Abstract

This study was aimed at describing the evolution of CD4 cell counts over time, for patients on antiretroviral therapy (ART) in Mildmay Uganda. It was also aimed at determining whether the evolution depends on selected patient characteristics.

Since repeated measures were taken from the patients, correlation has to be taken into account when analyzing these data. As such, this study illustrates the application of Linear Mixed Models to describe the changes in CD4 counts over time, for patients on ART. Separate models were fitted for patients with baseline age$\geq$15 years (presumed sexually active), for those with (6$\leq$Age$\leq$14) years, and for those with baseline age$\leq$5 years (whose absolute CD4 counts are not used in patient management).

Results show, that for patients who were aged $\geq$15 years at the start of ART, the evolution could be described by a cubic function of time. It was also noted that the effect of gender on the evolution depends on the patient's baseline CD4 category, also on the NNRTI drug that the patient uses. For the patients aged $\leq$5 years, also for those with (6$\leq$Age$\leq$14) years, linear functions of time were found appropriate to describe the evolutions, which depended on the baseline CD4 categories in either case.

In conclusion, the benefit of early treatment was shown in this study i.e. patients who started ART at higher baseline CD4 counts evolved higher than those who started at lower CD4 counts.

Keywords: CD4 cell count, Longitudinal Analysis, Linear Mixed Models, Antiretroviral Therapy (ART), Patients, HIV/AIDS

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For the unconditional love and unending support, from the Lubyayi family; dad, mum, brothers and sisters I dedicate my success to you. Heartfelt gratitude to our latest addition to the family, my dear wife Esther, thank you for being patient and encouraging me all the time.

Finally, I offer everything to God for without Him everything is nothing to me. I have had a miraculous journey to-date, all my achievements, opportunities, the precious gift of life, the list is endless. All I can say is, "Praise be unto Him."

Lawrence Lubyayi
September 9, 2014

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List of Abbreviations

3TC	Lamivudine
AIDS	Acquired Immuno-deficiency Syndrome
ART	Anti-retroviral Therapy
AZT	Azidothymidine / Zidovudine
d4T	Stavudine
EFV	Efavirenz
HBV	Hepatitis-B Virus
HIV	Human Immuno-deficiency Virus
MARPs	Most at risk Persons
ML	Maximum Likelihood
MUg	Mildmay Uganda
NEV	Nevirapine
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
REML	Restricted Maximum Likelihood
TB	Tuberculosis
TDF	Tenofovir
WHO	World Health Organization

1 Introduction

1.1 Background

Human Immunodeficiency Virus (HIV) is a lentivirus that causes Acquired Immunodeficiency Syndrome (AIDS) by reducing a person's ability to fight infection. HIV attacks an immune cell called the CD4 cell which is responsible for the body's immune response to infectious agents (Adams and Luguterah, 2013). As such, the number of CD4 cells per cubic millimeter of blood is widely used as an important biomarker for progression to AIDS when studying the efficacy of drugs to treat HIV-infected patients (Guo and Carlin, 2004). It is often measured repeatedly over follow-up periods in large-scale studies.

Since the beginning of the epidemic, almost 75 million people have been infected with the HIV virus, about 36 million people have died, 35.3 million [32.2-38.8 million] people were living with HIV at the end of 2012, an estimated 0.8% of adults aged 15-49 years worldwide are living with HIV, Sub-Saharan Africa remains most severely affected with 71% of the people living with HIV worldwide and nearly 1 in every 20 adults living with HIV (World Health Organization, 2014).

2012 HIV/AIDS statistics showed that 7.2 percent of Uganda's population was living with HIV. This amounted to an estimated 1.4 million people, which included 190,000 children. An estimated 62,000 people died from AIDS in 2011 and 1.1 million children had been orphaned by Uganda's devastating epidemic. HIV prevalence has been rising since its lowest rate of 6.4 percent in 2006. New infections are diagnosed in 150,000 people a year, of whom 20,600 are children (Avert, 2014).

Antiretroviral Therapy (ART) services have been available to HIV patients in Uganda for a while now. In May 2013, Uganda began the process of updating the ART guidelines to include recommendations from the 2013 WHO ART Guidelines which are aimed to dramatically increase access to ART and in turn provide new opportunities to save lives, improve clinical outcomes and reduce HIV incidence. The new guidelines recommend initiation of ART in all adults and adolescents with HIV at CD4 cut off of <500 cells/mm^3 regardless of clinical stage. The guidelines also recommend immediate ART initiation for patients with: HIV and active TB disease, HIV and HBV co-infection with evidence of severe chronic liver disease, HIV positive partner in a sero-discordant sexual relationship, Most at risk Persons (MARPs) in hot-spots (fisher folks, Commercial sex workers, long distance truck operators), Pregnant and Lactating Mothers and for all HIV positive children below 15 years of age (Ario, 2014). It is worth noting that these guidelines have been changing over time, for instance, the 2010 recommendations had a threshold of 350 CD4 cells/mm^3 (World Health Organization, 2014). Prior to 2010, there was a threshold of 250 CD4 cells/mm^3.

Mildmay Uganda (MUg) opened in 1998 to provide palliative outpatient care for people living with HIV/AIDS, and to act as a teaching and training centre for HIV/AIDS health care personnel in Uganda. MUg is primarily a treatment centre for persons living with HIV/AIDS and their families. It currently offers family centered care and support to approximately 24,000 clients. MUg has one central clinic located in Lweza, 12km outside Kampala, the capital of Uganda. MUg is also involved in a district health systems

strengthening programme to build HIV/AIDS health care capacity in existing health centres in 18 local districts (Funk *et al.*, 2012). Against this background, this study was conducted using routine data for patients who started ART between 2009 and 2012 at MUg. CD4 counts are normally taken for patients on ART on routine basis every 6 months.

Few studies have been conducted about longitudinal analysis of CD4 cell count data for patients on ART especially in Sub-Saharan Africa. For instance, Adams and Luguterah (2013) report that the pattern of growth in CD4 cell counts was not linear. Picat *et al.* (2013), use nonlinear mixed-effects models, and conclude that higher long-term CD4 counts were predicted for children starting ART younger, and with higher CD4 counts. Reda *et al.* (2013) use mixed models regression and report a substantial increment in weight and CD4 lymphocyte count among the patients who were taking ART in eastern Ethiopia.

This study was aimed at providing a longitudinal analysis of CD4 cell count data for patients who initiated ART between January 2009 and December 2012 at Mildmay Uganda. By considering changes over time, the longitudinal approach has the added advantage of observing changes more accurately, by increasing the power and validity of measuring the change in CD4 cell counts.

1.2 Study Objectives

The objectives of this study were two-fold:

1. To describe the evolution of CD4 cell counts for patients on ART in Mildmay Uganda.

2. To determine whether the evolution depends on selected patient characteristics.

1.3 The Data

Cleaned records for patients who started ART at Mildmay Uganda between 1st January 2009 and 31st December 2012, and were on first line regimen drugs formed the dataset for this study.

This dataset was split into 3 parts. Patients with baseline Age$\geq$15 years (n=3241) were analyzed separately because this population is presumed to be sexually active. Those with 6$\leq$Age$\leq$14 years (n=302) were also analyzed separately from those with baseline Age$\leq$5 years (n=259) because absolute CD4 counts are not used for clinical decisions for the patients with Age$\leq$5 years. Further still, the young patients (Age$<$15 years) were analyzed separately from the adults (Age$\geq$15 years) because in most cases, drug administration and adherence for the children depends on their guardians/caretakers.

The response variable (CD4 count) was log transformed because it was heavily skewed. Therefore, throughout this report the log transformed version is used. Table 1 below explains the variables that were considered in this report.

Table 1: *Summary of variables in the datasets*

Variable name	Description
logcd4	Natural logarithm of the CD4 counts
agecat	Categorization of the baseline age ($\leq$2, 3-5, 6-9, 10-14, 15-24, 25-34, 35-49, $\geq$50)
cd4cat	Baseline CD4 category ($\leq$200, 201-350, 351-500, >500)
gndr	Gender of patient (0=Female, 1=Male)
nrti	NRTI backbone used (d4t+3TC, TDF+3TC and AZT+3TC)
nnrti	NNRTI drug used (Nevirapine, Efavirenz)
change	Did the patient change drug combination (Yes/No)
yr	Year of starting ART (2009, 2010, 2011, 2012)
id	Unique identifier of patients

2 Methodology

2.1 Exploratory Data Analysis (EDA)

EDA was done in order to gain insight into the data prior to analysis. Descriptive statistics were generated and tabulated while histograms were constructed to show the distribution of number of measurements per patient, for each of the 3 datasets. χ^2 tests were used to compare baseline characteristics (in form of categorical variables) between patients treated with either efavirenz or nevirapine.

Individual profile plots were then constructed to gain some rough picture about how subjects evolve as well as to provide indications in terms of between and within subjects variability. Moreover, this exploration provided ideas about what random effects to start with.

Average profile plots were constructed to describe the mean evolution of CD4 counts, overall and according to different subgroups. From such exploration, indications were obtained, about the functional form of the evolution and also whether the evolution depends on given covariates. Smoothing using the Loess method was applied because the measurements were not equally spaced across the different subjects.

The variance structure was also explored for the 3 datasets. This was aimed at getting insight about how the variance evolves over time. This gave indications in terms of how the variance was to be modeled so that valid inferences could be made (Verbeke and Molenberghs, 2000). Smoothing techniques using the Loess method were again employed because of the unequal spacing of measurements.

2.2 The Linear Mixed Effects Model

Longitudinal designs which involve repeated measurements of a variable of interest in each of a series of individuals enable one to study changes within individual subjects over time or under varied conditions. However, the repeated measurements tend to be correlated, and this must be taken into account at the time of analysis or misleading conclusions may result. The Mixed Model is one of the statistical methods used to analyze repeated measurements in continuous longitudinal data in an easy, valid and flexible manner (Burton, Gurrin, and Sly 1998).

