The effects of longitudinal HIV viral load exposure on Immune outcomes, Mortality, and Opportunistic infections in people on ART in sub-Saharan Africa

By

Joseph Bukulu Sempa, B. Stat, MSc CEB

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Supervisor: Prof. Martin Nieuwoudt

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Sempa Joseph Bukulu

Date: **December 2017**

Abstract

Introduction: Longitudinal viral load monitoring is used as a cross-sectional marker for treatment failure in HIV infected people receiving antiretroviral therapy. Cumulative viral load, as quantified by area under the viral load curve during combination antiretroviral therapy, has been correlated with treatment outcomes in studies outside, but not within, sub-Saharan Africa. We investigate the effects of exposure to longitudinal viral load on, the incidence of opportunistic infections, mortality and immune recovery in local, previously combination antiretroviral therapy naïve, cohorts. Further, we systematically review statistically derived immune response models and use this to define *priors* for Bayesian models for application on a previously undescribed treatment cohort.

Methods: We analyze data from the Infectious Diseases Institute (IDI) cohort, Kampala-Uganda, and the Antiretroviral Clinic at Tshwane District Hospital in Gauteng-South Africa. For the systematic review, we use 'Preferred Reporting Items for Systematic Review and Meta-Analyses' guidelines. We also compare cumulative viral load as numerically estimated using two methods: area under the viral load curve, which is then log-transformed, named, 'untransformed cumulative viral load'; and area under the log-transformed viral load curve, above the kit-based detection limit of 400 copies/mL, named, 'transformed cumulative viral load'. We use Cox Proportional Hazards and Bayesian Generalized Mixed Effects to define treatment outcome models.

Results: In the IDI cohort most recent viral load, not cumulative viral load, is associated with a 1.34-fold (95% confidence interval: 1.12, 1.61) increase in the risk of opportunistic infections. Transformed, not untransformed, cumulative viral load is associated with mortality and immune response. Each $\log_{10}$ copy-yr/mL increase corresponds to a 1.63-fold (95% confidence interval: 1.02, 2.60) increase in risk of mortality. Systematic review of immune response statistical models also reveals many differences in the number and type of variables

adjusted-for, variable transformations and scales and scant details regarding the modelling methods employed. In the Tshwane cohort, using Bayesian methods, for the slope of longitudinal CD4 counts, each $\log_{10}$ copy-yr/mL increase cumulative viral load corresponds to a mean annual CD4 count decrease of -19.5 cells/μL (95% credible interval: -28.34, -10.72). Further, in the asymptote model, each $\log_{10}$ copy-yr/mL increase reduced the odds of having a CD4 count ≥500 cells/μL to 0.42 (95% credible interval: 0.242, 0.724). Modelling inherently variable absolute CD4 count using a Student's *t*-distribution produced better fits than assuming a Gaussian normal distribution.

Discussion: Transformed cumulative viral load is associated with both mortality and long-term immune response, while most recent viral load is associated with incidence of opportunistic infections. This thesis emphasizes the need for the review of existing literature prior to any statistical analyses, so that more comparable and robust statistical models than have been available to date will be constructed. In particular, comparing immunological outcomes (CD4 counts), statistical models for sub-Saharan African cohorts would benefit from the application of more uniform modelling techniques. Adjusting for transformed cumulative viral load and the use of appropriate distributional assumptions, improves the modelling of immune response to antiretroviral therapy. Future statistical immune response models would benefit from the use of Bayesian methods owing to their flexibility in the selection of prior distributions and hierarchical model designs.

Opsomming

Inleiding: Die monitering van longitudinale viruslading word gebruik as 'n biomerker vir behandelingsfaling in HIV-geïnfekteerde mense wat kombinasie antiretrovirale terapie ontvang. Kumulatiewe viruslading, gekwantifiseer as die area onder die viruslading kurwe tydens terapie, is gekorreleer met behandelingsuitkomste in studies elders, maar nie in Afrika suid van die Sahara. In hierdie studie word die effek van longitudinale viruslading, insluitend die voorkoms van opportunistiese infeksies, sterfte en die herstel van die immuunstelsel in plaaslik behandelde pasiënte ondersoek. Verder, word ’n sistematiese oorsig van statistiese immuunrespons modelle uitgevoer. Hierdie resultate word gebruik om Bayesiaanse modelle te definieer, vir toepassing op 'n voorheen onbeskrewe pasiënt groep.

Metodes: Kohorte van die Infectious Diseases Institute (IDI), in Kampala, Uganda, en van die Antiretrovirale Kliniek by die Tshwane Distrikshospitaal in Gauteng, Suid-Afrika is geanaliseer. Vir die sistematiese oorsig gebruik ons die sogenaamde ‘Preferred Reporting Items for Systematic Review and Meta-Analyses’ riglyne. Ons vergelyk ook kumulatiewe virusladings beraam met twee numeriese metodes: 1) die log-getransformeerde area onder die viruslading kurwe, genaamd 'ongetransformeerde kumulatiewe viruslading'; en 2) die area onder die log-getransformeerde viruslading kurwe, bo die toets-spesifieke limiet van deteksie van 400 kopieë/ml, genaamd die 'getransformeerde kumulatiewe viruslading'. Ons gebruik ‘Cox Proportional Hazards’ en ‘Bayesian Generalized Mixed Effects’ om behandelings-uitkoms modelle te definieer.

Resultate: Vir die IDI kohort is die mees onlangse viruslading, i.e. die nie-kumulatiewe viruslading, geassosieer met 'n 1.34-voud toename (95% vertrouensinterval: 1.12, 1.61) in die risiko van opportunistiese infeksies. Die getransformeerde kumulatiewe viruslading is geassosieer met sterfte en immuunrespons. Elke log10 kopie/jr/ml verhoging stem ooreen met 'n 1.63-voud toename (95% vertrouensinterval: 1.02, 2.60) in die risiko vir sterfte. Die

sistematiese oorsig van statistiese modelle vir immuunrespons het ook baie verskille getoon in die aantal en tipe veranderlikes waarvoor aangepas was, veranderlike transformasies en skale, en besonderhede was skaars oor die modelleringsmetodes wat gebruik was. In die Tshwane kohort, beraam met behulp van Bayesiaanse metodes, veroorsaak elke log10 kopie/jr/ml kumulatiewe viruslading toename 'n gemiddelde jaarlikse CD4-telling afname van -19.5 selle/µL (95% vertrouensinterval: -28.34, -10.72). Verder, in die asimptootmodel, verminder elke log10 kopie/jr/ml toename in viralelading die kans om 'n CD4-telling van meer as 500 selle/µL met 0.42 (95% vertrouensinterval: 0.242, 0.724). Die modellering van inherente veranderende CD4 telling het met behulp van 'n Student se t-verdeling beter modelle geproduseer as vir Gaussiese normaal verdelings.

Bespreking: Getransformeerde kumulatiewe viruslading is geassosieer met beide sterfte en langtermyn immuunrespons, terwyl die mees onlangse viruslading verband hou met die voorkoms van opportunistiese infeksies. Hierdie proefskrif beklemtoon die vereiste vir ’n oorskou van bestaande literatuur voordat enige statistiese ontledings onderneem word, sodat meer vergelykbare en robuuste statistiese modelle gebou sal word as wat tot dusver beskikbaar was. Die vergelyking van immuunrespons (CD4-telling) statistiese modelle in antiretrovirale terapie sal baat vind by die toepassing van meer eenvormige modelleringstegnieke. Sulke modelle is verbeter deur gebruik van getransformeerde kumulatiewe viruslading en meer akkurate verdelingsaannames. Toekomstige statistiese immuunrespons modelle sal ook baat vind by die gebruik van Bayesiaanse metodes as gevolg van hul aanpasbaarheid in terme van verdelings keuses en die implementasie van hiërargiese modelontwerpe.

Dedication

I dedicate this work to:

My parents, Mr and Mrs Sempa

for their hard work, sacrifice and dedication

to give me the best education.

My beautiful wife Doreen K. Sempa, the apple of my eye

for standing beside me through this amazing journey.

Your love has inspired me to work even harder!

Our lovely daughter Michaela:

you brighten up our lives, surely who is like Yahweh!

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I thank my PhD supervisor Prof. Martin Nieuwoudt for the enduring support throughout this period. From our first correspondence, I did not imagine the twists and turns that occurred prior to my arrival in Stellenbosch. In spite of this, your support remained resolute.

I am forever grateful to God for Doreen and Michaela, for the sacrifices they made so that we can achieve this milestone, for my parents (Mr and Mrs Sempa), siblings (Henry, Stella, Anne, Edward, and Jimmy), the family of Doctor and Professor Kizza, the Stellenbosch International Fellowship, and Mr and Mrs Goosen.

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List of Abbreviations

AIC—Akaike Information Criterion

AIDS—Acquired Immune Deficiency Syndrome

ANCOVA—Analysis of Covariance

ART—Antiretroviral Therapy

AHR—Adjusted Hazard Ratio

BGR—Brooks Gillman Rubin

CD4—Cluster of Differentiation 4

CI—Confidence Interval

CPH—Cox Proportional Hazards

DIC—Deviance Information Criterion

EFV—Efavirenz

GEE—Generalized Estimating Equations

GLME—Generalized Linear Mixed Effects

HAART—Highly Active Antiretroviral Therapy

HIV—Human Immune Virus

IQR—Interquartile Range

MCMC—Markov Chain Monte Carlo

MESH—Medical Subject Headings

NVP—Nevirapine

OI—Opportunistic Infections

OPenBUGS—Open Bayesian analysis Using Gibbs Sampler

PRISMA—Preferred Reporting Items for Systematic Reviews and Meta- Analyses

RLS—Resource Limited Settings

SSA—Sub-Saharan Africa

VL—Viral Load

WHO—World Health Organization

Author and co-author contributions

Chapter 2 was published in the American Journal of Epidemiology [1]. Data from the Infectious Diseases Institute in Kampala was used for this purpose. The data and some interpretation of clinical findings was provided by Dr's Barbara Castelnuovo and Agnes N. Kiragga. The International Clinics on Infectious Disease Dynamics and Data (ICI3D) program, facilitated by SACEMA, funded the statistical analysis and publication of this work. I, the author, Prof's Steve Bellan and Martin Nieuwoudt were instrumental in developing the study concept and design. The statistical analysis was primarily conducted by myself, with some advice from Prof's Jonathan Dushoff, Michael J. Daniels, Martin Nieuwoudt and Steven E Bellan. I also performed the literature review, composition of the draft and preparation of the final manuscript. All authors participated in editing and the approval of the final manuscript.

Chapter 3 was published in PLOS One [2]. The study concept was developed in collaboration with Prof Martin Nieuwoudt. All literature searches, publication selection and review were performed by myself. An article selection algorithm was employed with the help of co-authors, Ms Eva Ujeneza and Prof Martin Nieuwoudt, refer to chapter 3, section 3.2. All data analyses, and the composition of the initial draft and final manuscript were done by myself. All co-authors participated in editing the manuscript and approved the final publication.

Chapter 4 is intended for publication in an international journal in the very near future. The data for this study was from the Tswane District HIV treatment centre and provided by Prof's Theresa M Rossouw and Martin Nieuwoudt. The development of the study concept was done in collaboration with Prof Martin Nieuwoudt. All the statistical analysis was performed by myself, with some (Bayesian) technical advice and model interpretation provided by Prof Emmanuel Lesaffre. Prof Roussouw provided assistance with some clinical interpretation of results. I reviewed all the literature, performed the analyses, wrote the first

draft and prepared the final manuscript. All authors participated in editing and approving the final manuscript.

All the other sections of the dissertation were composed by myself. Prof Martin Nieuwoudt assisted with oversight on the overall composition and flow of the thesis, along with editorial input.

Chapter One

Introduction and Literature review

Global and sub-Saharan Africa (SSA) burden of HIV

The Human Immunodeficiency Virus (HIV) is arguably the third worst epidemic in the history of human kind, after Spanish flu and the Black Plague [3]. Further, at the end of 2013 SSA had 71% of the world's 35 million HIV infected population. In the same year there were 70% of 2.1 million HIV incident cases and 74% of 1.5 million deaths due to Acquired Immunodeficiency Syndrome (AIDS) in SSA [4]. According to the World Bank report of 2013, HIV/AIDS was the second largest cause of morbidity, and the largest contributor to low disability adjusted life years in SSA in 2010 [5].

The Impact of combination antiretroviral therapy (ART) on HIV

The introduction of ART in HIV-AIDS treatment has dramatically improved patient survival [6,7], and socio-economic outcomes in SSA [8]. Between 2004 and 2013, the use of ART led to a 39% reduction in AIDS mortality in SSA in spite of low treatment coverage [4]. Further, from the beginning of ART scale-up in SSA, the World Health Organization (WHO) initiation thresholds have progressively decreased to now immediate initiation [9–11]. This has been as a result of accumulating evidence demonstrating improved survival benefits in earlier ART initiation [12,13]. This, in turn, has increased the demand for ART in SSA. However, treatment naïve HIV-infected people are generally still initiated at very low CD4+ T-lymphocyte counts (CD4 count) [14–16], due mostly to structural bottlenecks to ART access [17]. From a population perspective, initiating and maintaining as many people with HIV on ART, given good adherence levels, reduces community viral load and reduces HIV incidence [18,19].

The incidence of HIV in SSA has declined since its peak in the early 1990's [20]. However, a number of factors continue to drive it, for example, sexually transmitted infections such as herpes simplex virus (HPV-2), multiple sexual partners and paid sex [21], among others. A number of HIV prevention strategies do exist but they are only effective when implemented as an integrated package [22,23]. For example, ART is effective in lowering HIV incidence by decreasing viral load at both individual and population level, however, it may not be as effective in the absence of consistent condom use. Using mathematical models, Cori et al. [24], have demonstrated that implementing a combination of strategies might reduce HIV incidence by more than 60% in SSA.

The role of biological markers in monitoring HIV

Normally, people on ART are monitored clinically for opportunistic infections (OIs), immunologically for CD4 count response, and virologically for HIV viral load. Viral load monitoring, though expensive, is the definitive marker for adherence or treatment failure in ART [9,25]. Previous studies of the implementation of viral load monitoring in SSA demonstrate mixed cost-effectiveness results [26–30]. However, prior to the expected arrival of more affordable point of care viral load tests [31,32], Van Zyl et al. [33] have suggested using sample pooling as a means of reducing the cost of viral load testing. Other authors suggest using dry blood spots [34,35]. Pooling involves combining 5 patient blood samples; then, if this tests virus-positive each patient sample is tested independently to ascertain the particular positive/s. Sample pooling is less expensive than traditional individual viral load testing, but its successful implementation is determined by ART adherence and the proportion of viral load suppression in each patient [33].

Organizations from North America and Europe have been instrumental in subsidizing viral load monitoring in SSA countries [36]. However, these organizations have adopted a

public health focus on viral suppression as a measure of ART efficacy. For this reason there has been a relative decline in interest in CD4 count monitoring [36]. However, in SSA 40% of patients initiated on ART develop sub-optimal immune responses [37]. Thus, CD4 count monitoring remains important. In spite of the dramatic scale up of ART, the majority of patients in SSA continue to initiate treatment at very low CD4 counts [14] and achieving CD4 counts >350 cells/μL often only occurs over a long duration [37]. CD4 count testing for such patients is also necessary at baseline, to benchmark the immediate risk of OIs and for long term monitoring in the event of their exceeding high OI risk thresholds [38–40]. Hemoglobin should also be monitored, particularly in the early stages after ART initiation [41], as HIV-infected people are at a high risk of anemia with up to a 50% decline in hemoglobin [42].

Previous studies of CD4 count trajectory after ART initiation have demonstrated a sharp increase of CD4 counts within the first two years compared to the period thereafter [43–45]. This increase during treatment depends on each patient's 'baseline' or initiating CD4 count [46]. Other factors also determine the CD4 count trajectory, for example, sex, age, protease inhibitor based ART regimen, ART adherence and the nadir CD4 count [43]. In virally suppressed people, CD4 increases are usually assured. However, particularly those with low baseline CD4 counts are prone to sub-optimal CD4 count recovery on therapy [47]. In a recent study of immune response of HIV infected people from East Africa, 40% of people had <350 cells/μL after 5-years on ART [37]. Protease inhibitors (PI) as first-line were also associated with a low risk of becoming sub-optimal, compared to non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimens [37]. As the majority of SSA HIV patients are initiated on NNRTI, not PI-based regimens, sub-optimal immune response remains a concern [48]. CD4 count recovery may also vary from country to country due to differing normal CD4 count ranges in local populations [49].

CD4 count is a marker for HIV disease progression [50]. In Resource Limited Settings (RLS) it has historically been used as part of the criteria to initiate ART and switch patients to second-line therapy [25]. However, switching patients to second-line ART using only CD4 counts has led to delayed switching or missed diagnoses of treatment failure [25,51,52]. As a result, the revised 'test and treat' public health policy [53] has ended mandatory CD4 count measurements at ART initiation. Unfortunately, many HIV patients in SSA present late into care [14] and in these a CD4 count test would improve clinical management against, for example, Immune Reconstitution Inflammatory Syndrome (IRIS) [54], OIs, and risk of suboptimal immune response [37].

Viral load monitoring in RLS, where available, is used as a cross-sectional measure to determine when to switch patients to second-line [55,56], and rarely used as a longitudinal measurement of disease progression on ART [57]. Previous studies outside of SSA have suggested that cumulative HIV-Viremia, which is the area under the curve of a patient's entire history of viral load measurements, is associated with clinical [58–60] and immunological outcomes [60]. Arguably, if HIV disease progression depends on the frequency of detectable viral loads, which is often the case, then cumulative HIV-Viremia may be used as a biomarker for inflammation, immune activation and associated with mortality. To date, the data of all prior cohorts that were analyzed using cumulative HIV-Viremia, were from resource-rich settings [58–60]. Generalizing such findings to SSA RLS is questionable as the treatment contexts are different. There have been no studies in SSA about the association of cumulative HIV-Viremia with the incidence of OIs, mortality and immune response. Further, whether cumulative HIV-Viremia is associated with such treatment outcomes depends on our understanding of the underlying ART and HIV disease dynamics. It is also dependent on the robustness and repeatability of the statistical methods. For example, although there are

increasing numbers of peer-reviewed articles describing immune response to ART in SSA, there are short comings in the cohesiveness and comparability of these studies.

1.1 Overall aim:

To use rigorous statistical methods to investigate the effects of longitudinal HIV viral load exposure on immune recovery, mortality and opportunistic infections among previously ART naïve patients in SSA.

1.2 Specific aims:

1. To compare most recent viral load versus cumulative HIV-Viremia, to predict OIs and mortality in an SSA ART cohort.
2. To conduct a systematic review of statistical, i.e. empirically-defined, models of immunological response to ART in SSA, and to define a 'meta-model' from this.
3. To prospectively implement the *prior*, meta-model resulting from specific aim 2 on the data of a previously undescribed SSA cohort, and then compare models with and without cumulative HIV-Viremia.

1.3 Study objectives:

In chapter two we review published methods used to estimate cumulative HIV-Viremia as a prognostic predictor. Further, we determine whether most recent viral load and cumulative HIV-Viremia are able to predict incident opportunistic infections and mortality in a SSA HIV ART cohort. We use data from a retrospective ART cohort, from the Infectious Diseases Institute Kampala, Uganda. We also review existing methods for numerical estimation of cumulative HIV-Viremia and compare this with a newly proposed method (cumulative log viral load). Further, we analyze time to mortality and incident opportunistic infections using Cox proportional hazards regression models corrected for repeated measures using Andersen-Gill standard errors.

In Chapter three we systematically review (SR) statistically-derived immune response models of HIV infected patients on ART in SSA cohorts. Further, we then describe and motivate methods that might be used to define future multivariate immune response models. In this SR we use 'Preferred Reporting Items for Systematic Review and Meta-Analyses' guidelines. We use 'Wordclouds' to identify and quantify the particular covariate sets for each type of outcome. We then define 'prior' knowledge that can be used to develop more robust statistical models in the future.

In Chapter four we implement multivariate Bayesian immune response models, using that prospectively defined in chapter three, to an as yet undescribed SSA ART cohort. Further, we then determine whether adjusting for cumulative log viral load provides better model fits than the crude model. We determine whether cumulative log viral load is associated immune response. We employ data from a retrospective ART cohort of patients from the Tshwane District Hospital, Gauteng, South Africa. We apply Bayesian generalized mixed effects models to analyze the contribution of cumulative log viral load on determining the slope CD4 count and asymptote models. Further, we perform a sensitivity analysis of the model by testing distributional assumptions of model parameters.

The material in chapter two has been published in American Journal of epidemiology, see reference 158. Material in chapter three has been published in PLOS ONE, see reference 157. Material in chapter four will be submitted in the near future to an appropriate peer-reviewed international journal.

Chapter Two

Reevaluating Cumulative HIV-1 Viral Load as a Prognostic Predictor: Predicting Opportunistic Infection Incidence and Mortality in a Ugandan Cohort

2.1 Introduction

Antiretroviral therapy (ART) for HIV-infected patients leads to CD4 T-cell count reconstitution, HIV RNA Viral Load reduction, reduced burden of opportunistic infections (OIs) and prolonged survival [6]. However, some treated patients never achieve complete CD4 count reconstitution or undetectable Viral Load [45] and consequently experience an increased risk of developing drug resistance[61], non-communicable diseases [62] and remain more infectious to their sexual partners relative to patients who respond to treatment more effectively [63].

Although CD4 count is used as a threshold criterion for ART initiation, Viral Load better indicates treatment failure, the subsequent need to switch regimens [64] and an individual's infectiousness [65]. High costs have limited use of Viral Load assays in resource-limited settings to date. However, this is changing with newer, cheaper technology, particularly point-of-care assays[66] and techniques like sample pooling [33]. Consequently, many countries, including Uganda, are scaling up Viral Load monitoring for all HIV patients on ART [67].

Further, recent research in resource-rich countries suggests new, potentially promising applications of Viral Load monitoring. In particular, several studies have found cumulative HIV-Viremia to better predict mortality [59,68,69], incident OIs [58,60,70] and immune recovery [60] than the most recent Viral Load measurement. These results appear biologically plausible given that accumulated exposure to high Viral Loads leads to inflammation, immune activation, and other etiological processes [43,59,68]. However, in view of diverse approaches

taken in prior analyses we review earlier studies of cumulative HIV-Viremia, investigating the consequences of varying assumptions and methodologies.

We then expand on prior work in several directions: We perform an analysis evaluating cumulative HIV-Viremia's prognostic utility in a resource-poor setting, where disease progression is often further along by the time patients receive treatment [71] and where Viral Load monitoring is being expanded [33,67]. Based on our review we explore the sensitivity of the results to *how* cumulative HIV-Viremias are accumulated, providing a formal comparison between accumulations on the linear vs. the logarithmic metrics. Since health outcomes are often observed more frequently than lab measurements, we implement a regression framework to estimate the declining prognostic utility of these measurements over time. We also highlight the clinical implications of this work and areas for future study.

2.2 Methods

Literature Review

We searched PubMed for publications containing the following keywords: "viremia copy-years", "viremia copy years", "cumulative HIV viremia", "cumulative viral load", during the period April 2014 until March 2015. We selected all studies in which cumulative HIV-Viremia methods were employed in analyzing data of HIV infected adults. Nine studies were identified as relevant.

Study setting and population

The Infectious Diseases Institute clinic is an urban HIV clinic, based at Mulago national referral hospital in Kampala, Uganda. A prospective cohort study of 559 HIV-1 patients initiated ART between April 2004 and April 2005 following World Health Organization [72] and national guidelines [73] (i.e. either CD4 <200 cells/μL irrespective of their WHO stage or clinically advanced symptoms), with up to 9 years of follow-up. Participants attended the clinic

for an in-depth examination approximately every 12 weeks with CD4 count, Viral Load and hemoglobin measured every other visit [74]. Baseline measurements were measured on the day of ART initiation. The primary Viral Load assay used throughout the study had lower and upper detection limits of 400 and 750,000 copies/mL, respectively. Despite the occasional use of assays with lower detection limits, we censored all measurements below 400 copies/mL for consistency. We excluded from the analysis 63 patients who died or were lost to follow-up before their second Viral Load measurement (including many who died within the first 12 weeks of ART [75]) and 7 patients lacking baseline CD4 or Viral Load measurements. Patients were also censored after they switched to second line regimens, died or were lost to follow-up. 14 patients had ART treatment stops due to toxicity and poor adherence.

Outcomes of interest

In our first analysis, we investigated predictors of OI risk, coding OI as a binary variable indicating the occurrence of ≥1 incident OIs since the last visit, since concurrent incident OIs were rare. We did not censor patients after their first incident OI and consequently each patient could contribute more than one incident OI outcome to the analysis. We included the following OIs: oropharyngeal and esophageal candidiasis, toxoplasmosis of the brain, unexplained chronic diarrhea, severe bacterial pneumonia, tuberculosis, herpes zoster, Pneumocystis jerovecii pneumonia, cryptococcal meningitis, Kaposi's sarcoma, prurigo and lymphoma. In our second analysis, we investigated predictors of mortality, excluding only cases where the stated cause of death was violence or accident. We did not differentiate between AIDS-related and non-AIDS deaths (such as e.g. cancer or heart disease) due to our small sample size.

Lab measurements and the calculation of cumulative HIV-Viremia

Most recent Viral Load and cumulative HIV-Viremia were the main predictors of interest. We avoid the often-used previous nomenclature of "copy-years", as cumulative HIV-

Viremia's unit depends on the method of calculation. Prior studies have calculated cumulative HIV-Viremia either by summing the area under the Viral Load curve and then taking the logarithm (log cumulative Viral Load) or, more rarely, by summing area under the log Viral Load curve (cumulative log Viral Load; Appendix 2.1 table 1, references).

Calculations were made using the trapezoidal rule [58]. Log cumulative Viral Load (time-updated area under the viral load curve) for patient *i* at their *j*-th visit was calculated as:

$$cVL_{1,ij} = log_{10}\left(\sum_{k=2}^{k=j}\left(t_{i,k} - t_{i,k-1}\right) \times \frac{\left(V_{i,k} + V_{i,k-1}\right)}{2}\right) \qquad 2.1$$

where $t_{i,k}$ and $V_{i,k}$ represent the time (in years) and (untransformed) Viral Load measurement of the *k*-th visit, respectively.

We chose to calculate cumulative log Viral Load as the time-updated area under the log Viral Load curve and above 400 copies/ml on the assumption that exposure to virus below this detection threshold does not contribute to OI or mortality risk. Thus, the cumulative log Viral Load for the *i*-th patient at their *j*-th visit to the cohort is given by,

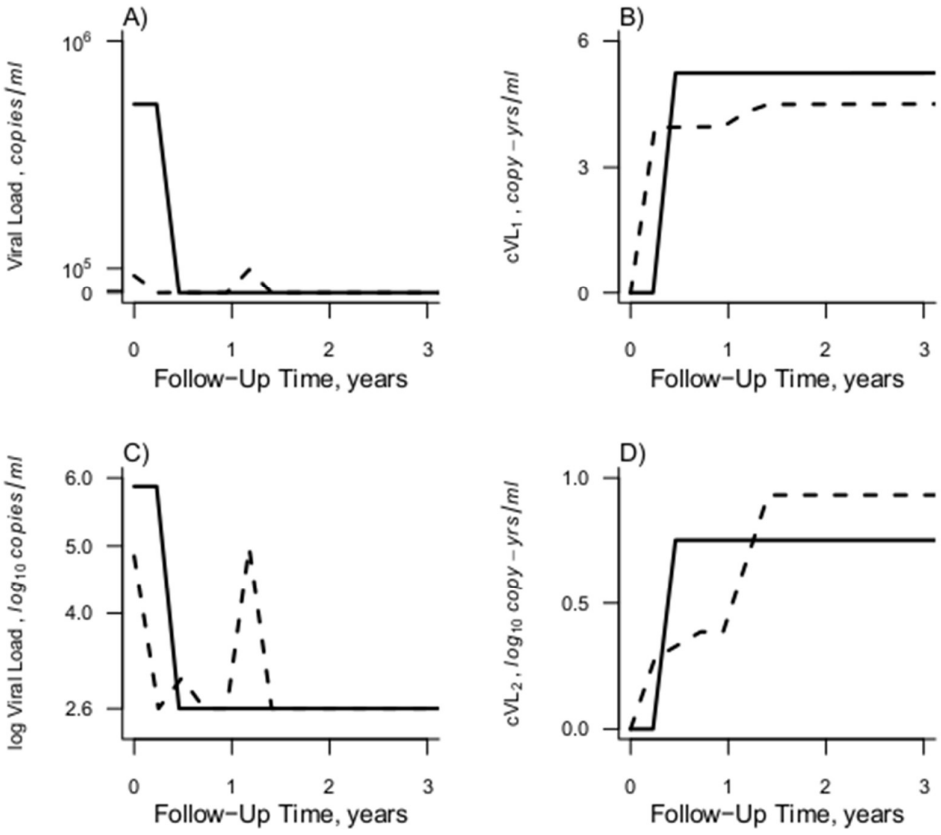
$$cVL_{2,ij} = \sum_{k=2}^{k=j}\left(t_{i,k} - t_{i,k-1}\right) \times \frac{\left(log_{10}\left(\frac{V_{i,k}}{400}\right) + log_{10}\left(\frac{V_{i,k-1}}{400}\right)\right)}{2} \qquad 2.2$$

We assumed that both cumulative HIV-Viremia measures were 0 at baseline visit. Through simulation we determined that using exact inter-visit durations biased survival regression coefficient estimates of cumulative HIV-Viremia. Since inter-visit durations were close to 12 weeks we rounded them to this value (see Appendix 2.1).

The two metrics produce very different cumulative HIV-Viremia trajectories (Figure 2.1). Accumulating Viral Load on a linear scale (Log cumulative Viral Load) gives more weight to a patient's largest Viral Load measurements. Summing on a log scale (cumulative

log Viral Load) gives more weight to intermediate measurements, such as transient rise in viral load, which occur during otherwise successful viral suppression. These two choices are not exhaustive: Viral measurements might also be accumulated on other transformed scales. The true scale is unknown and presumably dictated by mechanisms underlying HIV pathogenesis which, given available measurements, are imperfectly characterized. Consequently, we confined our analyses to the above two metrics. We also conducted two sensitivity analyses; first, using the WHO threshold for virologic failure (1000 copies/ml) for cumulative log Viral Load to account for the potential arbitrariness of the chosen threshold [67,76]; second, only analyzing data and accumulating cumulative HIV-Viremia after the 24 week visit.

