVP groups in Gondar university Hospital and reveals that the time of composite outcome of the EFV group is faster i.e., more patients who treated by EFV experienced the event compare to the NVP group.

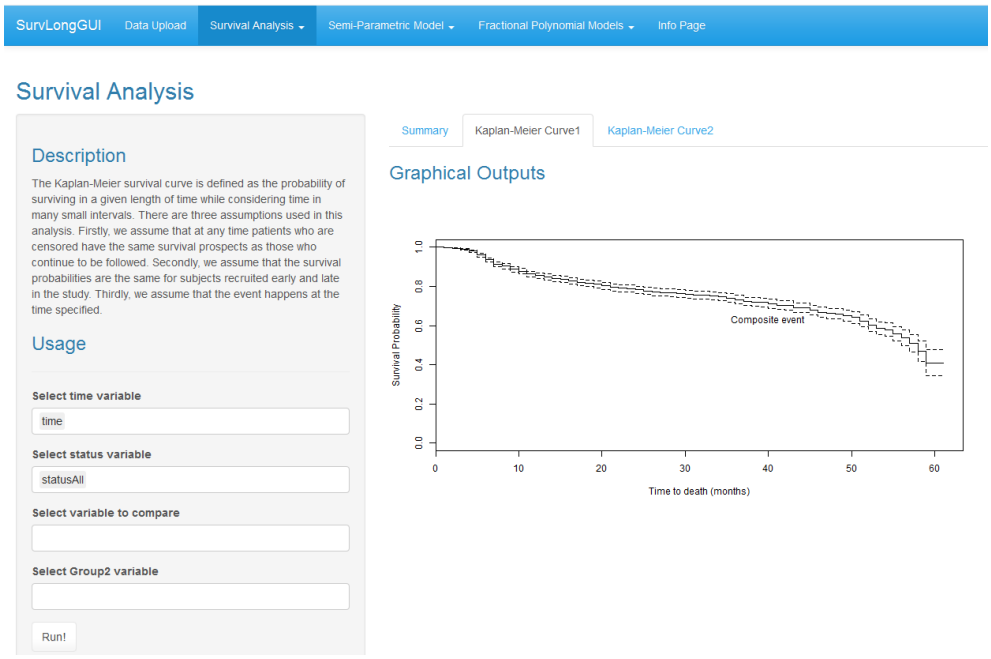


Figure 8.5. Gondar University Hospital. Kaplan-Meier survival curve for composite outcome.

The log-rank test specified above can be produced using the `R` function **`survdiff`**:

```
lrankNNRTI<-survdiff(Surv(time, comp)~NNRTI,
data = dataInput(), rho=0))
summary(lrankNNRTI)
```

The variable `NNRTI` is a factor variable and the time to event will be compared across the factor levels, i.e., EFV and NVP groups.

Cox Proportional Hazard Model

The ETART Shiny App allow to test the effect of NNRTI drug, NRTI backbone and other covariates at baseline on the event time using a Cox-PH model. The hazard for an event, conditional on the covariates of interest, is formulated as;

$$h_i(t|\mathbf{X_i}) = h_0(t)exp(\mathbf{X_i}\beta). \tag{8.2}$$

Here, $h_0(t)$ is the baseline hazard and $\mathbf{X_i}$ are the covariates of interest. For the analysis of time to composite outcome in Gondar University Hospital, it is assumed

Figure 8.6. Log-rank test between time to composite outcome of the EFV and NVP NNRTI groups.

to be depended on treatment, age and gender, that is

$$\mathbf{X_i}\beta = \beta_0 + \beta_1 \mathbf{NNRTI} + \beta_2 \mathbf{NRTI} + \beta_3 \mathbf{age} + \beta_5 \mathbf{sex}. \tag{8.3}$$

The specification of Cox-PH model in (8.2) and the output are presented in Figure 8.7. A similar model can be fitted using the `R` package `coxph` in the following code:

```
mysurv<-coxph(Surv(time, comp) ~as.character(NNRTI) +
as.character(NRTI) + age + as.character(sex), data = dataInput()))
   summary(mysurv)
```

For the model specified in (8.3), the variable `comp` is a censoring variable related to the event composite outcome. The variables `NNRTI`, `NRTI`, `age` and `sex` are the covariates in the dataset `1survdata.csv`, (see Figure 8.3) included in the model.

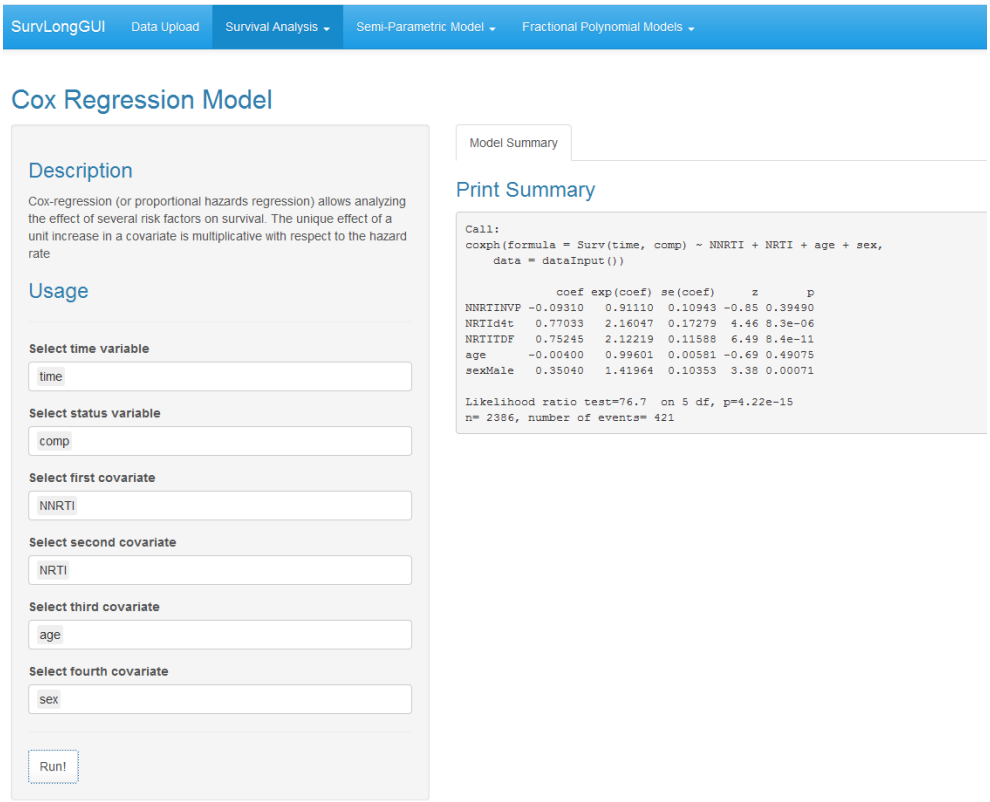


Figure 8.7. Cox-PH regression for selected covariates. Left panel: model's specification. Right panel: output.

8.3.2 A Longitudinal Analysis of Immunological Outcomes

Profile Plots

The second database contains information about immunological performance of the patients measured by CD4 cell counts. In addition to baseline variables, data are available at each patient's visit and can be used to compare patients immunological performance between treatment groups.
Patients profiles plots (by group) and the evolution of mean CD4 cell counts over tome is shown in Figure 8.8 and 8.9.

Semi-parametric Mixed Effect Model

The `ETART` Shiny App uses a penalized thin plate spline mixed effect model to model a subject specific change in CD4 cell counts over the treatment period. Let $\mathbf{Y_i}$ be

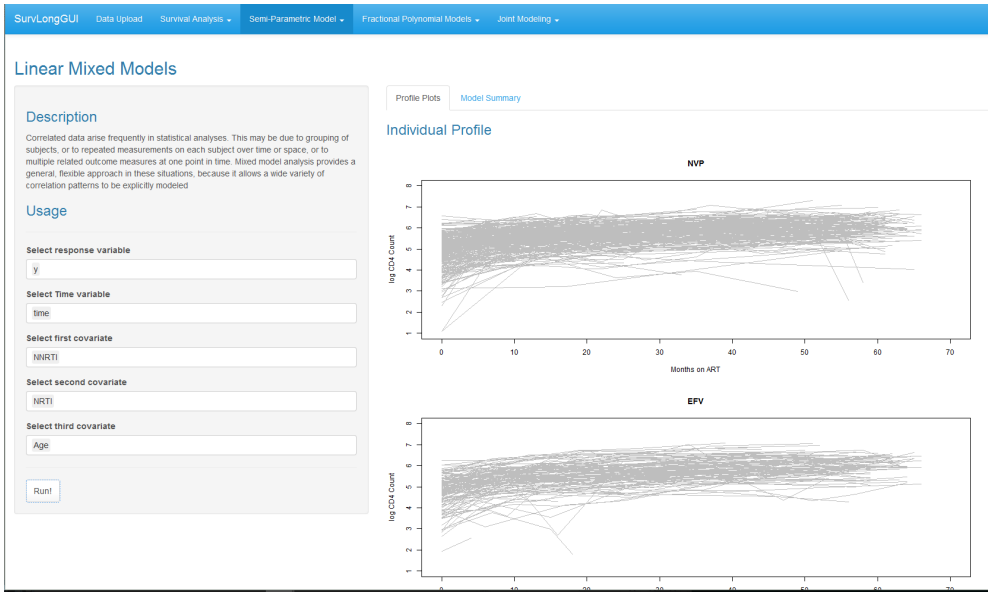


Figure 8.8. Gondar University Hospital. Patients' profiles of log(CD4) cell counts.

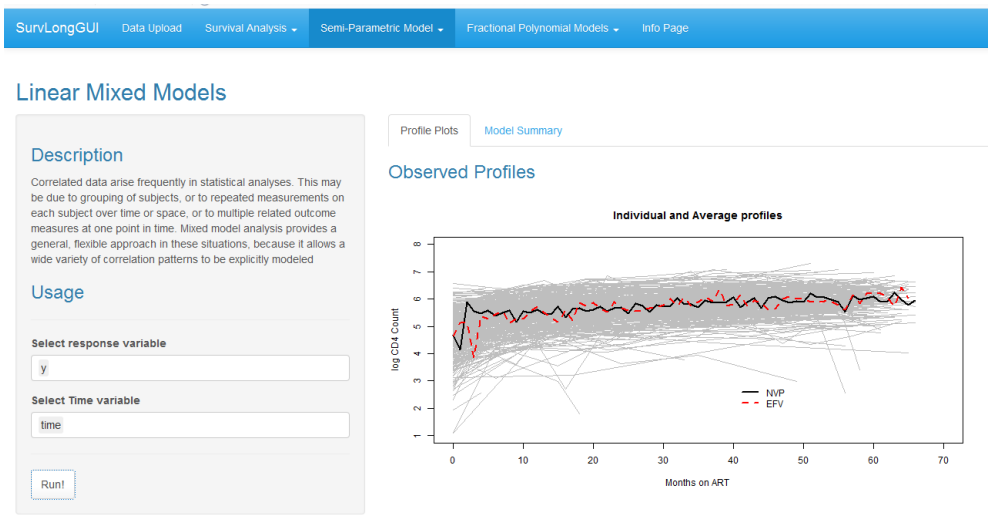


Figure 8.9. Gondar University Hospital. Observed individual and average profile of log(CD4) cell counts.

the patients CD4 cell counts. The penalized thin plate spline mixed effect can be expressed as a mixed model of the form

$$\mathbf{Y_i} = \underbrace{\mathbf{X}_i\beta_i}_{S(t_i)} + \mathbf{Z}_i b_i + \varepsilon_i. \tag{8.4}$$

Here, $\mathbf{X_i}$ is matrix of fixed effect covariate and $\mathbf{Z_i}$ is matrix of random effect covariates. For the CD4 cell counts, the following subject-specific model is formulated:

$$\mathbf{Y}_i(t_i) = S(t_i) + b_{0i} + b_{1i}t_i + b_{2i}t_i^2 + \varepsilon_{it_i}. \tag{8.5}$$

Here, $S(t_i)$ is the smoother to the log(CD4) evolution given by

$$S(t_i) = \beta_0 + \sum_{\iota=1}^{\nu} \beta_\iota f_\iota(t_i),$$

Where $f_\iota(t_i)$s are a set of thin plate spline basis functions. The `ETART` Shiny App uses a penalized thin plate spline mixed effect model to model a subject specific change in log(CD4) cell counts over the treatment period. Figure 8.10 shows the estimated mean profiles by treatment group, obtained for the penalized thin plate spline mixed model.
The semi-parametric model can be fitted using the R function `gamm` using the following `R` code:

```
GammFit<-gamm(y ~ as.character(NNRTI)+ s(time, by=NNRTI, bs="tp",
m=3), data=dataInput(),random=list(id=~1+ time + time*time))
summary(GammFit)
```

Here, `y` is the log(CD4) cell counts, `NNRTI` is the fixed effect for the group and `s(time, by=NNRTI, bs="tp", m=3)` is used to define a third order thin plate spline for which the data are smoothed with respect to time (`time`), taking into account the treatment group (`NNRTI`). The random effect configuration is defined using the `random` statement,

```
random=list(id=~1+time + time*time).
```

The rate at which the CD4 cell counts change over time can be estimated using the first derivative of the mean given by

$$\frac{dS_g(t_i)}{dt} + b_{1i} + 2b_{2i} \times t. \tag{8.6}$$

Figure 8.11 shows the first derivative and 95% confidence interval for the NNRTI

Figure 8.10. Gondar University Hospital. Estimated mean log(CD4) cell count over time by treatment group.

group. Note that whenever the first derivative cover the value of zero, it indicates that the mean CD4 cell count reach an asymptote. It shows that, for the NNRTI group in Gondar University Hospital, the patients are stabilized after approximately 10 months.

Pointwise Confidence Intervals

A point-wise confidence interval for the mean CD4 cell count can be computed as:

$$\hat{S}(t) \pm t_{1-\alpha/2} s.d(\hat{S}(t)). \tag{8.7}$$

Where $s.d(\hat{S}(t))$ is the square root of the diagonal of the variance covariance matrix $\mathbf{X}\hat{V}_\beta\mathbf{X}^t$, with $\hat{V}_\beta = (\mathbf{X}^t\hat{\mathbf{V}}^{-1}\mathbf{X} + \mathbf{Z})^{-1}$. Here, $\hat{\mathbf{V}}$ is the variance and covariance matrix and $\mathbf{Z}$ is the wiggliness penalty matrix.

Pairwise Comparison of Treatment Groups

The semi-parametric model implemented in the `ETART` Shiny App allows to compare between the patients immunological performance, i.e., the CD4 evolution of the

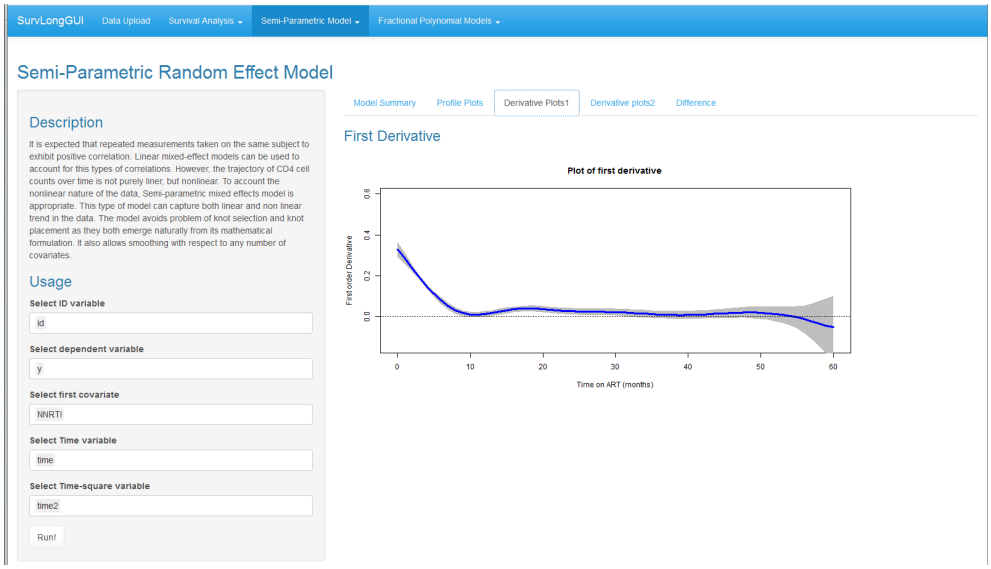


Figure 8.11. First derivative of CD4 cell counts. Plot with 95% point-wise confidence interval.

treatment group. This can be done using a group specific smoother,

$$Y_{ig}(t_i) = \underbrace{\beta_{0g} + \beta_1 treat_g + \sum_{\iota=1}^{\nu} \beta_{g\iota} f_\iota(t_i)}_{S_g(t)} + b_{0i} + b_{1i}t_i + b_{2i}t_i^2 + \varepsilon_{it_i}. \tag{8.8}$$

Where $Y_{ig}(t_i)$ is the response for the ith subject in the gth treatment group at time point t_i, $S_g(t)$ is a group specific smoother, and $f_\iota(t_i)$'s are a set of thin plate spline basis functions, $\beta_{\iota g}$ are the coefficients of the basis function. The first derivative is given by

$$\frac{dS_g(t_i)}{dt} + b_{1i} + 2b_{2i} \times t. \tag{8.9}$$

Here, $S_g^{'}(t_i) = \beta_g^t F^{'}(t_i)$ is a treatment group specific first order-derivatives of penalized thin-plate spline fit. Pointwise confidence interval for $S_g^{'}(t_i)$ can be constructed in the same way as described in Section 8.3.2.

Figure 8.12 shows that the point-wise confidence intervals for the EFV and NVP groups are overlapping indicating that there is no difference between the two group

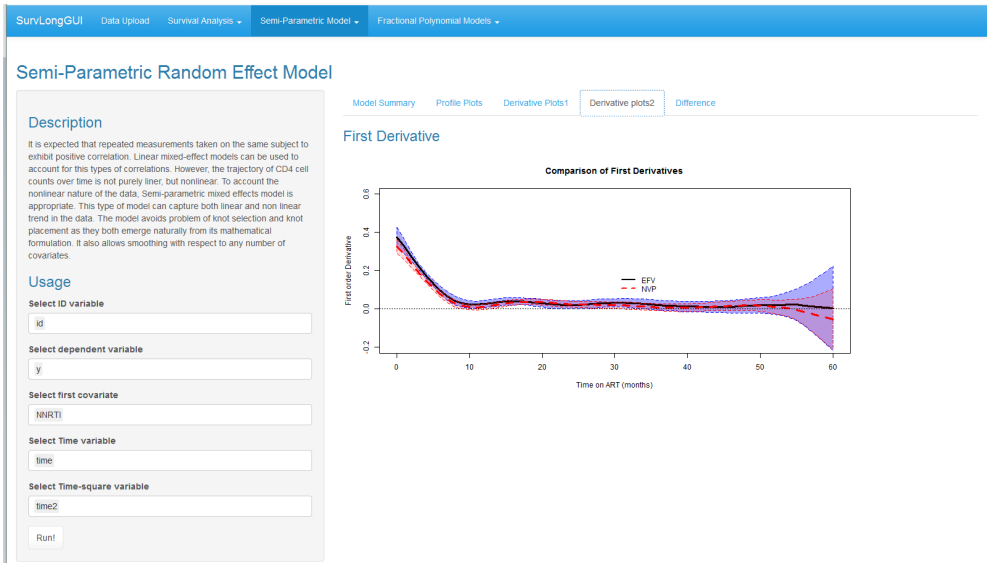


Figure 8.12. Treatment specific rate of change in CD4 cell counts over time and with 95% pointiwse confidence interval

in terms of the change in immunological performance of the patients.