The Linear mixed model handles longitudinal data analysis in the continuous case. Moreover, since it assumes a linear regression for each cluster separately, it can be used for data with unequal number of measurements per cluster (Verbeke and Molenberghs, 2000). Against this background a linear mixed-effects model was fitted. According to Verbeke and Molenberghs (2000), the linear mixed-effects model is defined as,

$$\begin{cases} \mathbf{Y}_i = \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b_i} + \boldsymbol{\varepsilon_i} \\ \mathbf{b}_i \sim N(\mathbf{0}, D), \\ \boldsymbol{\varepsilon}_i \sim N(\mathbf{0}, \boldsymbol{\Sigma}_i), \\ \mathbf{b}_i, \boldsymbol{\varepsilon}_i \qquad \text{are independent.} \end{cases}$$

where $\mathbf{Y}_i$ is the n_i dimensional response vector for the i^{th} subject, $1 \leq i \leq N$, N is the number of subjects, $\mathbf{X}_i$ and $\mathbf{Z}_i$ are $(n_i \times p)$ and $(n_i \times q)$ dimensional matrices of known covariates, $\boldsymbol{\beta}$ is a p-dimensional vector containing the fixed effects, $\mathbf{b_i}$ is a q-dimensional vector containing the random effects, $\boldsymbol{\varepsilon}_i$ is an n_i-dimensional vector of residual components, and D is a covariance matrix of random effects.

Using the general guidelines for model building proposed by Verbeke and Molenberghs (2000),the preliminary mean structure was first stated using a model with plausible combinations of covariates considering the cubic evolution of CD4 counts of patients, with random intercepts and random slopes (for the Age$\geq$15 dataset), for the other two datasets, linear functions of time were used to describe the evolutions. This was followed by the investigation of serial correlation, reduction of random effects, and reduction of mean structure consecutively.

Including serial correlation, if present, is far more important than correctly specifying it (Verbeke and Molenberghs, 2000). Hence to check for the appropriate serial correlation, likelihood based tests were used. Restricted Maximum Likelihood (REML) was preferred to Maximum Likelihood (ML) testing here, because it reduces the well-known finite sample bias in the estimation of the covariance (Fitzmaurice *et al.*, 2004).

The covariance estimates for the random effects were assessed using mixtures of chi-square distributions due to the boundary problem. This problem arises because these tests involve variances which cannot be negative. Thus a likelihood ratio test of the null hypothesis that a variance is zero is testing a null hypothesis that is on "the boundary of the parameter space" for a variance. One consequence is that the usual null distribution for the likelihood ratio test is no longer valid; instead, the null distribution is a mixture of chi-squared distributions (Fitzmaurice *et al.*, 2004).

Finally, to discover the most parsimonious mean structure, F-tests and Likelihood Ratio Tests were employed under ML estimation.

Since the random effects (b_i's) of the linear mixed effects model are stochastic, Bayesian methods were used to estimate them. The estimates, known as Empirical Bayes (EB) estimates, were presented in scatter plots to help identify outlying profiles.

2.3 Statistical Software

Statistical Analysis System (SAS) version 9.3 was used for statistical analysis and graphics. Some graphics were produced in R version 2.15.3. For statistical tests, a significance level of 5% was used.

3 Results

3.1 Exploratory Data Analysis

3.1.1 Descriptive statistics for the patients with Age≥15 years.

Table 2 below shows the baseline characteristics of the population studied with Age≥15 years (n=3241), at the point of ART initiation. Overall, there were more females (68%) than males (32%). Patients starting nevirapine were predominantly female, more likely to use zidovudine (than tenofovir or stavudine) in their NRTI backbone and had lower median CD4 counts (195 versus 209 cells/mm^3). The few patients who used stavudine in their NRTI backbone were not considered in the subsequent analysis, even so because that drug has been phased out of use.

Table 2: *Baseline characteristics for the patients Age≥15 years.*

Characteristic	Nevirapine, n=2058	Efavirenz, n=1183	P-value[a]
Gender, n(%)			
Female	1550(75.3)	651(55.0)	<0.0001
Male	508(24.7)	532(45.0)	
Age (years), median(IQR)	33(28 - 40)	34(28 - 40)	
Age at start of ART, n(%)			
15-24	260(12.6)	142(12.0)	0.0060
25-34	891(43.3)	450(38.0)	
35-49	773(37.6)	489(41.3)	
50+	134(6.5)	102(8.6)	
NRTI backbone, n(%)			
AZT+3TC	1356(65.9)	384(32.4)	<0.0001
TDF+3TC	692(33.6)	798(67.5)	
d4T+3TC	10(0.5)	1(0.1)	
Year of starting ART, n(%)			
2009	779(37.8)	239(20.2)	<0.0001
2010	314(15.3)	128(10.8)	
2011	602(29.3)	246(20.8)	
2012	363(17.6)	570(48.2)	
Baseline CD4 count (cells/mm^3), median(IQR)	195(112 - 253)	209(84 - 305)	
Baseline CD4 category, n(%)			
≤200	1072(52.1)	571(48.3)	<0.0001
201-350	926(45.0)	460(38.9)	
351-500	38(1.8)	86(7.3)	
>500	22(1.1)	66(5.6)	

AZT=Zidovudine, TDF=Tenofovir, d4T=Stavudine, 3TC=Lamivudine.
[a] *Pearson's χ^2 test for independence of rows and columns*

Figure 1 below shows the distribution of the number of measurements per patient for the Age≥15 years dataset. The largest number of patients (575) had two measurements, followed by those with four measurements (492) and least of all was one patient with 12 measurements.

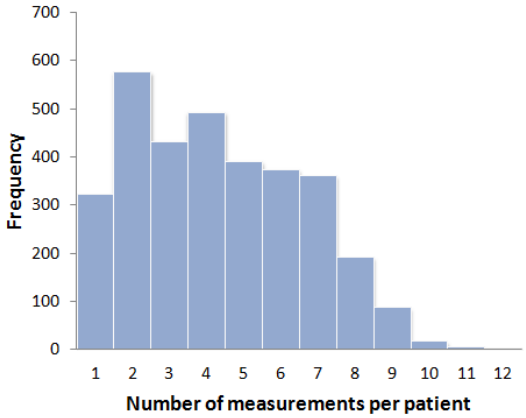

Figure 1: *Frequency of number of measurements per patient for the Age≥15 years dataset.*

Tables 3 and 4 below show the changes that happened within the first line regimen drugs used. It can be seen that 89 patients changed from nevirapine to efavirenz based ART (see Table 3) while 76 patients changed from zidovudine to tenofovir use (see Table 4).

Table 3: *Showing changes in NNRTI for patients with Age≥15 years.*

	Current NNRTI			
Original NNRTI	Nevirapine	Efavirenz	Others	Total
Nevirapine	1,921	89	48	2,058
Efavirenz	50	1,113	20	1,183
Total	1,971	1,202	68	3,241

Pearson chi2(2) = 2.6e+03 ; P-value <0.0001

Table 4: *Showing changes in NRTI backbone for patients with Age≥15 years.*

	Current NRTI backbone				
Original NRTI	d4T+3TC	TDF+3TC	AZT+3TC	Others	Total
d4T+3TC	11	0	0	0	11
TDF+3TC	0	1,432	43	15	1,490
AZT+3TC	0	76	1,611	53	1,740
Total	11	1,508	1,654	68	3,241

Pearson chi2(6) = 6.0e+03 ; P-value <0.0001

3.1.2 Descriptive statistics for the patients with (6≤Age≤14) years.

Table 5 below shows the baseline characteristics of the population studied with 6≤Age≤14 years (n=302), at the point of ART initiation. Overall, there were more females (57%) than males (43%). Patients starting nevirapine were predominantly female, more likely to use zidovudine (than tenofovir or stavudine) in their NRTI backbone and had lower median CD4 counts (225 versus 307 cells/mm^3).

Table 5: *Baseline characteristics for the patients with (6≤Age≤14) years.*

Characteristic	Nevirapine, n=225	Efavirenz, n=77	P-value[a]
Gender, n(%)			
Female	139(61.8)	33(42.9)	0.0040
Male	86(38.2)	44(57.1)	
Age (years), median(IQR)	10(8 - 12)	11(8 - 12)	
Age at start of ART, n(%)			
6-9	91(40.4)	31(40.3)	0.9770
10-14	134(59.6)	46(59.7)	
NRTI backbone, n(%)			
AZT+3TC	207(92.0)	66(85.7)	0.2620
TDF+3TC	9(4.0)	6(7.8)	
d4T+3TC	9(4.0)	5(6.5)	
Year of starting ART, n(%)			
2009	62(27.6)	17(22.1)	0.2880
2010	66(29.3)	17(22.1)	
2011	50(22.2)	22(28.6)	
2012	47(20.9)	21(27.2)	
Baseline CD4 count (cells/mm^3), median(IQR)	225(128 - 302)	307(194 - 409)	
Baseline CD4 category, n(%)			
≤200	94(41.8)	20(26.0)	<0.0001
201-350	114(50.7)	30(39.0)	
351-500	8(3.5)	13(16.9)	
>500	9(4.0)	14(18.1)	

AZT=Zidovudine, TDF=Tenofovir, d4T=Stavudine, 3TC=Lamivudine.
[a] *Pearson's χ^2 test for independence of rows and columns*

Figure 2 below shows the distribution of the number of measurements per patient for the 6≤Age≤14 years dataset. The largest number of patients (49) had five measurements, followed by those with three measurements (45) and least of all was one patient with 10 measurements.

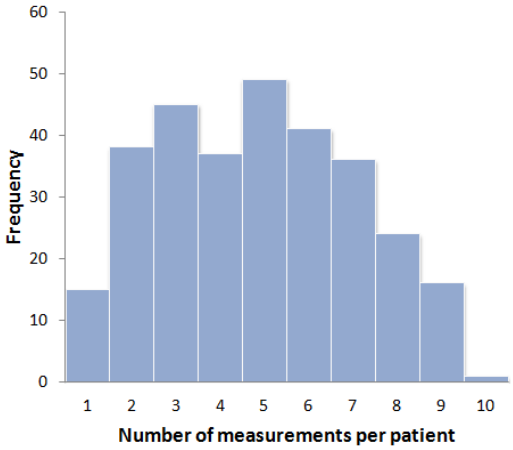

Figure 2: *Frequency of number of measurements per patient for the (6≤Age≤14) dataset.*

Tables 6 and 7 below show the changes that happened within the first line regimen drugs used. It can be seen that 7 patients changed from nevirapine to efavirenz based ART (see Table 6) while 5 patients changed from zidovudine to tenofovir use (see Table 7).