Figure 2.1: Comparison of Cumulative HIV-Viremia Derivations for two HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



Legend: Patients I—full line and Patient II—broken line. 2.6 mark on the y-axis of C) is the log of the detection limit of the Viral Load assay: (A) and(C) show two example patients' viral load and log viral load trajectories, respectively, as a function of time since cohort enrollment (i.e. ART initiation). (B) shows the log cumulative Viral Load for these patients, which is calculated by taking the logarithm of the time-updated area under the curve from (A). (D) shows their cumulative log Viral Load trajectories, which is calculated by taking the time-updated area under the curve from (C). Whether we accumulate viremia on a log or linear scale therefore determines which of these two patients we consider as having been exposed to the greatest cumulative HIV-Viremia since enrollment by three years.

Statistical Methods

We regressed time to OI and mortality outcomes against predictors using multivariate Cox proportional hazards models, which implicitly account for a temporally varying hazard as a function of time since ART initiation [77]. Gender, baseline age, baseline CD4 count and baseline Viral Load were included as constant predictors; time-varying predictors included most recent CD4 count, hemoglobin, Viral Load and either log cumulative Viral Load or cumulative log Viral Load. Viral Loads below 400 copes/ml were modeled as a separate categorical variable. While OI acquisition could occur over multiple observation intervals, we assumed that a patient's OI hazard was unaffected by their previous OI history. Since lab assays were only performed every other visit, we expected that the prognostic value of lab measurements would be greater for the interval immediately following measurement than for intervals with a larger time lapse since last measurement. We explicitly estimated this effect by including the most recent measurement of each lab predictor as a main effect and as an interaction with the time lag since its measurement. Further detail is provided in the Appendix 2.1.

Other than the main predictors, most recent Viral Load and cumulative HIV-Viremia, we chose to include other covariates as untransformed, log-scaled or categorical based on visual inspection of smoothed Martingale residual plots [77] (Appendix 2.1 figure 1). We report adjusted hazard ratios and, to account for inter-patient heterogeneity (i.e. in addition to that

accounted for by observed covariates), used Andersen-Gill robust standard errors [77] to calculate Wald 95% confidence intervals and P-values. By using the Andersen-Gill standard errors we avoid the need to specify a full probability model for the data. As a result, this precludes between-model comparisons based on likelihood based criterion and any statistics requiring a full model (i.e. R^2 and related statistics). For lab measurements made every other visit, we report adjusted hazard ratios both for the 0-12 week and 12-24 week intervals following their measurement. Statistical significance was defined as a P-value <0.05. Statistical analysis was done using R language version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

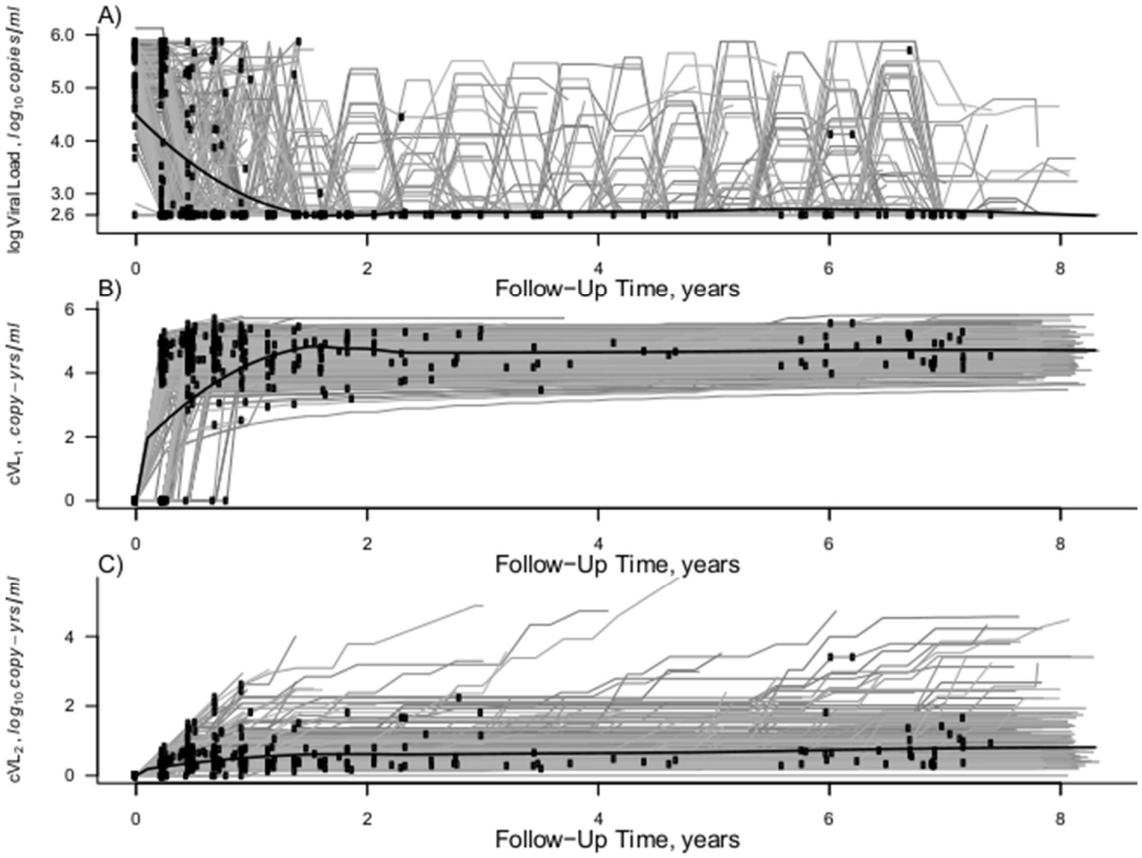
This study and analysis of the data was reviewed and approved by the Institutional Review Board of Makerere University and Uganda National Council for Science and Technology (MV 853). Written informed consent was sought from each study participant at cohort inception.

2.3 Results

Of 489 patients analyzed, 69.7% were female, median (IQR) baseline age was 35.3 years (30.2 – 41.8), baseline CD4 count was 100 cells/μL (30 – 168), baseline viral load was 5.4 $\log_{10}$ copies/ml (5.1 – 5.8) and follow-up time was 8.3 years (2.3 – 8.8; Appendix 2.1 table 2). Of the two cumulative HIV-Viremia measures, log cumulative Viral Load exhibited greater negative correlation with most recent Viral Load and a greater positive correlation with baseline Viral Load and peak Viral Load (Appendix 2.1 table3, Appendix 2.1 figure 2A).

Opportunistic Infection Model

Figure 2.2: Incident Opportunistic Infections versus Viral Load and Cumulative HIV-Viremia Trajectories for HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



Legend: Grey lines show each cohort participant's Viral Load (A) and log cumulative Viral Load (B) and cumulative log Viral Load (C) trajectory as a function of time since their cohort enrollment and initiation of antiretroviral therapy (ART); Thick black lines show a lowess trend line through these trajectories; Circles show the occurrence of an incident opportunistic infections along patients' trajectories. The 2.6 mark on the y-axis of A) is the log of the detection limit of the Viral Load assay.

The majority of incident OIs occurred during the first 2 years after ART initiation, with the Viral Loads approaching undetectable levels and the number of incident OIs decreasing substantially after 4 years (Figure 2.2). Based on inspection of smoothed Martingale residuals, we included hemoglobin, CD4 and baseline CD4 on the log scale, age on a linear scale and

baseline Viral Load as a categorical variable as possible predictors of OI occurrence. A 1og10 increase in Viral Load was associated with a statistically significant greater hazard of an incident OI for up to 12 weeks after their measurement (Table 2.1). Neither cumulative HIV-Viremia measure was a statistically significant predictor of incident OIs. Lower hemoglobin levels were a statistically significant predictor of OI risk for up to 24 weeks after measurement.

Table 2.1: Opportunistic Infection Model Results among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

Variable	Model with cVL_1		Model with cVL_2	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	1.28	1.090, 1.500 [b]	1.34	1.120, 1.610 [c]
predicting 0-24 weeks ahead	1.18	0.870, 1.590	1.21	0.969, 1.500
per log10 increase in cumulative viremia, *log_{10} copy-yrs/ml*				
predicting 0-12 weeks ahead	0.97	0.859, 1.090	0.78	0.523, 1.150
predicting 0-24 weeks ahead	1.00	0.905, 1.100	1.00	0.679, 1.480
per 2-fold increase in CD4 count, *cells/μL*				
predicting 0-12 weeks ahead	0.90	0.805, 1.000	0.90	0.804, 0.998 [a]
predicting 0-24 weeks ahead	0.92	0.759, 1.110	0.91	0.755, 1.110
per 10% increase in hemoglobin, *g/dl*				
predicting 0-12 weeks ahead	0.91	0.860, 0.961 [b]	0.91	0.859, 0.959 [c]
predicting 0-24 weeks ahead	0.89	0.805, 0.976 [a]	0.89	0.819, 0.971 [b]
per 2-fold increase in baseline CD4 count, *cells/μL*	0.98	0.897, 1.070	0.98	0.898, 1.080
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	0.97	0.699, 1.350	0.96	0.692, 1.320
3rd	1.23	0.871, 1.720	1.20	0.869, 1.640
4th	1.04	0.725, 1.500	1.01	0.715, 1.420
Gender				
Female	1			1
Male	0.78	0.601, 1.010	0.78	0.602, 1.010
per 10 year increase in baseline age	0.91	0.791, 1.040	0.91	0.791, 1.040

[a] Statistical significance: P <0.05; [b] P <0.01; [c] P <0.001

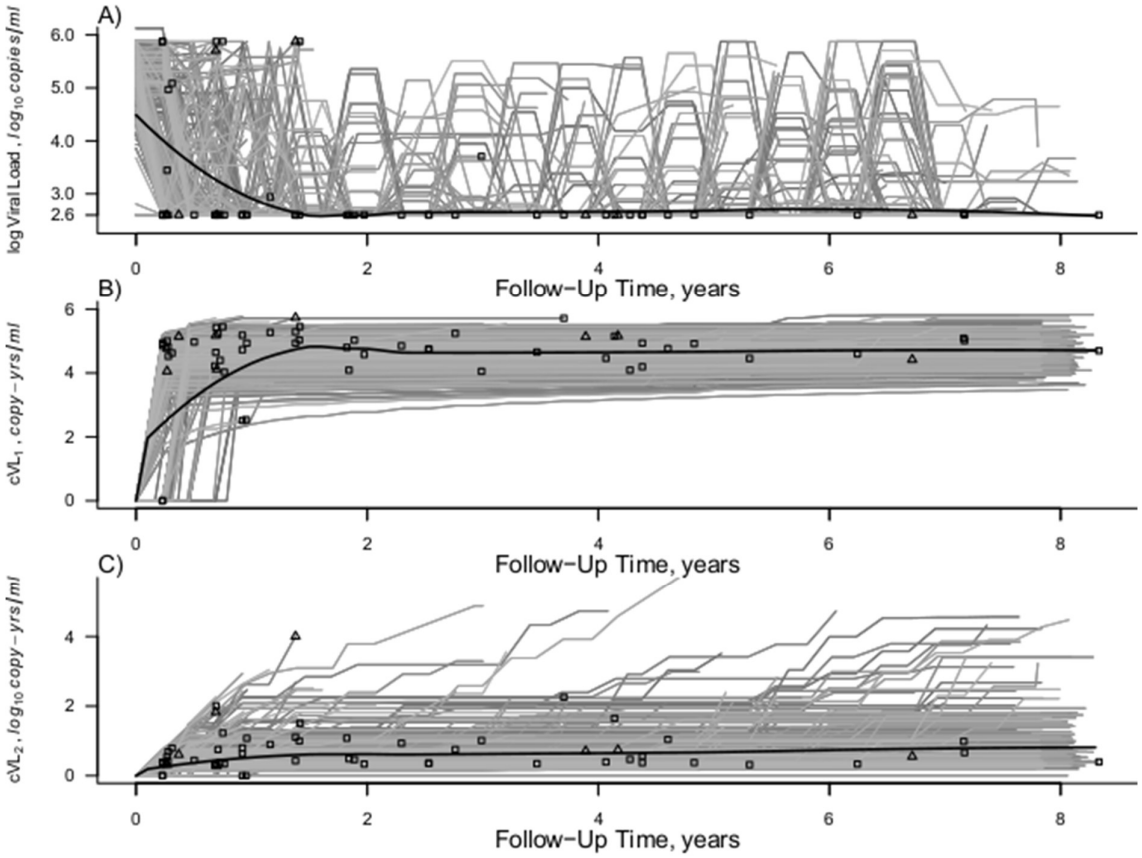
Quartiles: 1st— $\leq 10^{5.07}$; **2nd**— $10^{5.08}$ to $10^{5.44}$; **3rd**— $10^{5.45}$ to $10^{5.77}$; **4th**— $10^{5.78}$ to $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL_1**—log cumulative Viral Load; **cVL_2**—cumulative log Viral Load; **VL**— Viral Load. Values are adjusted hazard ratios and 95% confidence interval for the hazard of acquiring an incident opportunistic infection

from multivariate Cox proportional hazard models with cumulative viremia calculated as one of either log cumulative Viral Load or cumulative log Viral Load.

Mortality Model

Figure 2.3: Mortality versus Viral Load and Cumulative HIV-Viremia Trajectories for HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



Legend: Grey lines show each cohort participant's Viral Load (A), log cumulative Viral Load (B) and cumulative log Viral Load (C) trajectory as a function of time since their cohort enrollment and initiation of antiretroviral therapy (ART); Thick black lines show a loess trend line through these trajectories; Red-squares and black-triangles show the occurrence of an AIDS-related and non-AIDS-related mortality on patients' trajectories, respectively. The *2.6 mark* on the y-axis of A) is the log of the detection limit of the Viral Load assay.

62 patients died during follow-up, 33 of AIDS specific causes. As with OIs, most deaths occurred during the first 2 years after enrollment (Figure 2.3). Again, after examination of smoothed Martingale residuals, we included hemoglobin, current CD4 count and baseline CD4

count on the log scale and age and baseline Viral Load as categorical variables. Most recent Viral Load was not a statistically significant predictor of mortality (Table 2.2). In contrast, cumulative log Viral Load but not log cumulative Viral Load was a statistically significant predictor of mortality for up to 12 weeks after the most recent lab measurement. Low hemoglobin or CD4 counts were statistically significant predictors of high mortality risk for up to 24 weeks after measurement. Older age (>55 years) and baseline Viral Load ($\geq 10^{5.77}$ copies/ml) were also statistically significant predictors of mortality risk.

Table 2.2: All-cause Mortality Model Results among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

Variable	Model with cVL_1		Model with cVL_2	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	1.38	0.920, 2.050	1.13	0.722, 1.770
predicting 0-24 weeks ahead	0.98	0.554, 1.750	0.89	0.512, 1.550
per log10 increase in cumulative viremia, *log_{10} copy-yrs/ml*				
predicting 0-12 weeks ahead	0.97	0.648, 1.440	1.63	1.020, 2.600 [a]
predicting 0-24 weeks ahead	0.98	0.797, 1.220	0.50	0.168, 1.490
per 2-fold increase in CD4 count, *cells/μL*				
predicting 0-12 weeks ahead	0.58	0.457, 0.733 [c]	0.57	0.454, 0.723 [c]
predicting 0-24 weeks ahead	0.68	0.491, 0.957 [a]	0.69	0.514, 0.922 [a]
per 10% increase in hemoglobin, *g/dl*				
predicting 0-12 weeks ahead	0.77	0.703, 0.840 [c]	0.77	0.702, 0.832 [c]
predicting 0-24 weeks ahead	0.72	0.634, 0.815 [c]	0.73	0.650, 0.817 [c]
per 2-fold increase in baseline CD4 count, *cells/μL*	1.08	0.918, 1.280	1.08	0.920, 1.280
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	1.54	0.697, 3.400	1.51	0.682, 3.330
3rd	1.35	0.515, 3.530	1.28	0.527, 3.090
4th	3.94	1.610, 9.640 [b]	3.62	1.710, 7.640 [c]
Gender				
Female	1			1
Male	1.10	0.578, 2.100	1.07	0.556, 2.050
Baseline age, *years*				
≤35	1			1
36 – 45	1.22	0.676, 2.190	1.29	0.713, 2.340
46 – 55	1.61	0.766, 3.380	1.71	0.815, 3.600
≥56	2.94	1.260, 6.860 [a]	3.02	1.300, 6.970 [b]

[a]Statistical significance: P <0.05; [b]P <0.01; [c]P <0.001

Quartiles: 1st— ≤$10^{5.07}$; **2**nd— $10^{5.08}$ to $10^{5.44}$; **3**rd— $10^{5.45}$ to $10^{5.77}$; **4**th— $10^{5.78}$ to $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL$_1$**—log cumulative Viral Load; **cVL$_2$**—cumulative log Viral Load; **VL**— Viral Load. Values give adjusted hazard ratios and 95% confidence intervals for the hazard of dying of any cause from multivariate Cox proportional hazard models with cumulative viremia calculated either as log cumulative Viral Load or cumulative log Viral Load.

Sensitivity analyses

Changing cumulative log Viral Load to only accumulate Viral Load above 1000 copies/ml did not qualitatively affect results for either OI or mortality models (Appendix 2.1 tables 4 and 5). Excluding data prior to the 24-week visit decreased and increased log cumulative Viral Load and cumulative log Viral Load's respective correlations with most recent log Viral Load (Appendix 2.1 figure 2B). This sensitivity analysis did not qualitatively affect the results of the OI analysis (Appendix 2.1 table 6); however, in the mortality analysis, while cumulative log Viral Load remained significant, log cumulative Viral Load additionally became statistically significant (Appendix 2.1 table 7).

2.4 Discussion

In the data of this Ugandan cohort of HIV-infected patients on ART we found that most recent Viral Load, but neither cumulative HIV-Viremia metric, significantly predicted a patient's risk of acquiring a new OI. However cumulative log Viral Load, but neither Viral Load nor log cumulative Viral Load, significantly predicted their mortality risk. These significant associations persisted for only 12 weeks after the last measurement. In contrast, hemoglobin levels predicted risk of acquiring a new OI and both hemoglobin levels and CD4 counts predicted mortality risk for up to 24 weeks post-measurement. Our sensitivity analyses demonstrated that changing the detection limit from 400 to 1000 copies/ml did not qualitatively alter the results but that a cumulative HIV-Viremia metrics' predictive utility is sensitive to the inclusion of data within the first 24 weeks after ART initiation.

Previous studies have similarly shown that, after adjusting for CD4 count, most recent Viral Load is not associated with mortality [43,78,79] (see also Appendix 2.1 table 8). The lack of significant association between cumulative HIV-Viremia and OI risk may be explained by the early incidence of the majority of OIs in this cohort (Figure 2.2A), as driven by very low CD4 counts at ART initiation (Appendix 2.1 table 2) [80]. This cohort was intensely counselled regarding treatment adherence, which may explain the substantial reduction in incident OIs over time (Figure 2.2A). Since more incident OIs occurred earlier rather than later while on ART, they were less likely to be associated with cumulative HIV-Viremia than most recent Viral Load.

Within the 9 studies reviewed in Appendix 2.1 table 1, five studies [58–60,69,70] evaluated cumulative HIV-Viremia as a predictor of AIDS. One study [81] evaluated cumulative HIV-Viremia as a predictor of non-AIDS defining outcomes and 3 considered cumulative HIV-Viremia as a health outcome, regressing it against other predictors [82–84]. Among those evaluating AIDS-defining outcomes, 4 studies found statistically significant associations between cumulative HIV-Viremia and AIDS-related outcomes [58–60,70] and one did not [69]. However, methodological differences as described in Appendix 2.1 table 1 impede their direct comparability. We highlight these differences noting that, while subtle, they fundamentally affect the interpretation of cumulative HIV-Viremia and its relationship with health outcomes. We discuss several major characteristics of cumulative HIV-Viremia analyses that should be considered during the interpretations below.

A patient's cumulative HIV-Viremia summarizes the longitudinal history of Viral Load measurements that go back to a specified starting point. Thus, the cumulative HIV-Viremia starts from the patient's first Viral Load reading [81], from ART initiation [60,68,70,84] or after a specified delay, for example, 24 weeks [59] or 8 months [69] after initiation. However, cumulative HIV-Viremia has also been calculated from the date of sero-conversion [58].

Patients may enter studies at very different time-points post-infection, limiting the comparability of these metrics between individuals. Nonetheless, cumulative HIV-Viremia could be a useful proxy for treatment success as it reflects viral suppression patterns and the frequency, magnitude and duration of viral rebounds. Although, evidence from cellular studies suggest that viral replication during chronic infection drives certain aspects of HIV pathogenesis [85], cumulative HIV-Viremia accumulated after ART initiation cannot account for the association between pre-treatment Viral Load and AIDS-defining outcomes.

Another consideration includes the scale of accumulation. Eight of the 9 studies reviewed accumulated Viral Load on a linear scale (i.e. log cumulative Viral Load [58–60,68,69,81,82,84]) and two on a log scale (i.e. cumulative log Viral Load [70,82]). We demonstrated (Figure 2.1), that log cumulative Viral Load assigns greater weight to large Viral Load measurements while cumulative log Viral Load assigns greater weight to repeated intermediate measurements during otherwise successful viral suppression. For this reason log cumulative Viral Load is strongly correlated with the peak Viral Load and the Viral Load at treatment initiation (Appendix 2.1 table 3), unless the analysis is restricted to data after 24 weeks post-treatment initiation (Appendix 2.1 figure 2B). In our analysis log cumulative Viral Load was also strongly negatively correlated with most recent log Viral Load (Appendix 2.1 figure 2A) and positively correlated with CD4 count. The greater collinearity between log cumulative Viral Load compared to cumulative log Viral Load with other predictor variables likely indicates that linear accumulation provides less information regarding characteristics of patient Viral Load history than accumulations on the log scale. This is supported by our finding that cumulative log Viral Load rather than log cumulative Viral Load was predictive of mortality risk. Thus, statistical associations between log cumulative Viral Load and health outcomes, as previously found, might be artifacts of confounding as all variables may not have been appropriately controlled for (Appendix 2.1 table 1). Alternately such studies, including

ours, might underestimate the true magnitude of the effect of log cumulative Viral Load due to the difficulty in disentangling the effect of correlated variables.

In the choice of cumulative HIV-Viremia metric, the log-linear relationship between Viral Load and infectivity [86,87] should be considered. HIV infectivity and pathogenesis may arise from different mechanistic relationships with Viral Load. However, we are not aware of any clinical processes/outcomes that scale linearly with untransformed Viral Load. Indeed, many biological processes are log-linear and Viral Loads are commonly evaluated on a log scale. In addition, when calculating cumulative log Viral Load, by dividing each Viral Load by the Viral Load detection limit before log transformation, we assume that OI and mortality risk accumulates only due to detectable Viral Load. Without this approach a patient with Viral Load that has been suppressed for a long duration may have a similar cumulative HIV-Viremia to one who has a short follow up time but with viral rebounds. We consequently suggest that analyses should accumulate cumulative HIV-Viremia on a log scale and include the limit of detection.

Even after the accumulation starting point and scale transformation have been chosen, a cumulative HIV-Viremia metric may be included in the statistical analysis in a variety of ways. Marconi et al(2009) accumulated patient cumulative HIV-Viremia over the entire observation period and then used it as a constant predictor [60]. This amounts to using information from the future to predict past events and should be avoided. All other studies evaluating cumulative HIV-Viremia as a predictor, including our own, incorporated time-updated cumulative HIV-Viremia to predict the risk of an AIDS-defining outcome during a subsequent observation period. While most studies, including our own, included cumulative HIV-Viremia as a continuous predictor variable, both Chirouze et al(2015) and Marconi et al(2009) included cumulative HIV-Viremia as a binary variable because continuous measures of cumulative HIV-Viremia were not statistically significant in their analysis [60,69]. The need

to categorize cumulative HIV-Viremia to achieve statistical significance may indicate that the chosen scale of accumulation does not correspond well to an underlying linear relationship between cumulative HIV-Viremia and the study outcome.

As cumulative HIV-Viremia metrics, in particular log cumulative Viral Load, are highly collinear with many other covariates that are already well known to predict AIDS-defining outcomes, covariate adjustment will in large part determine whether any statistically significant association is epidemiologically meaningful. This suggests that cumulative HIV-Viremia analyses should adopt a principled approach to covariate adjustment and avoid exclusion of other covariates simply because they are collinear with cumulative HIV-Viremia, or because they are not significant in intermediate models, i.e. backward stepwise selection. Similarly, Kaplan-Meier curves stratified by cumulative HIV-Viremia or other univariate visualizations may also be misleading [68]. Some covariates that are collinear with cumulative HIV-Viremia metrics may themselves be better predictors of health outcomes. For instance, for studies accumulating cumulative HIV-Viremia starting at ART initiation, log cumulative Viral Load is highly correlated with the highest Viral Load post-ART initiation (0.88 in Cole et al. [58]; 0.34 here) because patient Viral Loads decline rapidly post-ART initiation and log cumulative Viral Load is disproportionately driven by large Viral Load measurements. Further, peak viral load may be a good proxy for chronic phase set-point viral load, which plays a known role in HIV pathogenesis [43]. Thus, previous findings that log cumulative viral load significantly predicts health outcomes may be driven by an association between set-point viral load and both log cumulative viral load and the health outcome, rather than a direct role of log cumulative viral load. Of the six reviewed studies that evaluate cumulative HIV-Viremia as a predictor, only one compared the predictive utility of log cumulative viral load with that of peak viral load. The study in question found that peak viral load and log cumulative viral load were highly collinear and performed equally well at predicting AIDS-defining outcomes [58].

The accuracy of a patient's measured cumulative HIV-Viremia is determined by the frequency of viral load monitoring and this is important when a patient's viral load varies substantially over time. During chronic untreated HIV infection, set-point viral load s are relatively stable [88] and accurate cumulative HIV-Viremia calculations may only require fairly infrequent measurement. Viral load trajectories are more variable during the acute, late or AIDS phases and for individuals on ART [89]. In treated individuals viral load trajectories usually remain below the detectability limit except for occasional short-lived blips that may arise from, e.g. imperfect adherence. For such patients, measurements only every few months are unlikely to identify blips and if they do, will fail to characterize their duration. Fung et al. 2012 noted the importance of sampling frequencies to studies of transient rise in viral load. cumulative HIV-Viremia, as a summary of a patient's viral load trajectory, including blips, is similarly susceptible to sampling frequency-related biases [90].

Sampling frequency also affects the interpretation of the prediction interval. The health outcome predictive accuracy of viral load and other lab measurements likely, decreases with increasing time. Since lab measurements in this cohort were made every other visit, every 24 weeks, our model explicitly allowed predictor coefficients to change with time following measurements. This enabled us to assess the change in their prognostic utility between 0-12 weeks and 12-24 weeks post-measurement. More *volatile* variables (e.g. viral load) were only statistically significant predictors of a new OI during the first interval. More stable predictors (e.g. hemoglobin, CD4 count) remained predictive for the full 24 weeks (Appendix 2.1 figure 3), with the exception of cumulative log viral load, which may reflect the difficulty in resolving its association due to the correlations identified above. We speculate volatility in viral load to arise from intrinsic viral dynamics and from the clinician's or patient's responses to measurements (e.g. increased adherence after a viral blip).

Thus, the use of cumulative HIV-Viremia as a prognostic predictor requires caution in both design and interpretation. We assume similar considerations apply in studies where cumulative HIV-Viremia is used as an outcome. Although we employed systematic strategies regarding cumulative HIV-Viremia metrics and the adjustment of covariates, our study does possess limitations. Similar to prior studies we were limited to viral load assays with lower and upper detection limits. Cumulative HIV-Viremia only approximates the true underlying cumulative HIV-Viremia as transient rise in viral load may occur at smaller temporal resolution than sampling frequency. We addressed, but did not eliminate, discrepant sampling frequency for explanatory and outcome variables. Our study also analyzed a resource-limited cohort with substantially lower CD4 counts and higher viral load at enrollment than previous cumulative HIV-Viremia analyses. Comparisons between studies should consider these differences. Kowalkowski et al(2014) focused on non-AIDS defining diseases [81] which are known to result from the accumulation of virus-induced inflammation [91]. However, aetiological mechanisms differ greatly within and between AIDS-defining and non-AIDS defining disease suggesting that the functional relationships between viral load trajectories and outcomes may be variable. Our study had a limited sample size to allow for multiple comparisons, therefore we have cautiously interpreted our results bearing that in mind.

In conclusion, we suggest that future work is necessary before deciding whether cumulative HIV-Viremia is indeed a useful prognostic measure in clinical settings. In particular, we recommend that future analyses be accompanied by simulations using within-host models of HIV replication and pathogenesis, in which the underlying parameters are known. This would help identify principles for choosing between cumulative HIV-Viremia metrics and covariate specification in cohorts with highly variable levels of left-censorship, infrequent measurements and collinear variables.

Chapter Three

Systematic review of statistically-derived models of immunological response in HIV-infected adults on antiretroviral therapy in Sub-Saharan Africa

3.1 Introduction

The successful roll out of antiretroviral therapy (ART) in Sub-Saharan Africa (SSA) has dramatically improved the survival of Human Immunodeficiency Virus (HIV) infected people in this region, which remains a focal point of the HIV epidemic [92]. In the majority of cases, the successful suppression of plasma viral load after ART initiation to below detection levels facilitates immunological recovery in the form of rising CD4 (+) T cell counts. However, 'residual viremia', involving the multiplication of the virus, within for example gut reservoirs, may continue even after circulating viral load has been suppressed [43]. As a result CD4 cell count depletion may continue in long term treatment [43,93]. Patients particularly at risk of secondary opportunistic infections include immune 'non-responders' who have low CD4 counts in spite of a suppressed viral load [43].

In resource limited settings (RLS) such as SSA, CD4 counts continue to be used for clinical decision making, e.g. when to initiate first-line, switch to second-line ART [11] and to benchmark the risk of incident clinical events [38,94]. In this region, patients who fail to reach >350 cells/μL after 5 years of ART [37] are common and ongoing immunological monitoring is necessary. CD4 count is more affordable than viral load monitoring and continues to be the only immunological biomarker recommended by the World Health Organization [10].

However, as a biomarker, CD4 counts are known to be inherently variable both within and between individuals [43,95]. Further, prior multivariate models of CD4 count response to ART have employed varying outcome measures and have consequently produced inconsistent results [25,44,51,96–100]. This variation in models complicates the effects of

inherent variation in CD4 counts and hinders the comparison of immunological responses to ART across different cohorts.

In this study we systematically review statistical, or empirically-derived rather than biological-mechanistic mathematical, models of immunological response (CD4 counts) in SSA cohorts. We highlight the similarities, differences and problems associated with the varying methodologies with the aim of defining prior knowledge, in the Bayesian sense, for prospective modeling exercises in the future.