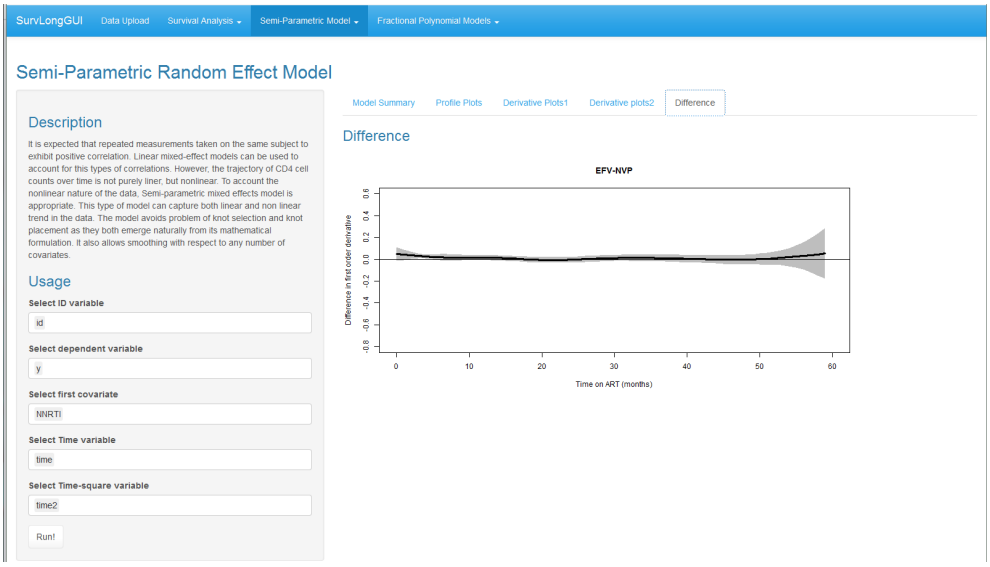


Figure 8.13. Difference between the rate of CD4 cell count change over time: NVP Vs. EFV. The smoothed line shows $S^{'}_{NVP} - S^{'}_{EFV}$.

8.3.3 Model Based Prediction of Time to Cross a Pre-Specified CD4 Threshold

The analysis presented in this section is based on initial modeling using fractional polynomials (FP) discussed in Chapter 4. It is assumed that the user fit a FP model and hence, in this stage, the powers are known. The mean structure of a second order mixed effects fractional polynomial is given by

$$f(t_{ij}) = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})t_{ij}^{p_1} + (\beta_2 + b_{2i})t_{ij}^{p_2}. \tag{8.10}$$

As shown in Chapter 4, the optimal values of the powers that lead to smallest value of AIC are (p1 = 0; p2 = 0.5) and (p1 = 0; p2 = 0) for NVP and EFV, respectively. The estimated subject-specific mean structure, for each treatment group, are given, respectively, by

$$\begin{aligned} \hat{f}(t_{ij}) &= (5.22 + \hat{b}_{0i}) + (0.05 + \hat{b}_{1i})log(t_{ij}) + (0.08 + \hat{b}_{2i})t_{ij}^{0.5}, \\ \hat{f}(t_{ij}) &= (5.05 + \hat{b}_{0i}) + (0.14 + \hat{b}_{1i})log(t_{ij}) + (0.024 + \hat{b}_{2i})(log(t_{ij}))^2. \end{aligned} \tag{8.11}$$

Figure 8.14 shows the estimated model for the NVP treatment. Note that, as explained above, the model is estimated assuming that the powers for the FP were estimated in the first stage of the analysis. Estimated mean CD4 cell counts for each group are shown in Figure 8.15.

As mentioned in Chapter 4, the mixed effect FP2 model allows to estimate and to predict a subject specific CD4 profile and to compare this to a pre specified threshold. Figure 8.16 shows the estimated CD4 profile (using an estimation period of 30 months) for selected subjects.

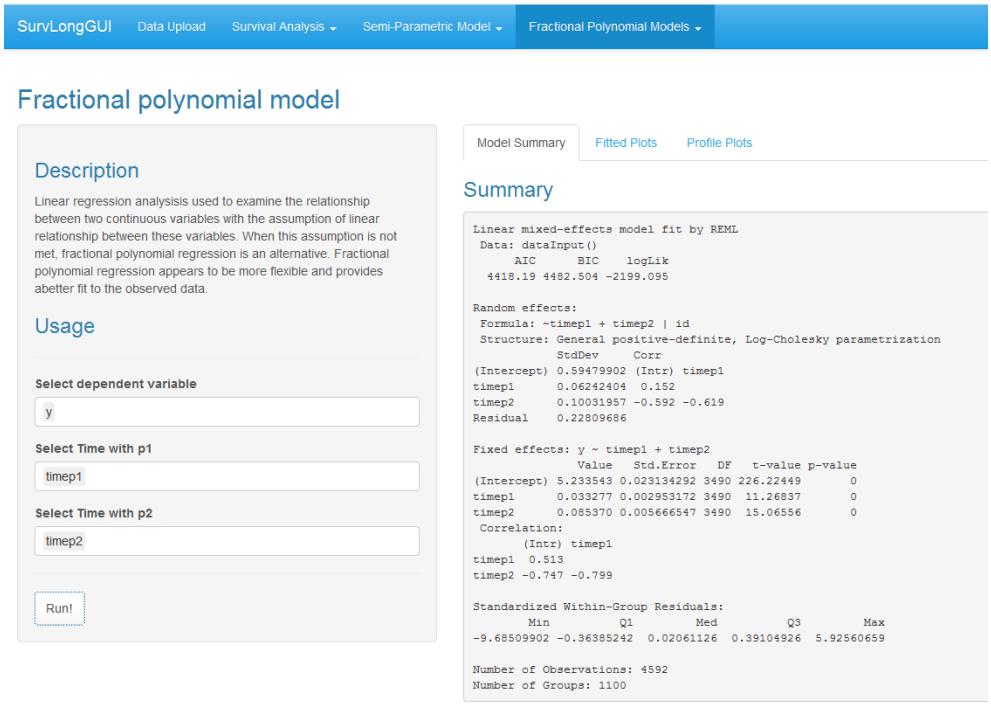


Figure 8.14. R output for the estimated fractional polynomial model of the NVP group.

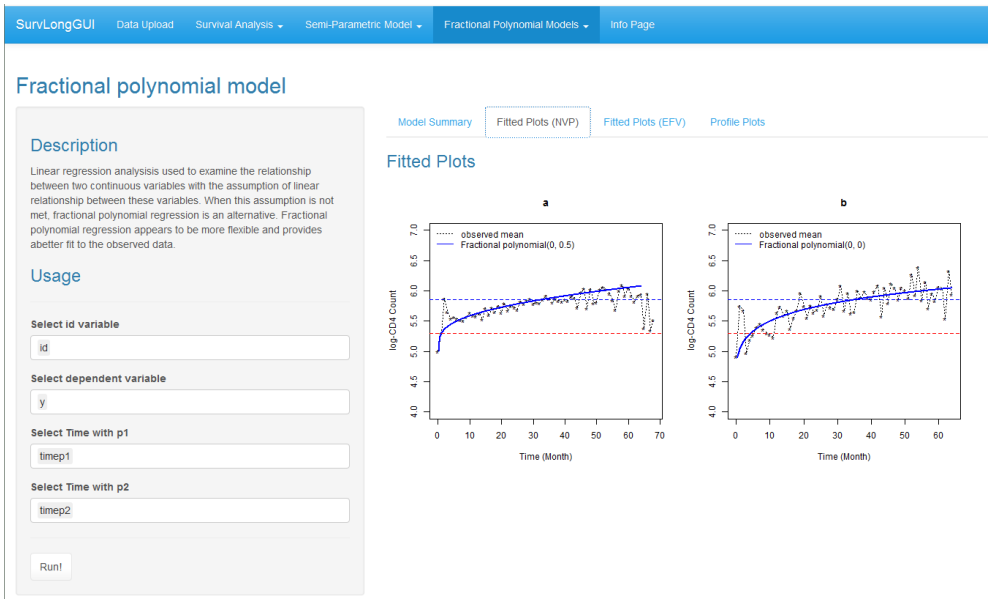


Figure 8.15. Estimated mean profiles using a fractional polynomial models. Panel a: NVP. Panel b: EFV.

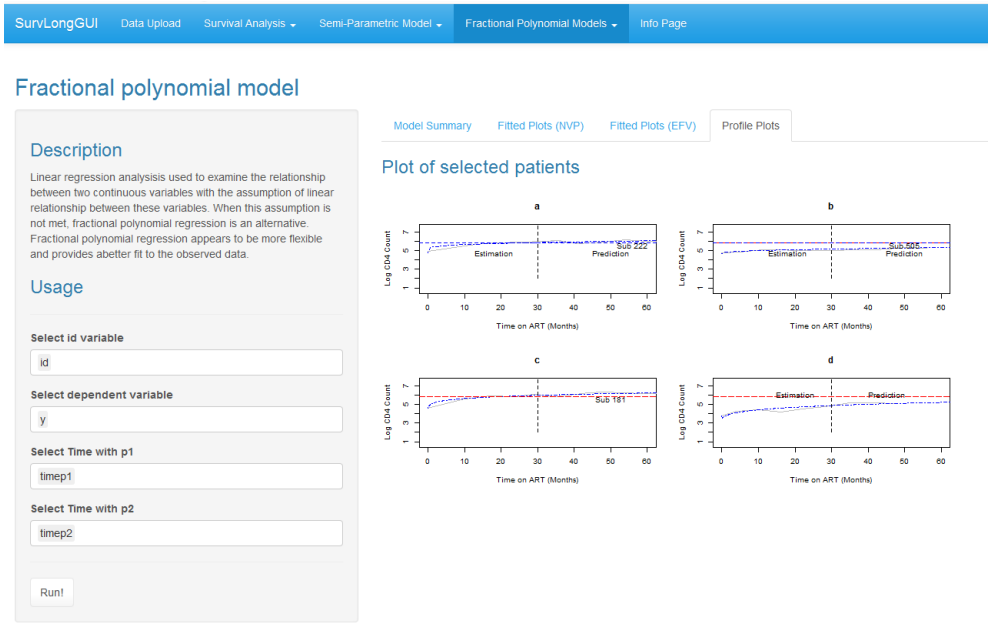


Figure 8.16. Observed and model based predicted log(CD4) cell counts values for selected individuals. Panel a and b: NVP group. Panel c and d: EFV group.

8.4 Discussion

In Ethiopian hospitals, HIV treatment centers are routinely collecting data on different characteristics of the patients since their time of enrolment in the center. These data are seldom analysed at a hospital level. Therefore, although the information is available, it is not usually used for a decision making and for monitoring the patient population in the treatment canter of the hospital. There is currently a gap between the capacity to collect the patients data and the capacity to analyse them and/or to produce a monitoring report about the patients' performance in treatment center. For example, the survival rate in a treatment center is typically does not estimate on a regular basis and over time.

This chapter is aimed to close this gap. We provide a publicly and user friendly Shiny App, the `ETART` that can be used to produce standard analysis of HIV patients under ART treatment. To conduct that analysis, the user should construct a standardized database for the HIV patients under treatment that include the clinical information and treatment allocation for the patient. Once the database is created, the user can conduct the analysis using a self-explanatory graphical user interface. An expert knowledge in R and/or statistical modelling is not required.

Chapter 9

Discussion and Future Research

9.1 Modeling HIV Data in Ethiopia

The emergence of HIV has been one of the biggest challenges the world faced for the last three decades. There is no cure for the diseases so far, however the introduction of ART transformed the disease manageable chronic disease type of illness. The primary goal of ART is to reduce HIV-related morbidity and mortality, prolong survival, improve the quality of life, restore and preserve immunologic function and prevent HIV-transmission (Günthard et al., 2014). Cohen et al. (2011) confirmed that if an HIV-positive person adheres to an effective ART regimen, the risk of transmitting the virus to their uninfected sexual partner can be reduced by 96%. A study in the US and Canada, reported in (Samji et al., 2013), that a person in his/her 20s who contracts HIV can now expect to live into the 70s if initiated ART early and adhere.

In chapter 2, we compared the effect of the choice of treatment combination during initiation of ART. First-line ART regimen should contain one NNRTIs (EFV or NVP) plus two NRTI, one of which is either AZT or TDF (WHO, 2006). The choice of treatment combinations for HIV-infected patients to initiate ART depends on cost and efficacy (Pandhi and Ailawadi, 2014). Identifying the long-term treatment outcomes of these drugs is very decisive for clinical decision. The systematic review and meta-analysis by Ayele et al. (2017) showed that initiating with EFV ART regimen is associated with a reduced risk of treatment failure compared to NVP regimen in resource limited settings. This is in line with previous meta-analysis by Pillay et al. (2013). In contrast, a Cochrane review of seven randomized clinical trials (Mbuagbaw et al., 2010) demonstrated that the two drugs provided comparable results in

suppression the viral load. In addition, the patients who initiated with EFV are less likely to switch treatment than NVP which is consistent with multicenter randomized non-inferiority trial (Bonnet et al., 2013).

The outcomes of first-line ART regimens are different in the long-run and a composite outcome was defined as NNRTI substitution, discontinuation, lost to follow up or death. In Chapter 3, we have shown that there is no statistical significant difference in the risk of composite outcome among patients who initiated with EFV or NVP regimens after adjusting for baseline covariates. This is contradicting with the result of systematic review and meta-analysis (Ayele et al., 2017). This might be due to the difference in the definition of the outcome of interest in which the current study defined outcome to be the combination of NNRTI substitution, discontinuation, lost to follow up or death.

Treatment selection is often influenced by subject characteristics at baseline in which selection bias is one of the major issues when we assess the treatment effect in observational studies. Propensity score matching method was performed to reduce the effect of selection bias. We have shown that the risk of composite outcomes between patients who were initiated with TDF regimen is higher compared to AZT regimen (after controlling for NNRTI drugs). The risk of composite outcome for TDF when combined with NVP is two times higher as compared to AZT. We have also shown that there was an increased risk of composite outcome for patients who were initiated with d4t containing regimen as compared to AZT regimen. The risk of composite outcomes was not statistically significant on d4t with any of the NNRTI drugs when lost patients were assumed censored. This indicated that the difference in risk on composite outcome on d4t versus AZT was due to lost to follow up cases. TDF has higher risk despite the types of events which is consistent with other studies (Benjamin et al., 2011; Rey et al., 2009). Those patients who initiated with d4t regimen had about two fold risk of lost to follow up as compared to those who initiated with AZT containing regimen. This might be due to the long-term irreversible side effects of d4t. Initiating with TDF backbone has also higher risk of NNRTI modification than those who initiated with AZT. This is in contrast with finding of the study in South Africa by Brennan et al. (2013) in which rate of substitution was lower among those who initiated with TDF than AZT or d4t.

The outcomes of treatment can be monitored using different markers of HIV-infected individual who initiated ART. From Chapter 3 onward, we focused on CD4 cell counts that has been used as a main surrogate marker of treatment response for HIV-infected individuals in order to predict long-term outcomes of treatment. In Chapter 3 we compared the rate of CD4 change between treatment groups at

initiation. We proposed a semi-parametric mixed effects model for the longitudinal evolution of CD4 cell counts in order to investigate treatment response. We assumed patient-specific random parameters for both the linear and quadratic time effects to capture different evolution of the CD4 cell count over time. The first derivative plot shows that the rate of CD4 increase in response to treatment is high during the first 10 months and stabilized later. The result also revealed that there was no difference in the trend of CD4 cell counts in the long-run among patients who initiated with EFV or NVP containing regimen which is in-line with previous study conducted in Ghana (Barry et al., 2013).

In Chapter 4, a flexible parametric approach is used to describe the dependency between a response of primary interest and continuous covariates. Further, the model was used for early prediction of CD4 cell counts under a specific treatment and to estimate the time to cross a given CD4 threshold under treatment. A cross-validation procedure was applied to evaluate the performance of the prediction accuracy of a fractional polynomial mixed effect model. Using the model, the time to attain a pre-specified CD4 threshold after ART initiation was estimated. The model allow us to estimate the distribution of the time to cross a pre specified CD4 cell count threshold of interest and to use this distribution to compare between treatments. Subsequently the probability to attained CD4 cell count above a pre-specified threshold is predicted for each individual. We have shown that more than half of the patients who initiated ART at CD4 cell counts less than 200 $cells/mm^3$ cross the threshold in six months period after initiation.

Different studies showed that the baseline CD4 cell count influences the rate of immune reconstitution (Jacobson et al., 2004; Kadima et al., 2014; Notermans et al., 1999; Smith et al., 2004). These studies indicated that markers of HIV disease stage at the time of ART initiation are critical determinants of the progression while under ART. The time it takes to reach the threshold is shorter for patients who were initiated with NVP regimen as compared to EFV. Similar trend was reported by other studies (Teeranaipong et al., 2016; Van Leth et al., 2005). The possible reason might be NVP has been used for patients with low CD4 level to reduce the side effect of EFV. This trend changed when treatment is initiated at higher CD4 cell counts which is also supported by other studies (Ford et al., 2017; Teeranaipong et al., 2016). This might be due to the high potency nature of EFV containing regiment.

In Chapter 5, the longitudinal process of CD4 cell count was used to predict the time to composite outcome (and death) using joint modelling proposed by Rizopoulos (2012b). The model with time dependent slope parametrization was found to be the best model. The results revealed that the hazard of death or composite outcome de-

pended on a longitudinal process of log-CD4 cell counts. The risk of death increased by 2.31-fold for a unit decrease in the current value of log(CD4) cell counts for patients who initiated with EFV. This is consistent with study conducted in Canada by Lim et al. (2013) in which significant association is observed between two processes. There was no significant difference among the interaction of drug and log(CD4) cell counts on the risk of death. The joint model provides a framework for performing individual predictions of the outcomes. The probability of developing the event process in the future time is predicted in the future for an individual with available baseline information and longitudinal outcomes.