Table 6: *Showing changes in NNRTI for patients with (6 ≤ Age ≤ 14) years.*

	Current NNRTI			
Original NNRTI	Nevirapine	Efavirenz	Others	Total
Nevirapine	215	7	3	225
Efavirenz	3	74	0	77
Total	218	81	3	302

Pearson chi2(2) = 252.8 ; P-value <0.0001

Table 7: *Showing changes in NRTI backbone for patients with (6 ≤ Age ≤ 14) years.*

	Current NRTI backbone				
Original NRTI	d4T+3TC	TDF+3TC	AZT+3TC	Others	Total
d4T+3TC	13	0	1	0	14
TDF+3TC	0	14	1	0	15
AZT+3TC	0	5	265	3	273
Total	13	19	267	3	302

Pearson chi2(6) = 482.0 ; P-value <0.0001

3.1.3 Descriptive statistics for the patients with Age≤5 years.

Table 8 below shows the baseline characteristics of the population studied with Age≤5 years (n=259), at the point of ART initiation. Overall, there were slightly more males (51%) than females (49%). Patients starting nevirapine were predominantly female, more likely to use stavudine (than tenofovir or zidovudine) in their NRTI backbone and had higher median CD4 counts (508 versus 451.5 cells/mm^3). The 3 patients using tenofovir in their NRTI backbone were dropped from the subsequent analysis.

Table 8: *Baseline characteristics for the patients Age≤5 years.*

Characteristic	Nevirapine, n=227	Efavirenz, n=32	P-value[a]
Gender, n(%)			
Female	118(52.0)	9(28.1)	0.0110
Male	109(48.0)	23(71.9)	
Age (years), median(IQR)	2(1 - 4)	4(4 - 5)	
Age at start of ART, n(%)			
≤2	122(53.7)	4(12.5)	<0.0001
3-5	105(46.3)	28(87.5)	
NRTI backbone, n(%)			
AZT+3TC	43(18.9)	3(9.4)	0.0080
TDF+3TC	1(0.4)	2(6.3)	
d4T+3TC	183(80.6)	27(84.4)	
Year of starting ART, n(%)			
2009	90(39.7)	14(43.7)	0.1220
2010	71(31.3)	5(15.6)	
2011	43(18.9)	6(18.8)	
2012	23(10.1)	7(21.9)	
Baseline CD4 count (cells/mm^3), median(IQR)	508(217 - 817)	451.5(150.5 - 639)	
Baseline CD4 category, n(%)			
≤200	50(22.0)	12(37.5)	0.1320
201-350	37(16.3)	2(6.3)	
351-500	26(11.5)	5(15.6)	
>500	114(50.2)	13(40.6)	

AZT=Zidovudine, TDF=Tenofovir, d4T=Stavudine, 3TC=Lamivudine.
[a] *Pearson's χ^2 test for independence of rows and columns*

Figure 3 below shows the distribution of the number of measurements per patient for the Age≤5 years dataset. The largest number of patients (49) had six measurements, followed by those with five measurements (43) and least of all were four patient with 10 measurements.

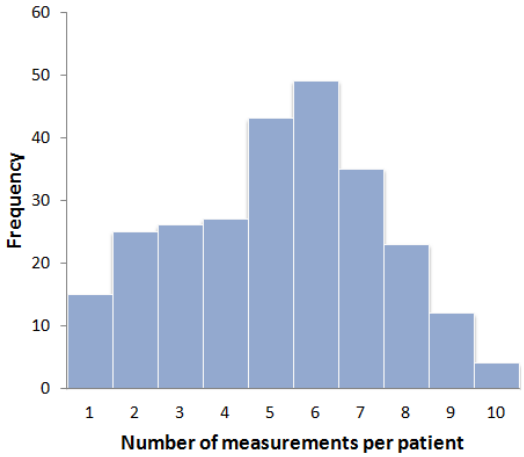

Figure 3: *Frequency of number of measurements per patient for the Age≤5 dataset.*

Tables 9 and 10 below show the changes that happened within the first line regimen drugs used. It can be seen that 6 patients changed from nevirapine to efavirenz based ART (see Table 9) while 7 patients changed from zidovudine to tenofovir use (see Table 10).

Table 9: *Showing changes in NNRTI for patients with Age≤5 years.*

	Current NNRTI			
Original NNRTI	Nevirapine	Efavirenz	Others	Total
Nevirapine	219	6	2	227
Efavirenz	4	28	0	32
Total	223	34	2	259

Pearson chi2(2) = 177.1 ; P-value <0.0001

Table 10: *Showing changes in NRTI backbone for patients with Age≤5 years.*

	Current NRTI backbone				
Original NRTI	d4T+3TC	TDF+3TC	AZT+3TC	Others	Total
d4T+3TC	44	0	2	0	46
TDF+3TC	0	3	0	0	3
AZT+3TC	0	7	201	2	210
Total	44	10	203	2	259

Pearson chi2(6) = 320.5 ; P-value <0.0001

3.1.4 Exploratory plots

Individual Profiles

Figure 4 depicts profiles for all patients split by our chosen 3 datasets. What can be noted from all the panels is that generally there is evidence of between subjects variability as well as within subject variability. The subjects have largely variable CD4 values at the start and also possibly different evolutions over time, this suggests that perhaps linear mixed models with random intercepts and slopes could be plausible starting points.

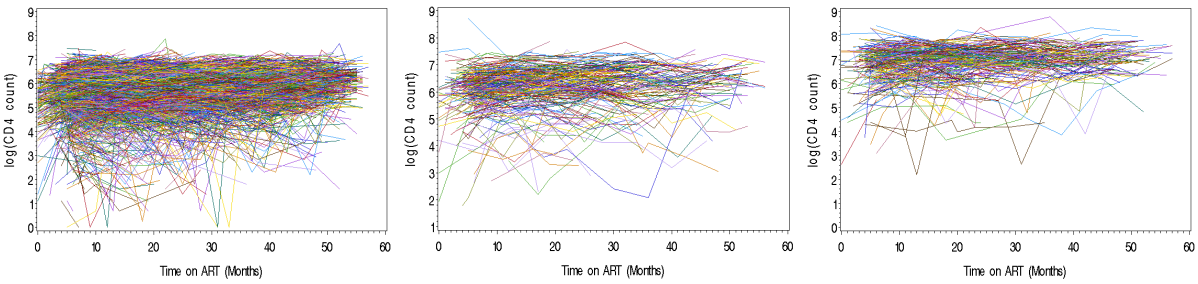

Figure 4: *Individual profiles for patients with Age≥15 years(left panel), those with (6≤Age≤14) years(middle panel) and for those with Age≤5 years (right panel)*

Mean structure

Figure 5 shows the average evolution of CD4 counts over time for the 3 datasets. For the Age≥15 years dataset we observe a sharp linear increase in the mean CD4 counts up to month 4, then a little dip at month 6 and from month 9 up to about month 40 it appears to flatten out, followed by a more gentle increase for the later time points. In this regard, we might have to consider a higher order polynomial for modeling the mean structure. For the 6≤Age≤14 years dataset, we observe a gentle increase in mean CD4 counts for the first 13 months and then it appears to flatten out for the remaining period. For the Age≤5 dataset, there appears to be a gentle increase in mean CD4 counts over time, a little bit more pronounced for the first 12 months.

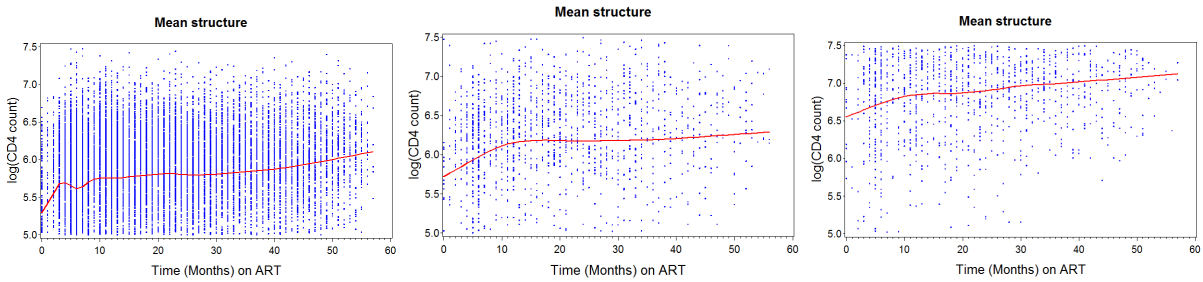

Figure 5: *Smoothed average evolution for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

In terms of gender (see Figure 6), females seem to have higher average CD4 counts than males at all time points for the Age≥15 years, and Age≤5 years datasets. For the 6≤Age≤14 years dataset, males have higher CD4 counts on average for the first 7 months from which point females rise above the males for the rest of the time.

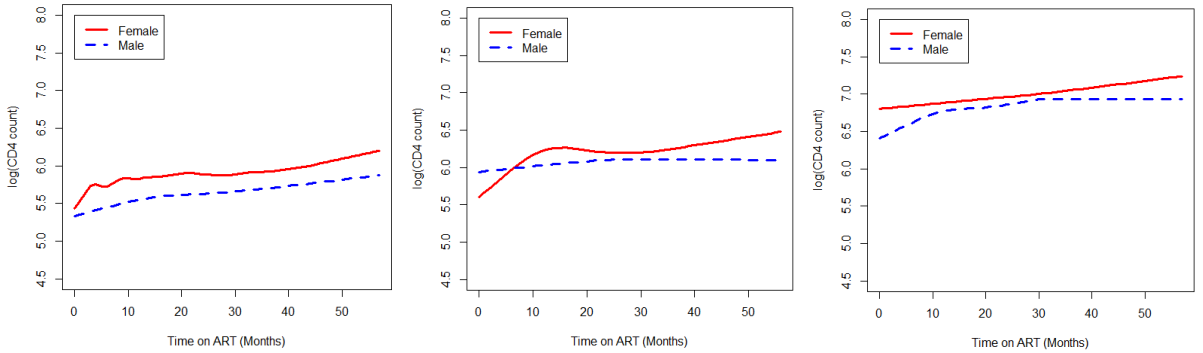

Figure 6: *Smoothed average evolution by gender for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

In terms of age categorization (see Figure 7), for the Age≥15 years dataset, apart from the first 10 months in which the subjects with (35-49)years seemed to evolve differently from the others, at the other time points all age categories seemed to evolve in a similar manner. It is worth noting though, that the patients above 50 years had the lowest CD4 counts on average at most of the time points.