3.2 Methods

Search Strategy

The guidelines from the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) (Appendix 3.1) [101]. The search syntax was constructed around 4 major terms, allowing for small variations within each. These included 'immune response', 'HIV antiretroviral treatment' or 'ART', 'Statistical model', and 'Sub Saharan Africa' or 'SSA'. Each term was defined based on Medical Subject Heading (MESH) terms or other common, published terminology. Online electronic databases were searched using SCOPUS© [102], from 1st January 2004 up to 2nd April 2015 (Appendix 3.2). This start date was selected as it corresponds to the commencement of ART scale-up in most of SSA [11]. Only studies published in peer-reviewed English-language journals, which existed in all four sets mentioned above were selected.

Study selection

Abstracts and full-texts of potentially relevant studies were reviewed by JBS and ELU. MN provided the deciding vote if consensus was not unanimous regarding the inclusion or exclusion of a study. Only studies of immunological response, measured as an outcome in any form, after ART initiation in adults were included. Although immune response is not

limited to CD4, our searches only returned modeling studies that employed it. Studies were excluded where: 1. there was no multivariate statistical model, 2. immune response was combined with any other treatment outcome, 3. data was analyzed that contained a combination of people from SSA with those from other regions, and 4. immune response prior to ART initiation was analyzed.

Model outcomes were categorized into 3 general groups, further sub-divided by the type of regression used:

1. the trajectory of CD4 counts within particular time-frames after ART initiation, or 'slope' models, with Generalized Estimating Equations (GEE), and Generalized Linear Mixed Effects (GLME),
2. the time to a particular immune response, or 'survival' models, with Cox Proportional Hazards (CPH) and
3. the specified overall gain in CD4 count, or 'asymptote' models, with Logistic, Simple Linear, Difference-in-Difference, Log-Binomial and Poisson regression.

Data extraction

The following data was extracted from each study: first author, year published, country, the sex/es studied, sample size, study design, ART follow-up years, initiating ART regimen (if reported), outcome/s analyzed, variable scale transformation methods, criteria for model variable/s selection (e.g. statistical methods and/or *a priori* clinical information), assessment of confounding and covariates adjusted for in the final model. For each of the final model variables, the unit and scale of measurement, effect sizes, 95% confidence intervals and, where available, standard deviations were noted. Effect sizes were rounded off to the nearest whole number and 95% confidence intervals and standard deviation to one decimal place. If

'immunologic failure' was mentioned, we checked if it was defined according to the WHO criteria [11].

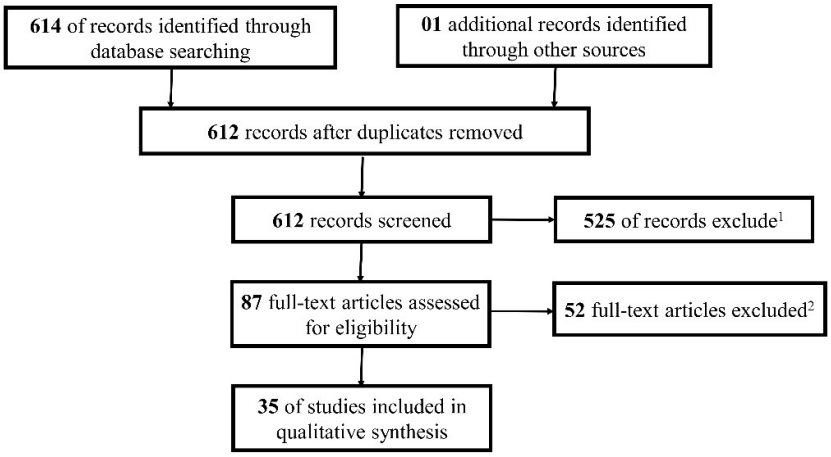
Risk of bias was also assessed in each study as follows: Low risk— covariate adjusted for in model based on its clinical/biological plausibility; medium risk—covariates included based on both biological and statistical significance; and high risk—model employed only statistical significance (p-value). The provision by authors of biological reasoning, including references, for their covariate adjustments was noted.

Statistical analysis

All data was collated in MS Excel (version 2013) and comparisons made as per the tables below. In R version 3.2.2 using package 'wordcloud' [103], variables adjusted for in the final multivariate models were presented. In wordclouds, the size and color of each word is determined by the frequency of its appearance in a list, in this case all covariates adjusted-for within a specified outcome. This enabled the comparison of variables with potentially different units and/or numeric scales. A minimal frequency cutoff of ≥3 was used to define the 'consensus' set of covariates across all models reviewed.

3.3 Results

Figure 3.1: systematic review flow chart



[1]Articles did not analyze immune response as the outcome-**490**, compared predictability of CD4 test – **35**
[2] Without immune response multivariate model - **46**, analyzed immune response in children and adults - **5**, analysis done in sero-negative patients - **1**

Of the 615 articles identified 580 were excluded based on the specified inclusion criteria (Figure 3.1). Of the remaining 35 the median sample size (and IQR) was 1002 (351-5448) with follow-up of 2 years (1-5). Across all models, 75 unique covariates were included in multivariate analysis, of which 69 were adjusted for in the final models. In the majority of cases the effect sizes of covariates were not directly comparable in view of the combination of different variables and varying scale transformations methods across models. However, the frequency of the occurrence of variables, independent of their scales, enabled the identification of a consensus set (Figure 3.2).

Figure 3.2: Wordclouds for the categorized immune response outcomes from SSA models.

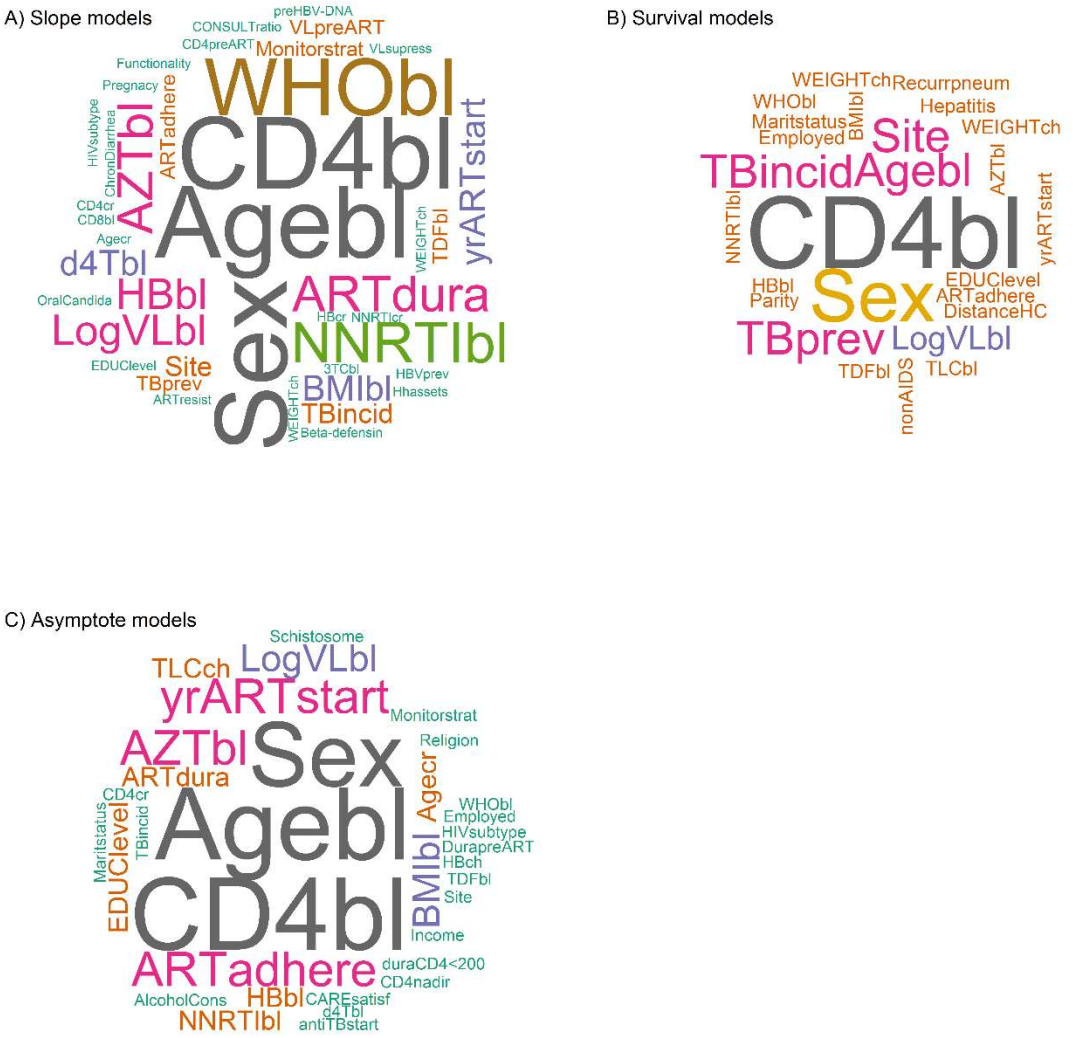


Figure 3.2A: Covariates adjusted for in the final slope models; *Figure 3.2B:* Covariates adjusted for in the final Survival models; and *figure 3.2C:* Covariates adjusted for in the final Asymptote models. The word size and color represents the frequency of covariates, hence the larger the size of the covariate, the higher its frequency in the list of adjusted covariates.

Legend: **Site** – location of the study; **KSincid** – Kaposis' sarcoma diagnosed after ART start; **HBVprev** – Hepatitis B virus diagnosed at ART start; TBprev – History of TB at ART start; **TDFbl** – treated with tenofovir at ART start; **3TCbl** – treated with lamivudine at ART start; **DistanceHC** – distance from health center; **Maritstatus** – marital status of the subject; **Season** – season of the tear when patient was initiated on ART; **ALTbl** – alanine aminotransferase at ART start; **sdNVP** – history of single does nevirapine; **Parity** – number of children; **CD8bl** – CD8 count at ART start; **CONSULTratio** – cadre levels at health center; **Hhassets** –

possession of any household assets; **OralCandida** – Oral candidiasis at ART start; **ChronDiarrhea** – Chronic diarrhea at ART start; **VLsupress** – ever had viral suppression; **NNRTIcr** – time-updated exposure to either nevirapine or efavirenz; **NRTI$_{cr}$** – time-updated exposure to **d4T$_{cr}$** (stavudine) or **AZT$_{cr}$** (zidovudine) or **TDF$_{cr}$** (tenofovir) or **3TC$_{cr}$** (lamivudine); **CD4preART** – pre-ART start CD4 count; **VLpreART** – pre-ART start viral load; **PreARTexp** – pre-ART exposure; **AlcoholCons** – consumption of alcohol; **DurapreART** – duration between ART start and diagnosis; **duraCD4<200** – duration while CD4 <200 cells/µL before ART start; and **antiTBstart** – patient initiated on anti-tuberculosis medicine. *For other variable definitions, please refer to the notes below tables 2, 3, and 4.*

For slope models this included, gender, baseline age, baseline CD4 count, baseline WHO stage, ART initiating or 'baseline' regimen, e.g. efavirenze vs nevirapine, baseline exposure to zidovudine or stavudine, ART duration, log viral load, baseline hemoglobin level, baseline Body Mass Index (BMI), year of ART start, study site and tuberculosis incidence. For survival models: baseline CD4 count, gender, baseline age and either prevalent or incident tuberculosis. For asymptote models, gender, baseline age, baseline CD4 count, baseline zidovudine exposure, year of ART start, ART adherence, log viral load and baseline BMI. Across all three types of models, Sex, Age, baseline log viral load, baseline CD4, ART initiation regimen and ART duration count were the most commonly adjusted for covariates and also those most often significantly associated with the immunological outcomes (Table 3.1).

Table 3.1: The high frequency (≥3) covariates adjusted for in multivariate models

Description	Slope models	Survival models	Asymptote models
Baseline CD4 count	13	7	9
Sex of the participants	13	5	8
Age at baseline	13	3	9
WHO stage at baseline	10	1	1
Type non-nucleoside reverse transcriptase Inhibitor (i.e efavirenze or nevirapine)	7	1	2
Initiated on zidovudine at baseline	6	1	4
Duration while on antiretroviral therapy	6	0	2
Log_{10} viral load at baseline	5	2	3
hemoglobin level at baseline	5	1	2
Calendar year of ART start	4	1	4
Body Mass Index at baseline	4	1	3
Initiated on stavudine at baseline	4	0	1
Location of treatment program or clinic	3	3	1
Incident tuberculosis diagnosis after ART start	3	3	1
History of TB at baseline	2	3	0
Antiretroviral therapy adherence	2	1	4

Notes:
'Baseline' - Refers to the measurement at ART initiation

Differences were found in the estimation of effect sizes and residuals across all 19 slope models (Table 3.2, below). Two authors reported using GEEs without additional details [96,104]. Hermans et al. 2010 used a GEE with robust standard errors and exchangeable correlation matrix [99]. Hawkins et al. 2011 applied GEE with step-wise restricted cubic splines to fit the non-linear CD4 count response [105]. Sudfeld et al. 2012 and Sudfeld et al. 2013 used GEE with restricted cubic splines and an m-dependent correlation matrix [106,107]. Hardwick et al. modeled slope of CD4 count using a GEE model with type 3 sums of squares and variance correction to correct for longitudinal CD4 count time points [108]. Boullé et al. 2013, Velen et al. 2013, Schomaker et al. 2013, Hamers et al. 2012, and Hamers et al. 2013 used GLMEs of slope of CD4 count [109–113]. Maman et al. 2012 and Reda et al. 2013 used GLME with random intercept and coefficients, while the former extended this by adding a second degree polynomial for time on ART [114,115]. Maskew et al. 2013 used GLME with

random slope and intercepts and specified an unstructured correlation matrix for repeated measures [116]. Mayanja et al. 2012 and Wandeler et al. 2013 used a GLME with functional polynomials [97,117]. Sarfo et al. 2014 used GLME with a log-link and assumed a Poisson distribution for CD4 count response [118], while De Beaudrap et al. 2009 applied a non-linear mixed effects model [119]. Vinikor et al 2014 used analysis of covariance (ANCOVA) [120].

Table 3.2: 'Slope' models of CD4 count trajectory in SSA

Authors	Location	Period	Study size	End point	Significant covariates
Hermans et al. 2010 [99]	Uganda	2003-09	5982	Mean CD4 count change from baseline	TBincid, $CD4_{bl}$, sex
Peterson et al. 2011 [104]	The Gambia	2004-09	359	Mean CD4 count change from baseline	$LogVL_{bl}$, $CD4_{bl}$, ART_{dura}
Hawkins et al. 2011 [105]	Tanzania	2004-08	12842	Mean CD4 count change between visits	Sex
Mayanja et al. 2012 [117]	Uganda	2004-09	88	Mean CD4 count response	ART_{dura}, Pregnancy and their interaction, CD4preg, TIMEpreg
Sudfeld et al. 2012 [107]	Tanzania	2006-10	875	Mean CD4 count change between visits	None reported
Hardwick et al. 2012 [108]	Ethiopia and Tanzania	No details	1002	Mean CD4 count response	Beta-defensin
Maman et al. 2012 [114]	Malawi, Uganda, Kenya	2001-09	12946	Mean CD4 count response	Sex, site, Age_{cr}, $CD4_{bl}$
Maskew et al. 2013 [116]	South Africa	2008-09	232	Mean CD4 count change from baseline	Sex, $CD4_{bl}$, Age_{curr}
Sempa et al. 2013 [96]	Uganda	2004-12	356	Mean CD4 count change from baseline	Sex, $CD4_{bl}$, log VL_{bl}, AZT_{bl}, ART_t, HB_{cr}
Boullé et al. 2013 [109]	Cameroon	2006-10	459	Mean CD4 count response	Sex, Age_{bl}, $logVL_{bl}$, ART_{dura}
Reda et al. 2013 [115]	Ethiopia	2005-10	1540	Mean CD4 count response	ART_{dura}
Sudfeld et al. 2013 [106]	Tanzania	2006-09	2145	Mean CD4 count change between visits	None reported
Velen et al. 2013 [110]	South Africa	2007-2009	6196	Mean CD4 count response	$d4T_{cr}$, AZT_{cr}, TDF_{cr}
Wandeler et al. 2013 [97]	Southern Africa	No details	72597	Mean CD4 count response	AZT_{cr}
Schomaker et al. 2013 [111]	South Africa	2003-10	15646	Mean CD4 count change between visits*	Sex, TBincid, $CD4_{bl}$, Age_{bl}, WHO_{st}
Sarfo et al. 2014 [118]	Ghana	2004-10	3990	Gains in CD4 count	$CD4_{bl}$, Age_{bl}, YrARTstart, Sex, WHO_{st}, $NRTI_{bl}$, $NNRTI_{bl}$, ART_{dura}
Vinikoor et al. 2014 [120]	Zambia	2004-10	43152	Mean CD4 count change from baseline	Age_{bl}
Hamers et al. 2012 [112]	Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe	2007-09	2439	Mean CD4 count response	ARTresist

Hamers et al. 2013 [113]	Zambia and South Africa	2007-08	1127	Mean CD4 count response	None reported
De Beaudrap et al. 2009 [119]	Senegal	1998-07	346	Mean CD4 count response**	$CD4_{bl}$, and $logVL_{bl}$

*cells/μL per 6 months; **Square root cells/μL

Note: site – study location; **Age_{bl}** – baseline age; **Age_{cr}** – current age; **WHO_{st}** – baseline WHO stage; **$Log_{10}VL_{bl}$** – baseline Log Viral Load; **$CD4_{bl}$** – baseline CD4count; **HB_{cr}** – current hemoglobin level; **YrARTstart** – year of ART start; **ART_{dura}** – duration on ART; **AZT_{bl}** exposure to zidovudine at baseline; **$NRTI_{bl}$** – exposure to **$d4T_{bl}$** (stavudine) or **$3TC_{bl}$** (lamivudine) at ART start; **$NNRTI_{bl}$** – exposure to either efavirenze or nevirapine at ART start; **ARTresist** – pre-ART drug resistance; **TBincid** – Incident tuberculosis diagnosis after ART start; **TIMEpreg** – duration between pregnancies; **CD4preg** – whether CD4 count was taken during pregnancy;

Table 3.3: 'Survival', or time-to immune response, models in SSA

Authors	Location	Period	sample size	End point * (criteria)	Significant covariates
Assefa et al. 2014 [100]	Ethiopia	2007-11	400	Time to immunologic failure (4)	Sex, $CD4_{bl}$
Kigozi et al. 2009 [121]	Uganda	2002-06	427	Time to CD4 increase ≥50 cells/μL	nonAIDS, $CD4_{bl}$, ARTadhere, TLC_{bl}
Palladino et al. 2013 [122]	Mozambique	2002-06	142	Time to immunologic failure (4)	$CD4_{bl}$, $Log_{10}VL_{bl}$
Alemu Melsew et al. 2013 [123]	Ethiopia	2007-12	509	Time to immunologic failure (4)	Recurrpneum, Employed, $WEIGHT_{ch}$, $CD4_{bl}$
Teshome et al. 2014 [124]*	Ethiopia	2004-12	268	Attain α, (4)	$CD4_{bl}$
Hawkins et al. 2011 [105]	Tanzania	2004-08	762	Time to immunologic failure (1, 2, 3, & 4)	Sex
Mudiope et al. 2013 [125]	Uganda	2003-11	289	Time to immunologic failure (4)	$CD4_{bl}$

*WHO criteria; 1) CD4 cell count falls below baseline in the absence of other concurrent infections, 2) CD4 cell count falls to less than 50% of peak levels without coexistent infections, or 3) CD4 cell count is persistently below 100 cells/μL, or 4) Any one of the 3 criteria above

Note: $Log_{10}VL_{bl}$ – baseline Log Viral Load; **$CD4_{bl}$** – baseline CD4count; **TLC_{bl}** – baseline total lymphocyte count; **ARTadhere** – Antiretroviral therapy adherence; **$WEIGHT_{ch}$** – change in weight from baseline; **Recurrpneum** – recurrent pneumonia; **Employed** – employment status; **nonAIDS** – AIDS or non-AIDS defining conditions

More similar methods were employed in Survival and Asymptote models to estimate effect sizes and their residuals. In survival models, six out of seven models estimated effect sizes using CPHs [100,105,121–123,125] and Teshome et al. 2014 used a stratified-CPH [124] (Table 3.3, above). Four variants of asymptote models, were found: 1. Factors associated with reaching a particular threshold CD4 count or not, analyzed using multivariate logistic regression [97,99,120,126–129]; 2. Overall change in CD4 count, using multivariate linear regression [98,104,130,131]; 3. Maskew et al. 2013 assumed the outcome followed a log-binomial distribution [132]; and 4. Takuva et al. 2012 assumed a Poisson distributed with robust standard errors [133], see Table 3.4, below.

Table 3.4: 'Asymptote' models in SSA

Authors	Location	Period	sample size	End point	Significant covariates
Anude et al. 2013 [126]	Nigeria	2008-09	596	CD4 count increase ≥50 cells/μL	Sex, Age_{bl},
Efraim et al. 2013 [129]	Tanzania	2009-11	351	Attain $^{\alpha, (1, 3)}$	Schistosome, BMI_{bl}, $CD4_{bl}$, EDUClevel
Hermans et al. 2010 [99]	Uganda	2003-09	5982	Attain $^{\alpha, (4)}$	TBincid, $CD4_{bl}$, AZT_{bl}
Diabaté et al. 2009 [127]	Ivory coast	2005	303	CD4 count increase ≥50 cells/μL	ARTadhere, TLC_{ch}
Wandeler et al. 2013 [97]	South Africa, Botswana, Zambia, and Lesotho	No details	14529	Attain $^{\alpha, (4)}$	AZT_{cr}, sex, Age_{bl}, $CD4_{bl}$, HB_{bl}, YrARTstart, Monitorstrat
Maskew et al. 2013 [132]	South Africa	2001-08	8676	CD4 count increase ≥50 cells/μL or ≥100 cells/μL	None reported
Nglazi et al. 2011 [128]	South Africa	2002-08	3162	CD4 ≤200 cells/μL at week 48	Sex, Age_{bl}, $CD4_{bl}$, VL_{bl}
Vinikoor et al. 2014 [120]	Zambia	2004-10	43152	Attain CD4 count ≥350 cells/μL	Age_{bl}
McKinnon et al. 2010 [131]	Kenya	2005-11	60	Overall change in CD4 count	CD4nadir
Alemu et al. 2012 [130]	Ethiopia	2009-10	1722	Overall change in CD4 count	Depression, SOCIALsup
Crawford et al. 2015 [98]	Uganda	2011	325	Overall increase in CD4 count	$CD4_{cr}$, ART_{dura}, Age_{bl}, CAREsatisf, and TLC_{ch} HB_{ch}
Peterson et al. 2011 [104]	The Gambia	2004-09	359	Overall increase in CD4 count	HIVsubtype, ART_{dura}, and their interaction
Takuva et al. 2012 [133]	South Africa	2004-09	1499	CD4 count increase ≥50 cells/μL	None reported

* Case-control study; $^{\alpha}$WHO criteria;
[1].CD4 cell count falls below baseline in the absence of other concurrent infections, or
[2].CD4 cell count falls to less than 50% of peak levels without coexistent infections, or
[3].CD4 cell count is persistently below 100 cells/μL, or
[4] Any one of the criteria above

Note: **Age_{bl}** – baseline age; **BMI_{bl}** – Body Mass Index; **EDUClevel** – level of education; **$CD4_{bl}$** – baseline CD4count; **$CD4_{cr}$** – current/most recent CD4 count; **HB_{bl}** – hemoglobin level at ART start; **HB_{ch}** – change in hemoglobin; **YrARTstart** – year of ART start; **ART_{dura}** – duration on ART; **AZT_{bl}** – exposure to zidovudine at baseline; **AZT_{cr}** – current exposure to zidovudine; **TBincid** – incident tuberculosis; **TLC_{ch}** – change in total lymphocyte count; **Monitorstrat** – monitoring strategy (clinical or immunological or virological); **Depression** – symptoms depression while on ART; **SOCIALsup** – perceived social support; **CAREsatisf** – patient satisfaction with care; **CD4nadir** – nadir CD4 count; **HIVsubtype** – HIV-1 subtype

For criteria used to select covariates for final multivariate models and assessment for confounding (table 3.5 below), 9 studies reported using significance cutoffs ranging from 0.05 to 0.25, and biological plausibility, i.e. the causal association between the immune response and the covariate, to generate this list of covariates [96,99,107,109,121,124–126]. Three authors used only statistical significance (p-values) as a basis for covariate selection in multivariate analysis [112,113,127]. Four of the above 12 studies employed step-wise regression [99,112,113,126], and two used step-wise regression and 'prior' reasoning to arrive at their final multivariate model [106,109]. Mayanja et al. 2012 listed model assumptions based on biological CD4 dynamics [117] and Sudfeld et al 2013 referred to prior studies [106]. Only two studies assessed covariates for confounding [108,116].

Table 3.5: Summary of different multivariate immune response modeling methods in SSA

Author	Criteria for selecting variables into the multivariate model			How they arrived at the Final model		Confounding
	Biological plausibility	Cutoff used	Cutoff	Stepwise selection only	Step-wise and a priori	Assessed confounding
Anude et al. 2013 [126]	✓	✓	0.20	✓	0	0
Assefa et al. 2014 [100]	✓	0		0	0	0
Efraim et al. 2013 [129]	✓	0		✓	0	0
Hermans et al. 2010 [99]	✓	✓	0.20	✓	0	0
Kigozi et al. 2009 [121]	✓	✓	0.05	0	0	0
Maman et al. 2012 [114]	✓	0		0	0	0
Maskew et al. 2013 [132]	✓	0		0	0	0
Maskew et al. 2013 [116]	✓	0		0	✓	✓
McKinnon et al. 2010 [131]	✓	0		✓	0	0
Palladino et al. 2013 [122]	0	0		0	0	0
Reda et al. 2013 [115]	✓	0		0	0	0
Sempa et al. 2013 [96]	✓	✓	0.20	0	0	0
Sudfeld et al. 2012 [107]	✓	✓	0.20	0	✓	0
Teshome et al. 2014 [124]	✓	✓	0.05	0	0	0
Velen et al. 2013 [110]	✓	0		0	0	0
Alemu Melsew et al. 2013 [123]	0	0		0	0	0
Alemu et al. 2012 [130]	0	0		0	0	0
Boullé et al. 2013 [109]	✓	✓	0.25	0	✓	0
Crawford et al. 2015 [98]	0	0		✓	0	0
Diabaté et al. 2009 [127]	0	✓	0.25	0	0	0
Hamers et al. 2012 [112]	0	✓	0.10	✓	0	0
Hamers et al. 2013 [113]	0	✓	0.15	✓	0	0
Hardwick et al. 2012 [108]	✓	0		0	✓	✓
Hawkins et al. 2011 [105]	✓	✓	0.20	0	0	0
Mayanja et al. 2012 [117]	✓	0		0	0	0
Mudiope et al. 2013 [125]	✓	✓	0.20	0	0	0
Peterson et al. 2011 [104]	✓	0		0	0	0
Sarfo et al. 2014 [118]	✓	0		0	0	0
Sudfeld et al. 2013 [106]	✓	0		0	0	0
Vinikoor et al. 2014 [120]	✓	0		0	0	0
Wandeler et al. 2013 [97]	✓	0		0	0	0
Nglazi et al. 2011 [128]	✓	0		0	0	0
Takuva et al. 2012 [133]	0	0		0	0	0
Schomaker et al. 2013 [111]	✓	0		0	0	0
De Beaudrap et al. 2009 [119]	✓	0		✓	0	0

3.4 Discussion

This study systematically reviewed recent statistical or empirically-defined models of CD4 count response in HIV-infected adults on ART in SSA. The aim was to arrive at a set of model covariates and outcomes that might allow the comparison of modeling results between cohorts. From the studies reviewed, Sex, Age, baseline log viral load, baseline CD4, ART initiation regimen and ART duration were the most commonly adjusted covariates and also those most often significantly associated with the different metrics of immune response across all models reviewed. Many permutations were found, in fact, the majority of the models were different with respect to variable transformations and scales, varying model assumptions, modeling strategies, model reporting methods and the use of different covariates, even if the same outcomes had been studied. In particular:

In the CPH models studied, authors did not adjust for time-updated variables [121–123,125]. It was assumed that patients remained on their initiation regimen throughout the period of follow-up. It is known from studies of ART regimen durability and tolerability that drug toxicity will often occur in the period soon after initiation, necessitating drug substitutions [134,135]. Such switches are obviously important in understanding CD4 responses, particularly if more potent drugs are subsequently employed. 'Joint' time-to-event and longitudinal (or repeated) measure models may be used for time-updated covariates, in which a 2-phase process involves combining the model/s of the endogenous longitudinal covariate/s with a CPH model [136].

All seven studies which analyzed time to immunological failure did so for only the time to the first failure episode [100,121–124]. However, it has been conjectured that multiple failures may actually occur and be hidden by the normal variability seen in adult CD4 counts [95]. CPH models are not appropriate for multiple failure-time points since the outcome

terminates after the first event. Further, the assumption of the independence of outcomes is violated since events within an individual are correlated [137]. Corrections to such models for correlated failure time points have been implemented in the form of Andersen-Gill, Marginal Wei-Lin-Weissfeld or Prentice-Williams-Peterson methods [77,137]. If multiple episodes of immunologic failure are present, as defined by the WHO criteria, then the Andersen-Gill method would appear to be a good choice [77].

In selecting regression methods, considerations regarding covariate distributions and the mathematical assumptions regarding their relationship/s with the outcome are important. These assumptions can be tested *a priori* using the dataset at hand. Only 4 of the studies reviewed indicated that such tests had been used to confirm that the particular covariates fulfilled the model assumptions [98,106,115,124]. If the assumptions are violated it is not possible to estimate the effect of the covariates on the outcome with both precision and accuracy [138].

Six studies used GEEs to model the slope of the CD4 count response. Three defined the outcome as the change in CD4 count from baseline, i.e. from ART initiation [96,99,104] and the others used the change in CD4 count between each subsequent visit [105–107]. Of the 11 studies that used GLMEs, 2 used the increase from the baseline as outcome [115,116], one used change in CD4 count between subsequent visits [110] and 8 used absolute change in CD4 counts over time [97,108,109,111–114,117]. Only one study used non-linear mixed effects regression [119]. Selecting either GEE - population averaged effects, or GLMM - individual averaged effects, is possible using tests of assumptions regarding the underlying mechanisms of CD4 count response [139]. CD4 counts vary due to both individual patient characteristics and laboratory procedures [140–142]. Given the individual effects, GLMEs may be preferable to GEEs in this context. Non-Linear Mixed Effect models (NLMEs) may also be used since they take into consideration mechanistic biological assumptions and both the underlying

subject-specific longitudinal responses (CD4) and the variation of these across the study group over time [139].