The findings in all the chapters should be interpreted with insight of the following limitations. (1) The studies are based on retrospective data in which many covariates were not measured. (2) The model used in Chapter 4 considered only one covariate to reduce model complexity. (3) For some models, possible confounding variables were not controlled neither methodologically nor in the analysis part. This is also related to the first limitation.

In conclusion, initiation of ART with EFV containing regimen has reduce risk of treatment failure as compared to NVP containing regimen. The long-term outcomes are comparable between NNRTI treatment groups. Significant difference was observed for NRTI backbones chosen to initiate HIV-infected patients in Ethiopia. The maximum benefit of ART is obtained during the first 10 months after initiation. For higher CD4 cell counts, EFV containing regimen improves CD4 cells counts of the patient quicker than NVP containing regimen. Thus, those who have higher baseline CD4 cells count can be initiated with EFV containing regimen. There is a strong association between the risk of composite outcome and rate of change in CD4 cell counts. Early prediction on the change in CD4 cell count could be an evidence to make decision by the health professional on the type of regimen the patients should take.

Based on our findings in the first part of the dissertation, several recommendations for future research/treatment procedures formulated:

- **Modeling recommendations for future research**
  The CD4 cell count was used for the analysis presented in the first part of the thesis. However, recently routine viral load measurements are available in resource-limited countries as well. Hence, the models implemented in this dissertation can be extended to address similar research questions based on viral load as a single marker or a joint model in which CD4 cell counts and viral load are used jointly to predict treatment failure.

- **Treatment procedures recommendations**:
  - We have shown that the first 10 months after initiation of ART were the period the patients can get the maximum benefit of the treatment with regard to CD4 improvement. The health providers have to take this in to account, monitor the patients progress within the 10 months period more closely and advice their clients accordingly.
  - The result indicated that EFV regimen improves CD4 cells counts of the patient faster than NVP regimen for higher baseline CD4 cell counts. Hence, those who have higher baseline CD4 cells count should be initiated with EFV regimen.
- **Public health recommendation**
  The analyses presented in this dissertation can be reproduce in other hospitals and the methodology presented in the dissertation can be applied to similar dataset from other treatment centers. This will allow public health authorities to compare between treatment centers and to monitor closely the patients progress within and between treatment canters.

9.2 Modeling VL-HIV Co-infection in Ethiopia

In the second part of the dissertation, we proposed a joint model for longitudinal measurements, applied to the prediction of time to VL relapse in VL-HIV co-infected patients under treatment. Results of the model revealed strong association between WBC and Hgb trajectories and VL relapse. Different parametrization of the longitudinal process were considered. Rate of change in Hgb level was found to be significant predictor for the incidence of relapse. Similarly, the incidence of relapse can be also predicted by the current value of WBC. The probabilities of relapse free survival for each individual was computed and updated dynamically when additional informations on the biomarker are included. The biomarkers measurements from an estimation period of 84 days were used to predict the probability of relapse in the later follow-up periods. The more the numbers of measurements of the biomarker, the precise the predicted probability as evidenced by the 95% confidence interval. The predictive ability of Hgb is better than WBC based on AUCs values. Lower values of Hgb has higher discriminating ability between patients who will experience relapse and who will not.
The analysis presented in the second part of the dissertation was conducted to each

biomarker separately. For a future research, it is recommended to develop a joint model in which both WBC and Hgb are used jointly to predict the time to relapse. Furthermore, based on the results presented Chapter 7, we recommend to develop a predictive tool in which WBC and Hgb of future patients will be used to determine the patients probability for relapse.

9.3 The `ETART` Shiny App

We are living in the era of data. The capacity to collect and to store detailed data was increases substantially in the last decade and in the same time, the cost decreased. As a result, big datasets can be constructed and stored. Data about HIV patients under ART in Ethiopia, and Africa, are collated routinely as part of the treatment procedure. Unfortunately, these data are either not analysed or analysed by external sponsor(s). In both cases, information about patients performance is not used by local clinicians or local researchers and, although available, typically ignored when decision is made about the treatment procedure

The `ETART` Shiny App, developed as a part of this PhD, is a first step to create major change in HIV patients data usage. The `ETART` Shiny App allows for both local clinicians and local researchers to analyse and monitor the treatment procedure for HIV patients using a standardized database. Furthermore, since the standardized database consists of measurements which are currently collected routinely, the cost for constructing the standardize database is minimal. Once the database is completed, it can be used for a "within treatment center" analysis or, when combined with similar databases from several treatment centers, to produce a "between center analysis.

The `ETART` Shiny App is a first step. Further work should be done to develop a protocol for the construction of the standardized database so it can be easily implemented within each treatment center. A construction of the standardized ART database can lead to a revolution in HIV data analysis and monitoring and can lead to an improvement in ART treatment allocation and further reduction of treatment failure.

The current findings can be translated to policy and program implications. The first implication is on HIV/AIDS treatment guidelines. The findings of this study revealed that initiating ART at a higher CD4 cell counts supported the treat all approach. The second implication is on the supply of drugs for HIV-infected adults. Our findings suggest that the long-term effect of EFV and NVP are comparable. Comparing the price of the drug, EFV is more expensive than NVP. Based on our finding, NVP can be made available. The third very important implication is on

VL relapse in VL-HIV co-infected patients. The rate of change in Hgb label or current values of WBC can be used to predict VL relapse. The forth very important implication is in utilizing available database for decision making by the local clinicians. The application we developed can be localized to other similar hospitals in the region.

Bibliography

Abah, I. O., Darin, K. M., Ebonyi, A. O., Ugoagwu, P., Ojeh, V. B., Nasir, N., Falang, K. D., Olaitan, O., Agbaji, O., Idoko, J., et al. (2015). Patterns and predictors of first-line antiretroviral therapy modification in hiv-1-infected adults in a large urban outpatient cohort in nigeria. *Journal of the International Association of Providers of AIDS Care (JIAPAC)*, 14(4):348–354.

Abebe, T., Takele, Y., Weldegebreal, T., Cloke, T., Closs, E., Corset, C., Hailu, A., Hailu, W., Sisay, Y., Corware, K., et al. (2013). Arginase activity-a marker of disease status in patients with visceral leishmaniasis in ethiopia. *PLoS neglected tropical diseases*, 7(3):e2134.

Adeyinka, D. A. and Ogunniyi, A. (2012). Predictors of clinical failure in hiv/aids patients on antiretroviral therapy in a resource limited setting, nigeria: A comparative study. *HIV & AIDS Review*, 11(1):20–24.

AIDSinfo (2014). Guidelines for the use of antiretroviral agents in hiv-1-infected adults and adolescents: https://aidsinfo.nih.gov/. accessed on 19/02/2018.

Akaike, H. (1973). Problems of control and information. In *2nd International Symposium on Information Theory, Budapest: Akademiai Kaido*, pages 267–281.

Akinbo, B. D., Atere, A. D., Fatunade, H. B., and Iyabor, N. O. (2015). Haematological indices and absolute cd4 counts of apparently healthy population in ondo state, nigeria. *British Journal of Medicine and Medical Research*, 8(8):717–723.

Alave, J., Paz, J., Gonzlez, E., Campos, M., Rodrguez, M., Willig, J., and et al (2013). Risk factors associated with virologic failure in hiv- infected patients receiving antiretroviral therapy at a public hospital in peru. *Rev Chilena Infectol*, 30(1):42–8.

Alemu, A. W. and Sebastián, M. S. (2010). Determinants of survival in adult hiv patients on antiretroviral therapy in oromiyaa, ethiopia. *Global health action*, 3(1):5398.

Alvar, J., Aparicio, P., Aseffa, A., Den Boer, M., and Moreno, J. (2008). The relationship between leishmaniasis and aids: the second 10 years. *Clinical microbiology reviews*, 21(2):334–359.

Alvar, J., Canavate, C., Gutierrez-Solar, B., Jimenez, M., Laguna, F., Lopez-Velez, R., Molina, R., and Moreno, J. (1997). Leishmania and human immunodeficiency virus coinfection: the first 10 years. *Clinical microbiology reviews*, 10(2):298–319.

Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., den Boer, M., Team, W. L. C., et al. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PloS one*, 7(5):e35671.

Alvar, J., Yactayo, S., and Bern, C. (2006). Leishmaniasis and poverty. *Trends in parasitology*, 22(12):552–557.

Anlay, D. Z., Alemayehu, Z. A., and Dachew, B. A. (2016). Rate of initial highly active anti-retroviral therapy regimen change and its predictors among adult hiv patients at university of gondar referral hospital, northwest ethiopia: a retrospective follow up study. *AIDS research and therapy*, 13(1):10.

Antolini, L., Boracchi, P., and Biganzoli, E. (2005). A time-dependent discrimination index for survival data. *Statistics in medicine*, 24(24):3927–3944.

Aregay, M., Shkedy, Z., Molenberghs, G., David, M.-P., and Tibaldi, F. (2013). Model-based estimates of long-term persistence of induced hpv antibodies: a flexible subject-specific approach. *Journal of biopharmaceutical statistics*, 23(6):1228–1248.

Argaw, D., Mulugeta, A., Herrero, M., Nombela, N., Teklu, T., Tefera, T., Belew, Z., Alvar, J., and Bern, C. (2013). Risk factors for visceral leishmaniasis among residents and migrants in kafta-humera, ethiopia. *PLoS Negl Trop Dis*, 7(11):e2543.

Assefa, Y., Gilks, C. F., Lynen, L., Williams, O., Hill, P. S., Tolera, T., Malvia, A., and Van Damme, W. (2017). Performance of the antiretroviral treatment program in ethiopia, 2005-2015: strengths and weaknesses toward ending aids. *International Journal of Infectious Diseases*, 60:70–76.

Awoke, T., Worku, A., Kebede, Y., Kasim, A., Birlie, B., Braekers, R., Zuma, K., and Shkedy, Z. (2016). Modeling outcomes of first-line antiretroviral therapy and rate of

cd4 counts change among a cohort of hiv/aids patients in ethiopia: a retrospective cohort study. *PloS one*, 11(12):e0168323.

Ayele, T. A., Worku, A., Kebede, Y., Alemu, K., Kasim, A., and Shkedy, Z. (2017). Choice of initial antiretroviral drugs and treatment outcomes among hiv-infected patients in sub-saharan africa: systematic review and meta-analysis of observational studies. *Systematic Reviews*, 6(1):173.

Barry, O., Powell, J., Renner, L., Bonney, E. Y., Prin, M., Ampofo, W., Kusah, J., Goka, B., Sagoe, K. W., Shabanova, V., et al. (2013). Effectiveness of first-line antiretroviral therapy and correlates of longitudinal changes in cd4 and viral load among hiv-infected children in ghana. *BMC infectious diseases*, 13(1):476.

Barth, R. E., Tempelman, H. A., Moraba, R., and Hoepelman, A. I. (2011). Long-term outcome of an hiv-treatment programme in rural africa: viral suppression despite early mortality. *AIDS research and treatment*, 2011.

Bates, P. A. (2007). Transmission of leishmania metacyclic promastigotes by phlebotomine sand flies. *Int J Parasitol.*, 37(10):10971106.

Benjamin, H., Mwango, A., Giganti, M. J., Sikazwe, I., Moyo, C., Schuttner, L., and et al (2011). Comparative outcomes of tenofovir- and zidovudine-based antiretroviral therapy regimens in lusaka, zambia. *J Acquir Immune Defic Syndr*, 58(5):47581.

Bern, C., Maguire, J. H., and Alvar, J. (2008). Complexities of assessing the disease burden attributable to leishmaniasis. *PLoS Neglected Tropical Diseases*, 2(10):e313.

Blas-García, A., Apostolova, N., and Esplugues, J. V. (2011). Future perspectives in nnrti-based therapy: Bases for understanding their toxicity. In *Recent Translational Research in HIV/AIDS*. InTech.

Blattner, W., Gallo, R. C., and Temin, H. (1988). Hiv causes aids. *Science*, 241(4865):515–516.

Bock, P., Fatti, G., and Grimwood, A. (2013). Comparing the effectiveness of efavirenz and nevirapine for first-line antiretroviral therapy in a south african multicentre cohort. *International health*, 5(2):132–138.

Boettiger, D. C., Sudjaritruk, T., Nallusamy, R., Lumbiganon, P., Rungmaitree, S., Hansudewechakul, R., Kumarasamy, N., Bunupuradah, T., Saphonn, V., Truong,

K. H., et al. (2016). Non-nucleoside reverse transcriptase inhibitor–based antiretroviral therapy in perinatally hiv-infected, treatment-naïve adolescents in asia. *Journal of Adolescent Health*, 58(4):451–459.

Bongiovanni, M. and Tordato, F. (2014). Tenofovir plus didanosine as nrti backbone in hiv-infected subjects. *Current Medicinal Chemistry*, 23(39):2789–5.

Bonnet, M., Bhatt, N., Baudin, E., Silva, C., Michon, C., Taburet, A.-M., Ciaffi, L., Sobry, A., Bastos, R., Nunes, E., et al. (2013). Nevirapine versus efavirenz for patients co-infected with hiv and tuberculosis: a randomised non-inferiority trial. *The Lancet infectious diseases*, 13(4):303–312.

Boulle, A., Orrel, C., Kaplan, R. F., van Cutsem, G., McNally, M. M., Hilderbrand, K., Myer, L., Egger, M., Coetzee, D. J., Maartens, G., and Wood, R. G. (2007). Substitutions due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral therapy in a large south african cohort. *Antiviral therapy*, 12 5:753–60.

Bowman, A. W. and Azzalini, A. (1997). *Applied smoothing techniques for data analysis: the kernel approach with S-Plus illustrations*, volume 18. OUP Oxford.

Braitstein, P., Brinkhof, M. W., Dabis, F., Schechter, M., et al. (2006). Mortality of hiv-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. *The lancet*, 367(9513):817.

Branca, F., Pastore, G., Demissie, T., and Ferro-Luzzi, A. (1993). The nutritional impact of seasonality in children and adults of rural ethiopia. *European journal of clinical nutrition*, 47(12):840–850.

Brennan, A. T., Maskew, M., Ive, P., Shearer, K., Long, L., Sanne, I., and Fox, M. P. (2013). Increases in regimen durability associated with the introduction of tenofovir at a large public-sector clinic in johannesburg, south africa. *Journal of the International AIDS Society*, 16(1).

Brinkhof, M., Pujades-Rodriguez, M., and Egger, M. (2009). Mortality of patients lost to follow-up in antiretroviral treatment programmes in resource-limited settings: systematic review and meta-analysis. *PLoS One*, 4(6):e5790.

Broder, S. (2010). The development of antiretroviral therapy and its impact on the hiv-1/aids pandemic. *Antiviral research*, 85(1):1–18.

Burza, S., Mahajan, R., Sinha, P. K., van Griensven, J., Pandey, K., Lima, M. A., Sanz, M. G., Sunyoto, T., Kumar, S., Mitra, G., Kumar, R., Verma, N., and Das,

P. (2014). Visceral leishmaniasis and hiv co-infection in bihar, india: Long-term effectiveness and treatment outcomes with liposomal amphotericin b (ambisome). *PLoS Negl Trop Dis*, 8(8):e3053.

Cain, L. and Hernn, M. (2013). The effect of efavirenz versus nevirapine-containing regimens in the hiv-causal collaboration: reply to llibre and podzamczer and additional results. *AIDS*, 27(13):2169–70.

Calmy, A., Ford, N., Hirschel, B., Reynolds, S. J., Lynen, L., Goemaere, E., de la Vega, F. G., Perrin, L., and Rodriguez, W. (2007). Hiv viral load monitoring in resource-limited regions: optional or necessary? *Clinical infectious diseases*, 44(1):128–134.

Calza, L., Marinacci, G., Manfredi, R., Colangeli, V., Fortunato, L., and Chiodo, F. (2001). Pentamidine isethionate as treatment and secondary prophylaxis for disseminated cutaneous leishmaniasis during hiv infection: case report. *Journal of chemotherapy*, 13(6):653–657.

CDC (2013). Parasites-leishmaniasis: Available in http://www.cdc. gov/parasites/leishmaniasis. accessed on 12/12/2017.

Chen, D. and Peace, K. (2013). *Applied meta-analysis with R*. CRC Press.

Clumeck, N., Mwamba, C., Kabeya, K., Matanda, S., Vaira, D., Necsoi, C., Kadiebwe, D., Delforge, M., Kasamba, E., Milolo, C., et al. (2014). First-line antiretroviral therapy with nevirapine versus lopinavir-ritonavir based regimens in a resource-limited setting. *AIDS (London, England)*, 28(8):1143.

Cohen, M. S., Chen, Y. Q., McCauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., Hakim, J. G., Kumwenda, J., Grinsztejn, B., Pilotto, J. H., et al. (2011). Prevention of hiv-1 infection with early antiretroviral therapy. *New England journal of medicine*, 365(6):493–505.

COHERE et al. (2012). Cd4 cell count and the risk of aids or death in hiv-infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from cohere. *PLoS medicine*, 9(3):e1001194.

Collaboration, H.-C. et al. (2011). When to initiate combined antiretroviral therapy to reduce mortality and aids-defining illness in hiv-infected persons in developed countries: an observational study. *Annals of internal medicine*, 154(8):509.

Collaboration, H.-C. et al. (2012). The effect of efavirenz versus nevirapine-containing regimens on immunologic, virologic and clinical outcomes in a prospective observational study. *AIDS (London, England)*, 26(13):1691.

Collin, S., Davidson, R., Ritmeijer, K., Keus, K., Melaku, Y., Kipngetich, S., and Davies, C. (2004). Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern sudan. *Clinical Infectious Diseases*, 38(5):612–619.

Cota, G. F., de Sousa, M. R., de Mendonça, A. L. P., Patrocinio, A., Assunção, L. S., de Faria, S. R., and Rabello, A. (2014). Leishmania-hiv co-infection: clinical presentation and outcomes in an urban area in brazil. *PLoS neglected tropical diseases*, 8(4):e2816.

Cota, G. F., De Sousa, M. R., and Rabello, A. (2011). Predictors of visceral leishmaniasis relapse in hiv-infected patients: a systematic review. *PLoS neglected tropical diseases*, 5(6):e1153.

Cox, D. R. (1972). Regression models and life-tables (with discussion). *J Roy Statist Soc*, 34:187–220.

Crampin, A., Mwaungulu, F., Ambrose, L., Longwe, H., and French, N. (2011). Normal range of cd4 cell counts and temporal changes in two hiv negative malawian populations. *The open AIDS journal*, 5:74.

Croft, S. L., Sundar, S., and Fairlamb, A. H. (2006). Drug resistance in leishmaniasis. *Clinical microbiology reviews*, 19(1):111–126.

CSA (2012). Ethiopia demographic and health survey 2011. *Central Statistical Agency and ICF International. Available at http://dhsprogram.com/pubs/pdf/FR255/FR255.pdf*, 430.