For the 6≤Age≤14 dataset, the patients with (6-9)years had higher CD4 counts on average than those with (10-14)years, at all time points. For the Age≤5 dataset, the patients with (≤2)years had higher CD4 counts on average than those with (3-5)years, at all time points.

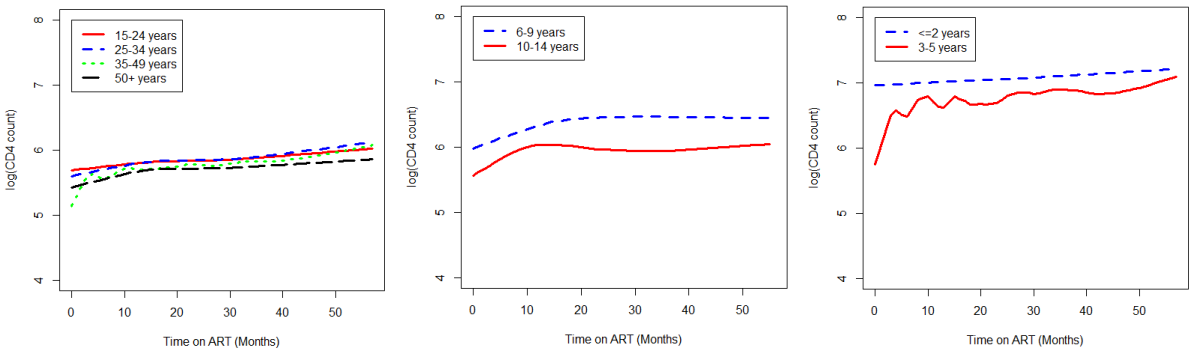

Figure 7: *Smoothed average evolution by Age group for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

In terms of the NRTI backbone (see Figure 8), for the Age≥15 years dataset, patients using AZT seemed to evolve in a similar way to those using TDF. The patients using d4T evolved in a different way, however, since these were few, they were not included in further analysis.

For the 6≤Age≤14 years dataset, patients using d4T start with lower CD4 counts on average but beyond 35 months on ART, they have higher CD4 counts than those using AZT or TDF. For the Age≤5 dataset, patients using AZT seemed to evolve in a similar way to those using d4T, but those using d4T had higher values on average at all times. There were very few patients using TDF, and these were excluded form further analysis.

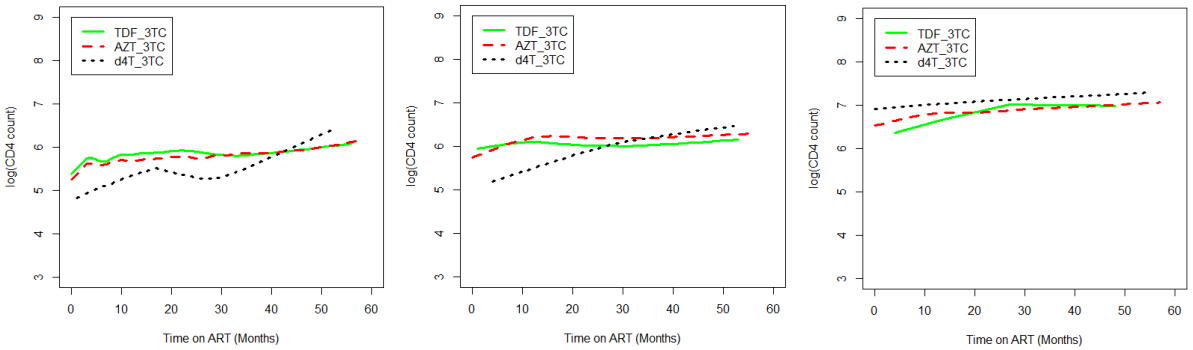

Figure 8: *Smoothed average evolution by treatment backbone for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

In terms of the NNRTI drug (see Figure 9), for the Age≥15 years dataset, for the first 7 months, patients on nevirapine and those on efavirenz seemed to evolve in a similar way, between 7 to 20 months, those on efavirenz seemed to have higher CD4 counts while beyond 20 months those on nevirapine had higher counts.

For the 6≤Age≤14 years dataset, patients on efavirenz seemed to have higher CD4 counts for the first 40 months, beyond which point those on nevirapine had higher counts. For the Age≤5 dataset, patients on nevirapine had higher CD4 counts at all time points.

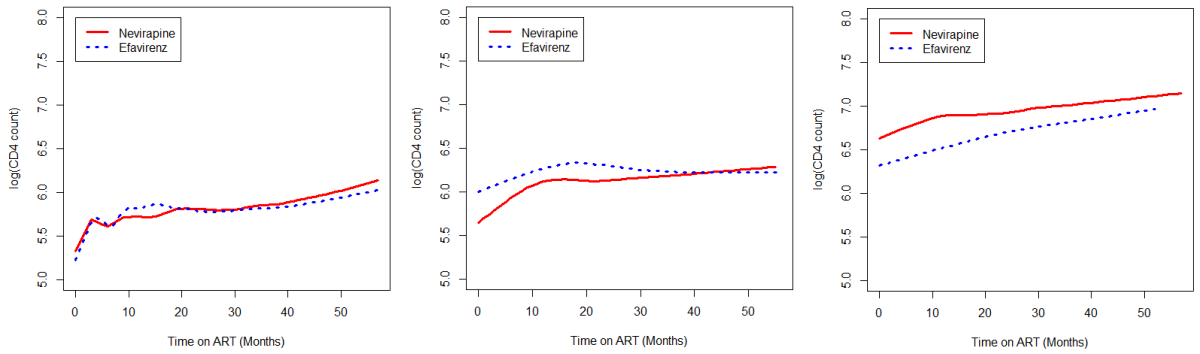

Figure 9: *Smoothed average evolution by NNRTI for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

In terms of Baseline CD4 categorization (see Figure 10), it is evident that the evolution differs depending on the baseline CD4 categorization for all the 3 datasets. It is also seen that, patients who start in higher CD4 categories remain with higher CD4 counts at almost all time points.

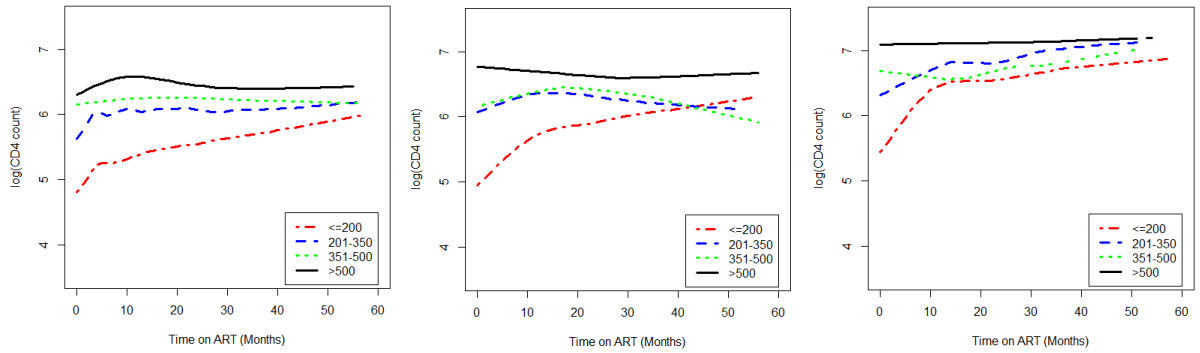

Figure 10: *Smoothed average evolution by Baseline CD4 for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

From Figure 11, we observe that there are minor variations in the average profiles between the patients who changed treatment and those who did not change. Whether these variations are significant will have to be tested using the fitted models.

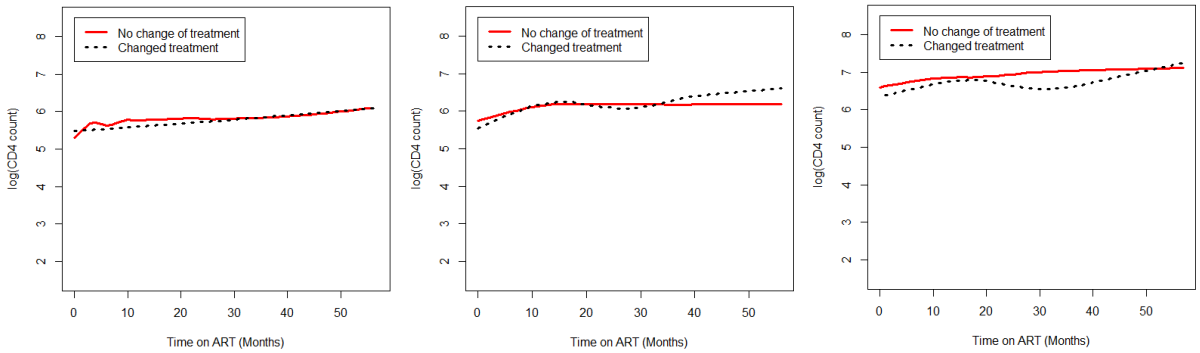

Figure 11: *Smoothed average evolution by change of treatment for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

Variance Structure

Figure 12 shows the observed variance functions for the 3 datasets, which are changing over time, indicating the need for other random effects in addition to the random intercepts.