Sarfo et al 2014 [118] modelled CD4 count response using a GLMM with a Poisson distribution. Baseline CD4 counts, being female, increasing ART duration and baseline WHO stage (stage 1 and stage 2) where associated with increasing CD4 counts, while initiating ART on efavirenz and zidovudine based regimens and higher baseline age were associated with decreasing CD4 counts. These results apparently support prior studies [43,45]. However, the incident rate ratios (IRR) in their final model were close to null. The Poisson distribution assumption may have biased the results towards the null and presumably explains their rounding off IRR to 3 decimal places of [118]. The Poisson model assumes that the probability of the occurrence of any two events $p(x \cap y)$ is negligible, and the probability of the occurrence of an event $p(x)$ is constant throughout the interval, Δt. In [118] the sampling frequency for CD4 count was 6 monthly, thus, the probability of having another CD4 count measurement was never negligible. Further, the probability of increasing CD4 counts throughout the sampling interval is variable due to adherence, opportunistic infections, and drug resistance [90].

There was also variation in approaches to adjustment for confounding between covariates. Confounding usually refers to a ≥10% change in the coefficient estimate of the main predictor after adjusting for the effect of a covariate [138]. It does not relate to the significance of the p-values for covariates in the model. Four studies did not report the criteria used to select covariates to be adjusted for in the multivariate models [122,123,130,133]. In others [119,121,124,129], covariates were excluded from the final model since they were not statistically significant. This practice may exacerbate confounding [143,144]. Directed Acyclic Graphs (DAGs) can be used as a non-statistical modeling strategy for multivariate analysis [145]. Such causal diagrams, which are based on clinical or biological assumptions, are useful

for deciding on the minimal set of covariates to adjust for (we discuss this further in appendix 3.3 using the model described in Chapter 2). Some studies [100,123,125,126,129] did not adjust for covariates, such as age and baseline CD4 count, even if appropriate data had been collected. Prior reviews by Pinzone et al 2012 [43] and Corbeau et al. 2011 [45] have shown that both baseline age and baseline CD4 count are associated with immunological response to ART.

Covariate scale transformations were reported to have been assessed in only five studies [97,107,108,115,117]. Others, report a square root transformation of CD4 counts [97,115,119]. Variable transformations are obviously important in meeting the distributional assumptions of the model/s [138]. Reda et al. [115] investigated a wide range of variable transformations for all variables in their model, while Sudfeld et al 2012 [107] transformed only the main predictor—Vitamin D levels. Other studies employed polynomial transformations of time on treatment [97,108,117] or regression splines on time [105–107,111]. Graphical inspection of the effect of covariate transformation are possible prior to modelling, while statistical tests such as Akaike's Information Criterion (AIC) and the Bayesian Information Criterion (BIC) are useful afterwards [146]. It is also possible to apply Martingale residuals [77] for CPHs. Caution is always required in variable transformation since, for example, categorizing continuous variables may result in residual confounding [147,148]. Further, the interpretation or translation of results into practice becomes problematic as it is no longer direct.

In terms of model validation, only 5 out of 34 studies provided goodness of fit metrics. These included the AIC [98], Hosmer-Lemeshow test [126], and the Log-likelihood ratio [114,115,128] goodness of fit tests. Other possible techniques include cross-validation, i.e. regressing the model on the training dataset to see if it still predicts the outcome, and graphical methods, i.e. analyzing whether model residuals are random by plotting predicted versus observed values. Without such validations there is a risk of overfitting to data [138].

Similarly, the dissemination of results also has a bearing on the comparability of models. Six studies reported only p-values without beta coefficients or confidence intervals [105–108,129,131] and two studies reported only model coefficients and p-values [117,129]. Ideally, both model coefficients and confidence intervals should be reported. Significant p-values continue to be commonly employed in modeling practice, but these do not indicate clinical significance nor the precision of parameter estimates [149].

Criticism of routine CD4 monitoring in ART has occurred due to the innate biological variation in these counts [95]. However, the value of such criticism seems questionable when it is presented in the absence of suggestions for alternatives, particularly given the fact that HIV is a disease which targets the immune system. Arguably, the limitation of immunological monitoring to only CD4, particularly in SSA, has been based more on considerations of public-health affordability than individual patient welfare. Alternative biomarkers, though considered as indirect immune markers [93], have existed for some time, including among others: Natural Killer (NK) cells, which secrete interferon activating macrophages, which in turn feed off infected and stressed cells and Plasmacytoid Dendritic Cells, which secret type-1 antiviral interferons [93]; β-defensins, which aid in the production of NK cells have also been associated with immunologic response [108,150]; and Co-stimulatory CD28 or co-inhibitory cytotoxic T-lymphocyte antigen 4 proteins, which are expressed by all T-cells in HIV infected people [43,93,151]. The possibility obviously exists to use a combination of CD4 and alternative biomarkers to provide a robust description of the immune system in ART.

This study has limitations. Publication bias may be present in view of the inclusion of only studies published in peer reviewed journals. While specified in the inclusion criteria, only statistical or empirically-derived models were reviewed. This excluded those originating

in mechanistic biological theory but did include those expressly incorporating assumptions regarding biological causality. All data collected regarding the models was contingent on the information provided in each study, and based on the assumption that these models should be reproducible using other similar datasets. In comparing the frequency of variables across models, we used a threshold of ≥3 which may have excluded 'rare' covariates in SSA cohorts. Only a small number of studies analyzed covariates on comparably transformed or untransformed scales. This negated the possibility of a meta-analysis, i.e. direct quantitative comparisons, since the models adjusted for varying sets of covariates. This situation may be understandable in terms of the facts that certain studies aimed at elucidating particular treatment effects, and that authors are incentivized to publish unique results.

In conclusion, for purposes of comparing immunological, i.e. CD4 count, outcomes across cohorts in SSA, statistical models would benefit from the application of more uniform modelling techniques. The value of the historic models to public health in SSA is questionable since the modeling was apparently performed in the absence of *a priori* comparisons across studies. That is, since such efforts have produced results that are anecdotal to individual cohorts only. However, this study was able to define 'prior' knowledge, in the Bayesian sense. Qualitative and semi-quantitative, rather than quantitative and completely comparable effect sizes, for variables in models of immunological response to ART were defined. Such information has value in terms of prospective modeling efforts in the future.

Chapter Four

A Bayesian interpretation of immune response to antiretroviral therapy in resource limited South African settings using cumulative HIV log Viral Load

4.1 Background

This chapter uses the defined 'slope' and 'asymptote' models as described in chapter 3. Reproducible methods for model building are used to define immune response models which are then prospectively employed on the data of an as yet undescribed HIV-cohort from South Africa. Linear mixed models are employed with specified parameter distributions (qualitative effect sizes) and estimated informative priors (semi-quantitative effect sizes) from the reviewed models. We also adjust for some of the covariates listed in table 3.1. We use the definition of transformed cumulative log viral load (i.e. cumulative log viral load), from Chapter 2, to define and adjust for this variable in our analysis.

4.2 Introduction

The roll out of effective combination antiretroviral therapy (ART) has markedly improved the survival of sub-Saharan African (SSA) Human Immunodeficiency Virus (HIV)-infected populations [6]. However, resource-poor settings have been characterized by late initiation of ART [14] and limited ongoing immunologic or virologic monitoring [152,153]. Since thymic CD4+ T-cell production declines with age [154], HIV-infected people are likely to have difficulty in recovering the CD4+ T-cell quantities destroyed by the direct and indirect effects of chronic viral infection [155]. Further, in part due to its recent roll-out in SSA, the long term immunological consequences of ART remain incompletely understood [43,93,156].

Statistical modelling of the relationship of HIV viral load to CD4 counts holds challenges [145]. Mechanistic models, which make particular assumptions regarding underlying biological processes, have mostly been used for this purpose [157]. In a previous

review of statistical models of immune response to ART in SSA we revealed an immense diversity in the methodologies employed [2]. Previous studies demonstrated that the distribution of CD4 counts is not normally distributed, i.e. right-skewed [140–142]. Despite this, assumptions of Gaussian normality were common [2]. This may in part have been due to the applied software requiring this assumption. Bayesian methods in combination with Markov Chain Monte Carlo (MCMC) computational techniques allow for more flexibility than Frequentist estimation methods, for example, alternative distributions to Gaussian normals. In addition, prior information can be included into the model and posterior outputs provide direct statements regarding the unknown model parameters. For a general overview of the Bayesian methodology please refer to Lesaffre and Lawson 2012 [149] and Gelman 2014 [158].

Cumulative HIV-1 Log viral load (cumulative log viral load), is a readily definable predictor [1]. It involves the numerical summation of the area under the log viral load curve above the viral load detection limit. It can be used as an indicator of long-term *in vivo* exposure to a detectable virus. We previously found that cumulative log viral load was associated with mortality [1]. Few studies have investigated it as a covariate associated with immune response. Marconi et al 2011 [60] used log cumulative viral load (i.e. log of total area under the viral load) as a main-predictor of immune response in the data of a North American cohort. However, the methods they employed have statistical weaknesses [1] and the relevance of their findings to resource-constrained SSA settings may be limited. Cumulative log viral load has not previously been used as a covariate in statistical models of immune response to ART.

In this study we define statistical models of immune response on ART based on a consensus of those previously systematically reviewed [2]. We employ Bayesian MCMC methods to examine the effect of non-standard distributions on the random part of the models. We then prospectively apply these models to the data of an as yet unpublished SSA ART cohort and simultaneously determine if cumulative log viral load is an important covariate. This study

is an effort to encourage reproducible modelling practices which have been lacking to date in historical models, as previously described [2].

4.3 Methods

Ethics statement

This study was approved by the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria (13/2010) and the Tshwane Metsweding Region Research Committee (TMREC 2011/05).

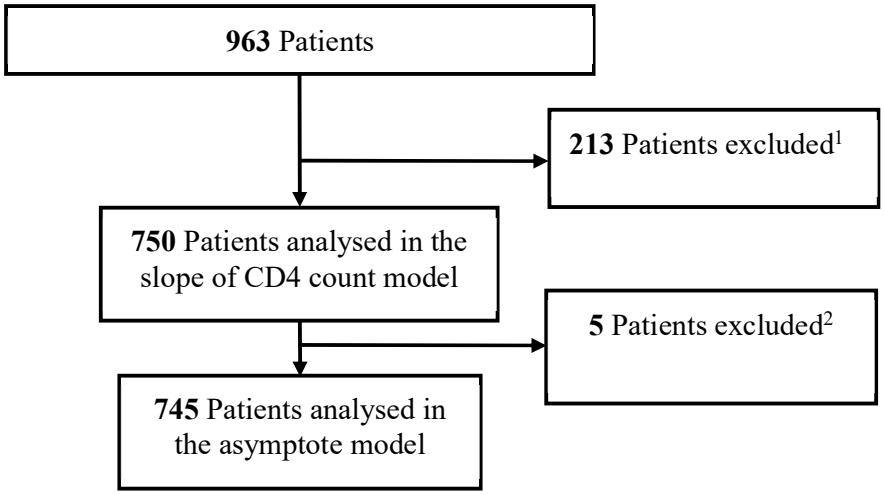
Study setting and patient population

The comprehensive demographic and long-term treatment data of the first, consecutive, 963 patients older than 18 years of age who presented for ART at the Tshwane District Hospital in Gauteng, South Africa during 2004 and 2005 were selected for analysis. All patients had started HAART after 2004 as part of the South African national HIV treatment plan and were treated according to the National Department of Health HIV guidelines (2004) operative during this time, i.e. eligibility for ART was CD4 <200 cells/μL or WHO stage 4 disease regardless of CD4 count. Treatment was initiated using a standardized triple-drug regimen consisting of two nucleoside reverse transcriptase inhibitors (mostly d4T and 3TC) and one non-nucleoside reverse transcriptase inhibitor (either NVP or EFV). CD4 and HIV-1 viral load monitoring was performed at baseline and then 6-monthly, according to the national protocol. Demographic, anthropometric, clinical, ART and 5 year longitudinal treatment response data were collected. We excluded all second-line ART visits for all patients who were switched to second-line therapy.

Data collection and inclusion criteria

Data was collated in Microsoft Excel spreadsheets. Of an initial 963 patients, 213 patients were excluded as they only had a baseline visit, which prevents the calculation of cumulative viral load. Of the 750 analysed, 59 patients had missing data on either sex, baseline CD4 count or baseline log viral load and model outcomes. Missing data for baseline CD4 count and baseline log viral load was imputed through a linear regression of baseline age, sex, while sex was imputed through a logistic regression of baseline age, baseline CD4 count and baseline log viral load. Missing data on the outcomes in the Bayesian approach is imputed directly in each model iteration [149] (see also Appendix 4.2). We analysed data of 750 patients for the slope model, and 745 in the asymptote model as 5 patients had a CD4 count ≥500 cells/μL at baseline (Figure 4.1).

Figure 4.1: Data selection diagram



[1] Patients had only one visit, at baseline

[2] Patients had a baseline CD4 count ≥500 cells/μL

Statistical methods

Models

Two types of statistical models were investigated:

Model 1: 'Slope model', the mean annual increase in CD4 counts, and the dependent variable was the absolute CD4 count at each 6 monthly visit following initiation of ART, and

Model 2: 'Asymptote model', the odds of having a CD4 count ≥500 cells/μL, where the dependent variable was coded '1' if at a particular visit a patient had a CD4 count ≥500 cells/μL, and 0 if they did not. Patients were not excluded after they had a CD4 count ≥500 cells/μL. This value was chosen as it is the described lower end of the healthy reference range of CD4 count for the SSA population at the time [159]. Previous studies have shown that patients with CD4 count ≥500 cells/μL have an almost equal life expectancy to the general population [44,160]. For both types of models we also investigated the utility of cumulative log viral load as an important covariate in immune response models.

The detection limit for the viral load assay used in this cohort was 50 copies/mL. However, time-updated log viral load was used to calculate cumulative log viral load, using a detection threshold of 400 copies/mL as reported in our initial publication [1]. This enabled us to exclude episodes of transient viremia [161], and since 1000 copies/mL had no detectable quantitative change in the results [1].

Cumulative log viral load, measured as copy-yr/mL, for the *i*-th patient at their *j*-th visit in the cohort is given by,

$$cVL_{2ij} = \sum_{k=2}^{k=j} \left(t_{i,k} - t_{i,k-1}\right) \times \frac{\left(log_{10}\left(\frac{V_{i,k}}{400}\right) + log_{10}\left(\frac{V_{i,k-1}}{400}\right)\right)}{2} \qquad (4.1)$$

where $t_{i,k}$ and $V_{i,k}$ represent the time (in years) and log viral load measurement of the k-th visit, respectively. Since the baseline viral load is measured before starting ART, cumulative log

viral load at that visit was set to 0. Other covariates adjusted for in the model include baseline CD4 count and viral load, sex, baseline age, and time on treatment as per consensus of the previously reviewed models [2].

We used linear mixed effects models and incorporated random intercept and slope as absolute CD4 counts vary between and within patients [43,162]. Further, CD4 measurements taken further apart in time were less correlated than those closely adjacent. Nash et al 2008 [44] demonstrated that CD4 counts on treatment reach a peak at approximately 2.5 years following initiation in patients with <200 cells/μL, which represents 91.7% of the current cohort, and then declines after 3.5 years. Following this trend, our slope models (*equation 4.2*) were defined as second-degree polynomials. These are denoted as 'crude' or $M_{1.10}$ when they are not corrected for cumulative log viral load and 'adjusted' or $M_{1.20}$, when they are:

$$\begin{cases} \boldsymbol{y}_i = \boldsymbol{x}_i\,\boldsymbol{\beta} + \boldsymbol{z}_i\,\boldsymbol{b}_i + \boldsymbol{\varepsilon}_i\,, \\ \boldsymbol{b}_i \sim \boldsymbol{N}(0, \Sigma), \qquad \boldsymbol{\varepsilon}_i \sim \boldsymbol{N}(0, \sigma_\varepsilon^2), \end{cases} \tag{4.2}$$

for $i = (1, \dots, n)$ patients and $\boldsymbol{y}_i = \left(y_{i1}, y_{i2}\,, \dots, y_{im_i}\right)$ $m_i\ X\ 1$ matrix of response variable i.e. absolute CD4 count is normally distributed '$\boldsymbol{N}$' such that $\boldsymbol{y}_i \sim \boldsymbol{N}(x_i\,\beta + z_i\,b_i, \sigma^2)$ with variance σ^2. $\boldsymbol{x}_i = \left(x_{i1}^T, x_{i2}^T, \dots, x_{im_i}^T\right)^T$ is a $(m_i\ X\ (p+1))$ matrix of p fixed effects including sex (0 for males and 1 for females), baseline age, baseline CD4 count, baseline log viral load, time on treatment, and cumulative log viral load. $\boldsymbol{\beta} = (\beta_1, \beta_2, \dots, \beta_p)^T$ is $((p+1)\ X\ 1)$ matrix of unknown fixed regression coefficients. $\boldsymbol{z}_i = \left(z_{i1}^T, z_{i2}^T, \dots, z_{im_i}^T\right)^T$ is a $(m_i\ X\ q)$ design matrix for the *i-th* $(q\ X\ 2)$ vector of $\boldsymbol{b}_i = (b_{i0}, b_{i1})$ random effects for the intercept and slope on time on treatment). We assume that $\boldsymbol{b}_i$ have zero-mean with bivariate Gaussian normal distribution and that Σ is a $(q\ X\ q)$ covariance matrix. The measurement error $\boldsymbol{\varepsilon}_i = \left(\varepsilon_{i1}, \varepsilon_{i2}, \dots, \varepsilon_{im_i}\right)^T$ is

$(m_i\ X\ 1)$ matrix with mean zero and variance σ_ε^2 and are assumed to be independent of the random effects.

An alternative implementation involved replacing the second-degree polynomial in *equation 4.2* with a cubic B-spline with 3 inner knots at (-0.001, 1.230, 2.5, 3.751, 5.001), and also 5 inner knots (-0.001, 0.833, 1.666, 2.5, 3.334, 4.167, 5.001) (see spline models in Appendix 2). This model assumes that the measurement errors and random effects are normally distributed. However, this is not usually the case in HIV populations as CD4 count distribution and response to treatment is usually skew-normal distributed (see for example Fig 4.4 below). Skew-normal distributions are a class of normal probability distributions that are extended to have a skewness shape parameter, or dispersion from the mean [163]. To address this we proceeded as follows:

a. We changed the CD4 count distribution from a Gaussian normal to a Student's-*t* with 3 degrees of freedom. The latter has longer tails and will sample values that fall further from the mean [149], e.g. $CD4\ count \sim dt(x_i\ \beta + z_i\ b_i, \sigma^2, 3)$. While doing so we assumed that model errors have Student's *t*-distributions, but the random-effects were still Gaussian normal.

b. We assumed that only the random-effects had a skew-normal distribution '$\boldsymbol{SN}$' [163,164], i.e. $\boldsymbol{b}_i \sim \boldsymbol{SN}_q(0, \Sigma, \Delta_b)$, where Δ_b is the dispersion from normality in the random-effects, and Σ the covariance matrix;

c. We assumed skew-normal distributions '$\boldsymbol{SN}$' for: CD4 count measurement error $\boldsymbol{\varepsilon_i} \sim \boldsymbol{SN}_p\ (0, R, \Delta_{\varepsilon_i})$, where Δ_{ε_i} is the dispersion term in the CD4 count measurement error and R the covariance matrix; and random-effects such that $\boldsymbol{y}_i|\boldsymbol{b}_i \sim \boldsymbol{SN}_p(\boldsymbol{x}_i\ \boldsymbol{\beta} + \boldsymbol{z}_i\boldsymbol{b}_i, R, \Delta_{\varepsilon_i})$.

The asymptote models (*equation 4.3*) were also defined as second-degree polynomials, and all variables used in the slope model were also adjusted for. The 'crude' model is denoted by $M_{2.10}$ and the 'adjusted' by $M_{2.20}$.

$$\begin{cases} logit(\pi_{ij}) = x_i\,\beta + z_i\,b_i + \varepsilon_i\,, \\ b_i \sim N_i(\mu,\tau), \qquad \varepsilon_i \sim N(0,\sigma_\varepsilon^2), \end{cases} \tag{4.3}$$

We assume that response y_{ij} , is 0 if CD4 count <500 cells/μL and 1 if CD4 count ≥500 cells/μL for patient '*i*' at visit '*j*', and follows a Bernoulli distribution, $bern(\pi_{ij}) = x_i\,\beta + z_i\,b_i$. All remaining variables are defined in the same way as in *equation 4.2*. Sensitivity analysis was performed in the same way as for the slope models, but we excluded *option c*.

Refer to Appendix 4.2 for the OpenBUGS model implementations.

Prior distributions

Let $\phi = \{\beta, \Sigma, \tau\}$ be the unknown parameters in the models 2 and 3. Their vague prior distributions are specified as follows: $\beta \sim \boldsymbol{N}(\beta_K, \sigma_K^2)$ for '*K*' number of covariates, and both τ and Σ had $\boldsymbol{W}(R,k)$, where the independent Gaussian normal (***N***) and Wishart (***W***) vague prior distributions were used to facilitate computation. We assumed *R* to be diagonal matrices.

We also investigated the effect of using informative priors for female-sex, baseline age, baseline log viral load and polynomial terms on model outputs. These were estimated from historical models [109,114,115] as previously discussed [2]. Variance s^2 was calculated from $\bar{x} = 1.96 * \sqrt{\left(s^2/n\right)}$, where $\bar{x}$ is the mean or effect size and n the study size. We assumed that the variance of CD4 count recovery in both female and male was the same. Variance for female-gender, however, is not available in historical models. In the asymptote models,

historical studies used different CD4 count thresholds, meaning that informative priors were not possible. Refer to Appendix 4.2, Table 1.

Data analysis

R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria, https://cran.r-project.org/) and OpenBUGS (http://www.openbugs.net) were used. With the exception of time on treatment, all continuous covariates were standardized to have mean zero and standard deviation equal to 1. In both models we employed 3 MCMC chains. For the second degree polynomial slope of CD4 count models, we used a burn-in of 20k, followed by a further 45k iterations. For other variations of the slope and asymptote models, we used a burn-in of 80k followed by 100k iterations. The skew-normal random-effects asymptote model had a 250k burn-in followed by 250k iterations. To confirm convergence the Brooks-Gelman-Rubin (BGR) diagnostic was used.

Model selection employed the Deviance Information Criterion (DIC) [165]. Better models have smaller DICs but should differ with at least 5. Parameter means and 95% credible intervals are reported.

4.4 Results

Descriptive results

In the data of the 750 patients analysed, 69.6% (n = 522) were female, with a median baseline age of 36 years (IQR: 31, 42). Patients had a median baseline CD4 count of 89 cells/μL (IQR: 43, 143) and median baseline log viral load of 5.1 $\log_{10}$ copies/mL (IQR: 4.64, 5.48). In these patients, the overall increase in cumulative log viral load was 0.3 $\log_{10}$ copy-yr/mL (IQR: 0.30, 0.43). Patients who were initiated on ART at baseline CD4 counts >200 cells/μL (n = 43) experienced a 278 cells/μL median increase starting at 261 cells/μL (IQR: 219, 298) ending at

529 cells/μL (IQR: 420, 633). Those with CD4 counts 101-200 cells/μL (n = 291) at baseline had a 241 cells/μL median increase from 143 cells/μL (IQR: 120, 173) to 386 cells/μL (IQR: 253, 525) at the end of follow-up. Patients with ≤100 cells/μL (n = 416) at baseline had a 270 cells/μL median increase in CD4 counts from 47 cells/μL (IQR: 20, 72) to 317 cells/μL (IQR: 188, 496). The 213 patients excluded from the analysis were most likely to have a higher median baseline log viral load (5.3 $\log_{10}$ copies/mL (IQR; 4.76, 5.54) vs 5.07 $\log_{10}$ copies/mL (IQR: 4.64, 5.48), p = 0.003) and older (39years (IQR: 34, 47) vs 36 years (IQR: 31, 42), p<0.001)

Patients with baseline log viral load >5 $\log_{10}$ copies/mL (n = 411) experienced a CD4 count median increase of 268 cells/μL during follow-up, from 78 cells/μL (IQR: 41, 131) to 367 cells/μL (IQR: 232, 515), while those with ≤5 $\log_{10}$ copies/mL (n = 339) had a 237 cells/μL increase in CD4 counts from 103 cells/μL (IQR: 47, 161) to 365 cells/μL (IQR: 226, 512). The increase in cumulative log viral load by baseline CD4 count category was 0.6 $\log_{10}$ copy-yr/mL (IQR: 0.44, 0.82), 0.7 $\log_{10}$ copy-yr/mL (IQR: 0.50, 0.90), and 0.7 $\log_{10}$ copy-yr/mL (IQR: 0.55, 1.28) for patients with baseline CD4 count >200, 101-200, and ≤100 cells/μL, respectively. Only 33.8% of the patients, in the data used in the asymptote model, ever reached a CD4 count ≥500 cells/μL.

In bivariate analysis, in the data used for the slope models, patients with baseline CD4 count >200 cells/μL, and those with 101 – 200 cells/μL had greater increases in CD4 counts than those with ≤100 cells/μL. Those with >200 cells/μL and 101 – 200 cells/μL had 247 cells/μL (95%CI: 221.0, 272.7), and 101 cells/μL (95%CI: 87.9, 112.7) increases, respectively, compared to ≤100 cells/μL. Females had an increase of 31 cells/μL (95%CI: 19.6, 52.7) greater than males. Patients with a baseline log viral load >5 $\log_{10}$ copies/mL experienced an annual decrease in CD4 count of 16 cells/μL (95%CI: 30.8, 0.1) compared to those with ≤5 $\log_{10}$ copies/mL during follow-up. There was no statistically significant difference in CD4 count

increase by age categories, i.e. <50 years and ≥50 years, during follow-up. Only baseline CD4 count and sex were significantly associated with having CD4 counts ≥500 cells/μL in the bivariate analysis of the asymptote model. There was a weak correlation, ranging from -0.41 to 0.01, between cumulative log viral load and other covariates in both slope of CD4 counts and asymptote models (Appendix 4.1, Figure 1).

Slope of CD4 count models

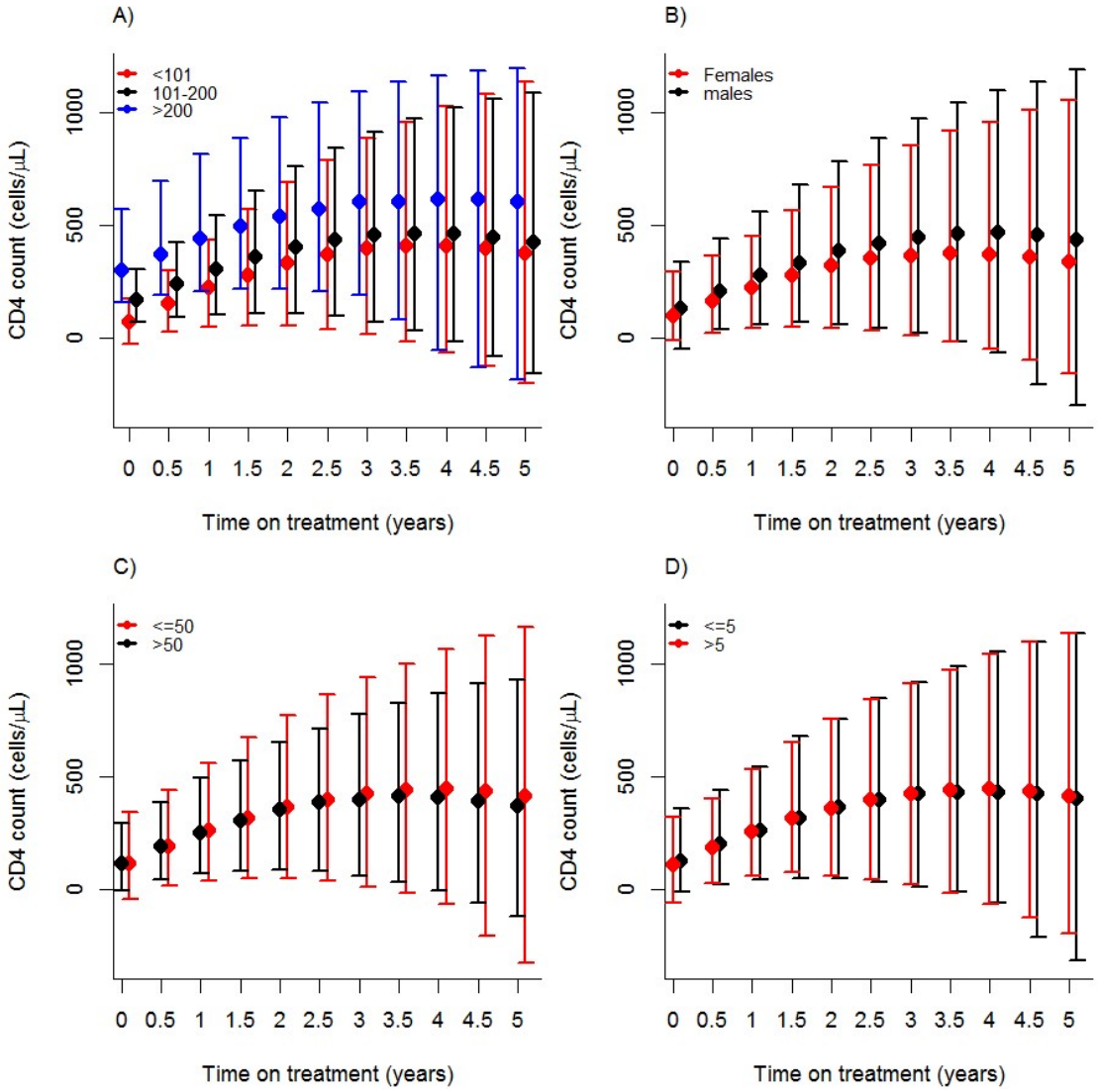
After adjusting for sex, baseline age, baseline CD4 count, baseline log viral load and time on treatment a $1\log_{10}$ copy-yr/mL increase in cumulative log viral load was associated with a -19.2 cells/μL (95% credible interval:-27.82, -10.48) decrease in absolute CD4 count, (Table 4.1). Other than baseline log viral load all other covariates were associated with slope of CD4 counts. The adjusted (with-cumulative log viral load) model had a substantially lower DIC compared to the crude (without-cumulative log viral load) model ($M_{1.20}$ = 47480 vs $M_{1.10}$ = 47510) (Table 4.3). The predicted median CD4 counts response for those with baseline CD4 counts ≤100 cells/μL, was low compared to those with >200 cells/μL, and remained low though similar to those in the 101 – 200 cells/μL category, especially towards the 5th year of ART (Figure 4.2A). Female-sex demonstrated a slightly higher increase in CD4 than males (Figure 4.2B). The predicted median CD4 count in baseline age, more so by baseline $\log_{10}$ viral load, remained similar during follow-up (Figure 4.2C and Figure 4.2D).