Daher, E. F., Fonseca, P. P., Gerhard, E. S., Leito, T. M. J. S., , and Jnior, G. B. S. (2009). Clinical and epidemiological features of visceral leishmaniasis and hiv co-infection in fifteen patients from brazil. *Journal of Parasitology*, 95(3):652–655.

de Castilla, D. L., Verdonck, K., Otero, L., Iglesias, D., Echevarría, J., Lut, L., Gotuzzo, E., and Seas, C. (2008). Predictors of cd4+ cell count response and of adverse outcome among hiv-infected patients receiving highly active antiretroviral therapy in a public hospital in peru. *International Journal of Infectious Diseases*, 12(3):325–331.

De La Hoz, J. M., Bolaño, L., Cárdenas, O., González, R., Sabbag, J., Palacio, L., Alonso, L. M., San-Juan-Vergara, H., and Cervantes-A, G. (2014). Characterization of treatment failure in hiv positive patients in the colombian caribbean region. *Colombia Médica*, 45(4):162–167.

Den Boer, M., Argaw, D., Jannin, J., and Alvar, J. (2011). Leishmaniasis impact and treatment access. *Clinical Microbiology and Infection*, 17(10):1471–1477.

DerSimonian, R. and Kacker, R. (2007). Random-effects model for meta-analysis of clinical trials: an update. *Contemporary clinical trials*, 28(2):105–114.

Desjeux, P. (2004). Leishmaniasis: current situation and new perspectives. *Comparative immunology, microbiology and infectious diseases*, 27(5):305–318.

Desjeux, P. (2006). Nature reviews microbiology. *Nature Reviews*, 2:692–693.

Diro, E., Lynen, L., Gebregziabiher, B., Assefa, A., Lakew, W., Belew, Z., Hailu, A., Boelaert, M., and Griensven, J. (2015a). Clinical aspects of paediatric visceral leishmaniasis in north-west ethiopia. *Tropical Medicine & International Health*, 20(1):8–16.

Diro, E., Lynen, L., Ritmeijer, K., Boelaert, M., Hailu, A., and van Griensven, J. (2014). Visceral leishmaniasis and hiv coinfection in east africa. *PLoS Negl Trop Dis*, 8(6):e2869.

Diro, E., Ritmeijer, K., Boelaert, M., Alves, F., Mohammed, R., Abongomera, C., Ravinetto, R., De Crop, M., Fikre, H., Adera, C., et al. (2015b). Use of pentamidine as secondary prophylaxis to prevent visceral leishmaniasis relapse in hiv infected patients, the first twelve months of a prospective cohort study. *PLoS Negl Trop Dis*, 9(10):e0004087.

Egger, M., Smith, G. D., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *Bmj*, 315(7109):629–634.

EMA (2011). Outcome of a renewal procedure. questions and answers on the review of zerit (stavudine). european medicines agency. http://www.ema.europa.eu/docs/en gb/documentlibrary/medicineqa/human/000110/wc500102227.pdf. accessed on 21/2/2018. Technical report.

EMH (2010). Standard treatment guideline for primary hospital: Drug administration and control authority of ethiopia contents ethiopia. Technical report, Ethiopian Minstry of Health.

EPHI (2017). Hiv related estimates and projections for ethiopia. *Addis Ababa: EPHI*, pages 6–14.

Faucett, C. L. and Thomas, D. C. (1996). Simultaneously modelling censored survival data and repeatedly measured covariates: a gibbs sampling approach. *Statistics in Medicine*, 15:16631685.

Ford, N., Meintjes, G., Vitoria, M., Greene, G., and Chiller, T. (2017). The evolving role of cd4 cell counts in hiv care. *Current Opinion in HIV and AIDS*, 12(2):123–128.

Frehiwot, N., Mizan, K., Seble, M., Fethia, K., Tekalign, M., Zelalem, T., et al. (2014). National guidelines for comprehensive hiv prevention, care and treatment. *Addis Ababa: Ministry of Health*.

Geck, C. (2016). The world factbook. *The Charleston Advisor*, 19(1):58–60.

Georgiadou, S. P., Makaritsis, K. P., and Dalekos, G. N. (2015). Leishmaniasis revisited: Current aspects on epidemiology, diagnosis and treatment. *J Transl Int Med.*, 3(2):4350.

Gilks, C. F., Crowley, S., Ekpini, R., Gove, S., Perriens, J., Souteyrand, Y., Sutherland, D., Vitoria, M., Guerma, T., and De Cock, K. (2006). The who public-health approach to antiretroviral treatment against hiv in resource-limited settings. *The Lancet*, 368(9534):505–510.

Gizaw, B. et al. (2017). National level implications of sdg implementation in ethiopia. Technical report, Southern Voice.

Goswami, R. P., Goswami, R. P., Basu, A., Ray, Y., Rahman, M., and Tripathi, S. K. (2017). Protective efficacy of secondary prophylaxis against visceral leishmaniasis in human immunodeficiency virus coinfected patients over the past 10 years in eastern india. *The American Society of Tropical Medicine and Hygiene*, 96(2):285–291.

Goto, H. and Prianti, M. d. G. (2009). Immunoactivation and immunopathogeny during active visceral leishmaniasis. *Revista do Instituto de Medicina Tropical de São Paulo*, 51(5):241–246.

Grafféo, N., Castell, F., Belot, A., and Giorgi, R. (2016). A log-rank-type test to compare net survival distributions. *Biometrics*, 72(3):760–769.

Group, I. S. S. et al. (2015a). Initiation of antiretroviral therapy in early asymptomatic hiv infection. *N Engl J Med*, 2015(373):795–807.

Group, T. A. . S. et al. (2015b). A trial of early antiretrovirals and isoniazid preventive therapy in africa. *N Engl j med*, 2015(373):808–822.

Gsponer, T., Petersen, M., Egger, M., Phiri, S., Maathuis, M. H., Boulle, A., Musondad, P., Tweya, H., Peter, K., Chi, B. H., et al. (2012). The causal effect of switching to second–line art in programmes without access to routine viral load monitoring. *AIDS (London, England)*, 26(1):57.

Günthard, H. F., Aberg, J. A., Eron, J. J., Hoy, J. F., Telenti, A., Benson, C. A., Burger, D. M., Cahn, P., Gallant, J. E., Glesby, M. J., et al. (2014). Antiretroviral treatment of adult hiv infection: 2014 recommendations of the international antiviral society–usa panel. *Jama*, 312(4):410–425.

Hassan, A. S., Nabwera, H. M., Mwaringa, S. M., Obonyo, C. A., Sanders, E. J., de Wit, T. F. R., Cane, P. A., and Berkley, J. A. (2014). Hiv-1 virologic failure and acquired drug resistance among first-line antiretroviral experienced adults at a rural hiv clinic in coastal kenya: a cross-sectional study. *AIDS research and therapy*, 11(1):9.

Higgins, J. P. and Green, S. (2011). *Cochrane handbook for systematic reviews of interventions*, volume 4. John Wiley & Sons.

Hladik, W., Shabbir, I., Jelaludin, A., Woldu, A., Tsehaynesh, M., and Tadesse, W. (2006). Hiv/aids in ethiopia: where is the epidemic heading? *Sexually transmitted infections*, 82(1):i32–i35.

Hurissa, Z., Gebre-Silassie, S., Hailu, W., Tefera, T., Lalloo, D. G., Cuevas, L. E., and Hailu, A. (2010). Clinical characteristics and treatment outcome of patients with visceral leishmaniasis and hiv co-infection in northwest ethiopia. *Tropical Medicine & International Health*, 15(7):848–855.

Jackson, D., Bowden, J., and Baker, R. (2010). How does the dersimonian and laird procedure for random effects meta-analysis compare with its more efficient but harder to compute counterparts? *Journal of Statistical Planning and Inference*, 140(4):961–970.

Jacobson, L. P., Phair, J. P., and Yamashita, T. E. (2004). Virologic and immunologic response to highly active antiretroviral therapy. *Current HIV/AIDS Reports*, 1(2):74–81.

Jiang, H.-Y., Zhang, M.-N., Chen, H.-J., Yang, Y., Deng, M., and Ruan, B. (2014). Nevirapine versus efavirenz for patients co-infected with hiv and tuberculosis: a systematic review and meta-analysis. *International Journal of Infectious Diseases*, 25:130–135.

Jin, Z.-C., Wu, C., Zhou, X.-H., and He, J. (2014). A modified regression method to test publication bias in meta-analyses with binary outcomes. *BMC medical research methodology*, 14(1):132.

Kadima, J. N., Mukanyangezi, M. F., and Uwizeye, C. B. (2014). Effectiveness and safety of concurrent use of first-line antiretroviral and antituberculous drugs in rwanda. *Journal of tropical medicine*, 2014.

Kaplan, J. E., Benson, C., Holmes, K. K., Brooks, J. T., Pau, A., and Masur, H. (2009). Guidelines for prevention and treatment of opportunistic infections in hiv-infected adults and adolescents. *MMWR Recomm Rep*, 58(RR-4):1–198.

Keiser, O., Chi, B. H., Gsponer, T., Boulle, A., Orrell, C., Phiri, S., Maxwell, N., Maskew, M., Prozesky, H., Fox, M. P., et al. (2011). Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in southern africa. *AIDS (London, England)*, 25(14):1761.

Keiser, O., Tweya, H., Braitstein, P., Dabis, F., MacPhail, P., Boulle, A., Nash, D., Wood, R., Lüthi, R., Brinkhof, M. W., et al. (2010). Mortality after failure of antiretroviral therapy in sub-saharan africa. *Tropical medicine & international health*, 15(2):251–258.

Kestens, L. (2013). The immune system and hiv: Virologic and immunologic basis of hiv infection + hiv pathogenesis. available in http://www.aidsmap.com. accessed 10/14/2014. Technical report.

Kiguba, R., Byakika-Tusiime, J., Karamagi, C., Ssali, F., Mugyenyi, P., and Katabira, E. (2007). Discontinuation and modification of highly active antiretroviral therapy in hiv-infected ugandans: prevalence and associated factors. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 45(2):218–223.

Kleinbaum, D. and Klein, M. (2005). *Survival Analysis: A Self-Learning Text*. Springer, New York, second edition edition.

Kryst, J., Kawalec, P., and Pilc, A. (2015). Efavirenz-based regimens in antiretroviral-naive hiv-infected patients: a systematic review and meta-analysis of randomized controlled trials. *PloS one*, 10(5):e0124279.

Kwobah, C. M., Mwangi, A. W., Koech, J. K., Simiyu, G. N., and Siika, A. M. (2012). Factors associated with first-line antiretroviral therapy failure amongst hiv-infected african patients: A case-control study. *World Journal of AIDS*, 2(04):271.

Laurent, C., Bourgeois, A., Mpoudi-Ngol, E., Ciaffi, L., Kouanfack, C., Mougnutou, R., and et al (2008). Tolerability and effectiveness of first-line regimens combining nevirapine and lamivudine plus zidovudine or stavudine in cameroon. *AIDS Res Hum Retroviruses*, 24(3):393–9.

Lawton, M., Tilling, K., Robertson, N., Tremlett, H., Zhu, F., Harding, K., Oger, J., and Ben-Shlomo, Y. (2015). A longitudinal model for disease progression was developed and applied to multiple sclerosis. *Journal of clinical epidemiology*, 68(11):1355–1365.

Leta, S., Dao, T. H. T., Mesele, F., and Alemayehu, G. (2014). Visceral leishmaniasis in ethiopia: an evolving disease. *PLoS neglected tropical diseases*, 8(9):e3131.

Leta, S., Dao, T. H. T., Mesele, F., and Alemayehu, G. (2015). Visceral leishmaniasis in ethiopia: An evolving disease. *PLoS Negl Trop Dis*, 8(9):e3131.

Leth, F., Phanuphak, P., Ruxrungtham, K., Baraldi, E., Miller, S., Gazzard, B., Cahn, P., Lalloo, U., Van Der Westhuizen, I., Malan, D., et al. (2004). Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2nn study. *The Lancet*, 363(9417):1253–1263.

Li, T., Vedula, S. S., Hadar, N., Parkin, C., Lau, J., and Dickersin, K. (2015). Innovations in data collection, management, and archiving for systematic reviews-data collection innovations for systematic reviews. *Annals of internal medicine*, 162(4):287–294.

Lim, H. J., Mondal, P., and Skinner, S. (2013). Joint modeling of longitudinal and event time data: application to hiv study. *Journal of Medical Statistics and Informatics*, 1(1):1.

Lindoso, J. A. L., Cunha, M. A., Queiroz, I. T., and Moreira, C. H. V. (2016). Leishmaniasis-hiv coinfection: current challenges. *HIV/AIDS (Auckland, NZ)*, 8:147.

Little, R. J. and Rubin, D. B. (2014). *Statistical analysis with missing data*, volume 333. John Wiley & Sons.

Lowenthal, E. D., Ellenberg, J. H., Machine, E., Sagdeo, A., Boiditswe, S., Steenhoff, A. P., Rutstein, R., Anabwani, G., and Gross, R. (2013). Association between efavirenz-based compared with nevirapine-based antiretroviral regimens and virological failure in hiv-infected children. *Jama*, 309(17):1803–1809.

Lubyayi, L., Shkedy, Z., Kajungu, D., Mwehire, D., Kirungi, C., Kawuma, E., Odiit, M., Mukasa, B., and Janssen, P. (2015). Evolution of cd4 cell counts over time for hiv/aids patients on antiretroviral therapy (art) in mildmay uganda. *Biom Biostat Int J*, 2(2):00025.

Luma, H. N., Choukem, S., Temfack, E., Ashuntantang, G., Joko, H., and Koulla-Shiro, S. (2012). Adverse drug reactions of highly active antiretroviral therapy (haart) in hiv infected patients at the general hospital, douala, cameroon: a cross sectional study. *Pan African Medical Journal*, 12(1).

Luz, P., Grinsztejn, B., Velasque, L., Pacheco, A., Veloso, V., Moore, R., and et al (2014). Long-term cd4+ cell count in response to combination antiretroviral therapy. *PLoS ONE*, 9(4):e93039.

Mahajan, R., Das, P., Isaakidis, P., Sunyoto, T., Sagili, K. D., Lima, M. A., Mitra, G., Kumar, D., Pandey, K., and geertruyden, J.-P. V. (2015). Combination treatment for visceral leishmaniasis patients coinfected with human immunodeficiency virus in india. *Clin Infect Dis*, 61(8):1255–1262.

Maizels, R. M., Hewitson, J. P., and Smith, K. A. (2012). Susceptibility and immunity to helminth parasites. *Current opinion in immunology*, 24(4):459–466.

Maman, D., Pujades-Rodriguez, M., Nicholas, S., McGuire, M., Szumilin, E., Ecochard, R., and Etard, J.-F. (2012). Response to antiretroviral therapy: improved survival associated with cd4 above 500 cells/μl. *Aids*, 26(11):1393–1398.

Manosuthi, W., Mankatitham, W., Lueangniyomkul, A., Prasithsirikul, W., Tantanathip, P., Suntisuklappon, B., Narkksoksung, A., Nilkamhang, S., and Sungkanuparph, S. (2010). Renal impairment after switching from stavudine/lamivudine to tenofovir/lamivudine in nnrti-based antiretroviral regimens. *AIDS research and therapy*, 7(1):37.

Marcos, A., Nova, E., and Montero, A. (2003). Changes in the immune system are conditioned by nutrition. *European journal of clinical nutrition*, 57(S1):S66.

Mbuagbaw, L., Irlam, J. H., Spaulding, A., Rutherford, G. W., and Siegfried, N. (2010). Efavirenz or nevirapine in three-drug combination therapy with two nucleoside-reverse transcriptase inhibitors for initial treatment of hiv infection in antiretroviral-naive individuals. *Cochrane Database Syst Rev*, 12.

McArthur, J. W. (2014). The origins of the millennium development goals. *SAIS Review of International Affairs*, 34(2):5–24.

McManus, H., Hoy, J. F., Woolley, I., Boyd, M. A., Kelly, M. D., Mulhall, B., Roth, N. J., Petoumenos, K., Law, M. G., et al. (2015). Recent trends in early stage response to combination antiretroviral therapy in australia. *Antiviral therapy*, 20(2):131.

McManus, H., OConnor, C. C., Boyd, M., Broom, J., Russell, D., and et al, K. W. (2012). Long-term survival in hiv positive patients with up to 15 years of antiretroviral therapy. *PLOS ONE*, 7(11):e48839.

Mengistu, G. and Ayele, B. (2007). Visceral lieshmaniasis and hiv co-infection in patients admitted to gondar university hospital, northwest ethiopia. *Ethiopian Journal of Health Development*, 21(1):53–60.

Meyerhoff, A. (1999). Food and drug administration approval of ambisome (liposomal amphotericin b) for treatment of visceral leishmaniasis. *Clin Infect Dis*, 28(1):42–8.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., Group, P., et al. (2009). Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. *PLoS medicine*, 6(7):e1000097.

Molina, I., Falcó, V., Crespo, M., Riera, C., Ribera, E., Curran, A., Carrio, J., Diaz, M., Villar del Saz, S., Fisa, R., et al. (2007). Efficacy of liposomal amphotericin b for secondary prophylaxis of visceral leishmaniasis in hiv-infected patients. *Journal of antimicrobial chemotherapy*, 60(4):837–842.

Morquin, D., Le Moing, V., Mura, T., Makinson, A., Klouche, K., Jonquet, O., Reynes, J., and Corne, P. (2012). Short-and long-term outcomes of hiv-infected patients admitted to the intensive care unit: impact of antiretroviral therapy and immunovirological status. *Annals of intensive care*, 2(1):25.

Mpondo, B. C., Kabangila, R., Lucheri, E., Ernest, A., and Kidenya, B. R. (2015). Immune recovery among hiv-infected patients in northwestern tanzania after 2 years of anti-retroviral therapy use: a retrospective cohort study. *Tanzania Journal of Health Research*, 17(4).

Mugavero, M., May, M., Harris, R., Saag, M., Costagliola, D., Egger, M., and et al (2008). Does short-term virologic failure translate to clinical events in antiretroviral-nave patients initiating antiretroviral therapy in clinical practice? *AIDS*, 22(18):248192.

Munn, Z., Tufanaru, C., and Aromataris, E. (2014). Jbi's systematic reviews: data extraction and synthesis. *AJN The American Journal of Nursing*, 114(7):49–54.

Murray, C. J., Barber, R. M., Foreman, K. J., Ozgoren, A. A., Abd-Allah, F., Abera, S. F., Aboyans, V., Abraham, J. P., Abubakar, I., Abu-Raddad, L. J., et al. (2015). Global, regional, and national disability-adjusted life years (dalys) for 306 diseases and injuries and healthy life expectancy (hale) for 188 countries, 1990–2013: quantifying the epidemiological transition. *The Lancet*, 386(10009):2145–2191.