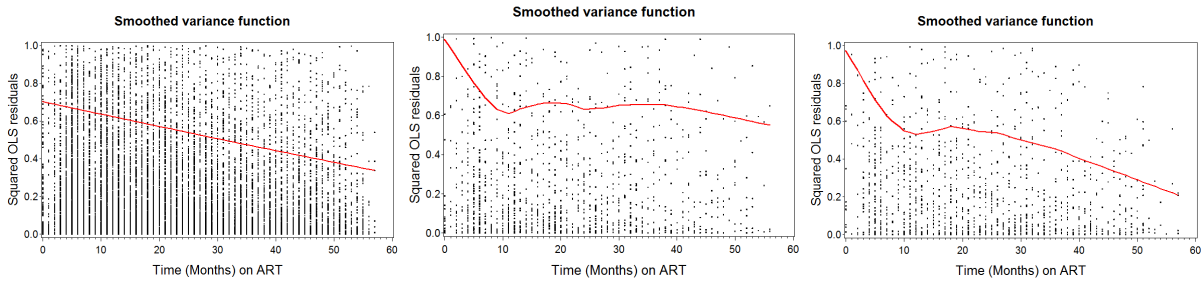

Figure 12: *Smoothed variance functions for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

3.2 Model Building

3.2.1 Selection of Preliminary Mean and Random effects Structures

Following from the observations in the exploratory analysis, good models that best describe the observed average trends and also reflect the observed variance structures, were sought for the 3 datasets. Verbeke and Molenberghs (2000) suggest that in case of highly unbalanced datasets with many covariates, it is necessary to use the most elaborate model one is prepared to consider for the mean structure, making it as extensive to answer the research question. In this case, all main effects and their two-way interactions were deemed sufficient to answer the research question. Several polynomials were then fitted on the same graphs with the observed average trends and the polynomials(functions of time) that seemed to best reflect the observed average trends were chosen. Figure 13 below shows the various fits. The decision on which polynomial to use was however taken after exploration of subject-specific regression models elucidated in the next subsection.

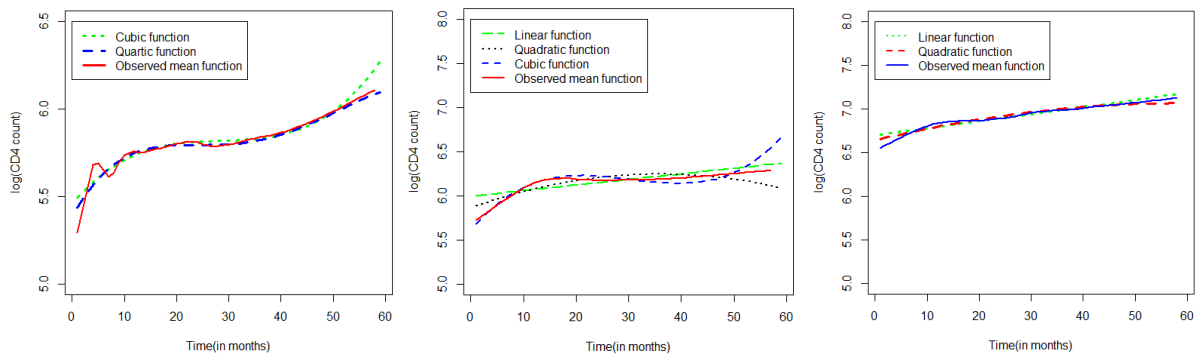

Figure 13: *Observed and fitted mean functions for the Age≥15 years dataset(left panel), for the (6≤Age≤14) years dataset (middle panel) and for the Age≤5 dataset (right panel)*

The choice of random effects structures was based on the observed variance functions as highlighted in the exploratory data part.

3.2.2 Exploration of subject-specific regression models

To further explore subject-specific evolutions, subject-specific regression models were fitted. Polynomial models with increasing powers were fitted to capture the varied trends of the individual evolutions. For the Age≥15 dataset, Table 11 shows that R^2_{meta} for the regression model with the linear effect of time was low at 0.4196, and it radically increased when the time covariate was raised to higher powers. Tests for model extension were conducted and p-values are also shown. Models with higher powers of time proved to be significant improvements from models with lower powers as shown by the significant p-values for the tests. However, with the aim of generating a model which is not only flexible but also parsimonious, the cubic polynomial model was chosen.

Table 11: R^2_{meta} *and tests for model extension for the Age≥15 dataset*

Model	R^2_{meta}	F_{meta}	P-value
Linear	0.4196		
Quadratic	0.5809	0.6539	<0.0001
Cubic	0.6623	0.4122	<0.0001
Quartic	0.7444	0.4045	<0.0001
Quintic	0.7976	0.2847	<0.0001

For the 6≤Age≤14 and the Age≤5 datasets, though the linear regression models had relatively low R^2_{meta} values (see Tables 12 and 13 below), tests for model extension to higher order powers proved insignificant. Therefore, for these datasets, going forward, linear functions of time were chosen to describe the average evolutions.

Table 12: R^2_{meta} *and tests for model extension for the 6≤Age≤14 dataset*

Model	R^2_{meta}	F_{meta}	P-value
Linear	0.4388		
Quadratic	0.6883	1.0776	0.2348
Cubic	0.8288	0.9271	0.7288

Table 13: R^2_{meta} *and tests for model extension for the Age≤5 dataset*

Model	R^2_{meta}	F_{meta}	P-value
Linear	0.3272		
Quadratic	0.4954	0.7479	0.9942
Cubic	0.7190	0.7460	0.9996

3.3 The Linear Mixed Effects Models

3.3.1 Results from the Mixed Effects Model fitted to the Age≥15 dataset

A linear mixed-effects model was fitted, using the repeated measurements of the CD4 counts of each person as a response. They were fitted against a cubic function of time, including all the 2-way interactions of all the covariates. On top of that, random effects for the intercept and linear slope were included to account for the variability between the different subjects. Extending the random effects structure to the quadratic and cubic slopes led to failure of convergence; because of this, robust inference was considered in the final model to circumvent problems of possible misspecification.

To assess the need for serial correlation, models with the same mean structures and random effects but different serial correlation, like Exponential, Power, Gaussian and Simple, were assessed. As shown in Table 14, there was no improvement in the log-likelihood for the models that considered serial correlation. Henceforth, for the proceeding models, the simple correlation structure was taken into account.

Table 14: *Models with several serial correlations and the associated values for the log-likelihood using REML (for the Age≥15 dataset)*

Residual covariance structure	REML log-likelihood
Measurement error	22466
Measurement error + Exponential	22466
Measurement error + Power	22466
Measurement error + Gaussian	22466

The need for reduction of the random effects structure was assessed using a mixture of chi-square distributions as mentioned in the methodology. Hence, the p-value under REML estimation for the comparison of Model 1 (with both random intercepts and slopes) versus Model 2 (with only random intercepts) (see Table 15) can then be calculated as:

$$\text{P-value} = \text{P}(\chi^2_{2:1} > 554.24) = 0.5\ \text{P}(\chi^2_2 > 554.24) + 0.5\ \text{P}(\chi^2_1 > 554.24) < 0.0001$$

From the above result it was concluded that the variance structure should not be simplified by dropping the random linear slope effect from the model.

Table 15: *Random Effects Models with the associated values for the log-likelihood using REML estimation (for the Age≥15 dataset)*

Random effects	REML log-likelihood
Model 1: Intercept, time	22501.27
Model 2: Intercept	22778.39

Finally, the most parsimonious model in terms of the mean structure was assessed using the likelihood ratio test. The model was found to have a significant fit based on the null model likelihood ratio test, implying that it is indeed necessary to model the covariance structure of the data. Summary of test results are shown in Table 16.

Table 16: *Null model likelihood ratio test (for the Age≥15 dataset)*

DF	Chi-square	p-value
3	6587.74	<0.0001

The final model with only significant effects was thus formulated as:

$$\begin{aligned} log(CD4count_{ij}) &= \beta_0 + b_{0i} + \beta_1 Gender_i + \beta_2 cd4cat_i + \beta_3 change_i + \beta_4 year_i \\ &+ \beta_5 nnrti_i + (\beta_6 + b_{1i}) time_{ij} + \beta_7 time^2_{ij} + \beta_8 time^3_{ij} \\ &+ \beta_9 Gender_i * time_{ij} + \beta_{10} cd4cat_i * time_{ij} + \beta_{11} nnrti_i * time_{ij} \\ &+ \beta_{12} nnrti_i * time^2_{ij} + \beta_{13} Gender_i * cd4cat_i + \beta_{14} Gender_i * nnrti_i \end{aligned} \quad (1)$$

Where: $log(CD4count_{ij})$ is the natural log of the CD4 count of the ith patient at the jth time, $time_{ij}$ is the time (in months) at which the jth CD4 measurement is taken

for the ith patient and $\beta_0, \beta_1, \ldots, \beta_{14}$ are regression coefficients while b_{0i} and b_{1i} denote subject specific deviations from the common intercept and linear slope. The latter are assumed to follow a multivariate normal distribution (see Molenberghs and Verbeke (2005)).

Table 17 below shows the covariance parameter estimates from the final model. It shows that the highest variability came from the random intercepts. It also shows that the variance of the random intercepts was higher than that of the random slopes, pointing to higher between patient variability than the within patient variability. The covariance of random effects is negative, implying that patients with high baseline CD4 counts tend to have smaller slopes than patients with low CD4 counts at baseline.

Table 17: *Covariance parameter estimates (for the Age≥15 dataset)*

Parameter	Estimate
$\text{Var}(b_{0i})$	0.3511
$\text{Cov}(b_{0i}, b_{1i})$	-0.00201
$\text{Var}(b_{1i})$	0.00013
Measurement error	
$\text{Var}(\varepsilon_i)$	0.1580

Results from the final model (see Table 18) show that there was a significant cubic evolution of the CD4 counts. The effect of gender on the evolution depends on the patient's baseline CD4 category (see Figure 14), also on the NNRTI drug that the patient uses (see Figure 15).