Table 4.1: Model for factors associated with slope of CD4 count (Posterior mean and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95% CI	Estimate	95% CI
Female-gender	23.5	(12.35, 34.65)	23.0	(11.90, 34.23)
Per one year increase in baseline age, years	-6.4	(-11.57, -1.26)	-5.9	(-11.11, -0.67)
Per one cell increase in baseline CD4 count, cells/µL	77.6	(72.21, 83.10)	78.1	(72.56, 83.57)
Per log10 increase in baseline log viral load , log10 copies/mL	1.8	(-0.95, 4.75)	1.8	(-0.95, 4.70)
Time on treatment	52.2	(45.43, 58.87)	55.5	(48.46, 62.58)
Time on treatment-squared	-22.9	(-24.93, -20.84)	-22.7	(-24.79, -20.70)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**-19.2**	**(-27.82, -10.48)**

Legend: Model without and with cumulative log viral load (cVL_2) are $M_{1.10}$ and $M_{1.20}$ respectively. In these models, CD4 counts outcome have a Gaussian normal distribution.

Figure 4.2: Predicted median CD4 counts trajectory by covariate strata from slope of CD4 count model



Legend: A. baseline CD4 count, **B.** sex, **C.** Baseline age, **D.** baseline $\log_{10}$ viral load. Median predicted CD4 counts from model $M_{1.20}$, is plotted without adjusting for the variable by which it is stratified. The red, black or blue dots represent predicted median CD4 count at different time point, and the bars are the whiskers. The red, black and blue lines, those connecting the different dots, show the trend of predicted CD4 counts across visits.

CD4 asymptote models

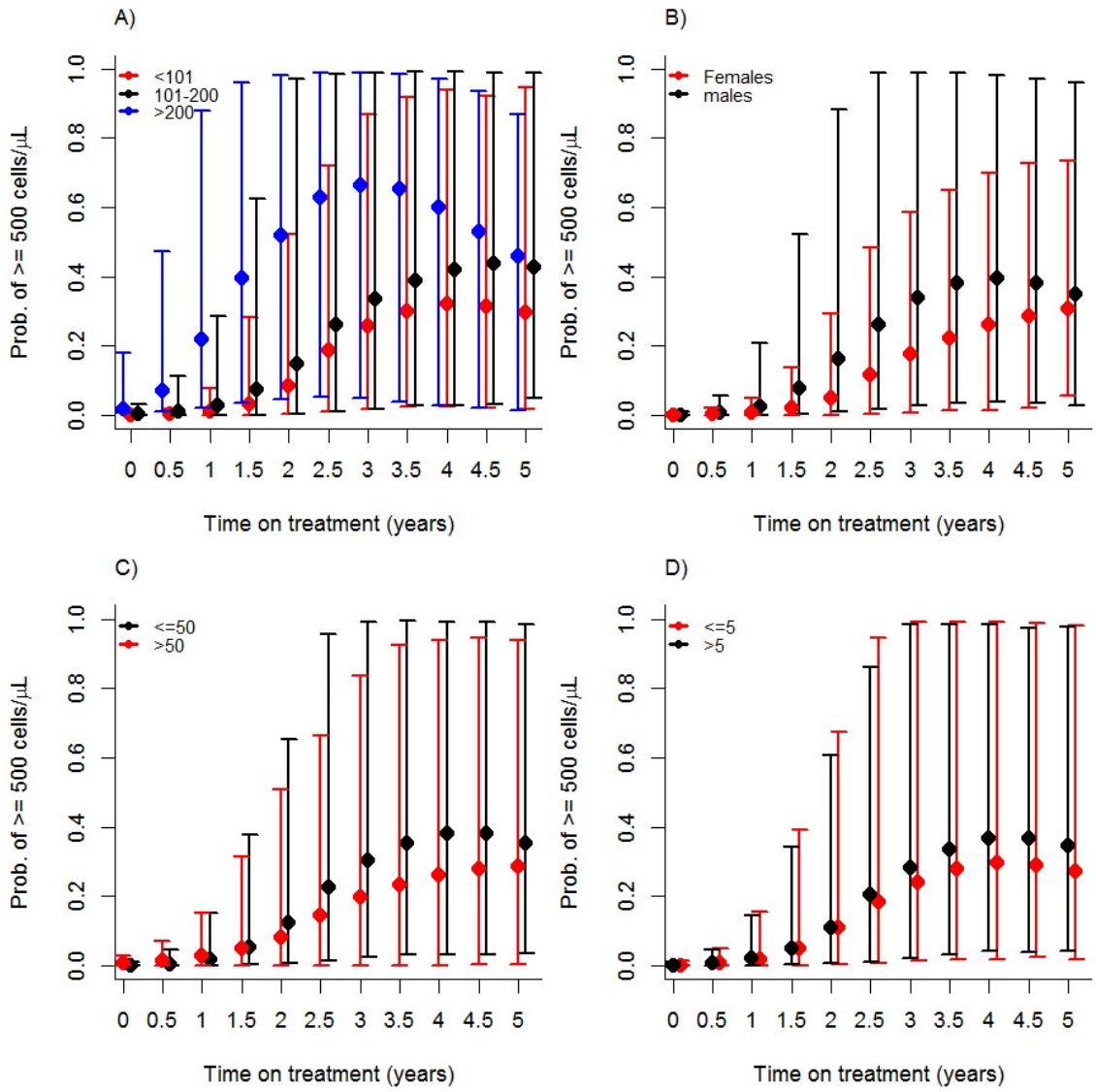
After adjusting for sex, baseline age, baseline CD4 count, time on treatment and baseline log viral load, each 1$\log_{10}$ copy-yr/mL increase in cumulative log viral load reduces the odds to 0.42 odds (95% CI: 0.242, 0.725) of having a CD4 count ≥500 cells/μL during 5 years of follow-up (Table 4.2). Apart from baseline log viral load, all other variables were associated with having CD4 counts ≥500 cells/μL. The adjusted compared to the crude model, had a significantly smaller DIC, respectively $M_{2.20}$ = 1695 and $M_{2.10}$ = 1724 (Table 4.5). Thus adjusting for cumulative log viral load provided a better model fit to the data. With the exception of patients whose baseline CD4 count was >200 cells/μL, the median probability of having a CD4 count ≥500 cells/μL remained less than 0.5 throughout the 5 years on ART (Figure 4.3A). Female-sex had a higher median predicted probability of having a CD4 count ≥500 cells/μL compared to males, however, this difference disappeared after 5-years of ART (Figure 4.3B). Following 1.5-years of treatment, patients with a baseline age ≤50 years old had a higher predicted median probability of having a CD4 count ≥500 cells/μL compared to those >50 years old (Figure 4.3C). Patients with higher baseline $\log_{10}$ viral load (≤5) had a lower predicted median probability of having CD4 counts ≥500 cells/μL after 4 years (Figure 4.3D).

Table 4.2: Model for factors associated with having a CD4 count ≥500 cells/µL (Posterior odds ratios and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95%CI	Estimate	95%CI
Female-gender	5.81	(2.793, 12.846)	6.11	(2.793, 12.846)
Per one year increase in baseline age, years	0.57	(0.412, 0.774)	0.57	(0.412, 0.774)
Per one cell increase in baseline CD4 count, cells/µL	2.45	(1.793, 3.310)	2.53	(1.793, 3.310)
Per log10 increase in baseline log viral load , log10 copies/mL	1.09	(0.874, 1.362)	1.09	(0.874, 1.362)
Time on treatment	3.53	(2.656, 4.707)	4.18	(2.656, 4.707)
Time on treatment - squared	0.66	(0.573, 0.763)	0.64	(0.573 0.763)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**0.42**	**(0.242, 0.724)**

Legend: **cVL_2**—cumulative log viral load; ***$M_{2.10}$*** and ***$M_{2.20}$*** are the crude and adjusted models, respectively where random effects are Gaussian normally distributed.

Figure 4.3: Predicted median probability trajectory of having a ≥500 cells/μL CD4 count by covariate strata



Legend: A) baseline CD4 count, **B)** sex, **C)** Baseline age, **D)** baseline $\log_{10}$ viral load. Median predicted probability of having CD4 count ≥500 cells/μL from model $M_{2.20}$, for each for the strata are plotted above. The red, black or blue dots represent predicted probability of having CD4 count ≥500 cells/μL at different time point, and the bars are the whiskers. The red, black and blue lines, those connecting the different dots, show the trend of predicted median probability across visits.

Sensitivity analysis

In the slope of CD4 count, changing model structure from a second-degree polynomial to cubic splines ($M_{1.21}$ and $M_{1.23}$), yielded significantly higher DICs (Table 4.3), indicating that the polynomial model, i.e. $M_{1.20}$ was still the better fit. The skew distributional plot of the random-effects (Figure 4.4) emphasised the requirement for changing assumptions. The change to a skew-normal distribution led to a significantly smaller DIC for the unadjusted and adjusted compared to Gaussian normal models. The greatest change could be seen between $M_{1.14}$ = 47270 vs $M_{1.20}$ = 47480. Further, when the distribution of the CD4 count was changed to Student's *t*, the DIC reduced from $M_{1.14}$ = 47270 in the unadjusted skew-normal model, to $M_{1.22}$ = 46490 in the adjusted model (Table 4.3). Since the difference in the DIC between the two models is ≥10 points, $M_{1.22}$ is the better fit. These changes improved the precision of model covariates, particularly for the Student's *t*-distribution. With the exception of the spline models (Appendix 4.1 Table 3 and 4), cumulative log viral load remained significantly associated with immune response (Table 4.4). Interestingly, models $M_{1.15}$ *and* $M_{1.25}$, which assumed skew-normal random-effects and measurement error had the smallest DICs, despite their multiple imputation models failing to converge. Using informative priors did improve the estimation of covariate effects (see $M_{1.20}^{*}$ and $M_{1.22}^{*}$ in Appendix 4.1, Table 5 and Table 6, respectively).

Table 4.3: Comparison of goodness of fit for slope of CD4 count models

Model	Crude	Model	Adjusted
	DIC		DIC
$M_{1.10}$	47510	$M_{1.20}$	47480
$M_{1.11}$	48120	$M_{1.21}$	48130
$M_{1.12}$	46510	$M_{1.22}$	46480
$M_{1.13}$	48120	$M_{1.23}$	48130
$M_{1.14}$	47270	$M_{1.24}$	47330
$M_{1.15}$	-3186	$M_{1.25}$	-29310

Legend: $M_{1.10}$ **:** $M_{1.20}$—models errors and random effects are Gaussian normally distributed; $M_{1.11}$ **:** $M_{1.21}$—models errors and random effects are Gaussian normally distributed and has cubic splines with 3 inner knots; $M_{1.12}$ **:** $M_{1.22}$—Student's *t*-distributed CD4 count outcome; $M_{1.13}$ **:** $M_{1.23}$—models errors and random effects are Gaussian normally distributed and has cubic splines with 5 inner knots ; $M_{1.14}$ **:** $M_{1.24}$—random-effects are Skew-normal distributed.

Figure 4.4: Histograms of estimated random intercept and slope obtained from the slope of CD4 count polynomial models

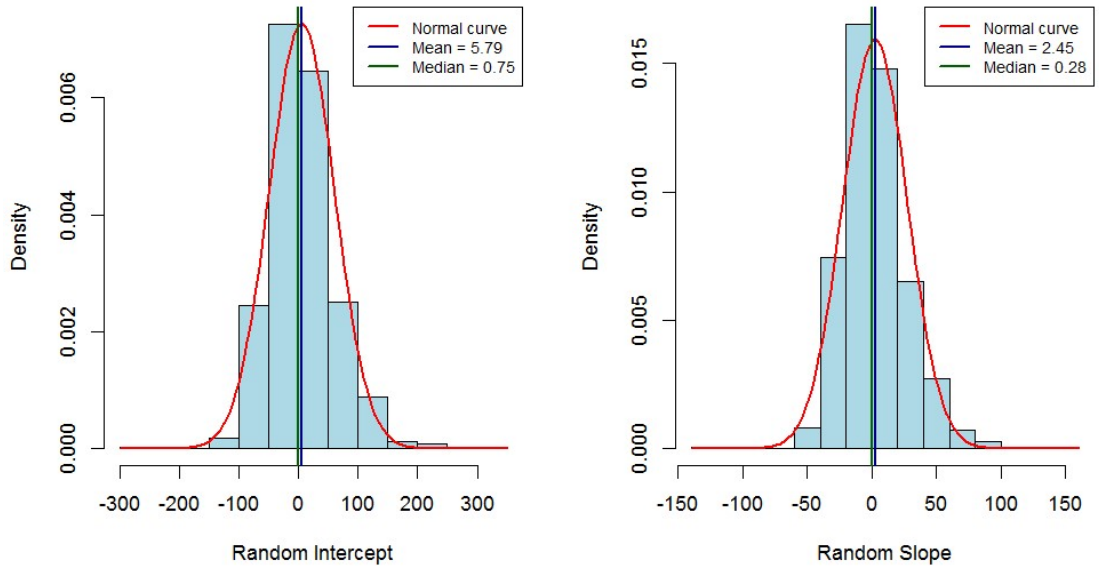


Table 4.4: The effect of changing distributional assumptions of parameters from Gaussian normal to skew-normal and Student's *t* on the precision of covariates in the slope model (Posterior mean and 95% credible intervals)

Parameter	Model $M_{1.20}$		Model $M_{1.24}$		Model $M_{1.22}$	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Female-gender	23.0	(11.99, 34.24)	23.2	(11.94, 34.31)	8.3	(1.59, 14.97)
Per one year increase in baseline age, years	-5.9	(-11.11, -0.69)	-5.95	(-11.15, -0.71)	-1.2	(-4.28, 1.94)
Per one cell increase in baseline CD4 count, cells/µL	78.1	(72.54, 83.56)	78.0	(72.48, 83.48)	80.8	(77.35, 84.17)
Per log10 increase in baseline log viral load , log10 copies/mL	1.8	(-0.95, 4.71)	1.8	(-0.98, 4.69)	0.98	(-0.89, 2.95)
Time on treatment	55.6	(48.45, 62.56)	55.6	(48.69, 62.44)	60.5	(53.14, 67.53)
Time on treatment-squared	-22.7	(-24.79, -20.7)	-22.8	(-24.78, -20.7)	-21.9	(-23.66, -20.24)
Per $\log_{10}$ increase in cumulative log viral load, $\log_{10}$ copy-year/mL	**-19.2**	**(-27.84, -10.49)**	**-19.2**	**(-27.85, -10.44)**	**-13.4**	**(-20.73, -6.00)**

Legend: $M_{1.20}$—cumulative log viral load adjusted model where model errors and random effects are Gaussian normally distributed slope model; $M_{1.24}$—cumulative log viral load adjusted model where model errors and random effects are skew-normally distributed slope model; $M_{1.22}$—cumulative log viral load adjusted model where CD4 count is Student's *t*-distributed.

In the asymptote model, the distribution of the random-effects of the polynomial model was also skew (Figure 4.5), which means that using a normal prior for the random effects is inappropriate. Using a skew-normal led to a significantly lower DIC compared to Gaussian normal distribution $M_{2.21} = 829$ vs $M_{2.20} = 1695$ in the adjusted model and similarly in the crude model $M_{2.11} = 1108$ vs $M_{2.10} = 1716$ (Table 4.5). The adjusted skew-normal model still had the lowest DIC in the asymptote models. Cumulative log viral load remained associated with having CD4 counts ≥500 cells/µL. However, this model has slightly larger credible intervals, implying a loss in precision of model coefficients in the model with a Gaussian normal versus skew-normal random effects (Table 4.6).

Figure 4.5: Histograms of estimated random intercept and slope obtained from the asymptote model

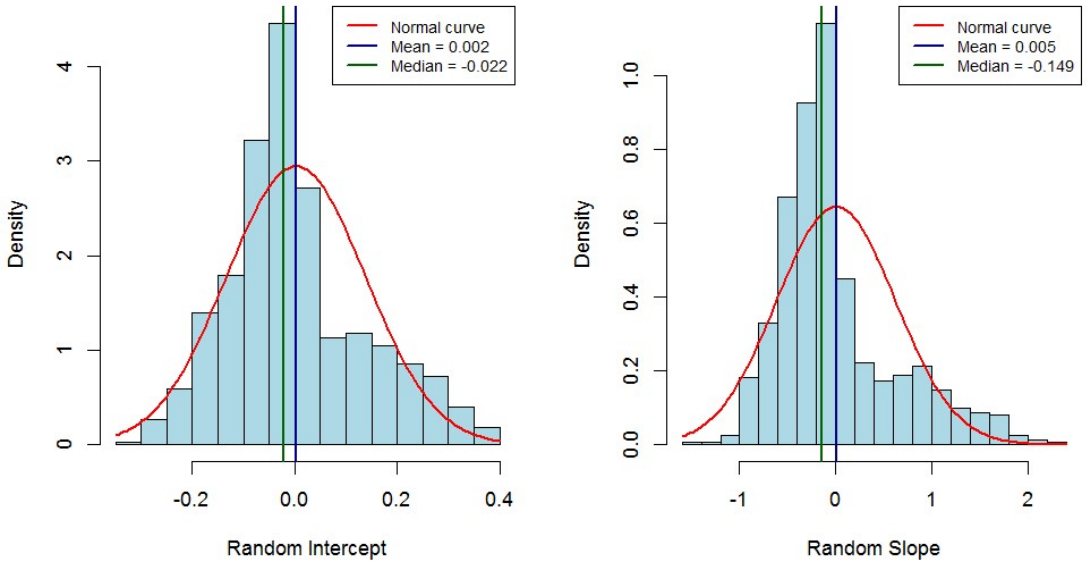


Table 4.5: Comparison of goodness of fit for asymptote models

Model	Crude	Model	Adjusted
	DIC		DIC
$M_{2.10}$	1716	$M_{2.20}$	1695
$M_{2.11}$	1108	$M_{2.21}$	829
$M_{2.12}$	1701	$M_{2.22}$	1695
$M_{2.13}$	1702	$M_{2.23}$	1689

Legend: $M_{2.10}$ and $M_{2.20}$—random effects are Gaussian normally distributed; $M_{2.11}$ and $M_{2.21}$ –random effects are skew-normally distributed; $M_{2.12}$ and $M_{2.22}$—random-effects are Gaussian normally distributed with cubic splines and 3 inner knots; $M_{2.13}$ and $M_{2.23}$—random-effects are Gaussian normally distributed with cubic splines and 5 inner knots.

Table 4.6: The effect of changing distributional assumptions of random-effects from Gaussian normal to skew-normal on precision of covariates in the asymptote models (Posterior odds ratios and 95% credible intervals)

Parameter	Model $M_{2.20}$		Model $M_{2.21}$	
	Estimate	95%CI	Estimate	95%CI
Female-gender	6.11	(2.793, 12.846)	6.36	(2.998, 13.722)
Per one year increase in baseline age, years	0.57	(0.412, 0.774)	0.57	(0.406, 0.783)
Per one cell increase in baseline CD4 count, cells/µL	2.53	(1.793, 3.310)	2.53	(1.855, 3.543)
Per log10 increase in baseline log viral load , log10 copies/mL	1.09	(0.874, 1.362)	1.11	(0.878, 1.377)
Time on treatment	4.18	(2.656, 4.707)	4.01	(3.056, 5.328)
Time on treatment - squared	0.64	(0.573 0.763)	0.65	(0.568, 0.747)
Per $\log_{10}$ increase in cumulative log viral load, $\log_{10}$ copy-year/mL	**0.42**	**(0.242, 0.724)**	**0.42**	**(0.245, 0.736)**

Legend: $M_{2.20}$—cumulative log viral load adjusted model where random effects are Gaussian normally distributed; $M_{2.21}$—cumulative log viral load adjusted model where random-effects are skew-normal distributed

4.5 Discussion

Viral load can usually only be suppressed to undetectable levels in plasma by complete ART adherence. When this is not achieved ongoing viral replication causes progressive depletion of CD4+ lymphocytes through direct and indirect mechanisms [43,45,93]. The extent of damage by a detectable viral load in the short term may not be readily apparent as CD4 counts are inherently biologically variable [140–142].

In addition to baseline log viral load, increasing baseline age, associated with a reduction in the size and functionality of the thymus [45,154], and lower baseline CD4 counts [44,46,96] are associated with ineffective CD4 count recovery. Lawn et al [166] have shown that late ART initiators, with baseline CD4 count <200 cells/ µL, equivalent to 94% of our cohort, often take longer than 5 years of follow-up before they reach CD4 count ≥500 cells/µL. Patients with baseline CD4 count ≤200 cells/µL never reached a 0.5 median predicted probability of having a CD4 count ≥500 cells/µL. Further, they are at risk of developing suboptimal immune response, i.e. failure to reach CD4 count >350 cells/µL after 5 years of ART [37]. Patients with >200 cells/µL at baseline experienced a decline in the median predicted probability of having CD4 count ≥500 cells/µL after 3 years. This may have been due to insufficient treatment adherence, possibly as a result of drug toxicity, as patients were initiated on Stavudine as one of two nucleoside reverse transcriptase inhibitors, and development of HIV drug resistances over time [167]. Our finding of increased CD4 count response in females compared to male appear to be consistent with other studies [43–45,96,114].

In a previously undescribed SSA cohort, we have demonstrated the long-term consequences of a history of detectable viral load, i.e. the cumulative log viral load biomarker, on immune response while on ART. Cumulative log viral load was strongly associated with and provides better model fits for both slope of CD4 count and having an asymptote of CD4

count ≥500 cells/μL. These combined CD4 count-viral load dynamics have not previously been demonstrated in causal statistical models and are in apparent agreement with prior biologically 'mechanistic' mathematical models [11]. After adjusting for sex, baseline age, baseline CD4 count, baseline $\log_{10}$ viral load and time on treatment, each $\log_{10}$ copy-yr/mL increase in cumulative log viral load was associated with a decrease in both slope of CD4 counts and lower odds of having CD4 count of ≥500 cells/μL while on ART. The median probability of having CD4 count ≥500 cells/μL was still <0.5 among patients with a baseline CD4 count ≤100 cells/μL after 5 years of follow-up. Having a CD4 count ≥500 cells/μL while on ART was previously associated with a similar life expectancy to that in the normal population [160]. Our findings provide evidence for the increased benefit of early ART initiation, as the chance of having a CD4 count ≥500 cells/μL is then increased.

For the slope of CD4 count, assuming a prior Student's *t*-distribution produced better model fits compared to using a Gaussian normal. The Student's *t*-distribution also has narrower and, by implication, more precise credible intervals. Despite a failure to converge, the slope of CD4 count skew-normal random-effects and measurements errors model had the lowest DIC, i.e. the best fit. Given that both the slope and asymptote models had skew random-effects distributions (Figure 4.4 and Figure 4.5), it was not surprising that using skew priors produced better fits compared to Gaussian normals. Due in part to inherent variation biological parameters often have non-linear functional forms [1].

A Bayesian approach enabled us to apply informative prior estimates from three historical models [109,114,115] as previously discussed [2]. The impact of informative priors is mostly visible in the variance of posterior distributions. Thus, we expected that in this case our outputs using non-informative and informative priors would be similar, and this was indeed observed (see Appendix 4.1 Table 5 and Table 6). It seems likely that in previous Frequentist approaches, the normality assumptions implicit in the analysis software may have contributed

to their inaccurate estimation of the underlying dynamics of CD4 count change over time. For example, models which assumed non-Gaussian distributions of either random-effects or CD4 counts had significantly smaller DICs compared to those with Gaussian normal.

This study has limitations. As for most Bayesian models using larger datasets, they were computationally intensive. As alluded to previously [1], estimation of cumulative log viral load may be affected by sampling frequency. Viral load was measured bi-annually in the current cohort, thus, possible unmeasured viral rebounds occurring between sampling intervals were excluded. This cohort included patients who were initiated on ART at very low CD4 counts, i.e. 87 cells/μL (IQR: 43, 144), potentially predisposing them to poor CD4 count responses (Figure 4.2) as compared to cohorts with early ART initiation in resource rich settings.

However, this study was successful at implementing Bayesian models in an as yet undescribed SSA cohort using prior CD4 count information. To facilitate model reproducibility we also explicitly state all covariate inclusions and distributional assumptions. Future efforts to model the slope and attaining a particular asymptote of recovery of CD4 count on ART should consider using Bayesian approaches owing to the flexibility in the choice of prior distributions, hierarchical model design and the incorporation of mixed effects. Quantifying the effect of viral rebounds occurring between sampling intervals will likely further improve estimates of cumulative log viral load. Cumulative log viral load might also be included in within-host models of HIV replication and pathogenesis, in which all parameters are defined.

Chapter Five

Discussion

In the complete IDI cohort there were many deaths in the first 2 years of ART [75]. This is similar to many other SSA cohorts [168,169]. Patients often suffered from incident OIs occurring after at least one detectable viral load measurement (Figure 2.3C). During the period of scale–up of ART in SSA from 2004, inadequate experience in medical management of advanced immunodeficiency and almost non-existent clinical resources contributed to rendering viral loads large enough to be detectable [168,169]. Any episode of detectable viral load triggers an increase in cumulative log viral load, hence the association. In addition, many patients have extensive inflammation before ART initiation (Appendix 2.1 table 2). In chronic infections like HIV inflammation may increase morbidity and mortality [93]. Inflammation is minimized during viral load suppression [43,45] and amplified during episodes of detectable viral load.

We found (in Chapter two) that most recent viral load, and not cumulative HIV-Viremia, was associated with incident OIs. It is likely that sub-optimal immune responders [47] remain at risk of incident OIs due to initially compromised immune systems [47,166] and the increased loss of CD4 cell counts due to detectable viral load (Figure 2.2C). Subsequent analysis of the IDI cohort [170] has demonstrated that viral suppression was associated with a remarkable reduction in incident OIs, which corroborates our findings. Our model censored individuals that died or were lost to follow-up regardless of the number of incident OIs in their history. Thus, the failure of cumulative HIV-Viremia to predict incident OIs may have been due to a statistical model artifact. With most OIs occurring within the first two years of follow-up, patients may have remained in care with a high, but stable, cumulative HIV-Viremia (Figure 2.2C). Subsequently, (in Chapter four) higher cumulative log viral load was strongly

associated with poor long-term immune response, and the association was significant. Patients with a history of detectable viral load stand a high risk of developing poor immune response [171]. Previously, detectable viral load was an indicator of poor adherence and treatment failure [9,55,56], but our findings now demonstrate that it also affects a patient's long term CD4 count response while on first-line ART. However, we assume that other incompletely understood factors may also cause a sub-optimal immune response in ART.

Hemoglobin and CD4 counts, in that order, were more reliable predictors of incident OIs (i.e. 0> slope <1) than most recent viral load (Appendix 2.1 figure 3). This may be due to patient-doctor responses to detectable viral load's, i.e. adherence counselling, in meticulously monitored cohorts [74]. Both hemoglobin and CD4 counts have been shown to be affected by long term exposure to HIV before ART and to have sub-optimal responses after initiation, hence they are linked to an increased risk of incident OIs [41,42,166,172–174].

We are not currently aware of a consensus definition for 'sub-optimal immune response' for people on ART. In a systematic review, Kelly et al. 2016 found a variety of differences in definitions, including for example, those based on CD4 increases or thresholds, viral suppression levels and durations of follow-up [175]. In this study we use the term, 'sub-optimal immune responders', to describe people who fail to reach the lower end of the normal CD4 count range, i.e. 500 cells/μL [159], after 5 years on ART. We found (in chapter four) that individuals with CD4 counts ≤100 cells/μL at initiation had sub-optimal immune responses, and failed to reach even a 50% probability of achieving CD4 counts ≥500 cells/μL after 5 years of ART (Figure 4.3A). Such findings suggest that ongoing CD4 count monitoring remains necessary in the treatment of HIV in SSA, particularly in individuals who have low CD4 counts at initiation.

Despite its inherent variability [140,142], CD4 count remains an appropriate marker of immune response for an immune impairing disease such as HIV [43,45]. CD4 counts are useful for benchmarking the initial risk of OIs [176] and for monitoring long term sub-optimal immune response [37]. Due to its variability there have been suggestions that CD4 counts are not a good predictor of OIs before ART initiation [49]. However, these findings should not be generalized to SSA where treatment initiation at low CD4 counts is common [14,177]. Further, it has been shown that at low levels CD4 count is a reliable indicator of prognosis [178].

The WHO guidelines recommend that CD4 count monitoring should not be mandatory for people who are virally suppressed on ART [9]. This makes sense from a public health perspective in terms of the reduction of laboratory detection costs. However, from a clinical perspective the guidelines do not cater for sub-optimal responders. Such people should ideally be monitored at least until they reach a lower end of uninfected-normal CD4 count level [39]. A recent cost-benefit analysis of different combinations of CD4 count and/or viral load monitoring has demonstrated that a combination of biannual CD4 count, with at least one viral load, is more cost effective than having no CD4 count at all [179]. In light of this, continued biannual CD4 count in addition to viral load monitoring makes sense. Particularly for sub-optimal immune responders, so that predicting risk and facilitating the management of OIs is possible. This also corroborates our findings (in chapter two) that CD4 count is a more reliable predictor of incident OIs than viral load [1].

Ying et al. 2016 [49] have suggested that CD4 count measurements should not be conducted at ART initiation, citing unnecessary costs and delays in receiving CD4 count results as an impediment to successful ART scale-up. However, baseline CD4 count has been shown to correlate with a variety of treatment outcomes, such as CD4 response after ART initiation, incident OIs and viral failure [50,180]. Its removal may make patient management difficult. For example, without the baseline initial CD4 count the subsequent impact on CD4 count

cannot be quantified, thus, suboptimal immune response cannot be identified [47]. In East Africa, 40% of ART patients with ≥95% self-reported adherence demonstrated sub-optimal immune responses during 5 years of ART [37]. Alternate options exist, such as streamlining service delivery to reduce waiting times. In the future, ART programs might also scale-up less expensive, more rapid and more accurate CD4 count point-of-care technology [181].

Our results do support the WHO guideline to initiate ART as early as possible after a positive HIV test [9]. We found (in chapter four) that patients initiated on ART with low baseline CD4 counts have difficulty in achieving even lower end of normal CD4 counts (figure 4.3A). While early ART is clearly beneficial [13,18], many countries in SSA struggle to meet the guideline due to limitations in funding and infrastructure [177]. Many ART programs in SSA countries depend on donor funding [182] which is dwindling [183]. Greater within-country advocacy to increase funding towards HIV programs is necessary [184].

An important aim of this thesis was to improve the reproducibility of our findings. For this reason, we explicitly provide information regarding model assumptions and structure, in contrast to that done in the reviewed historical models. We report inconsistences in the construction and reporting of multivariate immune response models in SSA (in chapter three). Unless comparable methods are used, the results of such models are not reproducible even when data from the same cohort is used. Modelling CD4 count response is not trivial. It is necessary to minimize 'noise,' in the form of its inherent variability [95,141], requiring robust statistical methods. The reviewed CD4 slope models (in chapter three) mostly assumed that outcomes were Gaussian normally distributed (Table 3.2). However, we found that the Student's *t*-distribution was more suitable owing to its broader tails [149]. It seems that modelling the asymptotic nature of CD4 count response on ART, in addition to using random intercept and slope models provide better approximations of the CD4 count dynamics. Further, we demonstrated that using *'prior'* knowledge in Bayesian model construction, on the data of

a previously undescribed cohort, led to the definition of a robust model with respect to its predictive abilities for immune response.