Murray, C. J., Ortblad, K. F., Guinovart, C., Lim, S. S., Wolock, T. M., et al. (2014). Global, regional, and national incidence and mortality for hiv, tuberculosis, and malaria during 1990–2013: a systematic analysis for the global burden of disease study 2013. *The Lancet*, 384(9947):1005–1070.

Mutasa-Apollo, T., Shiraishi, R., Takarinda, K., Dzangare, J., Mugurungi, O., Murungu, J., and et al (2014). Patient retention, clinical outcomes and attrition-associated factors of hiv-infected patients enrolled in zimbabwe's national antiretroviral therapy programme, 20072010. *PLoS One*, 9(1):e86305.

Nachega, J. B., Hislop, M., Dowdy, D. W., Gallant, J. E., Chaisson, R. E., Regensberg, L., and Maartens, G. (2008). Efavirenz versus nevirapine-based initial treatment of hiv infection: clinical and virological outcomes in southern african adults. *AIDS (London, England)*, 22(16):2117.

Narain, J. P., Dash, A., Parnell, B., Bhattacharya, S., Barua, S., Bhatia, R., and Savioli, L. (2010). Elimination of neglected tropical diseases in the south-east asia region of the world health organization. *Bulletin of the World Health Organization*, 88(3):206–210.

Nash, D., Katyal, M., Brinkhof, M., Keiser, O., May, M., Hughes, R., and et al (2008). Long-term immunologic response to antiretroviral therapy in low-income countries: a collaborative analysis of prospective studies. *AIDS*, 22(17):2291–302.

Njagi, E. N., Rizopoulos, D., Molenberghs, G., Dendale, P., and Willekens, K. (2013). A joint survival-longitudinal modelling approach for the dynamic prediction of re-

hospitalization in telemonitored chronic heart failure patients. *Statistical Modelling*, 13(3):179–198.

Notermans, D. W., Pakker, N. G., Hamann, D., Foudraine, N. A., Kauffmann, R. H., Meenhorst, P. L., Goudsmit, J., Roos, M. T., Schellekens, P. T., Miedema, F., et al. (1999). Immune reconstitution after 2 years of successful potent antiretroviral therapy in previously untreated human immunodeficiency virus type 1-infected adults. *The Journal of infectious diseases*, 180(4):1050–1056.

Nylén, S. and Sacks, D. (2007). Interleukin-10 and the pathogenesis of human visceral leishmaniasis. *Trends in immunology*, 28(9):378–384.

Okomo, U., Togun, T., Oko, F., Peterson, K., and Jaye, A. (2012). Mortality and loss to programme before antiretroviral therapy among hiv-infected children eligible for treatment in the gambia, west africa. *AIDS research and therapy*, 9(1):28.

Ortblad, K. F., Lozano, R., and Murray, C. J. (2013). The burden of hiv: insights from the global burden of disease study 2010. *AIDS (London, England)*, 27(13):2003.

Osborn, D., Cutter, A., and Ullah, F. (2015). Universal sustainable development goals. In *Understanding the transformational Challenge for Developed Countries. Available in http://www.un.org/sustainabledevelopment/sustainable-development-goals/. Accessed on 23/02/2018.*

Pandhi, D. and Ailawadi, P. (2014). Initiation of antiretroviral therapy. *Indian journal of sexually transmitted diseases*, 35(1):1.

Patel, A., Pujari, S., Patel, K., Patel, J., Shah, N., Patel, B., and Gupte+, N. (2006). Nevirapine versus efavirenz based antiretroviral treatment in naive indian patients: comparison of effectiveness in clinical cohort. *JAPI*, 54:915–18.

Patel, T. and Lockwood, D. (2009). Pentamidine as secondary prophylaxis for visceral leishmaniasis in the immunocompromised host: report of four cases. *Tropical medicine & international health*, 14(9):1064–1070.

Pavlia, A. and Maltezoub, H. C. (2010). Leishmaniasis, an emerging infection in travelers. *International Journal of Infectious Diseases*, 14(12):e1032e1039.

Pearson, R. and Queiroz, A. (1996). Clinical spectrum of leishmaniasis. *Clinical Infectious Diseases*, pages 1–11.

Perez-Molina, J., Lopez-Velez, R., Montilla, P., and Guerrero, A. (1996). Pentamidine isethionate as secondary prophylaxis against visceral leishmaniasis in hiv-positive patients. *Aids*, 10(2):237–238.

Peterson, I., Togun, O., De Silva, T., Oko, F., Rowland-Jones, S., Jaye, A., and Peterson, K. (2011). Mortality and immunovirological outcomes on antiretroviral therapy in hiv-1 and hiv-2-infected individuals in the gambia. *Aids*, 25(17):2167–2175.

Pillay, P., Ford, N., Shubber, Z., and Ferrand, R. A. (2013). Outcomes for efavirenz versus nevirapine-containing regimens for treatment of hiv-1 infection: a systematic review and meta-analysis. *PLoS One*, 8(7):e68995.

Poku, N. K. (2016). Hiv prevention: The key to ending aids by 2030. *The open AIDS journal*, 10:65.

Prentice, R. (1982). Covariate measurement errors and parameter estimation in a failure time regression model. *Biometrika*, 69(2):331342.

Quinn, T. C. (2008). Hiv epidemiology and the effects of antiviral therapy on long-term consequences. *AIDS (London, England)*, 22(Suppl 3):S7.

Rachel, H., Simon, C., Koert, R., Adey, B., and Robert, D. (2008). Concordant hiv infection and visceral leishmaniasis in ethiopia: The influence of antiretroviral treatment and other factors on outcome. *Clin Infect Dis*, 46(11):1702–1709.

Rajasekaran, S., Jeyaseelan, L., Raja, K., Vijila, S., Krithigaipriya, K., Kuralmozhi, R., et al. (2009). Increase in cd4 cell counts between 2 and 3.5 years after initiation of antiretroviral therapy and determinants of cd4 progression in india. *Journal of postgraduate medicine*, 55(4):261.

Reda, A., Biadgilign, S., Deribew, A., Gebre, B., and Deribe, K. (2013). Predictors of change in cd4 lymphocyte count and weight among hiv infected patients on antiretroviral treatment in ethiopia: A retrospective longitudinal study. *PLOS ONE*, 8(4):e58595.

Reddy, T., Molenberghs, G., Njagi, E. N., and Aerts, M. (2016). A novel approach to estimation of the time to biomarker threshold: applications to hiv. *Pharmaceutical statistics*, 15(6):541–549.

Rey, D., Hoen, B., Chavanet, P., Schmitt, M., Hoizey, G., Meyer, P., and et al (2009). High rate of early virological failure with the once-daily tenofovir/lamivudine/nevirapine combination in naive hiv-1-infected patients. *Antimicrob Chemother*, 62(2):380–8.

Richard, J. and Tim, H. (2014). The immune system, hiv and aging. available: http://www.treatmentactiongroup.org. Technical Report Version 2, Treatment action grou, New York.

Rizopoulos, D. (2010). Jm: An r package for the joint modelling of longitudinal and time-to-event data. *Journal of Statistical Software (Online)*, 35(9):1–33.

Rizopoulos, D. (2011). Dynamic predictions and prospective accuracy in joint models for longitudinal and time-to-event data. *Biometrics*, 67(3):819–829.

Rizopoulos, D. (2012a). Fast fitting of joint models for longitudinal and event time data using a pseudo-adaptive gaussian quadrature rule. *Computational Statistics & Data Analysis*, 56(3):491–501.

Rizopoulos, D. (2012b). *Joint Models for Longitudinal and Time-to-Event Data With Applications in R.* Chapman and Hall/CRC Biostatistics Series, 1st edition.

Roberts, T., Bygrave, H., Fajardo, E., and Ford, N. (2012). Challenges and opportunities for the implementation of virological testing in resource-limited settings. *Journal of the International AIDS Society*, 15(2).

Roche (2017). Taking aim at hiv/aids. available in https://www.roche.com/sustainability/. accessed on 6/11/2017.

Royston, P. and Altman, D. G. (1994). Regression using fractional polynomials of continuous covariates: parsimonious parametric modelling. *Applied statistics*, pages 429–467.

Royston, P., Altman, D. G., and Sauerbrei, W. (2006). Dichotomizing continuous predictors in multiple regression: a bad idea. *Statistics in medicine*, 25(1):127–141.

Royston, P. and Sauerbrei, W. (2008). *Multivariable model-building: a pragmatic approach to regression anaylsis based on fractional polynomials for modelling continuous variables*, volume 777. John Wiley & Sons.

Ruppert, D.and Wand, M., , and Carroll, R. (2003). *Semiparametric Regression.* Cambridge University Press, Cambridge.

Sabin, C. A. and Phillips, A. N. (2009). Should hiv therapy be started at a cd4 cell count above 350 cells/μl in asymptomatic hiv-1-infected patients? *Current opinion in infectious diseases*, 22(2):191–197.

Samji, H., Cescon, A., Hogg, R. S., Modur, S. P., Althoff, K. N., Buchacz, K., Burchell, A. N., Cohen, M., Gebo, K. A., Gill, M. J., et al. (2013). Closing the gap: increases in life expectancy among treated hiv-positive individuals in the united states and canada. *PloS one*, 8(12):e81355.

Sanne, I. M., Westreich, D., Macphail, A. P., Rubel, D., Majuba, P., and Rie, A. (2009). Long term outcomes of antiretroviral therapy in a large hiv/aids care clinic in urban south africa: a prospective cohort study. *Journal of the International AIDS Society*, 12(1):38.

Saravolatz, L. D., Bern, C., Adler-Moore, J., Berenguer, J., Boelaert, M., den Boer, M., Davidson, R. N., Figueras, C., Gradoni, L., Kafetzis, D. A., et al. (2006). Liposomal amphotericin b for the treatment of visceral leishmaniasis. *Clinical Infectious Diseases*, 43(7):917–924.

Sarfo, F. S., Sarfo, M. A., Kasim, A., Phillips, R., Booth, M., and Chadwick, D. (2013). Long-term effectiveness of first-line non-nucleoside reverse transcriptase inhibitor (nnrti)-based antiretroviral therapy in ghana. *Journal of Antimicrobial Chemotherapy*, 69(1):254–261.

Scarsi, K., Darin, K., Rawizza, H., Meloni, S., Chang, C., Olaitan, R., Agbaji, O., Gashau, W., Murphy, R., and Kanki, P. (2010). Tdf-3tc-nvp is inferior to azt-3tc-nvp in a large art program in nigeria. *International AIDS Society*.

Seid, A., Getie, M., Birlie, B., and Getachew, Y. (2014). Joint modeling of longitudinal cd4 cell counts and time-to-default from haart treatment: a comparison of separate and joint models. *Electronic Journal of Applied Statistical Analysis*, 7(5):292–314.

Self, S. and Pawitan, Y. (1992). Modeling a marker of disease progression and onset of disease. In *AIDS epidemiology*, pages 231–255. Springer.

Shearer, K., Brennan, A. T., Maskew, M., Long, L., Berhanu, R., Sanne, I., and Fox, M. P. (2014). The relation between efavirenz versus nevirapine and virologic failure in johannesburg, south africa. *Journal of the International AIDS Society*, 17(1).

Shearer, K., Fox, M. P., Maskew, M., Berhanu, R., Long, L., and Sanne, I. (2013). The impact of choice of nnrti on short-term treatment outcomes among hiv-infected

patients prescribed tenofovir and lamivudine in johannesburg, south africa. *PloS one*, 8(8):e71719.

Shipton, L., Wester, C., Stock, S., Ndwapi, N., Gaolathe, T., Thior, I., and et al (2009). Safety and efficacy of nevirapine- and efavirenz-based antiretroviral treatment in adults treated for tb-hiv co-infection in botswana. *Int J Tuberc Lung Dis*, 13(3):3606.

Shubber, Z., Calmy, A., Andrieux-Meyer, I., Vitoria, M., Renaud-Théry, F., Shaffer, N., Hargreaves, S., Mills, E. J., and Ford, N. (2013). Adverse events associated with nevirapine and efavirenz-based first-line antiretroviral therapy: a systematic review and meta-analysis. *Aids*, 27(9):1403–1412.

Siegfried, N., Uthman, O. A., and Rutherford, G. W. (2010). Optimal time for initiation of antiretroviral therapy in asymptomatic, hiv-infected, treatment-naive adults. *Cochrane Database Syst Rev*, 3.

Singh, O. P., Singh, B., Chakravarty, J., and Sundar, S. (2016). Current challenges in treatment options for visceral leishmaniasis in india: a public health perspective. *Infectious diseases of poverty*, 5(1):19.

Sinha, S., Raghunandan, P., Chandrashekhar, R., Sharma, S. K., Kumar, S., Dhooria, S., Ekka, M., Velpandian, T., Ranjan, S., Ahmad, H., et al. (2013). Nevirapine versus efavirenz-based antiretroviral therapy regimens in antiretroviral-naive patients with hiv and tuberculosis infections in india: a pilot study. *BMC infectious diseases*, 13(1):482.

Smith, C. J., Sabin, C. A., Youle, M. S., Kinloch-de Loes, S., Lampe, F. C., Madge, S., Cropley, I., Johnson, M. A., and Phillips, A. N. (2004). Factors influencing increases in cd4 cell counts of hiv-positive persons receiving long-term highly active antiretroviral therapy. *The Journal of infectious diseases*, 190(10):1860–1868.

Stephan, C., Hill, A., Xi, N., van Delft, Y., and Moecklinghoff, C. (2012). is monitoring for cd4 counts still needed for the management of patients with long-term hiv rna suppression? *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 61(5):e73–e75.

Stover, J., Johnson, P., Zaba, B., Zwahlen, M., Dabis, F., and Ekpini, R. (2008). The spectrum projection package: improvements in estimating mortality, art needs, pmtct impact and uncertainty bounds. *Sexually Transmitted Infections*, 84(Suppl 1):i24–i30.

Stringer, J. S., McConnell, M. S., Kiarie, J., Bolu, O., Anekthananon, T., Jariyasethpong, T., Potter, D., Mutsotso, W., Borkowf, C. B., Mbori-Ngacha, D., et al. (2010). Effectiveness of non-nucleoside reverse-transcriptase inhibitor-based antiretroviral therapy in women previously exposed to a single intrapartum dose of nevirapine: a multi-country, prospective cohort study. *PLoS medicine*, 7(2):e1000233.

Sudell, M., Kolamunnage-Dona, R., and Tudur-Smith, C. (2016). Joint models for longitudinal and time-to-event data: a review of reporting quality with a view to meta-analysis. *BMC medical research methodology*, 16(1):168.

Swaminathan, S., Padmapriyadarsini, C., Venkatesan, P., Narendran, G., Ramesh Kumar, S., Iliayas, S., Menon, P. A., Selvaraju, S., Pooranagangadevi, N. P., Bhavani, P. K., et al. (2011). Efficacy and safety of once-daily nevirapine-or efavirenz-based antiretroviral therapy in hiv-associated tuberculosis: a randomized clinical trial. *Clinical Infectious Diseases*, 53(7):716–724.

Sweeting, M. J. and Thompson, S. G. (2011). Joint modelling of longitudinal and time-to-event data with application to predicting abdominal aortic aneurysm growth and rupture. *Biometrical Journal*, 53(5):750763.

Tajebe, F., Getahun, M., Adem, E., Hailu, A., Lemma, M., Fikre, H., Raynes, J., Tamiru, A., Mulugeta, Z., Diro, E., et al. (2017). Disease severity in patients with visceral leishmaniasis is not altered by co-infection with intestinal parasites. *PLoS Neglected Tropical Diseases*, 11(7).

Takele, Y., Abebe, T., Weldegebreal, T., Hailu, A., Hailu, W., Hurissa, Z., Ali, J., Diro, E., Sisay, Y., Cloke, T., et al. (2013). Arginase activity in the blood of patients with visceral leishmaniasis and hiv infection. *PLoS neglected tropical diseases*, 7(1):e1977.

Takele, Y., Adem, E., Getahun, M., Tajebe, F., Kiflie, A., Hailu, A., Raynes, J., Mengesha, B., Ayele, T., Shkedy, Z., et al. (2016). Malnutrition in healthy individuals results in increased mixed cytokine profiles, altered neutrophil subsets and function. *PloS one*, 11(8):e0157919.

Takuva, S., Evans, D., Zuma, K., Okello, V., and Louwagie, G. (2013). Comparative durability of nevirapine versus efavirenz in first-line regimens during the first year of initiating antiretroviral therapy among swaziland hiv-infected adults. *Pan African Medical Journal*, 15(1).

Teeranaipong, P., Sirivichayakul, S., Mekprasan, S., Ohata, P. J., Avihingsanon, A., Ruxrungtham, K., and Putcharoen, O. (2016). Role of rilpivirine and etravirine in efavirenz and nevirapine-based regimens failure in a resource-limited country: a cross-sectional study. *PloS one*, 11(4):e0154221.

Tirfe, Z. M., Ahmed, T. A., Tedla, N. B., Debere, M. K., and Alamdo, A. G. (2013). Immunological responses of hiv/aids patients treated with nevirapine versus efavirenz based highly active anti-retroviral therapy in addis ababa, ethiopia: A retrospective cohort study. *Health*, 5(09):1502.

Tsiatis, A., , DeGruttola, V., and Wulfsohn, M. (1995). Modelling the relationship of survival to longitudinal data measured with error: application to survival and cd4 counts in patients with aids. *Journal of the American Statistical Association*, 90:2737.

Tvora, L. G. F., Nogueir, M. B., and Gomesb, S. T. (2015). Visceral leishmaniasis/hiv co-infection in northeast brazil: evaluation of outcome. *Braz J Infect Dis*, 19(6):651–6.

UN (2015). Millennium development goals. *United Nations. http://www. un. org/millenniumgoals/(accessed on 23 August 2011)*.

UNAIDS (2003). *Aids Epidemic Update. Available http://data.unaids.org/pub/report/2003/2003-epiupdate-en.pdf. Accessed on 20/12/2017*.

UNAIDS (2010). Getting to zero: 2011–2015 strategy. available in http://www.unaids.org/en/resources/documents/2010/20101221-jc2034-unaids-strategy. accessed on 21/2/2018.

UNAIDS (2015). On the fast-track to end aids by 2030: Focus on location and population. available in http://www.unaids.org/sites/default/files/media-asset/wad2015-report-en-part01.pdf. accessed on 23/2/2018. *Geneva: UNAIDS*, 2030.

UNAIDS (2016a). Global aids update 2016. available http://www.unaids.org/sites/default/files/media-asset/global-aids-update-2016-en.pdf. accessed 06/12/2017.

UNAIDS (2016b). Hiv and aids estimates: Country factsheets: Ethiopia. avaialble in http://www.unaids.org/en/regionscountries/countries/ethiopia. accessed on 13/12/2017.