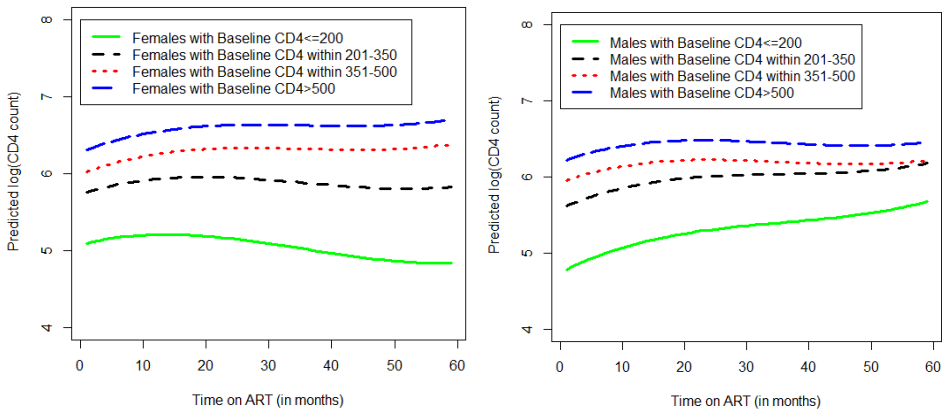

Figure 14: *Predicted CD4 count levels showing interaction of gender and baseline CD4 category, for the Age≥15 dataset*

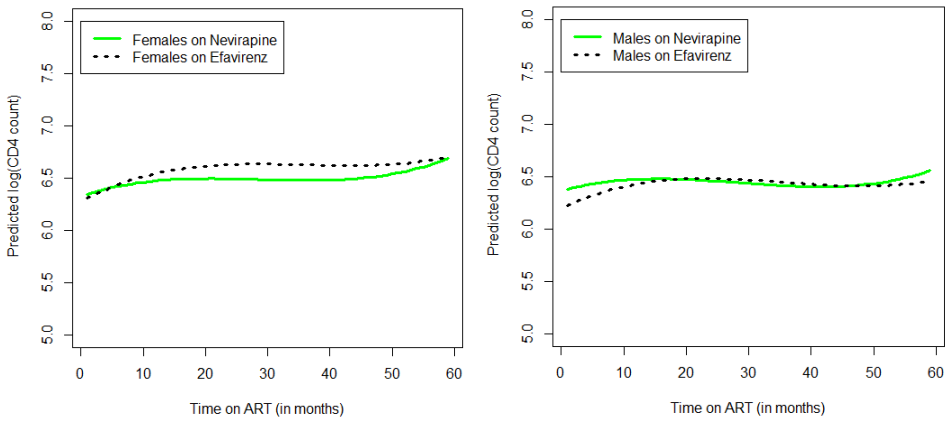

Figure 15: *Predicted CD4 count levels showing interaction of gender and NNRTI drug, for the Age≥15 dataset*

Table 18 also shows that patients who started ART between the years 2010 to 2012 had higher intercepts (CD4 starting values) than those who started ART in 2009. It also shows that patients who changed treatment drugs had lower intercepts (CD4 starting values) than those who didn't change.

Figure 21 in the Appendix shows the observed and predicted profiles plotted against each other for the Age≥15 dataset. It shows that our model fits relatively well to the observed data.

Table 18: *Parameter estimates and Robust Standard Errors (S.E.Robust) from the final model for the Age≥15 dataset.*

Effect	gender	cd4cat	change	Year	nnrti	Estimate (S.E.Robust)	P-value
Intercept						6.2460 (0.1515)	<0.0001
gender	F					0.0635 (0.1550)	0.6823
gender	M					0 (.)	.
cd4cat		≤200				-1.4551 (0.1501)	<0.0001
cd4cat		201-350				-0.6200 (0.1473)	<0.0001
cd4cat		351-500				-0.2953 (0.1504)	0.0497
cd4cat		>500				0 (.)	.
change			No			0.0957 (0.0604)	0.0172
change			Yes			0 (.)	.
Year				2009		-0.0835 (0.0283)	0.0032
Year				2010-2012		0 (.)	.
nnrti					NEV	0.1570 (0.0523)	0.0027
nnrti					EFV	0 (.)	.
time						0.0279 (0.0038)	<0.0001
time2						-0.0009 (0.0001)	<0.0001
time3						8.75E-06 (1.51E-06)	<0.0001
time*gender	F					0.0029 (0.0010)	0.0029
time*gender	M					0 (.)	.
time*cd4cat		≤200				0.0106 (0.0020)	<0.0001
time*cd4cat		201-350				0.0054 (0.0020)	0.0064
time*cd4cat		351-500				0.0005 (0.0022)	0.836
time*cd4cat		>500				0 (.)	.
time*nnrti					NEV	-0.0118 (0.0028)	<0.0001
time*nnrti					EFV	0 (.)	.
time2*nnrti					NEV	0.0002 (0.0001)	0.0007
time2*nnrti					EFV	0 (.)	.
gender*cd4cat	F	≤200				0.2513 (0.1588)	0.1137
gender*cd4cat	F	201-350				0.0718 (0.1554)	0.6442
gender*cd4cat	F	351-500				0.0138 (0.1612)	0.9320
gender*cd4cat	F	>500				0 (.)	.
gender*cd4cat	M	≤200				0 (.)	.
gender*cd4cat	M	201-350				0 (.)	.
gender*cd4cat	M	351-500				0 (.)	.
gender*cd4cat	M	>500				0 (.)	.
gender*nnrti	F				NEV	-0.1229 (0.0539)	0.0227
gender*nnrti	F				EFV	0 (.)	.
gender*nnrti	M				NEV	0 (.)	.
gender*nnrti	M				EFV	0 (.)	.

F=Female, M=Male; NEV=Nevirapine, EFV=Efavirenz

Empirical Bayes estimates for random effects were also calculated in order to check whether there were outliers or not. The scatter plot of random intercepts and slopes for the Age≥15 dataset (see Figure 16), showed that there were some potential outliers which may need to be investigated further.

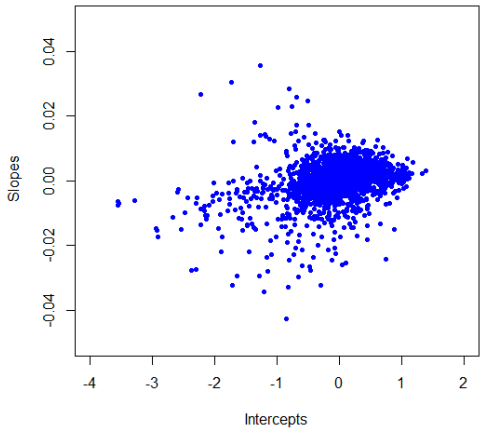

Figure 16: *Scatter plot of random intercepts and slopes for the Age≥15 years dataset.*

3.3.2 Results from the Mixed Effects Model fitted to the 6≤Age≤14 dataset

A linear mixed-effects model was fitted, using the repeated measurements of the CD4 counts of each person as a response. They were fitted against a linear function of time, including all the 2-way interactions of all the covariates. On top of that, random effects for the intercept and linear slope were included to account for the variability between the different subjects.

To assess the need for serial correlation, models with the same mean structures and random effects but different serial correlation, like Exponential, Power, Gaussian and Simple, were assessed. There was no improvement in the log-likelihood for the models that considered serial correlation. Henceforth, the simple correlation structure was taken into account.

The need for reduction of the random effects structure was assessed using a mixture of chi-square distributions as mentioned in the methodology. Hence, the p-value under REML estimation for the comparison of Model 1 (with both random intercepts and slopes) versus Model 2 (with only random intercepts) (see Table 19) can then be calculated as:

$$\text{P-value} = \text{P}(\chi^2_{2:1} > 187.2) = 0.5\ \text{P}(\chi^2_2 > 187.2) + 0.5\ \text{P}(\chi^2_1 > 187.2) < 0.0001$$

From the above result it was concluded that the variance structure should not be simplified by dropping the random linear slope effect from the model.

Table 19: *Random Effects Models with the associated values for the log-likelihood using REML estimation (for the 6≤Age≤14 dataset)*

Random effects	REML log-likelihood
Model 1: Intercept, time	2717.1
Model 2: Intercept	2810.7

Finally, the most parsimonious model in terms of the mean structure was assessed using the likelihood ratio test. The model was found to have a significant fit based on the null model likelihood ratio test, implying that it is indeed necessary to model the covariance structure of the data. Summary of test results are shown in Table 20.

Table 20: *Null model likelihood ratio test (for the 6≤Age≤14 dataset)*

DF	Chi-square	p-value
3	550.06	<0.0001

The final model with only significant effects was thus formulated as:

$$\begin{aligned} log(CD4count_{ij}) &= \beta_0 + b_{0i} + \beta_1 Gender_i + \beta_2 agecat_i + \beta_3 cd4cat_i + \beta_4 year_i \\ &+ \beta_5 nrti_i + (\beta_6 + b_{1i}) time_{ij} + \beta_7 cd4cat_i * time_{ij} + \beta_8 Gender_i * nrti_i \end{aligned} \quad (2)$$

Where: $log(CD4count_{ij})$ is the natural log of the CD4 count of the ith patient at the jth time, $time_{ij}$ is the time on ART (in months) at which the jth CD4 measurement is taken for the ith patient; $\beta_0, \beta_1, \ldots, \beta_8$ are regression coefficients while b_{0i} and b_{1i} denote subject specific deviations from the common intercept and linear slope respectively. The latter are assumed to follow a multivariate normal distribution.

Table 21 below shows the covariance parameter estimates from the final model. It shows that the highest variability came from the random intercepts. It also shows that the variance of the random intercepts was higher than that of the random slopes, pointing to higher between patient variability than the within patient variability. The covariance of random effects is negative, implying that patients with high baseline CD4 counts tend to have smaller slopes than patients with low CD4 counts at baseline.

Table 21: *Covariance parameter estimates (for the 6≤Age≤14 dataset)*

Parameter	Estimate
Var(b_{0i})	0.3791
Cov(b_{0i}, b_{1i})	-0.00642
Var(b_{1i})	0.00036
Measurement error	
Var(ε_i)	0.2095

Results from the final model (see Table 22) show that there was a significant linear evolution of the CD4 counts. The baseline CD4 category had an effect on the evolution (see Figure 17). It is seen that the patients who had baseline CD4 counts≤200 had steeper slopes than those who started in higher categories.