It is conceivable that cumulative log viral load may be implemented in within-host models of HIV replication and pathogenesis, such those of Perelson et al. [185] in which all the parameters are defined. This would require that appropriate units of measurements for cumulative log viral load be used, in order to be compatible with the other model parameters. However, a challenge remains in estimating cumulative log viral load, as it does not include the impact of viral rebounds, which may occur between sampling intervals. Unfortunately, viral load monitoring is expensive at finer resolutions than every 6 months.

It is understood that comprehensively monitoring cumulative log viral load would entail an increase, i.e. a scale-up, in the frequency of sampling, with associated increases in cost. In many resource constrained SSA settings, viral load testing was subsidized from 2015 [36]. This was driven by evidence from treatment as prevention trials that reducing community viral load reduces the risk of HIV transmission [186,187]. However, strategies have now become available, such as sample pooling [33] and finger-prick dried blood spot tests [34,35], that reduce both the cost and complexity of viral load testing methods [35,188]. Point of care viral load tests are also being developed, which will facilitate decentralized testing and reductions in waiting times for results [31,32]. Challenges remain in the scale-up of viral load testing, for example, an inadequate supply of suitably trained laboratory personnel along with the necessary equipment [188].

There are some limitations. Biannual viral load was used to estimate cumulative log viral load, which may have resulted in its underestimation, i.e. as unmeasured viral rebounds occurring between sampling intervals were by definition excluded. We used time lags between measurements as a means to overcome this (in chapter two), but this remains an

approximation. It was not possible to implement time lags (in chapter four) as exact visit dates were not available although measurement intervals were. We had missing data (<10%) but this was insufficient to alter the results from the analyses [189].

Sub-Saharan Africa, like many other resource constrained regions, lags in the implementation of WHO ART treatment polices for a variety of reasons [14,190–192]. CD4 count thresholds at ART initiation are still <200 cells/μL [14], representing over 90% of the patients whose data we analyzed in chapters 2 and 4. While some countries in SSA have been able to implement early ART start, the majority of patients still present into care with low CD4 counts [14]. Our findings do appear representative of many SSA first-line ART treatment cohorts in which a large fraction of patients initiate on ART with low CD4 counts and where viral load data is routinely collected. We were fortunate to analyze data from cohorts in which there was very little missing data. Good quality data in SSA is rare, despite its value in improving treatment outcomes and facilitating the decentralization of services [193–195]. It is foreseeable that our analysis could be extended to treatment programs with better quality data.

Chapter Six

Conclusions and Recommendations

Conclusions

A central theme of this thesis has been the (systematic) review of existing literature prior to any statistical analysis, the purpose being to construct more comparable and robust statistical models than have been available to date. In Chapter two we present the first peer-review published article on cumulative HIV-Viremia in SSA and the first to adjust for the detection limit of the viral load assay in the calculation of cumulative log viral load [1]. The systematic review [2] includes only published articles, thereby comparing only peer-reviewed statistical methods. We also analyzed data from two SSA cohorts, including one that was previously undescribed (in chapter four). To our knowledge, we describe the first model/s of immune response in SSA using Bayesian methods.

For purposes of comparing immunological, i.e. CD4 count, outcomes across cohorts in SSA, statistical models would benefit from the application of more uniform modelling techniques. Most historic multivariate immune response models from SSA appear to be anecdotal at best to the respective cohorts on which they were defined. There is tremendous variation in these models, such as the use of different covariates, variable transformations, scales, modelling assumptions and reporting methods, even for the same outcomes. This study succeeded in defining 'prior' knowledge, which has value for prospective modeling efforts in the future.

Cumulative log viral load is associated with both mortality and long term immune response, while most recent viral load is associated with incident OIs. Hemoglobin and CD4 counts are more reliable predictors of incident OIs compared to most recent viral load. Adjusting for

cumulative log viral load with the use of appropriate distributional assumptions improves the prediction of immune response. Assuming a Student's *t*-distribution provides better predictions compared to a Gaussian normal distribution. Patients who initiate ART at lower CD4 counts (i.e. ≤100 cells/μL) have <50% probability of reconstituting CD4 counts to ≥500 cells/μL during 5 years of ART. In the systematic review, qualitative and semi-quantitative, rather than quantitative and completely comparable effect sizes, for variables in models of immunological response to ART were defined.

Recommendations

To ensure generalizability, the construction of *de novo* statistical models requires prior review of existing models. Further, comparison across cohorts needs the application of more uniform modelling techniques.

The construction of ART mortality and immune response models ideally should adjust for cumulative log viral load, particularly when longitudinal viral load data is available. However, further investigation of causal relationships in cumulative log viral load is necessary. The measurement of viral rebounds occurring between sampling intervals will improve the numerical estimation and calibration of cumulative log viral load. This, in turn, will further resolve thresholds for heightened mortality risk. Cumulative log viral load might be included in dynamical within-host models of HIV replication and pathogenesis, in which all parameters are defined. Future models of immune response would benefit from the use of Bayesian methods owing their flexibility in the choice of prior distributions, hierarchical model designs and the incorporation of mixed effects.

Our results support the early initiation of ART in SSA. Continued monitoring of CD4 counts and hemoglobin levels are advisable, to enable targeted management of incident OIs, particularly in individuals with low initiating CD4 counts.

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Appendix 2.1

All-Cause Mortality Outcome

While we assessed all-cause mortality as an outcome, we censored one patient who died after a motor accident at their death, excluding this outcome *a priori* from the analysis since this was judged to be unrelated to exposure to HIV.

Cox Proportional Regression Equations

We used the following Cox proportional hazards model:

$$\log(\lambda_{i,t}) = H_0(t) + \boldsymbol{\beta}_{\text{lab}}\boldsymbol{X}_{\text{lab,i,t}} + \boldsymbol{\beta}_{\text{other}}\boldsymbol{X}_{other,i,t}$$

$$\boldsymbol{\beta}_{\text{lab}}\boldsymbol{X}_{\text{lab},i,t} = \sum_{\substack{\text{all } m \\ \text{laboratory} \\ \text{variables}}} \beta_m X_{m,i,t} + \beta_m^* X_{m,i,t} X_{\text{lag},i,t}$$

$$\boldsymbol{\beta}_{\text{other}}\boldsymbol{X}_{\text{other},i,t} = \sum_{\substack{\text{all } k \\ \text{other} \\ \text{variables}}} \beta_k X_{k,i,t}$$

for the hazard experienced by the i-th individual in the t-th time interval, allowing an interaction between laboratory measurements and the time since measurement $X_{lag,i,t}$ (governed by β^*). In this way, declining predictive utility of explanatory variables with time since measurement can be fit directly from the data. Laboratory variables included current and baseline viral load, current and baseline CD4, current hemoglobin and log cumulative Viral Load or cumulative log Viral Load. Non-laboratory variables included current age and sex. We dealt with the detection threshold of viral load by using a categorical dummy variable and associated coefficients for undetectable viral load measurements, such that the regression terms for time-updated viral load in the model were;

$$(1 - X_{\text{undetVL},i,t})(\beta_{\text{VL}} X_{\text{VL},i,t} + \beta_{\text{VL}}^* X_{\text{VL},i,t} X_{\text{lag},i,t}) + \beta_{\text{undetVL}} X_{\text{undetVL},i,t}$$

where $X_{\text{undetVL,i,t}}$ is an indicator variable (1 if undetectable and 0 if detectable). Thus, for example, the adjusted relative hazard for a 1 $\log_{10}$ difference in viral load over the 0-12 weeks post-measurement is given by

$$\text{ARH}_{\text{VL, 0-12 wk}} = \exp(\hat{\beta}_{VL} + \widehat{\beta^*}_{VL} \times 12)$$

Wald Chi Squared Confidence Intervals

We used Wald confidence interval calculations to assess the significance of the relationship between each predictor and outcome, after controlling a number of other variables. As noted above, we have used two coefficients for laboratory measurement variables (and three for viral load due to the detectability dummy variable). Confidence intervals were constructed based on linear combinations of fitted coefficients using their variance covariance matrix and the appropriate variance transformations.

Cox Proportional Hazards Models and Rounded Observation Intervals

We simulated lognormal viral load data for the patient observation time points for the 489 patients from the IDI cohort, calculating time-updated cumulative viral load according to the methods in the main text. We then simulated incident OI times based on time-varying hazards and an assumed causal relationship between viral load, but not cumulative viral load. Using actual start and end times and visit intervals, we simulated the dataset (adding incident OIs, viral load and cumulative HIV-Viremia) 1000-times. We analyzed the resulting simulated datasets (10,657 data points of 489 patients with an average of 1,162 OI events) with Cox proportional hazards model as in the main text and also with a Poisson regression model (cloglog link generalized linear mixed model; GLMM), using exact start and end points for each inter-visit observational interval. We found that results from the Cox proportional hazards model, but not from the Poisson GLMM to be biased. Specifically, Cox proportional hazards models consistently found cumulative HIV-Viremia to be significantly predictive of OI risk even when though was no underlying causal effect, while P-values for the association of

cumulative HIV-Viremia with the outcome were uniformly distributed from 0 to 1 for the Poisson GLMM (Appendix 2.1 figure 4). However, when we analyzed the simulations with a Cox proportional hazards model *in which inter-visit intervals were rounded* to their approximate 12 week values (as designated by the original study design), the analysis was unbiased, failing to spuriously attribute a significant association between cumulative HIV-Viremia and OI risk more than the nominal $\alpha = 0.05$ false positive rate. We therefore used rounded inter-visit observation intervals when using the Cox proportional hazards model to analyze the IDI cohort data in the main text. Please follow this link: https://Sempa@github.com/ICI3D/SempaetalAJE-00426-2015.git, to view or run the R-file "Coxph_glm.R" for simulation details.

Baseline regimen

Including baseline ART regimen (nevirapine or efavirenz based regimen) as covariates in survival models could cause confounding bias because at baseline patients were allocated to nevirapine or efavirenz based on aspects of clinical presentation that are already included via other covariates [196]. To avert this situation, we used regression trees to generate propensity scores [197] using baseline variables: viral load, CD4 count, hemoglobin, ART regimen, age, and gender to adjust for bias in treatment allocation (nevirapine or efavirenz) at ART initiation (see Appendix 2.1 figure 5). After pruning—removing highly specific nodes—there was only one root, which implied that these variables were not informative with regard to treatment allocation. We therefore completed the analysis without using propensity scores or ART regimen.

List of tables

Appendix 2.1 table 1: Review of all Published Studies Evaluating Cumulative HIV-Viremia as a Prognostic Predictor.

Study	Cumulative Viremia Calculation	Model[a]	Results[b]
Cole et al. 2010 [58] **MACS, USA, 1984-1998**	**cVL_1:** Calculated by summing under the **viral load** curve (6 monthly measurements), **starting from seroconversion dates**, which were known and assumed to correspond to a viral load of zero.	Cox proportional hazards model of **progression to AIDS or mortality** (combined) vs. viral load measures including viral set point and time-updated values of log viral load, cVL_1 and peak viral load to date. Adjusted for time-updated CD4 (spline).	Baseline CD4[c] (at seroconversion): 701 (513-916) All four viral load measures significantly associated with hazard of AIDS/death in univariate models. Univariate model with the cVL_1 model chosen as the best univariate model by AIC selection. No viral load predictors significant in full multivariate model, perhaps partly due to collinearity. CD4 effect not shown.
Zoufaly et al. 2009 [70] **ClinSurv, Germany, 1999-2006**	**cVL_2:** Calculated by summing under the **log viral load** curve (3 monthly measurements) and above the log (500copies/µl), **starting from ART initiation**; log (500) cutoff was chosen so that undetectable viral loads did not contribute to cumulative viremia. Baseline cumulative viremia assumed to be zero.	Cox proportional hazards model of **incident AIDS lymphoma** vs. time-updated viral load (categorized) and cVL_2 (continuous). Adjusted for baseline (< vs ≥200) and time-updated CD4 (<200, 201-350, ≥350).	Baseline CD4 (at ART initiation) lymphoma: 90 (38-220) Baseline CD4 (at ART initiation) no lymphoma: 204 (80-340) Both time-updated viral load and cVL_2 significantly associated with hazard of AIDS lymphoma in univariate models. cVL_2 was also significantly associated with hazard in a multivariate model, but this model excluded time-updated viral load. Low baseline and time-updated CD4 also predictive of increased risk in multivariate model.

Marconi et al. 2011 [60] **Military HIV Natural History Study, USA 1986-2008**	**cVL_1:** Calculated by summing under the **viral load** curve (6 monthly measurements) **starting from ART initiation**. cVL_1 was calculated only once per individual corresponding to their entire post-ART follow-up time (i.e. not time-updated).	Poisson regression of **progression to AIDS** vs. viral load decay rate over total follow-up time or within first year post-ART, viral load slope in first year post-ART and cVL_1. All four of these viral loads were dichotomized into binary variables based on their median values. Also included CD4 (continuous).	Baseline CD4 (at ART initiation): 278 (167-378) cVL_1, when included as a dichotomous but not continuous variable, was a statistically significant predictor of AIDS risk in either univariate or multivariate models. CD4 effect not shown.
Mugavero et al. 2011 [59] **CNICS, USA, 2000-2008**	**cVL_1:** Calculated by summing under the **viral load** curve (6 monthly measurements) and then taking the logarithm, **starting from 24 weeks post-ART initiation**. In sensitivity analyses, summed cumulative viremia starting from ART start, 48 weeks and 2-years post-ART start.	Cox proportional hazards model of **all-cause mortality** vs. log viral load, cVL_1, log viral load at ART initiation and log viral load at 24 weeks post-ART initiation. Used marginal structural models to account for time-dependent confounding between viral load measures and CD4 counts.	Baseline CD4 (at ART initiation): 222 (97-325) Of four viral load measures in the multivariate adjusted model, only increasing cVL_1 was significantly associated with increased mortality risk. Lower time-updated CD4 was also associated with increased mortality risk. Sensitivity analyses were consistent (data not shown).
Mugavero et al. 2012 [84] **UAB1917 clinic and UW Harborview Clinic, USA, 2007-2010**	**cVL_1;** Calculated by summing the area under the **viral load** curve (6 monthly measurements) and then taking the logarithm, starting from	Linear regression model of 2-year cVL_1 as an *outcome* variable as a function of clinic visit adherence, adjusting for baseline viral load and CD4, age, sex, race/ethnicity and health insurance.	Baseline CD4 at ART initiation: <200 (33%); 200-3500 (24%); and >350 (43%). Higher early retention rates were significantly associated with lower cVL_1 in a multivariate analysis.

	ART initiation and going up to two years.		
Saracino et al. 2013 [83] **Clinic of Infectious Diseases, Italy** **1997-present**	Calculated by summing the area under the **viral load** curve (other details not provided).	Mann-Whitney test of cVL_1 over total time followed up as an *outcome* variable as a function of HIV strain.	Found significant differences in cVL_1 between patients infected with different strains.
Lima et al. 2014 [82] **RCT NCT00162643, Mexico** **2005-2007**	Calculated as total area under linear and log viral load curves (median (IQR) 5 (4-5) viral load test) each cVL measure included either baseline or ≥6months, to week 48.	Wilcoxon rank sum test analysis was used to assess association between cVL_1 or cVL_2 with viral load at 48 weeks post-ART initiation. Also assessed both cVL metrics as an *outcome* of randomized treatment assignment.	Median baseline CD4 count: median (IQR) 56cells/mm^3 (25-117) cVL_2 correlated with viral load at 48 weeks, though the former was derived from the latter. Patients initiated on efavirenz had significantly lower cVL_2 compared to lopinavir/r. cVL_1 did not significantly correlate with viral load at 48 weeks (likely because this measure is closely correlated with peak viral load, which generally occurs earlier post-ART initiation) or treatment assignment.
Kowalkowski et al. 2014 [81] **HIV-CCR, USA** **1985-2010**	**cVL_1;** Calculated by summing the area under the **viral load** curve (inconsistent inter-measurement duration, but averaging 3 per year) and then taking the logarithm, **starting from first observation**. Included individuals who had ever initiated treatment, including in the analysis person-time at risk pre-ART initiation.	Cox proportional hazards model for incidence of non-AIDS events (Hepatocarcinoma, Hodgkin lymphoma and squamous cell carcinoma of the anus) vs cVL_1, time-updated and pre-ART nadir CD4 count, log viral load and time-updated cumulative % of measurements with undetectable viral loads and several other variables.	Nadir CD4 (pre-ART initiation)[e]:<200 (45%); 200-350 (29%); and >350 (18%) cVL_1 was associated with all three non-AIDS events in a univariate analysis, but only associated with Hodgkin lymphoma and squamous cell carcinoma of the anus in a multivariate analysis.

Chriouze et al. 2015 [69] **APROCO-COPILOTE** **France** **1997-2010**	**cVL_1;** Calculated by summing under the **viral load** curve **starting from 8 months post-baseline** (at baseline cohort included both pretreated and ART-naïve patients) and then taking the logarithm.	Cox proportional hazards model of **all-cause mortality** vs. dichotomized cVL_1. Adjusted for sex, age, ART status at baseline, history of AIDS event, baseline and time-updated CD4, baseline and time-updated log viral load.	Baseline CD4 (at ART initiation): 278 (125-416) cVL_1 (dichotomized) was only statistically significantly associated with all-cause mortality when time-updated log viral load was excluded from the analysis.
Sempa et al. 2015 (Current article) **IDI Cohort, Uganda** **2004-2013**	Calculated by summing under the **viral load** (cVL_1) or **log viral load** (cVL_2) curve (6 monthly measurements) and above the log (400cp/µl) detectability threshold **starting from ART initiation**; log (400) cutoff was chosen so that undetectable viral loads did not contribute to cumulative viremia. In a sensitivity analysis we used 1og (1000)	Cox proportional hazards model of **opportunistic infection**, **AIDS-related mortality**, or **all-cause mortality** vs. either cVL_1 or cVL_2. Adjusted for time-updated and baseline log viral load, time-updated and baseline CD4, baseline age and sex. Included interaction between laboratory measurements and time since measurement to include declining effect of measurement over time when outcomes observed more frequently than covariates.	Baseline CD4 (at ART initiation): 100 (38-168) Neither cVL measure was significantly associated with opportunistic infection risk, which was better predicted by time-updated viral load, hemoglobin levels and CD4 count. cVL_2, but neither cVL_2 nor time-updated log viral load, was significantly associated with mortality risk. Lower CD4 and lower hemoglobin were also significantly associated with increased mortality risk. Viral load measurements were only predictive of opportunistic infection or mortality risk for the 12 weeks post-measurement, while other variables were predictive of mortality (hemoglobin, CD4) or opportunistic infection (hemoglobin) risk for up to 24 weeks. cVL_2 remained a significant predictor

[a]Only covariates corresponding to viral load or CD4 measures are described in this table.
[b]We do not report hazard ratios because they are not directly comparable between studies that modeled viral loads and cumulative viremia calculated in different ways.

[d]All baseline CD4 given as median (IQR) except for [e].
[e]Breakdown of pre-ART CD4 nadirs by category

We use cVL_1 and cVL_2 to designate cumulative HIV-vremia metrics accumulated on a linear and log scale, respectively.

Appendix 2.1 table 2: Characteristics of the 489 for HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013 included in the analysis.

Variable	Median (IQR)
Baseline Age (years)	35.3 (30.2 – 41.8)
Gender: n (%)	
Female	341 (69.7)
Male	148 (30.3)
Baseline CD4 count (cells/µL)	100 (30-168)
Nevirapine based regimen at baseline: n (%)	363 (74.2)
Baseline viral load : Log10 copies/ml	5.4 (5.1 – 5.8)
Follow-up time (years)	8.3 (2.3 – 8.8)

Appendix 2.1 table 3: Spearman Correlation Matrix between Viral Load and CD4 variables among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

	log (VL)	cVL_1	cVL_2	baseline log (VL)	peak log (VL)	log (CD4)
log (VL)	1	-0.69	-0.034	0.045	0.07	-0.49
cVL_1		1	0.47	0.32	0.34	0.47
cVL_2			1	0.15	0.22	0.094
baseline log (VL)				1	0.89	0.0049
peak log (VL)					1	-0.0094
log (CD4)						1

Legend: Correlations displayed include 11819 observations of 489 patients where variables indicate either time-varying measurements (log (viral load, cVL_1—log cumulative viral load, cVL_2—cumulative log viral load, log (CD4)) or a single measurement for each patient (baseline log viral load, peak log viral load).

Appendix 2.1 table 4: Sensitivity analysis of Opportunistic Infection Model Results using different viral load detection thresholds among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

	Threshold for cVL_2 calculation			
	400 copies/ml		1000 copies/ml	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	1.34	1.120, 1.610 [c]	1.35	1.130, 1.620 [b]
predicting 0-24 weeks ahead	1.21	0.969, 1.500	1.21	0.968, 1.500
per log10 increase in cumulative viremia				
predicting 0-12 weeks ahead	0.78	0.523, 1.150	0.72	0.442, 1.180
predicting 0-24 weeks ahead	1.00	0.679, 1.480	0.99	0.610, 1.600
per 2-fold increase in CD4 count, *cells/μL*				
predicting 0-12 weeks ahead	0.90	0.804, 0.998 [a]	0.90	0.803, 0.999 [a]
predicting 0-24 weeks ahead	0.91	0.755, 1.110	0.91	0.754, 1.110
per 10% increase in hemoglobin				
predicting 0-12 weeks ahead	0.91	0.859, 0.959 [c]	0.91	0.859, 0.959 [c]
predicting 0-24 weeks ahead	0.89	0.819, 0.971 [b]	0.89	0.818, 0.971 [b]
per 2-fold increase in baseline CD4 count, *cells/μL*	0.98	0.898, 1.080	0.98	0.898, 1.080
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	0.96	0.692, 1.320	0.96	0.693, 1.320
3rd	1.20	0.869, 1.640	1.20	0.872, 1.650
4th	1.01	0.715, 1.420	1.01	0.718, 1.430
Gender				

Female	1			1
Male	0.78	0.602, 1.010	0.78	0.603, 1.010
per 10 year increase in baseline age	0.91	0.791, 1.040	0.91	0.791, 1.040

[a] Statistical significance: P <0.05; [b] P <0.01; [c] P <0.001

Quartile: 1st—$<10^{5.07}$; **2nd**—$10^{5.08}$ - $10^{5.44}$; **3rd**—$10^{5.45}$ - $10^{5.77}$; **4th**—$10^{5.78}$ - $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL_1**—log cumulative viral load; **cVL_2**—cumulative log viral load; **VL**— Viral Load. Values give adjusted hazard ratios (95% confidence interval) for the hazard of acquiring an incident opportunistic infection from multivariate Cox proportional hazard models with cVL_2 calculated using either viral load detection thresholds of either 400 or 1000 copies/ml.

Appendix 2.1 table 5: Sensitivity analysis of All-cause Mortality Model Results using different viral load detection thresholds among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

Variable	Threshold for cVL_2 calculation			
	400 copies/ml		1000 copies/ml	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	1.13	0.722, 1.770	1.11	0.710, 1.740
predicting 0-24 weeks ahead	0.89	0.512, 1.550	0.85	0.491, 1.490
per log10 increase in cumulative viremia, *log_{10} copy-yrs/ml*				
predicting 0-12 weeks ahead	1.63	1.020, 2.600 [a]	1.86	1.060, 3.260 [a]
predicting 0-24 weeks ahead	0.50	0.168, 1.490	0.28	0.0623, 1.210
per 2-fold increase in CD4 count, *cells/µL*				
predicting 0-12 weeks ahead	0.57	0.454, 0.723 [c]	0.57	0.453, 0.720 [c]
predicting 0-24 weeks ahead	0.69	0.514, 0.922 [a]	0.69	0.517, 0.926 [a]
per 10% increase in hemoglobin, *g/dl*				
predicting 0-12 weeks ahead	0.77	0.702, 0.832 [c]	0.76	0.702, 0.831 [c]
predicting 0-24 weeks ahead	0.73	0.650, 0.817 [c]	0.73	0.654, 0.822 [c]
per 2-fold increase in baseline CD4 count, *cells/µL*	1.1	0.920, 1.280	1.08	0.920, 1.280
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	1.51	0.682, 3.330	1.53	0.687, 3.390
3rd	1.28	0.527, 3.090	1.31	0.535, 3.220
4th	3.62	1.710, 7.640 [c]	3.71	1.740, 7.930 [c]

Gender				
Female	1			1
Male	1.07	0.556, 2.050	1.07	0.556, 2.050
Baseline age, *years*				
≤35	1			1
36 – 45	1.29	0.713, 2.340	1.31	0.719, 2.370
46 – 55	1.71	0.815, 3.600	1.74	0.828, 3.650
≥56	3.02	1.300, 6.970 [b]	3.09	1.340, 7.150 [b]

[a] Statistical significance: P <0.05; [b] P <0.01; [c] P <0.001

Quartile: 1st—$<10^{5.07}$; **2nd**—$10^{5.08}$ - $10^{5.44}$; **3rd**—$10^{5.45}$ - $10^{5.77}$; **4th**—$10^{5.78}$ - $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL_1**—log cumulative viral load; **cVL_2**—cumulative log viral load; **VL**— Viral Load. Values give adjusted hazard ratios (95% confidence interval) for the hazard of dying of any cause from multivariate Cox proportional hazard models with cVL_2 calculated using either viral load detection thresholds of either 400 or 1000 copies/ml.

Appendix 2.1 table 6: Sensitivity Analysis of Opportunistic Infection Model Results among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

Variable	Model with cVL_1		Model with cVL_2	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	1.30	1.030, 1.640 [a]	1.30	1.030, 1.640 [a]
predicting 0-24 weeks ahead	1.57	1.130, 2.180 [b]	1.53	1.090, 2.150 [a]
per log10 increase in cumulative viremia, *log_{10} copy-yrs/ml*				
predicting 0-12 weeks ahead	0.91	0.689, 1.190	0.76	0.416, 1.400
predicting 0-24 weeks ahead	0.89	0.659, 1.190	0.86	0.451, 1.630
per 2-fold increase in CD4 count, *cells/µL*				
predicting 0-12 weeks ahead	0.88	0.635, 1.230	0.89	0.639, 1.230
predicting 0-24 weeks ahead	0.98	0.645, 1.500	0.97	0.646, 1.460
per 10% increase in hemoglobin, *g/dl*				
predicting 0-12 weeks ahead	0.91	0.840, 0.987 [a]	0.91	0.838, 0.981 [a]
predicting 0-24 weeks ahead	0.85	0.749, 0.962 [a]	0.85	0.751, 0.966 [a]
per 2-fold increase in baseline CD4 count, *cells/µL*	1.01	0.716, 1.420	1.01	0.715, 1.420
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	0.87	0.551, 1.380	0.87	0.550, 1.370
3rd	1.10	0.705, 1.720	1.10	0.705, 1.720
4th	0.86	0.539, 1.360	0.85	0.537, 1.350
Gender				
Female	1			1

Male	0.88	0.588, 1.310	0.88	0.590, 1.310
per 10 year increase in baseline age	1.10	0.917, 1.330	1.10	0.916, 1.330

[a] Statistical significance: P <0.05; [b] P <0.01; [c] P <0.001

Quartile: 1st—<$10^{5.07}$; **2nd**—$10^{5.08}$ - $10^{5.44}$; **3rd**—$10^{5.45}$ - $10^{5.77}$; **4th**—$10^{5.78}$ - $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL_1**—log cumulative viral load; **cVL_2**—cumulative log viral load; **VL**— Viral Load. The sensitivity analysis involved recalculating cumulative HIV-Viremia by moving baseline viral load from baseline visit to 24 week measurement. Values give adjusted hazard ratios (95% confidence interval) for the hazard of acquiring an incident opportunistic infection from multivariate Cox proportional hazard models with cumulative viremia calculated as one of either cVL_1 or cVL_2.

Appendix 2.1 table 7: Sensitivity Analysis of All-cause Mortality Model Results among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

Variable	Model with cVL_1		Model with cVL_2	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	0.93	0.529, 1.630	1.20	0.671, 2.160
predicting 0-24 weeks ahead	1.17	0.684, 2.010	1.32	0.768, 2.270
per log10 increase in cumulative viremia, *log_{10} copy-yrs/ml*				
predicting 0-12 weeks ahead	1.81	1.270, 2.580 [b]	1.81	1.010, 3.230 [a]
predicting 0-24 weeks ahead	0. 90	0.601, 1.350	0.58	0.219, 1.520
per 2-fold increase in CD4 count, *cells/µL*				
predicting 0-12 weeks ahead	0.54	0.399, 0.733 [c]	0.55	0.402, 0.738 [c]
predicting 0-24 weeks ahead	0.67	0.410, 1.110	0.61	0.378, 0.980 [a]
per 10% increase in hemoglobin, *g/dl*				
predicting 0-12 weeks ahead	0.78	0.718, 0.856 [c]	0.79	0.718, 0.862 [c]
predicting 0-24 weeks ahead	0.74	0.634, 0.868 [c]	0.73	0.633, 0.848 [c]
per 2-fold increase in baseline CD4 count, *cells/µL*	1.00	0.710, 1.400	0.93	0.660, 1.310
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	1.50	0.630, 3.580	1.59	0.648, 3.900
3rd	1.09	0.416, 2.860	1.14	0.428, 3.020
4th	2.46	1.100, 5.490 [a]	2.73	1.200, 6.230 [a]
Gender				
Female	1			1

Male	1.06	0.495, 2.260	1.08	0.488, 2.390
Baseline age, *years*				
≤35	1			1
36 – 45	1.61	0.737, 3.510	1.68	0.752, 3.760
46 – 55	1.94	0.765, 4.920	1.90	0.742, 4.850
≥56	3.98	1.430, 11.100 [b]	3.88	1.390, 10.800 [b]

[a] Statistical significance: P <0.05; [b] P <0.01; [c] P <0.001

Quartile: 1st—<$10^{5.07}$; **2nd**—$10^{5.08}$ - $10^{5.44}$; **3rd**—$10^{5.45}$ - $10^{5.77}$; **4th**—$10^{5.78}$ - $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL_1**—log cumulative viral load; **cVL_2**—cumulative log viral load; **VL**— Viral Load. The sensitivity analysis involved recalculating cumulative HIV-Viremia by moving baseline viral load from baseline visit to 24 week measurement. Values give adjusted hazard ratios (95% confidence interval) for the hazard of dying of any cause from multivariate Cox proportional hazard models with cumulative viremia calculated either as cVL_1 or cVL_2.