UNAIDS (2017a). Fact shee: Latest statistics on the status of the aids epidemic. available on http://www.unaids.org/en/resources/fact-sheet. accessed on 27/06/2017.

UNAIDS (2017b). Global hiv/aids overview. available in www.hiv.gov. accessed on 12/11/2017.

UNICEF (2017). Hiv and aids: Ester and southern africa. available www.unicef.org/esaro/5482-hiv-aids.html. accessed on 21/8/2017.

van Dijk, J. H., Sutcliffe, C. G., Hamangaba, F., Bositis, C., Watson, D. C., and Moss, W. J. (2013). Effectiveness of efavirenz-based regimens in young hiv-infected children treated for tuberculosis: a treatment option for resource-limited settings. *PLoS One*, 8(1):e55111.

van Griensven, J., Diro, E., Lopez-Velez, R., Ritmeijer, K., Boelaert, M., Zijlstra, E. E., and Asrat Hailu, L. L. (2014a). A screen-and-treat strategy targeting visceral leishmaniasis in hiv-infected individuals in endemic east african countries: The way forward? *PLoS Negl Trop Dis*, 8(8):e3011.

van Griensven, J., Zijlstra, E. E., and Hailu, A. (2014b). Visceral leishmaniasis and hiv coinfection: time for concerted action. *PLoS neglected tropical diseases*, 8(8):e3023.

Van Leth, F., Andrews, S., Grinsztejn, B., Wilkins, E., Lazanas, M., Lange, J., Montaner, J., Group, N. S., et al. (2005). The effect of baseline cd4 cell count and hiv-1 viral load on the efficacy and safety of nevirapine or efavirenz-based first-line haart. *Aids*, 19(5):463–471.

van Zyl, G. U., van der Merwe, L., Claassen, M., Zeier, M., and Preiser, W. (2011). Antiretroviral resistance patterns and factors associated with resistance in adult patients failing nnrti-based regimens in the western cape, south africa. *Journal of medical virology*, 83(10):1764–1769.

Velen, K., Lewis, J., Charalambous, S., Grant, A., Churchyard, G., and Hoffmann, C. (2013). Comparison of tenofovir, zidovudine, or stavudine as part of first-line antiretroviral therapy in a resource-limited-setting: A cohort study. *PLoS One*, 8(5):e64459.

Verbeke, G. and Molenberghs, G. (2000). *Linear Mixed Models for Longitudinal Data*. Springer Series in Statistics.

Wainberg, M. A., Martinez-Cajas, J. L., and Brenner, B. G. (2007). Strategies for the optimal sequencing of antiretroviral drugs toward overcoming and preventing drug resistance. *Future HIV Therapy*, 1(7):291–313.

Wang, H., Wolock, T. M., Carter, A., Nguyen, G., Kyu, H. H., Gakidou, E., Hay, S. I., Mills, E. J., Trickey, A., Msemburi, W., et al. (2016). Estimates of global, regional, and national incidence, prevalence, and mortality of hiv, 1980-2015: the global burden of disease study 2015. *The lancet. HIV*, 3(8):e361–87.

Wells, G., Shea, B., Oconnell, D., Peterson, J., Welch, V., Losos, M., and Tugwell, P. (2013). The newcastle-ottawa scale (nos) for assessing the quality if nonrandomized studies in meta-analyses. 2009. *Epub Available from: URL: http://www. ohri. ca/programs/clinical_epidemiology/oxford. htm [cited 2009 Oct 19]*.

WHO (2006). Antiretroviral therapy for hiv infection in adults and adolescents: recommendations for a public health approach. available in http://www.who.int/hiv/pub/guidelines/adult/en/. accessed on 21/02/2018.

WHO (2010). Antiretroviral therapy for hiv infection in adults and adolescents 2010 revision. Technical report, World Health Organization. Available in http://www.who.int/hiv/pub/arv/adult2010/en/, Geneva, Switzerland.

WHO (2011). The 3 by 5 initiative. *Geneva: WHO. Available from: URL: http://www. who. int/3by5/en*.

WHO (2014). Control of the leishmaniases: report of a meeting of the 399 who expert committee on the control of leishmaniases. 2010.

WHO (2015). Global health sector response to hiv, 2000 to 2015: Focus on innovations in africa: progress report. Technical report, Geneva, Switzerland.

WHO (2016). Antiretroviral therapy (art) coverage among all age groups. available in http://www. who. int/gho/hiv/epidemic-response/art-text/en/(accessed 17/february 2014). *World Health Organization*.

WHO (2017). Leishmaniasis and hiv coinfection. available in http://www.who.int/leishmaniasis/burden/hiv-coinfection/burden-hiv-coinfection/en/. accessed on 10/12/2017.

Woldemedhin, B. and Wabe, N. T. (2012). The reason for regimen change among hiv/aids patients initiated on first line highly active antiretroviral therapy in southern ethiopia. *North American journal of medical sciences*, 4(1):19.

Wood, E., Hogg, R. S., Harrigan, P. R., and Montaner, J. S. (2005). When to initiate antiretroviral therapy in hiv-1-infected adults: a review for clinicians and patients. *The Lancet infectious diseases*, 5(7):407–414.

Wood, S. (2003). Thin plate regression splines. *Journal of the Royal Statistical Society*, 65(1):95114.

Wright, S., Petoumenos, K., Boyd, M., Carr, A., Downing, S., O'Connor, C., and et al (2013). Ageing and long-term cd4 cell count trends in hiv-positive patients with 5 years or more combination antiretroviral therapy experience. *HIV Medicine*, 14(4):208–16.

Wulfsohn, M. and Tsiatis, A. (1997). A joint model for survival and longitudinal data measured with error. *Biometrics*, 53(1):330–9.

Young, S. D. (2015). A big data approach to hiv epidemiology and prevention. *Preventive medicine*, 70:17–18.

Appendix A

Systematic Review and Meta-Analysis

This Appendix contains additional supporting information for the materials presented in Chapter 2.Sensitivity analysis to assess heterogeneity is presented in Figure A.1.

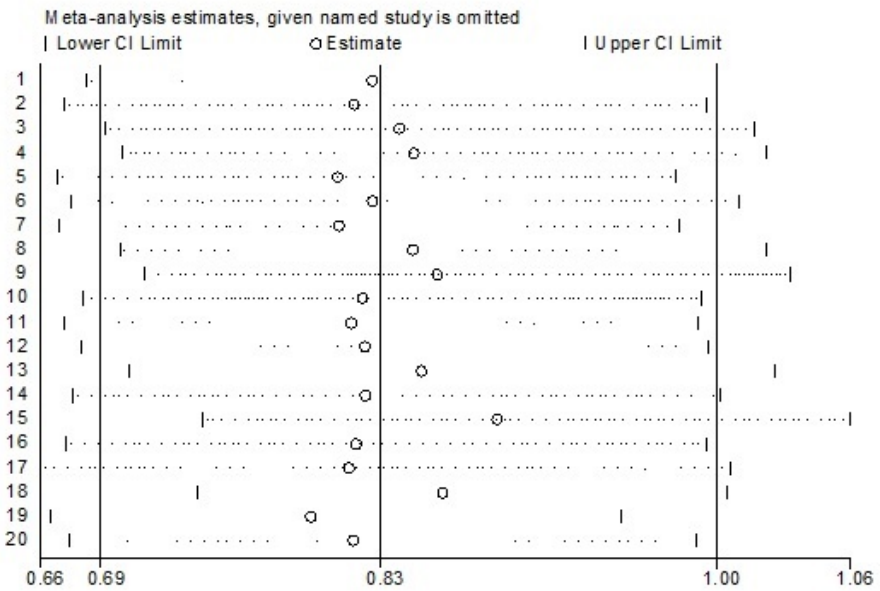


Figure A.1. Sensitivity analysis to assessing the influence of individual study.

Table A.1 provides the list of questions used to extract information from each paper included in the systematic review and meta analysis.

Table A.1. Data extraction form.

S.No	Question	Answer
1	General information	
	Study ID	
	First Author and year	
	Study site (setting)	
	Enrolment and follow up period	
2	Study Characteristics	
	Study design	
	Inclusion criteria of the study	
	Exclusion criteria of the study	
3	Sample size	
	Sampling methods	
	Intervention/exposed group	
	Control/unexposed group	
4	Statistical analysis	
5	Result	
	Length of follow up	
	Participants include in the analysis	
	Lost to follow up/drop	
	Number of total person-years	
6	Participants characteristics	
7	Outcome measures(Effect size)	
8	Quality Assessment	
	Selection	
	Comparability	
	Outcome	
9	Comment on methodology you have after reading	
10	Main findings	

Detail of the selected characteristics of the papers included in the systematic review and meta analysis are presented in Table A.2.

Table A.2. Selected characteristics of the included studies.

Authors	Outcomes	Intervention	Confounding adjusted	Main Findings	Newcastle-Ottawa-scale.
Stringer JS, et al	Treatment Failure	nNVP=355 nEFV =523	Month on ART, CD4 cell count viral load WHO stage, age, gb, BMI, Weight, NNRTI	-Month on ART, CD4 cell count viral load, WHO stage, age, Hgb, and BMI were significantly associated factors In total, 724 women (82%) completed 48 week of follow-up on an NNRTI-containing regimen	Selection 2 stars Comparability 1 star Outcome 2 stars
Kwobah CM,et al	Treatment Failure	nEFV =427 nNVP=2,633	Education level, CD4 category, WHO stage, BMI, Hemoglobin,Adherence, disclosure, travel time, NNRTI, and NRTI	-No association between the choice of NNRTI used (Nevirapine or Efavirenz) and treatment failure. -Low baseline CD4, AZT based NRTI, imperfect adherence are associated with first line ART failure NNRTI-containing regimen	Selection 3 stars Comparability 2 star Outcome 1 stars
Nachega JB, et al	Virologic Failure	nEFV =212 nNVP=103	Age, sex, race, baseline CD4, baseline VL, NRTI, year of ART, adherence	-Nevirapine was associated with greater risk of virologic failure compared to efavirenz -NNRTI, age, sex, baseline viral load, year on ART, and adherence were significantly associated	Selection 2 stars Comparability 2 star Outcome 2 stars
Boulle A, et al	NNRTI substitution	nEFV =1,612 nNVP=1,067	Weight, age, WHO stage per increment, CD4 Count, district	-Substantial difference in the tolerability of commonly used first line ART drugs. -Baseline weight, and Age, for NVP and Weigh and WHO stage for EFV	Selection 2 stars Comparability 2 star Outcome 2 stars
Shearer K, et al	Treatment Failure	nEFV =11,962 nNVP=878	NNRTI, ART, year, sex, age, baseline CD4, WHO stage, BMI,NRTI, baseline anaemia	-Patients with NVP-are more likely to experience virologif failure -NNRTI, years of ART initiation, age, and baseline CD4 cell counts	Selection 3 stars Comparability 2 star Outcome 1 stars
Sarfo FS, et al	Composite endpoint	nEFV =2,369 nNVP=1,621	Sex, age, NNRTI, NRTI, Baseline CD4, Baseline BMI, WHO stage, Adherence	-Treatment outcomes were comparable whether EFV or NVP is used. There was a 36% lower risk of all-cause discontinuation of EFV compared with NVP. NRTI, age, baseline CD4 counts, BMI, WHO stage, and adherence were associated factors of treatment failure	Selection 3 stars Comparability 2 star Outcome 2 stars
Shearer K, eta al	Treatment failure	nEFV =2,254 nNVP=131	NNRTI, sex, age, baseline CD4, WHO stage, Anemia, BMI	-Given TDF as NRTI, Nevirapine has higher risk of treatment failure as compared to EFV. Regimen, and Baseline CD4 cell counts were significantly associated with failure	Selection 3 stars Comparability 1 star Outcome 1 stars
Barth RE, et al	Treatment failure	nEFV =426 nNVP=309	Gender, age, BMI, Karnofsky score CD4 counts, Time since start ART, NNRTI, Occupation	-No difference between EFV and NVP in treatment failure -60% of patients showed of them were switched to second-line treatment. Gender, mean BMI, and baseline CD4 counts were associated in the univariate	Selection 2 stars Comparability 1 star Outcome 2 stars
Gsponer T, et al	Treatment failure	nEFV =186 nNVP=2,218	Age, Sex, Baseline CD4, WHO stage	-Mortality was lower among patients who switched compared to patients remaining on failing first-line ART -Current CD4 count was associated	Selection 2 stars Comparability 1 star Outcome 1 stars
Sarfo FS, et al	NNRTI Substitution	nEFV = 2,378 nNVP=1,621	NNRTI, Gender, age, BMI, WHO stage, CD4 counts, hepatitis B surface antigen status, ALT	-Patients starting nevirapine are more likely to develop rashes and then more likely to discontinue therapy than those starting efavirenz. -NNRTI, Gender, and low baseline CD4 counts were associated factors	Selection 3 stars Comparability 2 star Outcome 1 stars
Keiser O, et al	Treatment failure	nEFV=1,951 nNVP=2,325	Not controlled	-Compared to patients who remained on non-failing first-line therapy, mortality and loss from follow-up was higher y in patients who switched, and substantially higher in patients who remained on failing first-line therap	Selection 2 stars Comparability 1 star Outcome 2 stars
Anlay DZ, et al	NNRTI Substitution	nEFV=289 nNVP=121	Weight, WHO stage TB on initial regimen, NRTI, NNRTI, Co-medication	-There is no difference in regimen change between NVP and EFV. -WHO stage, TB status, co-medication, and side effect were associated factors	Selection 3 stars Comparability 2 star Outcome 2 stars
van Zyl GU, et al	NNRTI resistance	nEFV=82 nNVP=85	Age, gender, ART regimen most recent CD4 count, concurrent viral load and genotypic resistance	-Failure on NVP therapy may result in cross-resistance to ETV. NNRTI, and estimated period of failure were associated	Selection 3 stars Comparability 2 star Outcome 2 stars
Abah IO, et al	NNRTI Substitution	nEFV=558 nNVP=5,751	Sex, age, HBV, CD4 count, WHO stage, NNRTI, NRTI	-Drug substitutions of efavirenz were more likely than of nevirapine -Age, greater immunosuppression, EFV and drug toxicity were significant	Selection 3 stars Comparability 2 star Outcome 1 stars
Bock P, et al	NNRTI Substitution	nEFV=19,441 nNVP=7,909	NNRTI, NRTI gender, age, CD4, WHO stage, TB year of ART initiation	-Superior efficacy of EFV compared with NVP for first-line ART-NNRTI, gender, year of initiation, and province were associated	Selection 3 stars Comparability 2 star Outcome 1 stars
Tirfe ZM, et al	Treatment failure	nEFV=246 nNVP=246	NNRTI, Facility type, age sex, marital status education status, religion NRTI, presence of OIs	-NVP and EFV regimens were effective and comparable, in term of immunological responses.-Gender, eligibility criteriar WHO stage, provision of IPT, and baseline CD4 counts were associated	Selection 3 stars Comparability 2 star Outcome 1 stars

Parameters estimates for the random effect meta-regression, discussed in Section 2.4.4, are presented in Table A.3.

Table A.3. Parameter estimates of meta regression.

Covariate	Estimate	S.E	95%CI
Length of Follow up	-0.0138	0.0666	(-0.1322-0.1046)
Median CD4 count	-0.0034	0.0066	(-0.0150-0.0079)
Median Age	0.0415	0.0503	(-0.1013-0.0755)
Year of publication	0.0637	0.0815	(-0.1736-0.0980)
Female proportion	-0.5305	1.4575	(-3.6792- 2.6183)

Appendix B

Modeling Outcomes of ART and Rate of CD4 Change

This Appendix provides additional supporting informations for the analysis presented in Chapter 3.

Sensitivity Analysis for Composite Outcome

As a second sensitivity analysis, the definition of the composite outcome was modified and the new event is defined as follows: patients experienced either death, lost to follow up, treatment discontinuation, or NNRTI substitution. The results of the cox-regression model for the modified composite outcome are presented in Table B.1. There is no significant difference in the risk of modified composite outcome on NVP (AHR=1.02, 95%CI:0.82-1.27). TDF containing regimen at initiation has 1.73 (95%CI:1.42-2.07)times higher risk on modified composite event. Baseline covariates such as sex, WHO stage, functional status and ART start year were significantly associated with composite outcomes. The risk of the modified composite outcomes on male, WHO stage III or IV, ART start year since 2010 were 1.27 (94%CI:1.07-1.50), 1.35 (95%CI:1.12-1.63), and 1.7 5(95%CI:1.43-2.13), respectively.

Separate Analysis of Long-term Treatment Outcomes

From the separate analysis of events death, lost to follow up and NNRTI substitution (Table B.2), there was no significant difference in the hazard of death and lost to follow up among patients who initiated with EFV or NVP regimen after adjusting for other covariates (sex, WHO stage, and functional status). Though the hazard of lost to follow up was lower for patients who were initiated with NVP from bivariable

Table B.1. Parameter estimates for Cox-PH model of factors associated with the modified composite outcome among HIV/AIDS patients at Gondar University Hospital, in Northwest Ethiopia.

	Sensitivity analysis(Composite outcomes)		
Covariate	Unadjusted HR(95%CI)	Adjusted HR(95%CI)	p-value
Sex, n(%)			
Female	1	1	
Male	1.27(1.08-1.49)	1.27(1.05-1.50)	0.006
Age			
< 40 years	1		
≥ 40 years	1.02(0.85-123)	-	-
NNRTI			
Efavirenz	1	1	
Nevirapine	0.87(0.73-1.02)	1.02(0.82-1.27)	0.881
NRTI backbone			
Zidovudine	1	1	
Stavudine	1.33(0.98-1.81)	1.33(0.97-1.83)	0.072
Tenofovir	1.64(1.38-1.94)	1.73(1.42-2.07)	<.001
WHO stage			
I and II	1	1	
III and IV	1.48(1.24-1.77)	1.35(1.12-1.63)	0.002
Base CD4 cells			
<200 cells/mm3	1	1	
≥ 200 cells/mm3	0.86(0.71-1.04)	0.95(0.78-1.15)	0.5
Functional status			
Ambulatory/Bedridden	1	1	
Working	0.52(0.43-0.63)	0.58(0.47-0.71)	<0.001
ART start Year			
Before 2010	1	1	
Since 2010	1.45(1.20-1.76)	1.75(1.43-2.13)	<0.001

analysis, it turned out to be insignificant after adjustment for other covariates. The risk of NNRTI discontinuation was 2.55 (95%CI: 1.70-3.82) times higher on NVP compared to EFV after adjusting for other covariates. Initiating ART with TDF containing regimen had increased risk of death, lost to follow up and NNRTI change in reference to AZT backbone. The hazard of death, lost to follow up and NNRTI change increased by 1.62 (95%CI: 1.23 2.32), 1.92 (95%CI: 1.40-2.64), and 1.59 (95%:1.12-2.25) times for patients who initiated with TDF containing regimen in reference to AZT regimen, respectively. Initial ART regimen with D4T has 2.30 (95%CI:1.46-3.62) times higher risk of lost to follow up in reference to AZT initial regimen.