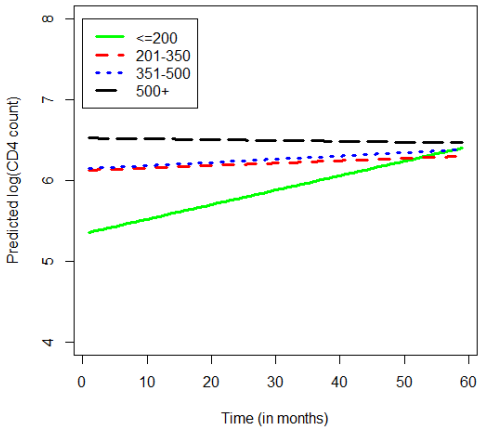

Figure 17: *Predicted CD4 count levels by baseline CD4 category, for the 6≤Age≤14 dataset*

From Table 22, we observe that the effect of gender on the CD4 level depends on the current NRTI backbone that the patient uses. It also shows that patients who started ART between the years 2010 to 2012 had higher intercepts (starting CD4 counts) than those who started ART in 2009. Further still, it is seen that patients who were aged 6 to 9 years at baseline had higher intercepts (starting CD4 counts) than those who were aged 10 to 14 years.

Figure 22 in the Appendix shows the observed and predicted profiles plotted against each other for the (6≤Age≤14) years dataset. It shows that the chosen model fits relatively well to the observed data.

Table 22: *Parameter estimates and Robust Standard Errors (S.E.Robust) from the final model for the (6≤Age≤14) years dataset.*

Effect	gender	agecat	cd4cat	Year	nrti	Estimate (S.E.Robust)	P-value
Intercept						6.524 (0.121)	<0.0001
gender	F					0.038 (0.069)	0.5869
gender	M					0 (.)	.
agecat		6-9				0.374 (0.068)	<0.0001
agecat		10-14				0 (.)	.
cd4cat			≤200			-1.167 (0.139)	<0.0001
cd4cat			201-350			-0.397 (0.112)	0.0004
cd4cat			351-500			-0.377 (0.146)	0.0098
cd4cat			>500			0 (.)	.
Year				2009		-0.210 (0.087)	0.0159
Year				2010-2012		0 (.)	.
nrti					d4T+3TC	-0.837 (0.349)	0.0168
nrti					TDF+3TC	0.308 (0.254)	0.2247
nrti					AZT+3TC	0 (.)	.
time						-0.001 (0.003)	0.7175
time*cd4cat			≤200			0.019 (0.004)	<0.0001
time*cd4cat			201-350			0.004 (0.003)	0.2015
time*cd4cat			351-500			0.005 (0.006)	0.4185
time*cd4cat			>500			0 (.)	.
gender*nrti	F				d4T+3TC	1.091 (0.380)	0.0042
gender*nrti	F				TDF+3TC	-0.292 (0.267)	0.2752
gender*nrti	F				AZT+3TC	0 (.)	.
gender*nrti	M				d4T+3TC	0 (.)	.
gender*nrti	M				TDF+3TC	0 (.)	.
gender*nrti	M				AZT+3TC	0 (.)	.

F=Female, M=Male; AZT=Zidovudine, TDF=Tenofovir, d4T=Stavudine, 3TC=Lamivudine.

Empirical Bayes estimates for random effects were also calculated in order to check whether there were some outlying profiles or not. The scatter plot of random intercepts and slopes for the 6≤Age≤14 dataset (see Figure 18), showed that there were some potential outliers which may need to be investigated further.

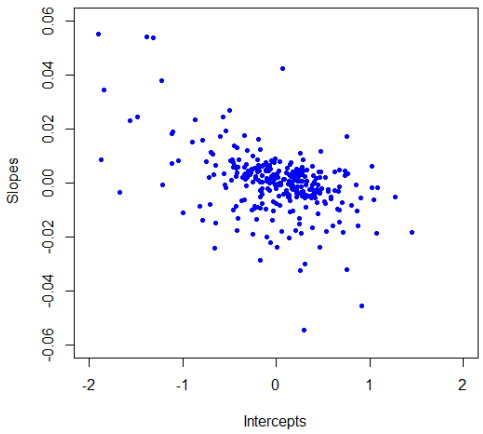

Figure 18: *Scatter plot of random intercepts and slopes for the (6≤ Age≤14) years dataset.*

3.3.3 Results from the Mixed Effects Model fitted to the Age≤5 dataset

A linear mixed-effects model was fitted, using the repeated measurements of the CD4 counts of each person as a response. They were fitted against a linear function of time, including all the 2-way interactions of all the covariates. Again, random effects for the intercept and linear slope were included to account for the variability between the different subjects.

To assess the need for serial correlation, models with the same mean structures and random effects but different serial correlation, like Exponential, Power, Gaussian and Simple, were assessed. There was no improvement in the log-likelihood for the models that considered serial correlation. Henceforth, the simple correlation structure was taken into account.

The need for reduction of the random effects structure was assessed using a mixture of chi-square distributions as mentioned in the methodology. Hence, the p-value under REML estimation for the comparison of Model 1 (with both random intercepts and slopes) versus Model 2 (with only random intercepts) (see Table 23) can then be calculated as:

$$\text{P-value} = \text{P}(\chi^2_{2:1} > 27.2) = 0.5\ \text{P}(\chi^2_2 > 27.2) + 0.5\ \text{P}(\chi^2_1 > 27.2) < 0.0001$$

From the above result it was concluded that the variance structure should not be simplified by dropping the random linear slope effect from the model.

Table 23: *Random Effects Models with the associated values for the log-likelihood using REML estimation (for the Age≤5 dataset)*

Random effects	REML log-likelihood
Model 1: Intercept, time	2485.1
Model 2: Intercept	2498.7

Again, the most parsimonious model in terms of the mean structure was assessed using the likelihood ratio test. The model was found to have a significant fit based on the likelihood ratio test. Summary of test results are shown in Table 24.

Table 24: *Null model likelihood ratio test (for the Age≤5 dataset)*

DF	Chi-square	p-value
3	353.37	<0.0001

The final model with only significant effects was thus formulated as:

$$\begin{aligned} log(CD4count_{ij}) &= \beta_0 + b_{0i} + \beta_1 agecat_i + \beta_2 cd4cat_i + \beta_3 nrti_i \\ &+ (\beta_4 + b_{1i}) time_{ij} + \beta_5 cd4cat_i * time_{ij} \end{aligned} \quad (3)$$

Where: $log(CD4count_{ij})$ is the natural log of the CD4 count of the ith patient at the jth time, $time_{ij}$ is the time on ART (in months) at which the jth CD4 measurement is taken for the ith patient; $\beta_0, \beta_1, \ldots, \beta_5$ are regression coefficients while b_{0i} and b_{1i} denote subject specific deviations from the common intercept and linear slope respectively. The latter are assumed to follow a multivariate normal distribution.

Table 25 below shows the covariance parameter estimates from the final model. It shows that the highest variability came from the random intercepts. It also shows that the variance of the random intercepts was higher than that of the random slopes, pointing to higher between patient variability than the within patient variability. The covariance of random effects is negative, implying that patients with high baseline CD4 counts tend to have smaller slopes than patients with low CD4 counts at baseline.

Table 25: *Covariance parameter estimates (for the Age≤5 dataset)*

Parameter	Estimate
Var(b_{0i})	0.2818
Cov(b_{0i}, b_{1i})	-0.00297
Var(b_{1i})	0.00009
Measurement error	
Var(ε_i)	0.2471

Results from the final model (see Table 26) show that there was a significant linear evolution of the CD4 counts. The baseline CD4 category had an effect on the evolution (see Figure 19), evident in lower categories having steeper slopes than higher categories.

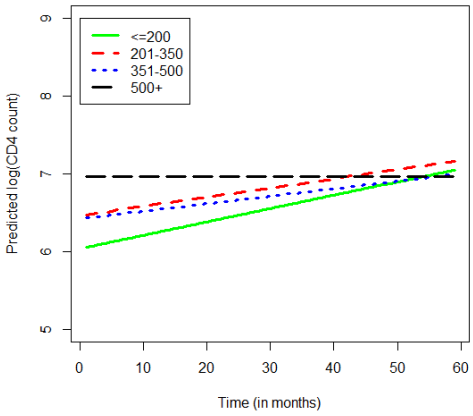

Figure 19: *Predicted CD4 count levels by baseline CD4 category, for the Age≤5 dataset*

Table 26 shows that patients who were aged ≤2 years had higher intercepts (starting CD4 counts) than those aged 3 to 5 years. It is also evident that patients who had d4T

in their current NRTI backbone had higher intercepts (starting CD4 counts) than those who had AZT in their current NRTI backbone.

Figure 23 in the Appendix shows the observed and predicted profiles plotted against each other for the Age≤5 years dataset. It shows that the chosen model fits relatively well to the observed data.

Table 26: *Parameter estimates and Robust Standard Errors (S.E.Robust) from the final model for the Age≤5 years dataset.*

Effect	agecat	cd4cat	NRTI	Estimate (S.E.Robust)	P-value
Intercept				6.9673 (0.0565)	<0.0001
agecat	≤2			0.1704 (0.0667)	0.0108
agecat	3-5			0 (.)	.
cd4cat		≤200		-0.9124 (0.1353)	<0.0001
cd4cat		201-350		-0.4942 (0.1388)	0.0004
cd4cat		351-500		-0.5365 (0.1156)	<0.0001
cd4cat		>500		0 (.)	.
NRTI			d4T+3TC	0.1778 (0.0720)	0.0138
NRTI			AZT+3TC	0 (.)	.
time				-0.0001 (0.0015)	0.9410
time*cd4cat		≤200		0.0173 (0.0033)	<0.0001
time*cd4cat		201-350		0.0119 (0.0047)	0.0101
time*cd4cat		351-500		0.0098 (0.0042)	0.0185
time*cd4cat		>500		0 (.)	.

AZT=Zidovudine, d4T=Stavudine, 3TC=Lamivudine.

Empirical Bayes estimates for random effects were also calculated in order to check whether there were some outlying profiles or not. The scatter plot of random intercepts and slopes for the Age≤5 dataset (see Figure 20), showed that there were some potential outliers which may need further investigation.