Appendix 2.1 table 8: HIV specific Mortality Model Results among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.

Variable	Model with cVL_1		Model with cVL_2	
	AHR	95% CI	AHR	95% CI
per log10 increase in VL, *log_{10} copies/ml*				
predicting 0-12 weeks ahead	1.05	0.580, 1.900	0.94	0.464, 1.920
predicting 0-24 weeks ahead	1.58	0.789, 3.150	0.93	0.446, 1.960
per log10 increase in cumulative viremia, *log_{10} copy-yrs/ml*				
predicting 0-12 weeks ahead	1.18	0.493, 2.820	1.34	0.601, 2.980
predicting 0-24 weeks ahead	1.74	1.000, 3.030 [a]	1.20	0.415, 3.440
per 2-fold increase in CD4 count, *cells/µL*				
predicting 0-12 weeks ahead	0.60	0.429, 0.831 [b]	0.58	0.416, 0.798 [c]
predicting 0-24 weeks ahead	0.65	0.435, 0.969 [a]	0.72	0.489, 1.060
per 10% increase in hemoglobin, *g/dl*				
predicting 0-12 weeks ahead	0.71	0.637, 0.792 [c]	0.71	0.632, 0.786 [c]
predicting 0-24 weeks ahead	0.59	0.459, 0.760 [c]	0.63	0.509, 0.777 [c]
per 2-fold increase in baseline CD4 count , *cells/µL*	1.00	0.822, 1.220	1.01	0.822, 1.230
Baseline viral load, *log_{10} copies/ml*				
1st	1			1
2nd	2.19	0.694, 6.890	2.24	0.754, 6.630
3rd	1.95	0.528, 7.200	2.13	0.646, 7.040
4th	5.93	1.840, 19.200 [b]	6.99	2.580, 18.900 [c]
Gender				
Female	1			1

Male	0.87	0.335, 2.250	0.85	0.321, 2.250
Baseline age, *in years*				
≤35	1			1
36 – 45	1.65	0.782, 3.480	1.58	0.719, 3.460
46 – 55	1.32	0.428, 4.060	1.33	0.432, 4.110
≥56	2.29	0.563, 9.340	2.32	0.599, 8.980

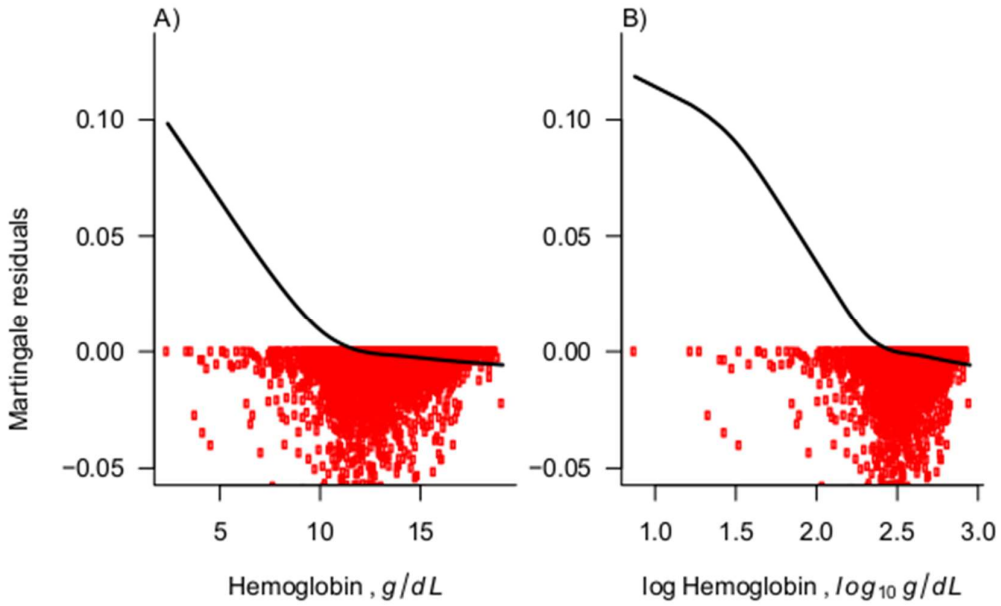
[a] Statistical significance: P <0.05; [b] P <0.01; [c] P <0.001

Quartile: 1st—<$10^{5.07}$; **2nd**—$10^{5.08}$ - $10^{5.44}$; **3rd**—$10^{5.45}$ - $10^{5.77}$; **4th**—$10^{5.78}$ - $10^{6.15}$

ART—Antiretroviral therapy; **AHR**—Adjusted Hazard Ratio; **HIV**—Human Immune Virus; **cVL_1**—log cumulative viral load; **cVL_2**—cumulative log viral load; **VL**— Viral Load. Values give adjusted hazard ratios (95% confidence interval) for the hazard of HIV-related causes from multivariate Cox proportional hazard models with cumulative viremia calculated either as cVL_1 or cVL_2

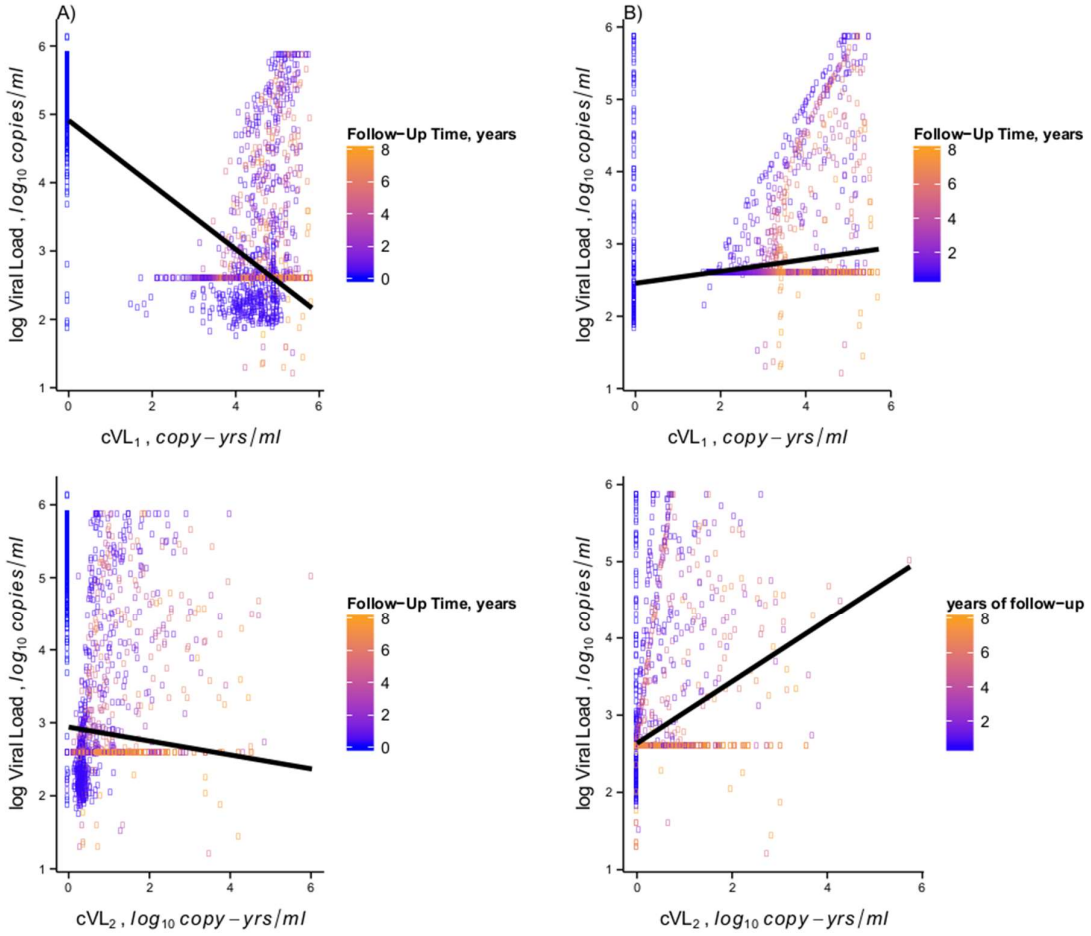
List of figures

Appendix 2.1 figure 1: Loess-smoothed Martingale Residuals for All-cause Mortality Outcomes versus Hemoglobin counts for HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



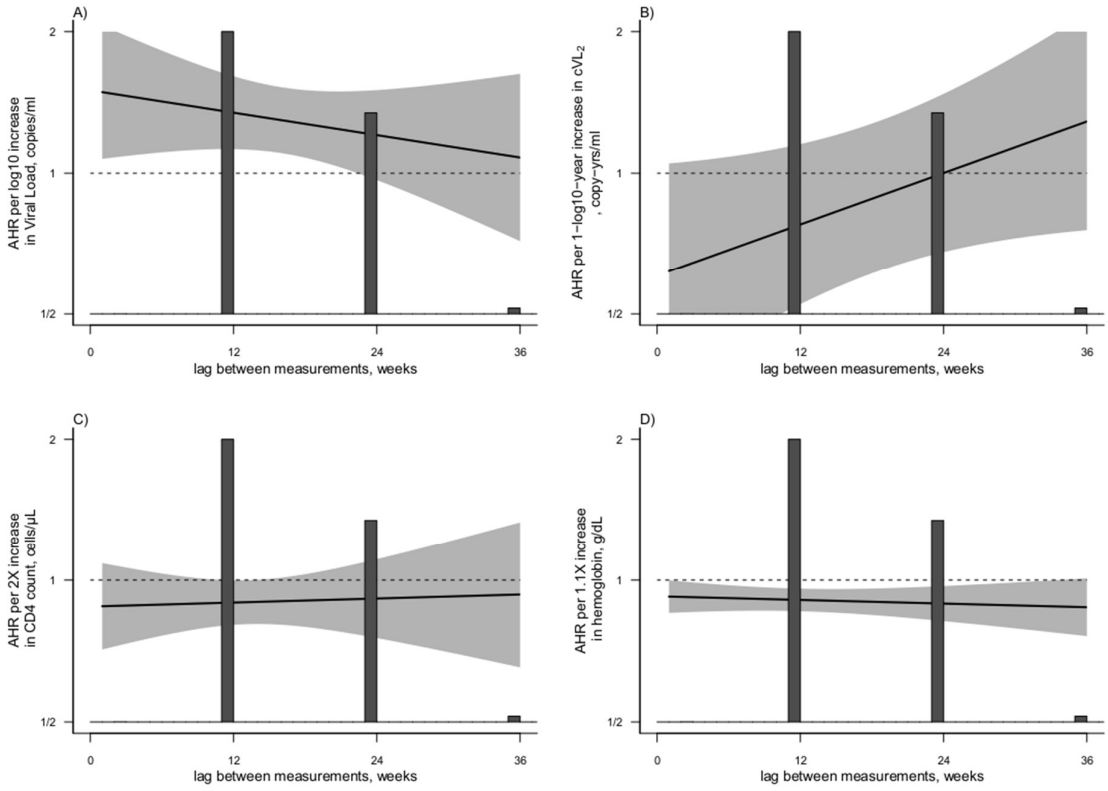
Legend: Multivariate Cox proportional hazards were fit with all variables as indicated in the main text except for time-updated hemoglobin. Martingale residuals were then plotted versus hemoglobin on a linear (A) and logarithmic scale (B), with a loess trend to visually inspect their functional relationship. The trend with hemoglobin on a log scale is better approximated by a linear relationship, justifying the inclusion of hemoglobin's inclusion in the model as log hemoglobin. Similar visual inspections were used to determine the specification of each covariate.

Appendix 2.1 figure 2: Correlation between Cumulative HIV-Viremia Metrics and log Viral Load for HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



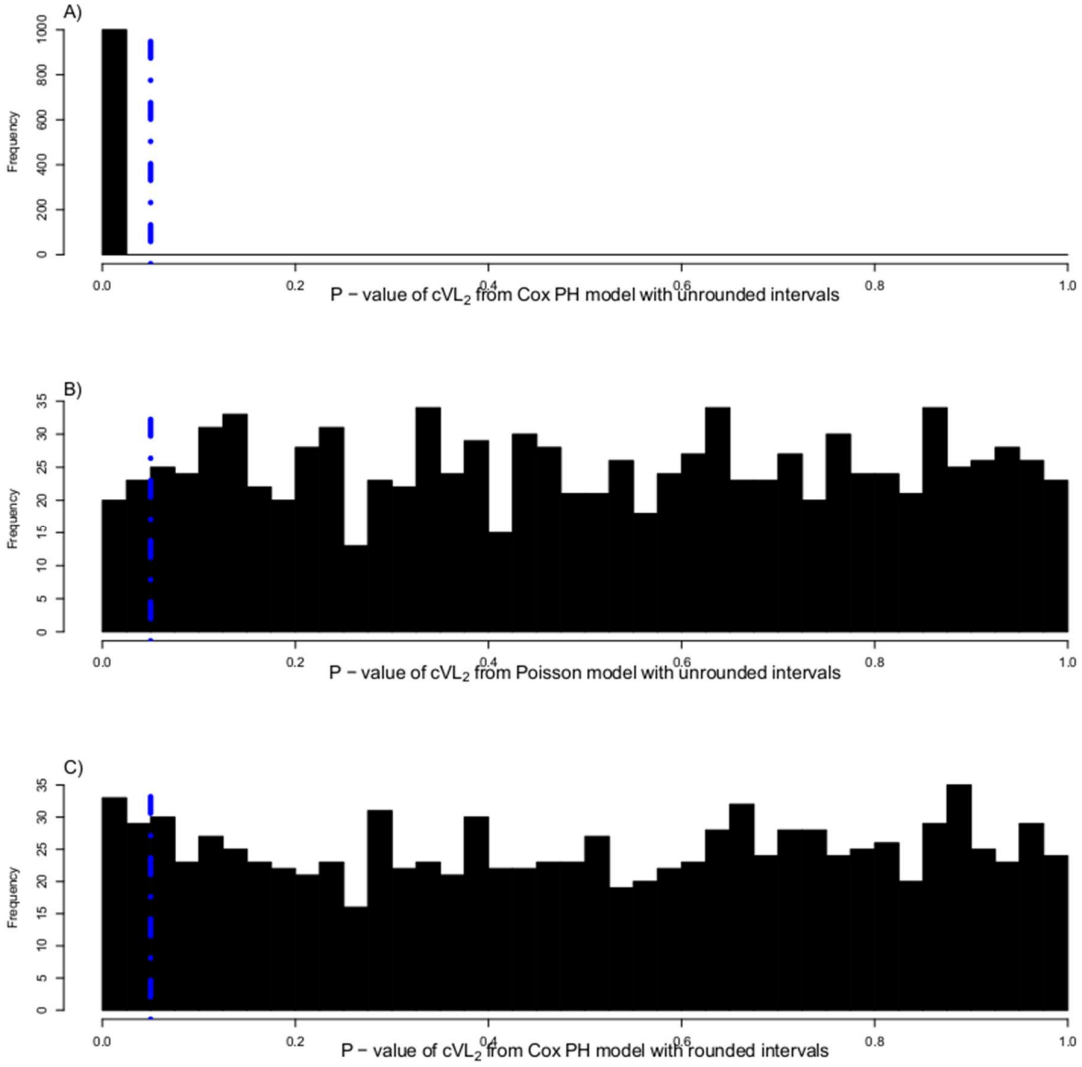
Legend: A— correlation between log viral load, and cumulative HIV-Viremia measures when baseline is at baseline visit. B— correlation between log viral load, and cumulative HIV-Viremia measures when baseline is shifted to week 24. Each point shows a single laboratory measurement, with color indicating the time since ART initiation (i.e. years of follow-up) for that measurement. A linear model (black line) is displayed to illustrate the correlation shown in Appendix 2.1 table 3. The strong negative correlation between log cumulative viral load and log viral load is driven by log cumulative viral load's rapid increases at the baseline visit when viral load is high. Because accumulation on a linear scale means that log cumulative viral load only increases slightly for subsequent intermediate viral load measurements.

Appendix 2.1 figure 3: Declining Prognostic Value with Increasing Time since Measurement among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



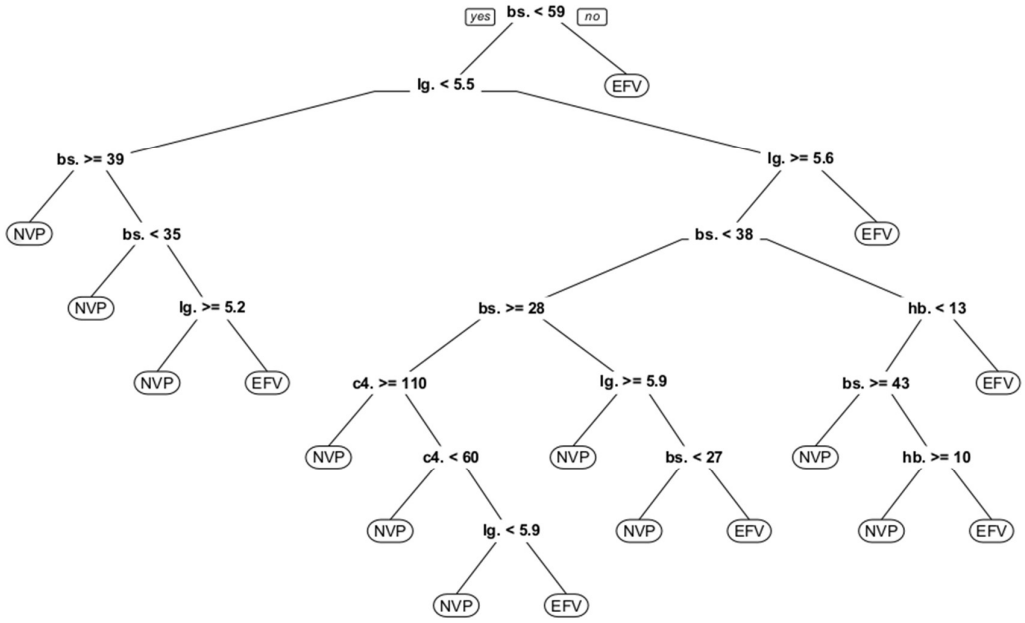
Legend: Lines and shaded regions show the adjusted hazard ratio (AHR) and 95% confidence intervals, respectively, for the hazard of acquiring an incident OI per log10 increase in viral load (A), per log10 increase in cumulative log viral load (B), per 50 increase in CD4 count (C) and per 1g/dl increase in hemoglobin (D). The AHR is modeled as a function of the time since the laboratory measurement was made, facilitating the estimation of how the predictive utility of each measurement declines over time. Inset histograms show the frequency distribution of time lags between a clinic visit and the time of the last laboratory measurement. Because most patients visited quarterly but only had laboratory assays performed every other visit, most time lags were at 12 and 24 weeks and visits are rounded to 12-week intervals in the analysis.

Appendix 2.1 figure 4: Distribution of P-values for the effect of cumulative HIV-Viremia on OI risk amongst 1000 simulations assuming no actual effect.



Legend: The graphs are in the order Cox proportional hazards model with unrounded inter-visit intervals, Poisson regression with unrounded inter-visit intervals and Cox proportional hazards model with rounded inter-visit intervals.

Appendix 2.1 figure 5: Probability of receiving nevirapine or efavirenz among HIV Patients on ART in the IDI cohort, Kampala, Uganda, 2004-2013.



Legend: NVP - nevirapine; EFV- efavirenz; base.age – Age at baseline; cd4.base – Baseline CD4 count, logv.l – Baseline HIV log viral load; hb.l – Baseline hemoglobin

Appendix 3.1

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) check list

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	**1**
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	**2, 3**
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	**4**
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	**5**
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	**N/A**
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	**5**
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	**5**
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	**5, Appendix 3.2**

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	**5, 6**
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	**6, 7**
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	**6**
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	**7**
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	**6**
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	**7**

Section/topic	**#**	**Checklist item**	**Reported on page #**
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	**7**
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	**7**
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	**7, 8**
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	**11, 12, & 14**
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	**15**
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	**8**

Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	**N/A**
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	**16**
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	**8**
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	**17**
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	**21, 22**
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	**22**
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	**23**

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Appendix 3.2

SCOPUS search strategy

Immune response: 402,690

TITLE-ABS-KEY (**"Immune response"** OR **"CD4 count change"** OR **"CD4 trajectory"** OR **"CD4 count"** OR **"CD4"** OR **"cd4 lymphocyte count"** OR **"CD4 counts"** OR **"immunity cellular"** OR **"cellular immune response"** OR **"cellular immune recovery"** OR **"cellular immune reconstitution"** OR **"cellular immune trajectory"** OR **"CD4 recovery"** OR **"CD4 trajectory"** OR **"CD4 response"** OR **"CD4 gains"**) AND (LIMIT-TO (LANGUAGE, **"English"**))

HAART: 54,159

TITLE-ABS-KEY (**"antiretroviral therapy"** OR **"HAART"** OR **"combination antiretroviral therapy"** OR **"cART"** OR **"highly active antiretroviral therapy"**) AND (LIMIT-TO (LANGUAGE , **"English"**))

Statistical Model: 1,022,199

TITLE-ABS-KEY (**"Likelihood Functions"** OR **"Linear Models"** OR **"Logistic Models"** OR **"Proportional Hazards Models"** OR **"Least-Squares Analysis"** OR **"Nomograms"** OR **"Models, Statistical"** OR **"regression"**) AND (LIMIT-TO (LANGUAGE, **"English"**))

SSA: 555,920

TITLE-ABS-KEY (**"Angola"** OR **"Benin"** OR **"Botswana"** OR **"Burkina Faso"** OR **"Burundi"** OR **"Cameroon"** OR **"Cape Verde"** OR **"Central African Republic"** OR **"Chad"** OR **"Comoros"** OR **"Congo"** OR **"Côte d'Ivoire"** OR **"Djibouti"** OR **"Equatorial Guinea"** OR **"Eritrea"** OR **"Ethiopia"** OR **"Gabon"** OR **"Gambia"** OR **"Ghana"** OR **"Guinea"** OR **"Guinea-Bissau"** OR **"Kenya"** OR **"Lesotho"** OR **"Liberia"** OR **"Madagascar"** OR **"Malawi"** OR **"Mali"** OR **"Mauritania"** OR **"Mauritius"** OR **"Mozambique"** OR **"Namibia"** OR **"Niger"** OR **"Nigeria"** OR **"Réunion"** OR **"Rwanda"** OR **"Sao Tome and Principe"** OR **"Senegal"** OR **"Seychelles"** OR **"Sierra Leone"** OR **"Somalia"** OR **"South Africa"** OR **"Sudan"** OR **"Swaziland"** OR **"Tanzania"** OR **"Togo"** OR **"Uganda"** OR **"Zambia"** OR **"Zimbabwe"** OR **"Sub-Saharan Africa"** OR **"Subsaharan Africa"** OR **"Africa, Sub-Saharan"** OR **"south of sahara"**) AND (LIMIT-TO (LANGUAGE, **"English"**))

Immune response AND HAART = 20,476

(_(_TITLE-ABS-KEY_(_**"Immune response"**_ OR_ **"CD4 count change"**_ OR_ **"CD4 trajectory"**_ OR_ **"CD4 count"**_ OR_ **"CD4"**_ OR_ **"cd4 lymphocyte count"**_OR_ **"CD4 counts"**_ OR_ **"immunity cellular"**_ OR_ **"cellular immune response"**_ OR_ **"cellular immune recovery"**_ OR_ **"cellular immune reconstitution"**_ OR_ **"cellular immune trajectory"**_OR_ **"CD4 recovery"**_ OR_ **"CD4 trajectory"**_ OR_ **"CD4 response"**_ OR_ **"CD4 gains"**_)_)_ AND_(_TITLE-ABS-KEY_(_**"antiretroviral therapy"**_ OR_ **"HAART"**_ OR_ **"combination antiretroviral therapy"**_ OR_ **"cART"**_ OR_ **"highly active antiretroviral therapy"**_)_)_)_ AND_(LIMIT-TO_(LANGUAGE_,_**"English"**_)_)_

(Immune response AND HAART) ***AND*** *SSA =2588*

(_(_TITLE-ABS-KEY_(_**"Immune response"**_ OR_ **"CD4 count change"**_ OR_ **"CD4 trajectory"**_ OR_ **"CD4 count"**_ OR_ **"CD4"**_ OR_ **"cd4 lymphocyte count"**_OR_ **"CD4 counts"**_ OR_ **"immunity cellular"**_ OR_ **"cellular immune response"**_ OR_ **"cellular immune recovery"**_ OR_ **"cellular immune reconstitution"**_ OR_ **"cellular immune trajectory"**_OR_ **"CD4 recovery"**_ OR_ **"CD4 trajectory"**_ OR_ **"CD4 response"**_ OR_ **"CD4 gains"**_)_)_ AND_(_TITLE-ABS-KEY_(_**"antiretroviral therapy"**_ OR_ **"HAART"**_ OR_ **"combination antiretroviral therapy"**_ OR_ **"cART"**_ OR_ **"highly active antiretroviral therapy"**_)_)_)_)_ AND_ (_TITLE-ABS-KEY_(_**"Angola"**_ OR_ **"Benin"**_ OR_ **"Botswana"**_ OR_ **"Burkina Faso"**_ OR_ **"Burundi"**_ OR_**"Cameroon"**_ OR_ **"Cape Verde"**_ OR_ **"Central African Republic"**_ OR_ **"Chad"**_ OR_**"Comoros"**_ OR_ **"Congo"**_ OR_ **"Côte d'Ivoire"**_ OR_ **"Djibouti"**_ OR_ **"Equatorial Guinea"**_ OR_**"Eritrea"**_ OR_ **"Ethiopia"**_ OR_ **"Gabon"**_ OR_ **"Gambia"**_ OR_ **"Ghana"**_ OR_ **"Guinea"**_ OR_**"Guinea-Bissau"**_ OR_ **"Kenya"**_ OR_ **"Lesotho"**_ OR_ **"Liberia"**_ OR_ **"Madagascar"**_ OR_ **"Malawi"**_ OR_ **"Mali"**_ OR_ **"Mauritania"**_ OR_ **"Mauritius"**_ OR_ **"Mozambique"**_ OR_ **"Namibia"**_OR_ **"Niger"**_ OR_ **"Nigeria"**_ OR_ **"Réunion"**_ OR_ **"Rwanda"**_ OR_ **"Sao Tome and Principe"**_ OR_**"Senegal"**_ OR_ **"Seychelles"**_ OR_ **"Sierra Leone"**_ OR_ **"Somalia"**_ OR_ **"South Africa"**_ OR_**"Sudan"**_ OR_ **"Swaziland"**_ OR_ **"Tanzania"**_ OR_ **"Togo"**_ OR_ **"Uganda"** OR_ **"Zambia"**_ OR_ **"Zimbabwe"**_ OR_ **"Sub-Saharan Africa"**_ OR_ **"Subsaharan Africa"**_ OR_ **"Africa, Sub-Saharan"**_ OR_ **"south of sahara"**_)_)_)_ AND_ (_LIMIT-TO_(_LANGUAGE_,_ **"English"**_)_)_

(Immune response AND HAART AND SSA) AND Statistical models = 614

(_(_TITLE-ABS-KEY_(_**"Immune response"**_ OR_ **"CD4 count change"**_ OR_ **"CD4 trajectory"**_ OR_ **"CD4 count"**_ OR_ **"CD4"**_ OR_ **"cd4 lymphocyte count"**_OR_ **"CD4 counts"**_ OR_ **"immunity cellular"**_ OR_ **"cellular immune response"**_ OR_ **"cellular immune recovery"**_ OR_ **"cellular immune**

reconstitution" OR **"cellular immune trajectory"** OR **"CD4 recovery"** OR **"CD4 trajectory"** OR **"CD4 response"** OR **"CD4 gains"**)) AND (TITLE-ABS-KEY (**"antiretroviral therapy"** OR **"HAART"** OR **"combination antiretroviral therapy"** OR **"cART"** OR **"highly active antiretroviral therapy"**)))) AND (TITLE-ABS-KEY (**"Angola"** OR **"Benin"** OR **"Botswana"** OR **"Burkina Faso"** OR **"Burundi"** OR **"Cameroon"** OR **"Cape Verde"** OR **"Central African Republic"** OR **"Chad"** OR **"Comoros"** OR **"Congo"** OR **"Côte d'Ivoire"** OR **"Djibouti"** OR **"Equatorial Guinea"** OR **"Eritrea"** OR **"Ethiopia"** OR **"Gabon"** OR **"Gambia"** OR **"Ghana"** OR **"Guinea"** OR **"Guinea-Bissau"** OR **"Kenya"** OR **"Lesotho"** OR **"Liberia"** OR **"Madagascar"** OR **"Malawi"** OR **"Mali"** OR **"Mauritania"** OR **"Mauritius"** OR **"Mozambique"** OR **"Namibia"** OR **"Niger"** OR **"Nigeria"** OR **"Réunion"** OR **"Rwanda"** OR **"Sao Tome and Principe"** OR **"Senegal"** OR **"Seychelles"** OR **"Sierra Leone"** OR **"Somalia"** OR **"South Africa"** OR **"Sudan"** OR **"Swaziland"** OR **"Tanzania"** OR **"Togo"** OR **"Uganda"** OR **"Zambia"** OR **"Zimbabwe"** OR **"Sub-Saharan Africa"** OR **"Subsaharan Africa"** OR **"Africa, Sub-Saharan"** OR **"south of sahara"**)) AND (TITLE-ABS-KEY (**"Likelihood Functions"** OR **"Linear Models"** OR **"Logistic Models"** OR **"Proportional Hazards Models"** OR **"Least-Squares Analysis"** OR **"Nomograms"** OR **"Models, Statistical"** OR **"regression"**)) AND (LIMIT-TO (LANGUAGE, **"English"**))

Appendix 3.3

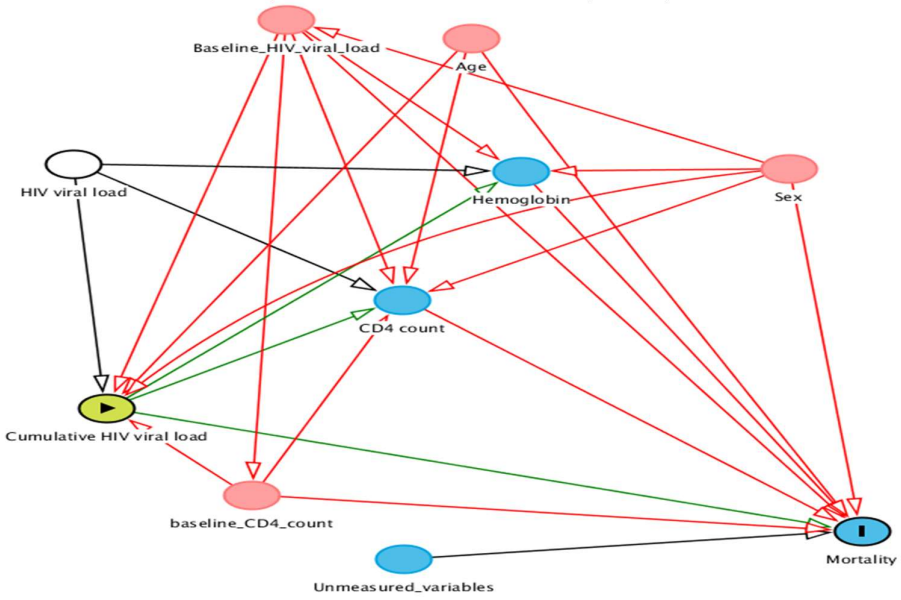
Time-dependent confounding and Marginal Structural Models

In chapter 2, using longitudinal data from an ART cohort, the effect of exposure to most recent viral load and cumulative HIV-Viremia on incidence of opportunistic infection and mortality was investigated. Cox-proportional hazards regression with Andersen-Gill standard errors were used (see Appendix 2.1). Time-dependent confounding is a potential danger in such analyses. This occurs if a covariate is a risk factor/ predictor for a particular outcome and its history predicts its current level [198]. For example, CD4 count can be a time-dependent confounder when investigating the effect of antiretroviral therapy on time to mortality. CD4 counts determine the baseline regimen, any subsequent regimens, and are also independently associated with mortality.