Table B.2. Parameter estimates for Cox-PH model for factors associated with treatment outcomes among HIV/AIDS patients at Gondar University Hospital, in Northwest Ethiopia.

Outcome	Treatment	Unadjusted HR(95%CI)	P-value	Adjusted HR(95%CI)	p-value
Death	**NNRTI**				
	Efavirenz	1		1	
	Nevirapine	0.75(0.55 - 1.02)	0.065	1.14(0.81 - 1.62)	0.45
	NRTI backbone				
	Zidovudine	1		1	
	Stavudine	1.97(1.14 - 3.42)	0.015	1.29(0.72 2.29)	0.38
	Tenofovir	2.03(147 - 2.80)	<0.001	1.62(1.23 2.32)	0.008
Lost to follow up	**NNRTI**				
	Efavirenz	1		1	
	Nevirapine	0.59(0.45- 0.76)	<0.001	0.88(0.85- 1.20)	0.41
	NRTI backbone				
	Zidovudine	1		1	
	Stavudine	2.65(1.72 -4.01)	<0.001	2.30(1.46- 3.62)	0.003
	Tenofovir	2.18(1.65- 2.89)	<0.001	1.92(1.40- 2.64)	<0.001
NNRTI substitution	**NNRTI**				
	Efavirenz	1		1	
	Nevirapine	1.94(1.35-2.79)	0.003	2.55(1.70-3.82)	<0.001
	NRTI backbone				
	Zidovudine	1		1	
	Stavudine	0.31(0.13-0.71)	0.005	0.43(0.18-1.01)	0.053
	Tenofovir	0.84(0.62-1.16)	0.31	1.59(1.12-2.25)	0.008

Log-rank Test

Log-rank test was performed for all treatment combinations. Kaplan-Meier curves are presented in Figure B.1. Significant difference was observed between the treatment combinations (p-value=0.001).

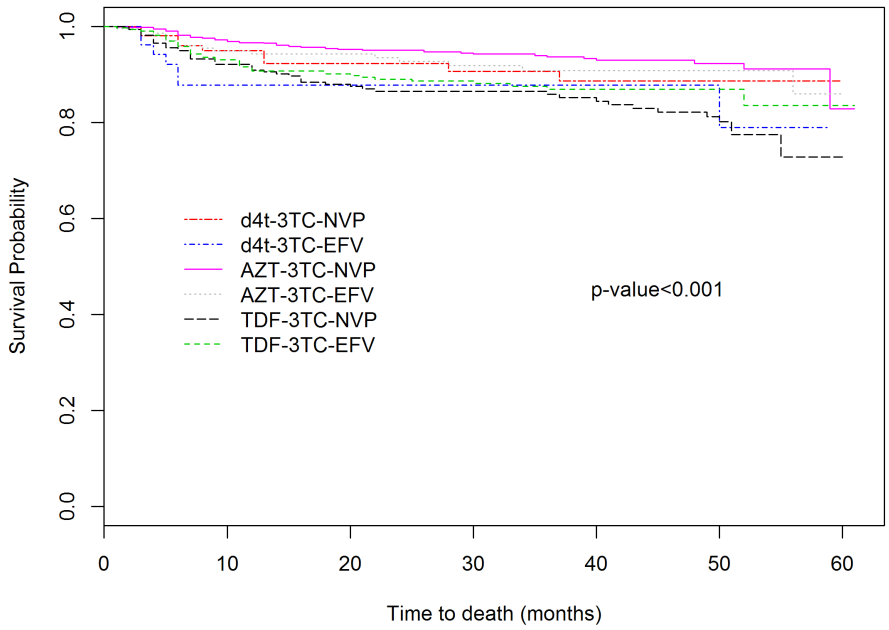


Figure B.1. Kaplan-Meier curves for all treatment regimens.

Similarly, log-rank tests were used to compare between sex, age, functional status and WHO (Figure B.2).

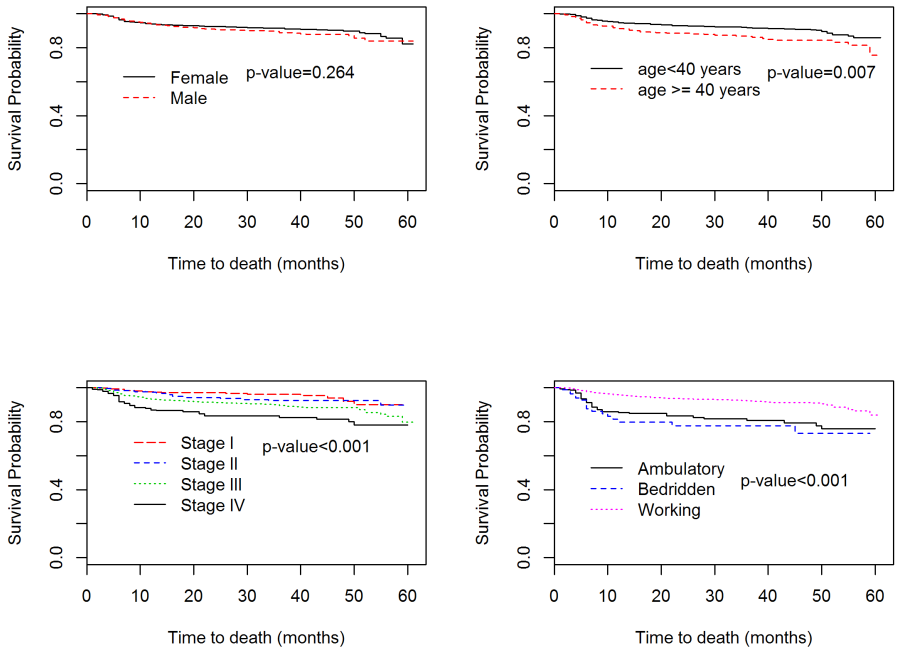


Figure B.2. Kaplan-Meier curves for selected baseline covariates.

Test of Proportionality Hazard Assumption

The proportional hazards (PH) assumption was checked using statistical tests based on the scaled Schoenfeld residuals. The function *cox.zph*() provides a test of proportional hazards assumption for each covariate included in a Cox-regression model. Table B.3 shows the statistical test of PH assumption for Cox-regression.

Table B.3. Test of proportionality hazard assumption.

Covariate	ρ	chi-square	p-value
as.factor(sex)Male	0.10267	2.01974	0.1553
as.factor(ageg)2	-0.06889	0.89916	0.3430
as.factor(NNRTI)NVP	0.11468	2.72739	0.0986
as.factor(NRTI)d4t	-0.13484	3.45644	0.0630
as.factor(NRTI)TDF	0.02006	0.08251	0.7739
as.factor(bcd4g2)2	-0.07467	1.06209	0.3027
as.factor(bwhosg)	-0.09699	1.82211	0.1771
as.factor(year2)2	-0.00604	0.00687	0.9340
GLOBAL	NA	12.88871	0.1157

Appendix C

Model-based Prediction of CD4 Cell Counts

This Appendix presented additional materials supporting the analysis presented in Chapter 4.

Power Selection for the FP Models

The powers for the first order fractional polynomial versus AIC values is presented in Figure C.1 for NVP and EFV. Similarly, the power of second degree fractional polynomial and AIC values is presented in Figure C.2.

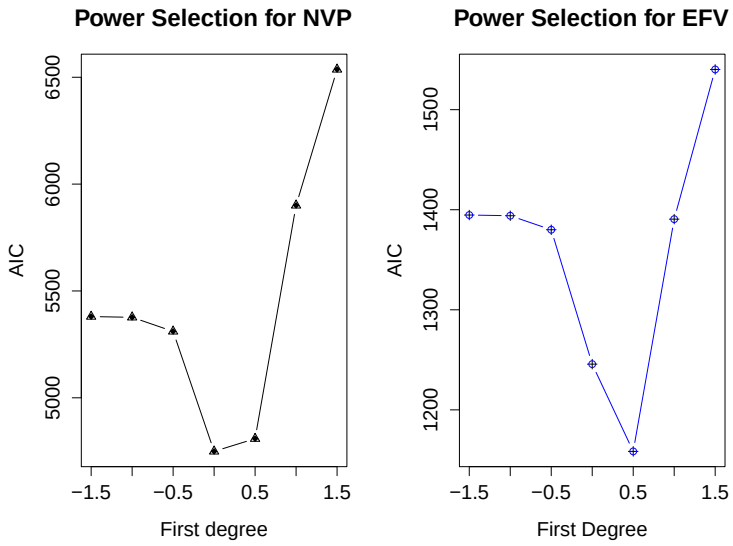


Figure C.1. Powers for the first order fractional polynomial versus AIC values. Left panel: NVP. Right panel: EFV.

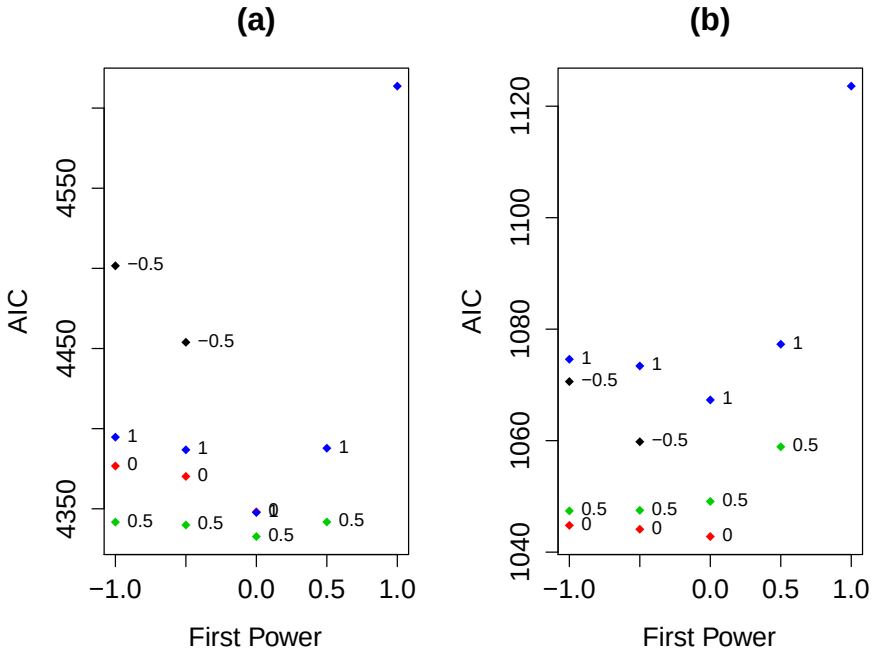


Figure C.2. Powers for the second order fractional polynomial versus AIC value from the model. Panel a: first and second powers against AIC for NVP. Panel b: for EFV.

Comparison of models is made using log-likelihood are presented in Table C.1.

Table C.1. Application of the functional selection procedure to NVP and EFV ART regimens.

Model	Power	Comparison	-LogLL	LogLL diff.	p-value
			NVP		
Null	-	Linear vs Null	8972.7	2901.1	<0.0001
Linear	1	FP1 vs Linear	6071.6	1334.4	<0.0001
FP1	0	FP21 vs FP1	4737.2	293.2	<0.0001
FP2	0 ; 0.5	FP21 vs FP22	4444.0	131.3	<0.0001
			EFV		
Null	-	Linear vs Null	1867.4	489	<0.0001
Linear	1	FP1 vs Linear	1378.4	259.9	<0.0001
FP1	0.5	FP21 vs FP1	1121.5	19.5	0.0002
FP2	0;0	FP21 vs FP22	1102.0	79.2	<0.0001

Table C.2 shows the fixed effect parameter estimates fo the two degrees fractional polynomial mixed effect model (NVP and EFV treatment group).

Table C.2. Parameter estimates and their associated standard error using FP22. The p-values are based on the Wald test.

Effect	Estimate	Estimate(s.e)	P-value
		NVP	
β_0	5.2224	0.02262	<0.0001
β_1	0.04857	0.004126	<0.0001
β_2	0.07766	0.006034	<0.0001
		EFV	
β_0	5.0473	0.05368	'<0.0001
β_1	0.1398	0.007194	<0.0001
β_2	0.02436	0.002888	<0.0001

Appendix D

Joint Modeling for Longitudinal and Time to Event Outcome

This appendix presents additional supporting information for the analyses presented in Chapter 5 and 7.

Cox-PH Model for Time to Death and Composite Outcome

The survival model (cox-PH) is fitted for both death and composite outcome discussed in Chapte 5. The result are presented in Table D.1.

Table D.1. Estimates of hazard ratio and 95% CI obtained for the Cox-PH model for death and composite outcome.

	Event					
	Death			Composite outcomes		
Covariate	HR	95%CI	P-value	HR	95%CI	P-value
NNRTI(NVP)	0.72	0.539; 0.962	0.0262	0.858	0.715; 1.031	0.102

Two Stage Procedure (Chapter 5)

To account the measurement error and biological variations, we used two stage procedure. In the first stage log(CD4) cell count is modelled using fractional polynomial mixed effect model and the maximum likelihood estimate and best linear unbiased predictors of the random effects are obtained. Then in a second stage, Cox-PH model is fitted to the survival data using the longitudinal fitted values at different time as

Table D.2. Estimates of hazard ratio and 95% CI using a two-stage procedure for death and composite outcomes.

	Event							
	Death				Composite outcomes			
Covariate	HR	SE	95%CI	P-value	HR	SE	95%CI	P-value
NNRTINVP	0.6761	0.166	0.4881; 0.9367	0.0186	1.142	0.0948	0.9484; 1.3752	0.161
yy1	0.3580	0.1068	0.2904; 0.4413	<0.0001	0.5227	0.0654	0.4598; 0.5943	<0.0001

covariates. The result revealed that the effect of fitted value of log(CD4) cell count at time 1, 3, 6, 9 and 12 months were significantly associated with death and composite outcome in the multivariate analysis (Table D.2).

Maximum Likelihood Estimation for Joint modeling

As pointed out in Rizopoulos (2012a), maximum likelihood estimation for joint models is based on the maximization of the log-likelihood corresponding to the joint distribution of the time-to-event and longitudinal outcomes $\{T_i, \delta, y_i\}$. In this section we briefly describe the estimation procedure for the joint model. The vector of time independent random effects $\mathbf{b_i}$ underlies both the longitudinal and survival processes. This means that these random effects account for both the association between the longitudinal and event processes, and the correlation between the repeated measurements in the longitudinal process. Strictly, we assumed that the longitudinal process and the survival process are conditionally independent given b_i:

$$p(T_i, \delta_i, y_i|b_i; \theta) = p(T_i, \delta_i|b_i; \theta)p(y_i|b_i; \theta),$$

with

$$p(y_i|b_i; \theta) = \prod_j p\{y_i(t_{ij}|b_i; \theta)\}.$$

Where $\theta = (\theta_t^T, \theta_y^T, \theta_b^T)$ denotes the full parametric vector for the event time outcome, the longitudinal outcome and for the random effect covariance matrix respectively. Due to the fact that the survival and longitudinal submodels share the same random effects, joint models of this type are also known as shared parameter models. Under the modeling and these conditional independence assumptions the joint log-likelihood contribution for the i^{th} subject can be formulated as;

$$log(p(T_i, \delta_i, y_i; \theta)) = log \int p(T_i, \delta_i|b_i\theta_t, \beta)[\prod_j p\{y_i(t_{ij})|b_i; \theta_y\}]p(b_i; \theta_b)db_i. \quad \text{(D.1)}$$

where the likelihood of the survival part is written as

$$p(T_i, \delta_i | b_i; \theta, \beta) = \{h_i(T_i | \mathcal{M}_i(T_i); \theta_t, \beta)\}^{\delta} S_i(T_i | \mathcal{M}_i(T_i; \theta_t), \beta). \tag{D.2}$$

with $h_i(.)$ and $S_i(.)$ obtained survival submodel. Whereas, the joint density for longitudinal responses together with the random effects is performed through the following expression

$$\begin{aligned} &\prod_j p\{y_i(t_{ij}) | b_i; \theta_y\} p(b_i; \theta_b) = (2\pi\sigma^2)^{n_i/2} exp\{-\|y_i - X_i\beta - Z_i b_i\|^2 \sigma^2\} \\ &\times (2\pi)^{-qb/2} det(D)^{-1/2} exp(-b^T D^{-1} b_i/2). \end{aligned} \tag{D.3}$$

Where qb and $\|.\|$ denotes the dimensionality of the random effect vector, and the euclidean vector norm respectively. The estimation procedure is discussed in details in Rizopoulos (2012a).

Model Formulation of the Hazard Function.

In Chapter 5 and 7, we used different model parametrizations for longitudinal process such as current value, lagged effect, time dependent slope and cumulative effects parametrization as covariates that might influence the hazard function.

1. Lagged effect parametrization: Assumes that the risk of relapse at time t depends on the value of the biomarker at time $t - c$, where c specifies the time lag of interest. The model is defined as

$$h_i(t | \mathcal{M}_i(t), \omega_i) = h_0(t) \exp(\gamma^\tau \omega_i + \alpha m_i(t_{ij}) \{max(t - c, 0)\}), t > 0.$$

2. Cumulative effect parametrization: The risk of relapse at time point t depends on the cumulative effect of the longitudinal outcome up to time point t. The model is formulated as

$$h_i(t | \mathcal{M}_i(t), \omega_i) = h_0(t) \exp\left\{\gamma^\tau \omega_i + \alpha \int_0^t m_i(s_{ij}) ds_{ij}\right\}, t > 0.$$

Additional Joint Models Fitted for the Analysis Presented in Section 5.3.3

In this section we present several joint models that were fitted within the analysis presented in Section 5.3.3

1. Current value

We assumed that the event of interest depends on the current value of the CD4 cell count evolution. There was no significant different on the risk of death or composite event whether the patient initiated with NVP or EFV. The parameter α measures the association between log(CD4) cell counts and death or composite outcome. The risk of death and composite event increased by 2.04 (95%CI:1.670;2.490) and 1.53 (95%CI:1.371;1.804) fold associated with one unit decrease in the current value of log(CD4) level, respectively (Table D.3). In the standard joint model, it is assumed

Table D.3. Joint modeling of CD4 cell counts evolution of current value and composite events.