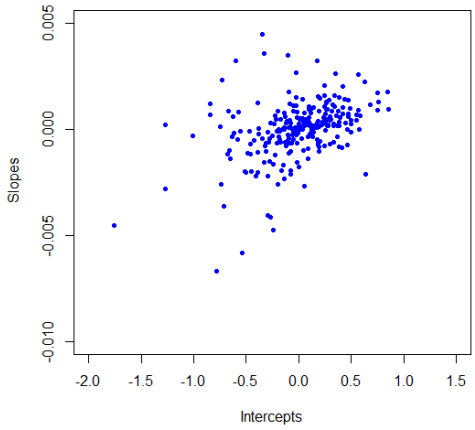

Figure 20: *Scatter plot of random intercepts and slopes for the Age≤5 years dataset.*

4 Discussion and Concluding Remarks

This project was aimed at describing the evolution of CD4 cell counts for patients on ART at Mildmay Uganda. It was also aimed at determining whether the evolution depends on selected patient characteristics. In view of this objective, exploratory data analysis was conducted to visualize the key hypotheses and to provide some initial basis for the subsequent statistical modeling.

For the Age$\geq$15 years dataset, results from the final model showed that there was a significant cubic evolution of the CD4 counts. This result differs from that of Adams and Luguterah (2013) who described the evolution as a logarithmic function of time. It also differs from the result of Reda *et al.* (2013) who used a linear function of time to describe the evolution.

For both the 6$\leq$Age$\leq$14 and the Age$\leq$5 years datasets, linear functions of time were found to be appropriate descriptions of the evolution of CD4 cell counts. This result differs from that of Picat *et al.* (2013) who used non-linear models to describe the evolution of CD4 cell counts in children.

In contrast to the results of Adams and Luguterah (2013) that report male patients responding to treatment better than females, our results for the Age$\geq$15 years dataset showed that the effect of gender on the evolution depends on the patient's baseline CD4 category, also on the NNRTI drug that the patient uses.

This study was in agreement with the studies of Reda *et al.* (2013) and that of Adams and Luguterah (2013), in showing the benefit of early treatment. A higher baseline CD4 cell count would result in a better rate of recovery of patients on ART. This is thus an additional voice to advocacy for early starting of treatment as opposed to waiting for dropping of CD4 counts to various thresholds.

For the Age$\geq$15 years dataset, patients who started ART between the years 2010 to 2012 had higher CD4 counts over time than those who started ART in 2009. This could be because of the change in treatment guidelines over time that have seen a shift to higher thresholds for starting ART unlike in the earlier years. For instance, the latest WHO recommendations encourage initiation of ART in adults living with HIV when their CD4 cell count falls to 500 cells/mm^3 or less, unlike the 2010 recommendations which had a threshold of 350 CD4 cells/mm^3 (World Health Organization, 2014). Prior to 2010, there was a threshold of 250 CD4 cells/mm^3.

Results for the Age$\geq$15 years dataset also show that patients who changed treatment drugs had lower CD4 counts over time than those who didn't change. This phenomenon is as a result of treatment failure, since changes are made for patients who perform poorly on certain drugs.

This study showed (for the Age$\geq$15 years dataset) that the age of patients did not have any significant effect on the evolution in contrast to the studies of Adams and Luguterah (2013) and that of Reda *et al.* (2013). This could be explained by the relatively young nature of the patients considered with median age (IQR) = 34 (28-40). Probably, the

behaviors of these people do not differ as much.

For both the 6≤Age≤14 and the Age≤5 years datasets, the baseline CD4 category had an effect on the evolution. Results from these 2 datasets also showed the benefit of early treatment i.e. the patients who started ART at higher baseline CD4 counts evolved higher than those who started at lower CD4 counts. This result was similar to that of Picat *et al.* (2013).

For the 6≤Age≤14 years dataset, the effect of gender on the CD4 level depended on the current NRTI backbone that the patient used. For instance, in as much as the females who were using d4T maintained higher values than females who used AZT or TDF, the males using d4T maintained lower values over time than their colleagues who used AZT or TDF.

For both the 6≤Age≤14 and the Age≤5 years datasets, the patients who started ART at younger ages maintained higher CD4 counts over time than those who started older. This result was similar to that of Picat *et al.* (2013) and further highlights the need for starting ART as early as possible even for children.

For the Age≤5 years dataset, it was observed that the patients who had d4T in their NRTI backbone maintained higher CD4 counts over time than those who used AZT. Despite the fact that use of d4T has been stopped in Uganda (see e.g. Ario, 2014), patients (with Age≤5 years) using this drug had higher CD4 counts over time than those using AZT in this study.

This study also explored Empirical Bayes estimates which are vital in identifying subjects with outlying profiles. For each of the 3 datasets, scatter plots of the random intercepts and slopes were constructed from which potential outliers can be identified and investigated further.

In conclusion, this study shows that evolution of CD4 cell counts over time could be sufficiently described by a cubic polynomial for the patients with Age≥15, whilst linear functions of time could be used to describe the evolutions for the patients with 6≤Age≤14 and those with Age≤5. The effects of several factors on the evolution were discussed and the influence of the baseline CD4 stood out in all three analyses.

It is recommended that further studies of this nature include other important covariates that were not included in this study. Such covariates include: viral load results, treatment failure, opportunistic infections and many others.

Another way in which the objectives of this research could be answered (especially for the Age≥15 dataset) is by use of linear spline models. Linear spline models provide a very useful and flexible way to accommodate many of the non-linear trends that can not be approximated by simple polynomials in time (Fitzmaurice *et al.*, 2004). Such a method has been used by Wools-Kaloustian *et al.* (2006) to determine the clinical and immunological outcomes of a cohort of HIV infected patients receiving ART.

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6 Appendix

Table 27: *Type 3 Tests of Fixed Effects for the final model for the Age≥15 years dataset.*

Effect	Num DF	Den DF	F-value	P-value
gndr	1	3303	1.50	0.2214
cd4cat	3	3401	286.15	<.0001
change	1	3735	5.68	0.0172
yr2	1	3019	10.84	0.0010
nnrti	1	5254	8.62	0.0033
time	1	11E3	106.66	<.0001
time2	1	11E3	53.07	<.0001
time3	1	11E3	39.02	<.0001
time*gndr	1	1449	9.46	0.0021
time*cd4cat	3	1342	23.04	<.0001
time*nnrti	1	11E3	26.42	<.0001
time2*nnrti	1	11E3	17.40	<.0001
gndr*cd4cat	3	2985	5.09	0.0016
gndr*nnrti	1	3097	5.57	0.0183

Table 28: *Type 3 Tests of Fixed Effects for the final model for the 6≤Age≤14 years dataset.*

Effect	Num DF	Den DF	F-value	P-value
gndr	1	873	3.96	0.0470
agecat	1	873	30.44	<.0001
cd4cat	3	873	26.72	<.0001
yr2	1	873	5.83	0.0159
nrti	2	873	1.88	0.1539
time	1	283	10.83	0.0011
time*cd4cat	3	873	7.00	0.0001
gndr*nrti	2	873	4.81	0.0083

Table 29: *Type 3 Tests of Fixed Effects for the final model for the Age≤5 years dataset.*

Effect	Num DF	Den DF	F-value	P-value
agecat	1	250	6.43	0.0118
cd4cat	3	259	24.69	<.0001
nrti	1	231	4.45	0.0359
time	1	173	42.61	<.0001
time*cd4cat	3	171	10.66	<.0001

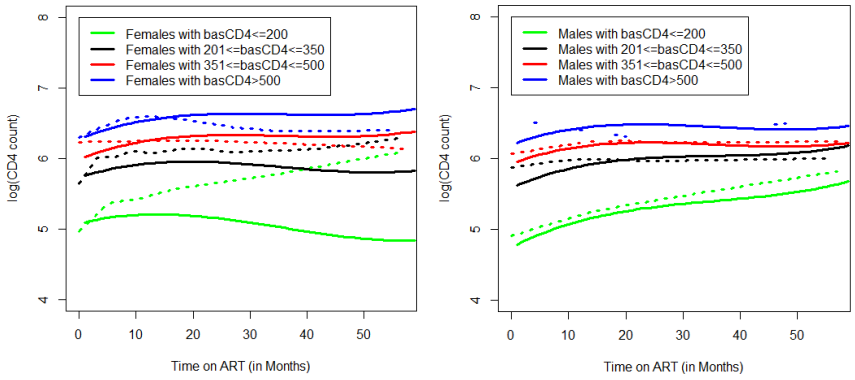

Figure 21: *Observed (dotted lines) and Predicted (connected lines) profiles for females (left pannel) and males (right pannel) disaggregated by baseline CD4 categories, for the Age≥15 dataset*

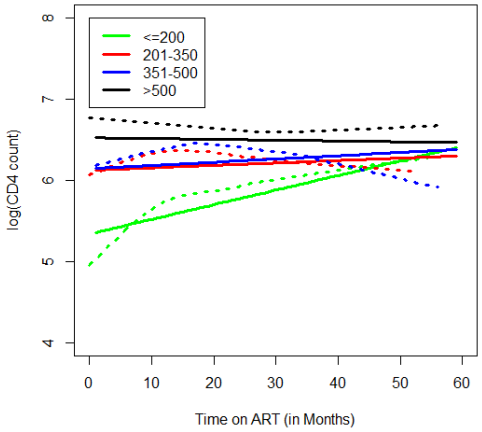

Figure 22: *Observed (dotted lines) and Predicted (connected lines) profiles disaggregated by baseline CD4 categories, for the (6≤Age≤14) dataset*

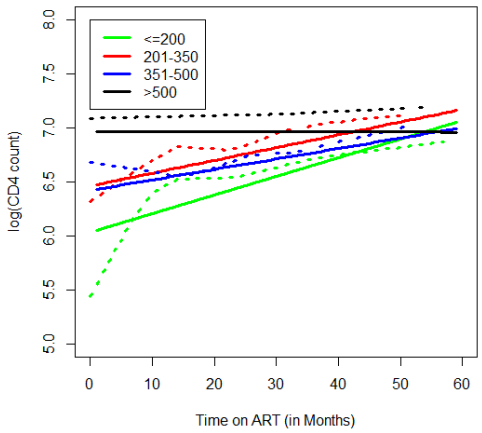

Figure 23: *Observed (dotted lines) and Predicted (connected lines) profiles disaggregated by baseline CD4 categories, for the Age≤5 dataset*

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Evolution of CD4 cell counts over time for HIV/AIDS patients on Antiretroviral Therapy (ART) in Mildmay Uganda

Richting: **Master of Statistics-Biostatistics**
Jaar: **2014**

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