	Death				Composite outcome			
Covariate	Estimate	SE	95%CI	P-value	Estimate	SE	95%CI	P-value
Longitudinal process								
(Intercept)	5.033	0.035	(4.963;5.102)	<0.0001	5.033	0.035	(4.964; 5.102)	<0.0001
NNRTINVP	0.102	0.035	(0.032;0.171)	0.004	0.102	0.035	(0.033; 0.171)	0.0039
log(time)	0.046	0.004	(0.039;0.053)	<0.0001	0.046	0.004	(0.039; 0.053)	<0.0001
$log(time)^2$	0.098	0.006	(0.086; 0.109)	<0.0001	0.098	0.006	(0.086;0.109)	<0.0001
Random Effect								
σ_{b_0}	0.676				0.675			
σ_{b_1}	0.060				0.060			
σ_{b_2}	0.037				0.037			
σ	0.299				0.299			
Event process								
NNRTINVP	0.816	0.1937	(0.558; 1.194)	0.2961	0.759	0.125	(0.549;1.050)	0.3715
α	2.039	0.102	(1.670;2.490)	<0.0001	1.528	0.070	(1.371;1.804)	<0.0001
log.Lik	-2969.60				-3821.76			

that the risk for an event at a particular time point t depends on the true level of the log(CD4) cell counts at the same time point t. The strength of the association between the current level of the log(CD4) counts and death or composite outcome is captured by the parameter α. However, this may not be the most appropriate in expressing for the correct relation between the two processes. This is because of the fact that time-dependent covariates can be much more challenging to handle than baseline covariates.

2. Interaction Effect

The joint model was refitted with interaction effects of drug and log(CD4) cell counts in addition to the main effect. The interaction of treatment with log(CD4) cell counts was statistically significant for composite outcome, but not for death. The risk of death and composite outcome increased by 2.31-fold (95%CI:(1.652; 3.235)) and 2.10-fold (95%CI(1.645; 2.653)) for a unit decrease in the current value of log(CD4) cell counts in patients who initiated with EFV, respectively. Whereas for patients who initiated with NVP, the risk of death and composite outcome were 1.942-fold and 1.385-fold, respectively (See Appendix D.4).

Table D.4. Joint modeling of CD4 cell counts evolution with interaction effect parametrization.

	Death				Composite outcome			
Covariate	Estimate	SE	95%CI	P-value	Estimate	SE	95%CI	P-value
Longitudinal process								
(Intercept)	5.032	0.035	4.963; 5.101	<0.0001	5.033	0.035	4.964; 5.102	<0.0001
NNRTINVP	0.101	0.035	0.032; 0.170	0.0041	0.102	0.035	0.032; 0.171	0.004
log(time)	0.0461	0.0035	0.039; 0.053	<0.0001	0.046	0.004	0.039; 0.053	<0.0001
$time^0.5$	0.0975	0.0059	0.086; 0.109	<0.0001	0.097	0.006	0.086; 0.109	<0.0001
Random Effect								
σ_{b_0}	0.676			0.675				
σ_{b_1}	0.060			0.060				
σ_{b_2}	0.037			0.037				
σ	0.2989			0.2989				
Event process								
NNRTINVP	0.361	0.9678	0.054; 2.404	0.2921	0.153	0.7061	0.038; 0.611	0.008
α	0.432	0.1714	0.309; 0.605	<0.0001	0.479	0.122	0.377; 0.608	<0.0001
Assoct:NNRTINVP	1.190	0.209	0.790; 1.793	0.4043	1.508	0.146	1.134; 2.006	0.005
AIC	5981.634					7681.763		

3. Legged Effect

The current value of log(CD4) cell counts may not determine the event process, but previous measurement of the marker. One way to take this into account is to use time-lagged covariates. The idea is the risk at time t depends on the true value of the longitudinal marker at time tc, where c specifies the time lag of interest. Table D.5 shows joint model results at lagged time 1. The association between log(CD4) cell counts at lag-time 1 and the risk for death was statistically significant. There was 2.07-fold (95%CI: 1.703; 2.511) increase in the risk of death for one unit decrease of log(CD4) cell counts at time $t-1$. We also observed that a unit decrease in log(CD4) cell count at time $t-1$ has 1.562-fold (95%CI:1.361; 1.793) increase in the risk of composite event. There was no statistical significant difference in the risk of either death or composite outcome among the two treatment groups at baseline (Table D.5). Higher Lagged time can be also considered, Table D.6 presents the model output for lagged time 2 $t-2$.

Table D.5. Joint modeling of CD4 cell counts evolution with lagged 1 effect.

	Death				Composite outcome			
Covariate	Estimate	SE	95%CI	P-value	Estimate	SE	95%CI	P-value
Longitudinal process								
(Intercept)	5.070	0.043	4.986; 5.153	<0.0001	5.080	0.043	4.996; 5.164	<0.0001
NNRTINVP	0.088	0.0426	0.005; 0.171	0.0387	0.086	0.043	0.002; 0.169	0.0441
log(time)	0.049	0.004	0.041; 0.057	<0.0001	0.050	0.004	0.042;0.058	<0.0001
$time^0.5$	0.090	0.008	0.074; 0.105	<0.0001	0.086	0.008	0.071; 0.102	<0.0001
Random Effect								
σ_{b_0}	0.719			0.722				
σ_{b_1}	0.074			0.072				
σ_{b_2}	0.106			0.106				
σ	0.288			0.2886				
Event process								
NNRTINVP	0.819	0.193	0.563; 1.20	0.314	1.129	0.125	0.884 1.442	0.3307
$\alpha_{(lag=1)}$	2.07	0.099	1.703;2.511	<0.0001	1.562	0.070	1.361; 1.793	<0.0001
AIC	6717.942					8420.485		

Table D.6. Joint modeling of CD4 cell counts evolution with lagged 2 effect.

	Death				Composite outcome			
Covariate	Estimate	SE	95%CI	P-value	Estimate	SE	95%CI	P-value
Longitudinal process								
(Intercept)	5.069	0.043	4.986; 5.153	<0.0001	5.080	0.043	4.995; 5.163	<0.0001
NNRTINVP	0.088	0.043	0.005; 0.171	0.0386	0.086	0.043	0.002; 0.169	0.0438
log(time)	0.048	0.004	0.041; 0.056	<0.0001	0.050	0.004	0.042;0.058	<0.0001
$time^0.5$	0.090	0.008	0.074; 0.105	<0.0001	0.086	0.008	0.071; 0.102	<0.0001
Random Effect								
σ_{b_0}	0.719			0.722				
σ_{b_1}	0.074			0.072				
σ_{b_2}	0.106			0.106				
σ	0.288			0.2886				
Event process								
NNRTINVP	0.819	0.193	0.561; 1.196	0.3015	1.129	0.125	0.884 1.442	0.3307
$\alpha_{(lag=2)}$	2.044	0.099	1.683;2.482	<0.0001	1.55	0.070	1.353; 1.780	<0.0001
AIC	6718.561					8420.929		

4. Cumulative Effect

To account the whole history of CD4 count up to time point t, the integral of the longitudinal trajectory is included in the relative risk submodel as a liner predictor. The parameter which measures the association is statistically insignificant.

Table D.7. Joint modeling of cumulative effect parametrization.

	Death				Composite outcome			
Covariate	Estimate	SE	95%CI	P-value	Estimate	SE	95%CI	P-value
Longitudinal process								
(Intercept)	5.0581	0.0427	4.974; 5.142	<0.0001	5.0563	0.0430	4.972; 5.141	<0.0001
NNRTINVP	0.0896	0.0474	0.007; 0.173	0.0343	0.088	0.042	0.006; 0.171	0.0363
log(time)	0.0474	0.004	0.039; 0.055	<0.0001	0.0470	0.0041	0.039; 0.055	<0.0001
$time^0.5$	0.094	0.0078	0.079; 0.109	<0.0001	0.095	0.008	0.080; 0.110	<0.0001
Random Effect								
σ_{b_0}	0.7287			)0.7347				
σ_{b_1}	0.0741			0.0737				
σ_{b_2}	0.1064			0.1059				
σ	0.2878			0.2880				
Event process								
NNRTINVP	0.865	0.189	0.597; 1.254	0.4447	1.140	0.1222	0.897; 1.448	0.2830
α	0.994	0.008	0.979; 1.009	0.4603	1.000	0.0039	0.993; 1.009	0.8317
AIC	5971.252					7668.195		

Table D.8. Comparison of models based on the different parametrization.

	Death			Composite outcome		
Parametrization	Log-Likelihood	AIC	BIC	Log-Likelihood	AIC	BIC
Current value	-3338.606	6717.213	6816.172	-4189.778	8419.555	8518.514
Interaction	-2969.817	5981.634	6085.54	-3819.882	7681.763	7785.67
Lag 1	-3338.971	6717.942	6816.901	-4190.242	8420.485	8519.443
Lag 2	-3339.28	6718.561	6817.519	-4190.465	8420.929	8519.888
Time-Dependent Slope	-2964.626	5971.252	6075.159	-3813.097	7668.195	7772.101
Cumulative effect	-3364.919	6769.837	6868.796	-4207.455	8454.909	8553.868

AUC and DDI for WBC and Hgb

Table D.9 and Table D.10 present additional materials for the analysis reported in Section 7.4.3. Table D.9 presents the area under the ROC curve and estimated DDI of WBC biomarker for patient 13 by considering simple and relative decrease prediction rules.

Table D.9. Area under the ROC curve and the estimated DDI of WBC (based on 1000 Monte Carlo samples for patient 13) for both of the prediction rules.

Parametrization		Simple value		20% Rel.decrease	
Δt	t	AUC(t)	DDI	AUC(t)	DDI
14	0	0.7049	0.7748	0.7253	0.7748
	28	0.7222		0.6854	
	56	0.6600		0.6836	
	84	0.7102		0.6720	
28	0	0.6905	0.654	0.6997	0.654
	28	0.7173		0.6852	
	56	0.6599		0.6907	
	84	0.7058		0.6732	
42	0	0.6811	0.7038	0.6934	0.7038
	28	0.7136		0.6850	
	56	0.6628		0.6897	
	84	0.7037		0.6743	
56	0	0.6749	0.681	0.6893	0.681
	28	0.7107		0.6857	
	56	0.6642		0.6887	
	84	0.7026		0.6754	

Table D.10 presents the area under the ROC curve and estimated DDI of Hgb biomarker for patient 20 by considering simple and relative decrease prediction rules.

Table D.10. Area under the ROC curve and the estimated DDI of Hgb marker (based on 1000 Monte Carlo samples for patient 20) for both of the prediction rules.

Parametrization		Simple value		20% Rel.decrease	
Δt	t	AUC(t)	DDI	AUC(t)	DDI
14	0	0.8638	0.7225	0.8858	0.7225
	28	0.8432		0.8816	
	57	0.8562		0.8309	
	86	0.9094		0.8522	
28	0	0.8278	0.6627	0.8531	0.6627
	28	0.8172		0.8642	
	57	0.8409		0.8144	
	86	0.9089		0.8438	
42	0	0.8012	0.6507	0.8283	0.6507
	28	0.7953		0.8536	
	57	0.8294		0.8036	
	86	0.9032		0.8399	
56	0	0.7791	0.6401	0.8088	0.6401
	28	0.7790		0.8465	
	57	0.8193		0.7933	
	86	0.8950		0.8378	

Samenvatting

De opkomst van HIV tijdens de laatste drie decennia is een van de grootste uitdagingen waarmee de wereld wordt geconfronteerd. De ziekte is voorlopig ongeneesbaar maar de invoering van Antiretroviral Therapy (ART) maakt van het virus een controleerbare chronische ziekte. Indien er reeds een besmetting is van het HIV virus, is een co-infectie met andere ziekten een extra zware last. Zo is Visceral leishmaniasis (VL) een endemische en mogelijk levensbedreigend ziekte. Een derde van alle HIV patinten leeft in regios waar leishmaniasis endemisch is en een co-infectie van VL-HIV verspreidt zich doorheen deze regios. In deze dissertatie, beoogden we het resultaat van behandelde genfecteerde HIV and co-genfecteerde V-HIVL patinten in Noordwest Ethiopi in kaart te brengen.

De hoofdzakelijke doelen van ART op korte termijn zijn de HIV gerelateerde ziekte- en sterftecijfers reduceren, de overlevingskansen verhogen, de levenskwaliteit verbeteren, het herstellen en het behouden van het immunologisch functioneren en het voorkomen van HIV-transmissie. De uitkomsten van ART op lange termijn zijn anders. Daarom werd een nieuw variabele gedefinieerd met de volgende categorien: een vervangend geneesmiddel werd toegediend, de patint wordt niet meer opgevolgd, stopzetting van de behandeling en sterfte. Er was geen significant verschil in het risico van deze samengestelde variabele tussen patinten die een behandeling begonnen met EFV of NVP ART na aanpassing van de nul status. De selectie van een juiste behandeling wordt vaak benvloed door de karakteristieken van de patint. Hierbij is vooringenomenheid voor een bepaalde behandeling een groot probleem indien we de effect van een behandeling willen bestuderen in observatie studies. Een geneigdheidsscore werd opgesteld voor elke behandeling om zo de selectiebias te reduceren. Gelijkaardige resultaten werden gevonden.

Een significant verschil werd geobserveerd in het risico tussen patinten gestart

met een TDF behandeling die ART bevat and patinten die startte met AZT na het controleren voor NNRTI medicijnen. Het risico voor TDF gecombineerd met NVP is in vergelijking met AZT twee keer zo hoog. De studie toonde ook dat er een verhoogd risico was voor patinten gestart met d4t in vergelijking met AZT. Het risico was niet significant verschillend voor d4t en de NNRTI medicijnen waarbij werd aangenomen dat niet meer gevolgde patinten gecenseerd werden. Dit toonde aan dat het verschil in risico tussen d4t en AZT werd veroorzaakt door de verloren opgevolgde patinten. Patinten wiens behandeling startte met d4t hadden twee keer het risico niet opgevolgd te worden in vergelijking met zij die begonnen met AZT. Een mogelijke verklaring zijn de onomkeerbare bijwerkingen van d4t op lange termijn.

De effecten van de behandeling op de CD4 aantallen variren over tijd en er wordt aangenomen dat herhaalde metingen van eenzelfde patint geassocieerd zijn. De data toont een niet lineaire trend en het opleggen van een parametrische functie voor de gemiddelde evolutie kan ongewenste resultaten opleveren. We namen patint specifieke random parameters aan voor zowel het lineaire en kwadratische tijdseffect om zo de verschillende evoluties van het CD4 aantal over de tijd te modelleren. De eerste afgeleide plot toont dat de snelheid van de CD4 stijging als reactie op de behandeling hoog is gedurende de eerste 10 maanden en stabiliseert na een jaar. Het resultaat onthulde ook dat er op lange termijn geen verschil was in de trend van de CD4 aantallen tussen patinten met een EVF of NVP behandeling.

Een flexibele parametrisch benadering werd gebruikt voor de vroege predictie van CD4 aantallen voor een specifieke behandeling en om de nodige tijd te schatten dat het CD4 aantal een bepaalde drempelwaarde zal overschrijden. We pasten een cross validatie toe om de prestatie van een fractioneel polynomiaal gemengd model te evalueren in voorspellingsnauwkeurigheid. Het model wordt vervolgens gebruikt om de verwachtte tijd te voorspellen die nodig is om de CD4 drempelwaarde te overschrijden na het opstarten van ART. Zo kan de verdeling van de tijd nodig om de vooropgestelde CD4 drempelwaarde worden opgesteld en kan deze verdeling worden gebruikt om behandelingen te vergelijken.

Vervolgens wordt de kans om een voldoende hoog CD4 aantal te bereiken voorspeld voor elk individu. Meer dan de helft van de patinten met een ART behandeling en CD4 aantallen minder dan 200 $cells/mm^3$ overstijgen de drempelwaarde binnen zes maanden na het opstarten van de behandeling. De tijd nodig om de drempel te overschrijden is korter voor patinten met een NVP behandeling in vergelijking met een EFV behandeling. Het verschil werd ook vergeleken gebruik makend van een log-rank test door de tijd die het kost om de drempelwaarde te overschrijden te nemen als de survival tijd. Het resultaat toonde aan dat de mediane tijd die het kost om de

drempelwaarde van 200 CD4 $cells/mm^3$ korter is voor patinten met een NVP dan met een EFV behandeling. Een mogelijke reden is dat NVP wordt gebruikt voor patinten met een laag CD4 aantal om de bijwerking van EFV te reduceren. Deze trend is anders indien de behandeling begint met hogere CD4 aantallen. Dit kan mogelijk veroorzaakt worden door de sterke natuur van de EFV behandeling.

De heropleving van VL in VL-HIV co-genfecteerde patinten is zeer hoog. Een vroege detectie van herval kan worden gemaakt aan de hand van biomarkers. Dit impliceert het testen van verschillende klinische, laboratorische en immunologische opgevolgde variabelen voor het mogelijk gebruik als biomarker voor terugval. We implementeerde een joint model voor longitudinale metingen, toegepast op het voorspellen van de tijd tot VL herval in VL-HIV co-genfecteerde patinten onder behandeling. Resultaten toonden aan dat sterke WBC en hemoglobine kunnen worden gebruikt als een biomarker voor het voorspellen van een VL herval. De snelheid van de verandering in hemoglobine niveaus werd een significante predictor bevonden voor het voorkomen van een herval. Gelijkaardig kan het voorkomen van een herval kan ook worden voorspeld door de huidige waarde van WBC.

De kansen op een terugval vrije overleving werden bepaald voor elk individu en dynamisch gepdatet door het toevoegen van bijkomstige informatie van de biomarker. De kans op herval in latere opvolg periodes werden voorspeld aan de hand van de metingen van de biomarkers gedurende 84 dagen. Hoe meer metingen van de biomarkers, hoe nauwkeuriger de kans kon worden voorspeld zoals aangetoond door het 95% betrouwbaarheidsinterval. De voorspellende kracht van de hemoglobine marker is groter dan deze van WBC. Dit is gebaseerd op de AUC waarden van voor herval. Lagere waarden van hemoglobine heeft een hogere discriminerend vermogen tussen patinten die een terugval zullen ondervinden en zij die terugval vrij zullen blijven.

Ter besluit, de lange termijn effecten van ART zijn vergelijkbaar in EFV en NVP behandelingsgroepen. Een significant verschil werd geobserveerd voor de NNRTI behandeling voor HIV patinten. Het maximum voordeel van ART wordt bereikt gedurende de eerste 10 maanden na de opstart van de behandeling. Bij hogere CD4 aantallen, verbetert het CD4 aantal sneller met een EFV behandeling in vergelijking met een NVP behandeling. Er is een sterke associatie tussen het risico van de eind variabele en de snelheid van de verandering in het CD4 aantal. Een vroege voorspelling in de verandering van het CD4 aantal kan een argument zijn voor het type behandeling. De kans op VL herval kan worden voorspeld door de verandering van het hemoglobine niveau en de huidige waarde van de WBC aantallen. In het maken van het onderscheid tussen patinten met een terugval en zij zonder terugval, heeft hemoglobine meer kracht dan WBC. Een daling van 20% in hemoglobine kan een on-

derscheid maken met patinten die binnen 14 dagen een terugval zullen ondervinden.