Directed Acyclic Graphs (DAGs) are useful for visually demonstrating covariate-outcome relationships, with potential time-dependent confounding. A confounder is causal to (i.e. is an ancestor of) the outcome, is associated with the exposure but is not causal to (i.e. not a descendant of) exposure or outcome [145]. Figure 1 below is a DAG for the outcome of mortality as investigated in Chapter 2. In this case CD4 counts and hemoglobin were not time-dependent confounders as they did not confound the relationship of cumulative HIV-viremia to mortality. Adjustment was made for the following potential confounders: age, baseline HIV viral load, sex and baseline CD4 count, as well as time-updated CD4 count and hemoglobin. We also adjusted for HIV viral load as one of the two main predictors under study (Appendix 3.3 Figure 1).

Marginal Structural Models (MSM's) or Structural Nested Models (SNMs) are useful when analyzing data containing potential time-dependent confounding. The parameters of MSMs can be estimated using the inverse probability of treatment, with censored weights [198], while for SNMs G-parameter estimation is done [199]. To obtain the combined inverse probability of treatment and censoring, the two inverse probabilities are multiplied by each other. The former is the conditional probability of treatment, given baseline covariates and past treatment history divided by the conditional probability of receiving treatment given the past treatment history. The latter is the conditional probability of not being censored, given baseline covariates and past treatment history, divided by the conditional probability of not being censored, given previous treatment history and covariate history. It is possible to adjust for these in Cox proportional hazards models [198].

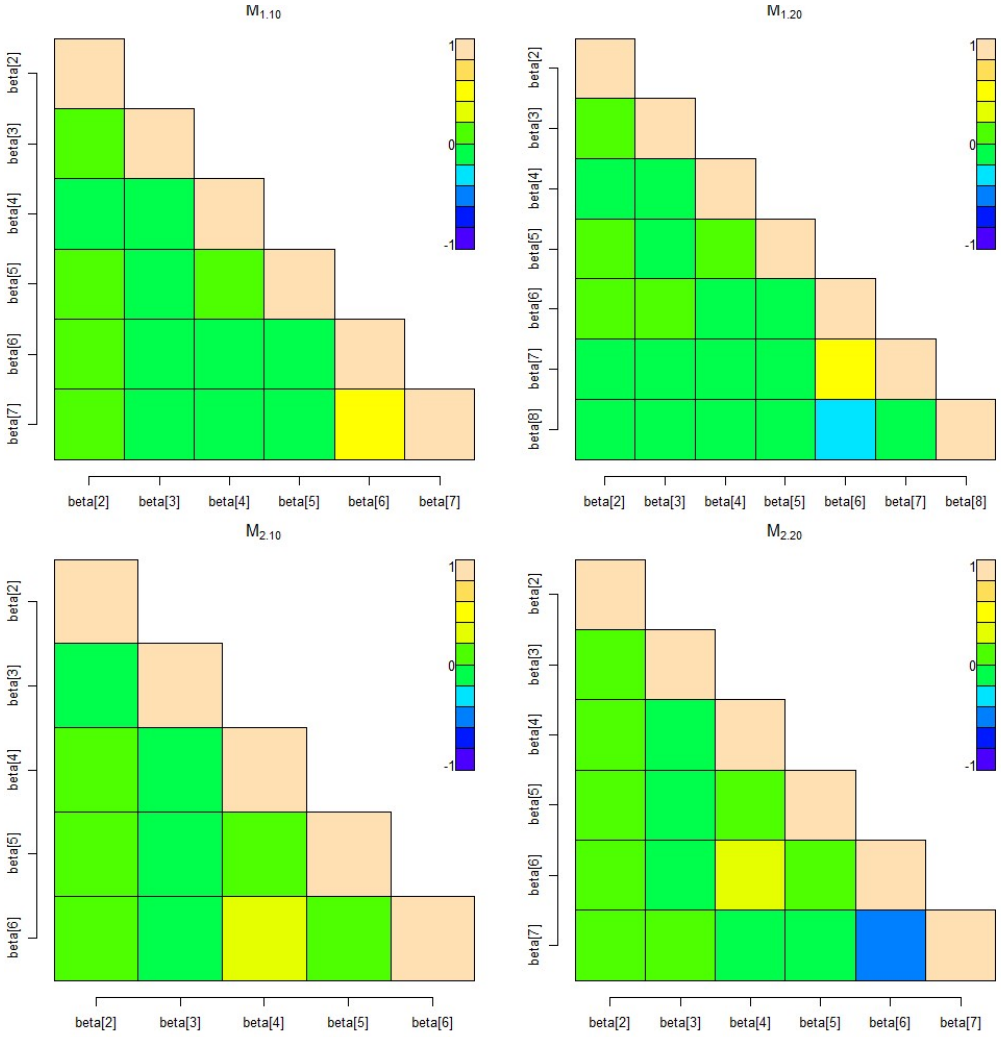
Appendix 3.3 Figure 1: Directed acyclic graph showing the different relationships between covariates adjusted for in the mortality analysis



Legend: blue with black boarder is the outcome which is mortality; the white circle with a black border is for the variable we *'forcefully'* adjust for which is HIV viral load; yellow circle with black boarder and black arrow is the main exposure which is cumulative HIV-Viremia; blue circles represents ancestors of exposure and outcome; and pink circles represent ancestor of exposure and outcome. Green lines represent causal path; pink arrows represent the relationships that need to adjust for to avoid biased estimates and; black line is for unmeasured makers.

Appendix 4.1

Appendix 4.1 Figure 1: Cross correlation plots between corresponding parameters adjusted for in the primary models



Legend: $M_{1.10}$ and $M_{1.20}$ are models without or with cumulative log viral load, respectively. In these models, CD4 counts outcome in the likelihood follows normal distribution, while beta priors follow independent Gaussian normal distribution; and $\boldsymbol{M_{2.10}}$ and $\boldsymbol{M_{2.20}}$ are models without or with cumulative log viral load, respectively. In these models, are having ≥500 cells/μL follows Bernoulli distribution, beta priors follow independent Gaussian normal distribution. Beta[2] up to beta[6] represents sex, baseline age, baseline CD4 count, baseline log viral load and time on treatment. For $\boldsymbol{M_{1.10}}$ and $\boldsymbol{M_{1.20}}$ beta[7] and beta[8] are time on treatment-squared and cumulative log viral load, respectively, while beta[7] was for cumulative log viral load in $\boldsymbol{M_{2.10}}$ and $\boldsymbol{M_{2.20}}$ and beta[6] is cumulative log viral load.

Appendix 4.1 Table 1: Effect of using a Student's *t*-distributed CD4 count in the slope of CD4 count model (Posterior mean and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95% CI	Estimate	95% CI
Female-gender	8.77	(2.148, 15.4)	8.301	(1.588, 14.97)
Per one year increase in baseline age, years	-1.36	(-4.452, 1.749)	-1.181	(-4.278, 1.939)
Per one cell increase in baseline CD4 count, cells/µL	80.56	(77.11, 83.94)	80.79	(77.35, 84.17)
Per log10 increase in baseline log viral load , log10 copies/mL	0.97	(-0.933, 2.938)	0.9843	(-0.8866, 2.949)
Time on treatment	58.74	(51.7, 65.83)	60.51	(53.14, 67.53)
Time on treatment-squared	-21.97	(-23.7, -20.25)	-21.94	(-23.66, -20.24)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**-13.39**	**(-20.73, -6.001)**

Legend: cVL_2—cumulative log viral load

Appendix 4.1 Table 2: Implementing the model with cubic splines and 3 inner knots for the slope of CD4 counts model (Posterior mean and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95% CI	Estimate	95% CI
Female-gender	16.8	(5.549, 28.1)	16.85	(5.658, 28.08)
Per one year increase in baseline age, years	-6.453	(-11.62, -1.287)	-6.501	(-11.65, -1.322)
Per one cell increase in baseline CD4 count, cells/µL	79.16	(73.67, 84.69)	79.12	(73.63, 84.61)
Per log10 increase in baseline log viral load , log10 copies/mL	1.661	(-1.09, 4.496)	1.667	(-1.095, 4.534)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	-0.2658	(-9.299, 8.803)

Legend: cVL_2—cumulative log viral load

Appendix 4.1 Table 3: Effect of using cubic splines with 5 inner knots for the slope of CD4 counts model (Posterior mean and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95% CI	Estimate	95% CI
Female-gender	16.83	(5.604, 28.0)	16.92	(5.692, 28.18)
Per one year increase in baseline age, years	-6.484	(-11.62, -1.363)	-6.525	(-11.66, -1.364)
Per one cell increase in baseline CD4 count, cells/µL	79.13	(73.66, 84.6)	79.07	(73.62, 84.58)
Per log10 increase in baseline log viral load , log10 copies/mL	1.694	(-1.066, 4.539)	1.692	(-1.061, 4.524)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	-0.2218	(-9.273, 8.861)

Legend: cVL_2—cumulative log viral load

Appendix 4.1 Table 4: Effect of using skew-normal random effect in the slope of CD4 count model (Posterior mean and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95% CI	Estimate	95% CI
Female-gender	24.08	(13.0, 35.21)	23.19	(11.94, 34.31)
Per one year increase in baseline age, years	-6.605	(-11.79, -1.409)	-5.941	(-11.15, -0.7084)
Per one cell increase in baseline CD4 count, cells/µL	77.26	(71.78, 82.73)	77.98	(72.48, 83.48)
Per log10 increase in baseline log viral load , log10 copies/mL	1.867	(-0.9285, 4.747)	1.808	(-0.9761, 4.692)
Time on treatment	52.11	(45.13, 58.95)	55.63	(48.69, 62.44)
Time on treatment-squared	-22.9	(-24.94, -20.86)	-22.75	(-24.78, -20.7)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**-19.15**	**(-27.85, -10.44)**

Legend: cVL_2—cumulative log viral load

Appendix 4.1 Table 5: Effect of using informative priors in the slope of CD4 count model where parameters followed Gaussian normal distribution (Posterior mean and 95% credible intervals)

Parameter	Model $M_{1.20}$		Model $M_{1.20}^{*}$	
	Estimate	95% CI	Estimate	95% CI
Female-gender	23.03	(11.99, 34.24)	23.8	(12.37, 35.34)
Per one year increase in baseline age, years	-5.892	(-11.11, -0.6867)	-5.894	(-11.11, -0.6808)
Per one cell increase in baseline CD4 count, cells/µL	78.05	(72.54, 83.56)	78.01	(72.5, 83.49)
Per log10 increase in baseline log viral load , log10 copies/mL	1.821	(-0.9488, 4.705)	1.787	(-0.9821, 4.627)
Time on treatment	55.55	(48.45, 62.56)	56.22	(49.26, 63.16)
Time on treatment-squared	-22.74	(-24.79, -20.7)	-22.65	(-24.69, -20.6)
Per $\log_{10}$ increase in cumulative log viral load, $\log_{10}$ copy-year/mL	**-19.16**	**(-27.84, -10.49)**	**-19.37**	**(-28.03, -10.66)**

Legend: $M_{1.20}$ – Gaussian normally distributed CD4 count in the likelihood; *and* $M_{1.20}^{*}$ – Gaussian normally distributed CD4 count in the likelihood but with informative priors.

Appendix 4.1 Table 6: Effect of using informative priors in the slope of CD4 count model where CD4 count had Student's t-distribution (Posterior mean and 95% credible intervals)

Parameter	Model $M_{1.22}$		Model $M_{1.22*}$	
	Estimate	95% CI	Estimate	95% CI
Female gender	8.301	(1.588, 14.97)	8.326	(1.574, 15.15)
Per one year increase in baseline age, years	-1.181	(-4.278, 1.939)	-1.196	(-4.3, 1.894)
Per one cell increase in baseline CD4 count, cells/µl	80.79	(77.35, 84.17)	80.8	(77.34, 84.2)
Per log10 increase in baseline log viral load , log10 copies/mL	0.9843	(-0.8866, 2.949)	0.992	(-0.889, 2.963)
Time on treatment	60.51	(53.14, 67.53)	61.39	(53.93, 68.47)
Time on treatment-squared	-21.94	(-23.66, -20.24)	-21.84	(-23.58, -20.12)
Per $\log_{10}$ increase in cumulative log viral load, $\log_{10}$ copy-year/mL	**-13.39**	**(-20.73, -6.001)**	**-13.55**	**(-20.96, -6.119)**

Legend: $M_{1.22}$ – Student's *t*-distributed CD4 count; *and* $M_{1.22}^{*}$ – Student's *t*-distributed CD4 count with informative priors.

Appendix 4.1 Table 7: Effect of using skew-normal for random effect in the asymptote model (Posterior odds ratios and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95%CI	Estimate	95%CI
Female-gender	5.82	(1.044, 12.466)	6.36	(2.998, 13.722)
Per one year increase in baseline age, years	0.57	(0.410, 0.773)	0.57	(0.406, 0.783)
Per one cell increase in baseline CD4 count, cells/µL	2.44	(1.812, 3.391)	2.53	(1.855, 3.543)
Per log10 increase in baseline log viral load , log10 copies/mL	1.10	(0.881, 1.360)	1.11	(0.878, 1.377)
Time on treatment	3.49	(2.689, 4.522)	4.01	(3.056, 5.328)
Time on treatment - squared	0.67	(0.584, 0.761)	0.65	(0.568, 0.747)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**0.42**	**(0.244, 0.736)**

Legend: cVL_2—cumulative log viral load

Appendix 4.1 Table 8: Effect of using cubic splines with 3 inner knots in the asymptote model (Posterior odds ratios and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95%CI	Estimate	95%CI
Female-gender	4.58	(2.214, 11.12)	4.495	(2.201, 9. 964)
Per one year increase in baseline age, years	0.66	(0.498, 0.857)	0.66	(0.502, 0.864)
Per one cell increase in baseline CD4 count, cells/µL	2.08	(1.647, 2.616)	2.10	(1.668, 2.678)
Per log10 increase in baseline log viral load , log10 copies/mL	1.03	(0.886, 1.194)	1.03	(0.885, 0.839)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**0.76**	**(0.470, 1.258)**

Legend: cVL_2—cumulative log viral load

Appendix 4.1 Table 9: Effect of using cubic splines with 5 inner knots in the asymptote model (Posterior odds ratios and 95% credible intervals)

Parameter	Model without cVL_2		Model with cVL_2	
	Estimate	95%CI	Estimate	95%CI
Female-gender	4.48	(2.292, 9.125)	4.24	(0.7587, 8.645)
Per one year increase in baseline age, years	0.66	(0.497, 0.858)	0.66	(0.507, 0.867)
Per one cell increase in baseline CD4 count, cells/µL	2.06	(1.637, 2.603)	2.08	(1.652, 2.633)
Per log10 increase in baseline log viral load , log10 copies/mL	1.03	(0.886, 1.195)	1.03	(0.889, 1.195)
Per log_{10} increase in cumulative log viral load, log_{10} copy-year/mL	–	–	**0.77**	**(0.476, 1.281)**

Legend: cVL_2—cumulative log viral load

Appendix 4.2

OpenBUGS code for slope of CD4 count models:

Model $M_{1.20}$: primary cumulative log viral load model

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        CD4[i , j] ~ dnorm(mu[i , j], tau.int)
                        mu[i , j] <- beta[1] + beta[2] * sex.f[i] + beta[3] * age_count[i]
+ beta[4] * base.cd4[i] + beta[5] * base.logvl[i] + beta[6] * (yrs.fu[j] - 2.5) + beta[7] *
pow(yrs.fu[j] - 2.5, 2) + beta[8] * cumvl[i , j] * cumvlFlag[i , j] + U[i , 1] + U[i , 2] *
yrs.fu[j]
                }
                U[i , 1:2] ~ dmnorm(U0[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age_count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age_count[i]
                sex.f[i] ~ dbern(p[i])
                p[i] <- max(1.0E-5, min(0.99999, ptemp[i]))
                logit(ptemp[i]) <- beta3[1] + beta3[2] * age_count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age_count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
                mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age_count[i]
                for( j in 1 : M ) {
                        cd4.p1[i , j] ~ dnorm(mu[i , j], tau.int)
                        r12[i , j] <- CD4[i , j] - cd4.p1[i , j]
                        sqr[i , j] <- r12[i , j] * r12[i , j]
                }
        }
        mspe <- mean(sqr[ , ])
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        cor ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau.int <- pow(sigma1,  -2)
        sigma1 ~ dunif(10, 1000)
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
```

```
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 8 ) {
                beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
                beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betab[k] ~ dnorm(0, 0.001)
        }
}
```

Model with cumulative log viral load – $M_{1.21}$: cubic b-splines with 3 inner knots

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        CD4[i , j] ~ dnorm(mu[i , j], tau.int)
                        mu[i , j] <- beta[1] * sex.f[i] + beta[2] * age_count[i] + beta[3]
* base.cd4[i] + beta[4] * base.logvl[i] + beta[5] * cumvl[i , j] * cumvlFlag[i , j] + U[i ,
1] + U[i , 2] * yrs.fu[j] + bspline[1] * time1[i , j] + bspline[2] * time2[i , j] +
bspline[3] * time3[i , j] + bspline[4] * time4[i , j] + bspline[5] * time5[i , j] +
bspline[6] * time6[i , j] + bspline[7] * time7[i , j] + bspline[8] * time8[i , j]
                }
                U[i , 1:2] ~ dmnorm(U0[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age_count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age_count[i]
                sex.f[i] ~ dbern(p[i])
                p[i] <- max(1.0E-5, min(0.99999, ptemp[i]))
                logit(ptemp[i]) <- beta3[1] + beta3[2] * age_count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age_count[i]
```

```
            basecd4[i] ~ dnorm(mub[i], taub.int)
            mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age_count[i]
            for( j in 1 : M ) {
                cd4.p1[i , j] ~ dnorm(mu[i , j], tau.int)
                r12[i , j] <- CD4[i , j] - cd4.p1[i , j]
                sqr[i , j] <- r12[i , j] * r12[i , j]
            }
        }
        mspe <- mean(sqr[ , ])
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        cor ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau.int <- pow(sigma1,  -2)
        sigma1 ~ dunif(10, 1000)
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 5 ) {
            beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
            beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            betab[k] ~ dnorm(0, 0.001)
        }
        for( d in 1 : 8 ) {
            bspline[d] ~ dnorm(0, 1.0E-6)
        }
}
```

Model with cumulative log viral load – $M_{I.22}$: CD4 count with Student's t-distribution

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        CD4[i , j] ~ dt(mu[i , j], tau.int, 3)
                        mu[i , j] <- beta[1] + beta[2] * sex.f[i] + beta[3] * age_count[i]
+ beta[4] * base.cd4[i] + beta[5] * base.logvl[i] + beta[6] * (yrs.fu[j] - 2.5) + beta[7] *
pow(yrs.fu[j] - 2.5, 2) + beta[8] * cumvl[i , j] * cumvlFlag[i , j] + U[i , 1] + U[i , 2] *
yrs.fu[j]
                }
                U[i , 1:2] ~ dmnorm(U0[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age_count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age_count[i]
                sex.f[i] ~ dbern(p[i])
                p[i] <- max(1.0E-5, min(0.99999, ptemp[i]))
                logit(ptemp[i]) <- beta3[1] + beta3[2] * age_count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age_count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
                mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age_count[i]
                for( j in 1 : M ) {
                        cd4.p1[i , j] ~ dnorm(mu[i , j], tau.int)
                        r12[i , j] <- CD4[i , j] - cd4.p1[i , j]
                        sqr[i , j] <- r12[i , j] * r12[i , j]
                }
        }
        mspe <- mean(sqr[ , ])
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        cor ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau.int <- pow(sigma1,  -2)
        sigma1 ~ dunif(10, 1000)
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
```

```
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 8 ) {
                beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
                beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betab[k] ~ dnorm(0, 0.001)
        }
}
```

Model with cumulative log viral load – $M_{1.23}$: cubic B-splines with 5 inner knots

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        CD4[i , j] ~ dnorm(mu[i , j], tau.int)
                        mu[i , j] <- beta[1] * sex.f[i] + beta[2] * age_count[i] + beta[3]
* base.cd4[i] + beta[4] * base.logvl[i] + beta[5] * cumvl[i , j] * cumvlFlag[i , j] + U[i ,
1] + U[i , 2] * yrs.fu[j] + bspline[1] * time1[i , j] + bspline[2] * time2[i , j] +
bspline[3] * time3[i , j] + bspline[4] * time4[i , j] + bspline[5] * time5[i , j] +
bspline[6] * time6[i , j] + bspline[7] * time7[i , j] + bspline[8] * time8[i , j] +
bspline[9] * time9[i , j] + bspline[10] * time10[i , j]
                }
                U[i , 1:2] ~ dmnorm(U0[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age_count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age_count[i]
                sex.f[i] ~ dbern(p[i])
                p[i] <- max(1.0E-5, min(0.99999, ptemp[i]))
                logit(ptemp[i]) <- beta3[1] + beta3[2] * age_count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age_count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
```

```
            mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age_count[i]
            for( j in 1 : M ) {
                cd4.p1[i , j] ~ dnorm(mu[i , j], tau.int)
                r12[i , j] <- CD4[i , j] - cd4.p1[i , j]
                sqr[i , j] <- r12[i , j] * r12[i , j]
            }
        }
        mspe <- mean(sqr[ , ])
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        cor ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau.int <- pow(sigma1,  -2)
        sigma1 ~ dunif(10, 1000)
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 5 ) {
            beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
            beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
            betab[k] ~ dnorm(0, 0.001)
        }
        for( d in 1 : 10 ) {
            bspline[d] ~ dnorm(0, 1.0E-6)
        }
}
```

Model with cumulative log viral load – $M_{1.24}$: Skew-normal random-effects

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        CD4[i , j] ~ dnorm(mu[i , j], tau.int)
                        mu[i , j] <- beta[1] + beta[2] * sex.f[i] + beta[3] * age_count[i]
+ beta[4] * base.cd4[i] + beta[5] * base.logvl[i] + beta[6] * (yrs.fu[j] - 2.5) + beta[7] *
pow(yrs.fu[j] - 2.5, 2) + beta[8] * cumvl[i , j] * cumvlFlag[i , j] + U[i , 1] + U[i , 2] *
yrs.fu[j]
                }
                U0.t[i , 1] ~ dnorm(0, 1)
                U0.t[i , 2] ~ dnorm(0, 1)
                U0[i , 1] <- deltab.abs[1] * U0.t[i , 1]
                U0[i , 2] <- deltab.abs[2] * U0.t[i , 2]
                U1[i , 1:2] ~ dmnorm(meanb[1:2], tau[1:2 , 1:2])
                for( ii in 1 : 2 ) {
                        U[i , ii] <- U0[i , ii] + U1[i , ii]
                }
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age_count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age_count[i]
                sex.f[i] ~ dbern(p[i])
                p[i] <- max(1.0E-5, min(0.99999, ptemp[i]))
                logit(ptemp[i]) <- beta3[1] + beta3[2] * age_count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age_count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
                mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age_count[i]
                for( j in 1 : M ) {
                        cd4.p1[i , j] ~ dnorm(mu[i , j], tau.int)
                        r12[i , j] <- CD4[i , j] - cd4.p1[i , j]
                        sqr[i , j] <- r12[i , j] * r12[i , j]
                }
        }
        mspe <- mean(sqr[ , ])
        Om[1 , 1] <- a * Omega[1 , 1]
        Om[1 , 2] <- a * Omega[1 , 2]
        Om[2 , 1] <- a * Omega[2 , 1]
        Om[2 , 2] <- a * Omega[2 , 2]
        Q[1:2 , 1:2] ~ dwish(Om[1:2 , 1:2], 3)
        tau[1 , 1] <- pow(D1,  -2) * Q[1 , 1]
        tau[1 , 2] <- pow(D1,  -1) * pow(D2,  -1) * Q[1 , 2]
        tau[2 , 1] <- pow(D2,  -1) * pow(D1,  -1) * Q[2 , 1]
        tau[2 , 2] <- pow(D2,  -2) * Q[2 , 2]
```

```
        sigmab2[1:2 , 1:2] <- inverse(tau[ , ])
        sigmab[1] <- sqrt(sigmab2[1 , 1])
        sigmab[2] <- sqrt(sigmab2[2 , 2])
        corrb <- sigmab2[1 , 2] / sigmab[1] * sigmab[2]
        D1 ~ dunif(0.001, 100)
        D2 ~ dunif(0.001, 100)
        deltab[1] ~ dnorm(0.0, 1.0E-6)
        deltab[2] ~ dnorm(0.0, 1.0E-6)
        deltab.abs[1] <- abs(deltab[1])
        deltab.abs[2] <- abs(deltab[2])
        tau.int <- pow(sigma1,  -2)
        sigma1 ~ dunif(10, 1000)
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 8 ) {
                beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
                beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betab[k] ~ dnorm(0, 0.001)
        }
}
```

Appendix 4.2 Table 1: estimated informative priors used for slope of CD4 count model

Variable	Same model Coef.[a]			Informative priors			
	Historical study	Current study	$M_{1.20}$ Coef.	Change[b]	Coef.[c]	Variance	Precision
Female-gender [109]	35,2	36,17	23	-13,17	22,03	12038,79	8,31E-05
Baseline $\log_{10}$ viral load (copies/mL) [109]	13,9	-10,09	1,8	-11,89	2,01	340,7415	0,002935
Baseline age (years) [115]	-0,97	-6,283	-5,9	-0,383	-1,353	733,8447	0,001363
Time on treatment (years) [114]	65	56,18	55,5	-0,68	64,32	13941694	7,17E-08
Time on treatment (years)-squared [114]	-6	-22,97	-22,7	-0,27	-6,27	132482,5	7,55E-06

[a] list coefficients from historical models as published in the respective articles (historical study) and from the same reanalysed models using our data (current study); [b] obtained by subtracting current study coefficients from full model ($M_{1.20}$); [c] obtained by adding both Change and historical study columns.

OpenBUGS code for Asymptote models:

Model without cumulative log viral load – $M_{2.20}$: primary model

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        response[i , j] ~ dbern(p[i , j])
                        p[i , j] <- max(1.0E-5, min(0.99999, ptemp[i , j]))
                        logit(ptemp[i , j]) <- beta[1] + beta[2] * sex.f[i] + beta[3] *
age.count[i] + beta[4] * base.cd4[i] + beta[5] * base.logvl[i] + beta[6] * (yrs.fu[j] -
2.5) + beta[7] * pow(yrs.fu[j] - 2.5, 2) + beta[8] * cumvl[i , j] * cumvlflag[i , j] +
b[id[i] , 1] + b[id[i] , 2] * yrs.fu[j]
                }
                b[i , 1:2] ~ dmnorm(meanb[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age.count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age.count[i]
                sex.f[i] ~ dbern(phi[i])
                phi[i] <- max(1.0E-5, min(0.99999, phitemp[i]))
                logit(phitemp[i]) <- beta3[1] + beta3[2] * age.count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age.count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
                mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age.count[i]
```

```
        }
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        cor ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 8 ) {
                beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
                beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betab[k] ~ dnorm(0, 0.001)
        }
}
```

Model with cumulative log viral load – $M_{2.21}$: skew-normal random-effects distribution model

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        response[i , j] ~ dbern(p[i , j])
                        p[i , j] <- max(1.0E-5, min(0.99999, ptemp[i , j]))
                        logit(ptemp[i , j]) <- beta[1] * sex.f[i] + beta[2] * age.count[i] +
beta[3] * base.cd4[i] + beta[4] * base.logvl[i] + beta[5] * cumvl[i , j] * cumvlflag[i , j]
+ b[id[i] , 1] + b[id[i] , 2] * yrs.fu[j] + bspline[1] * time1[i , j] + bspline[2] * time2[i ,
```

```
j] + bspline[3] * time3[i , j] + bspline[4] * time4[i , j] + bspline[5] * time5[i , j] +
bspline[6] * time6[i , j] + bspline[7] * time7[i , j] + bspline[8] * time8[i , j]
                }
                b[i , 1:2] ~ dmnorm(meanb[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age.count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age.count[i]
                sex.f[i] ~ dbern(phi[i])
                phi[i] <- max(1.0E-5, min(0.99999, phitemp[i]))
                logit(phitemp[i]) <- beta3[1] + beta3[2] * age.count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age.count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
                mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age.count[i]
        }
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        corrb ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 5 ) {
                beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
                beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betaa[k] ~ dnorm(0, 0.001)
        }
```

```
        for( k in 1 : 3 ) {
                betab[k] ~ dnorm(0, 0.001)
        }
        for( d in 1 : 8 ) {
                bspline[d] ~ dnorm(0, 1.0E-6)
        }
}
```

Model with cumulative log viral load – $M_{2.23}$: cubic B-splines with 5 inner knots

```
model{
        for( i in 1 : N ) {
                for( j in 1 : M ) {
                        response[i , j] ~ dbern(p[i , j])
                        p[i , j] <- max(1.0E-5, min(0.99999, ptemp[i , j]))
                        logit(ptemp[i , j]) <- beta[1] * sex.f[i] + beta[2] * age.count[i] +
beta[3] * base.cd4[i] + beta[4] * base.logvl[i] + beta[5] * cumvl[i , j] * cumvlflag[i , j]
+ b[id[i] , 1] + b[id[i] , 2] * yrs.fu[j] + bspline[1] * time1[i , j] + bspline[2] * time2[i ,
j] + bspline[3] * time3[i , j] + bspline[4] * time4[i , j] + bspline[5] * time5[i , j] +
bspline[6] * time6[i , j] + bspline[7] * time7[i , j] + bspline[8] * time8[i , j] +
bspline[9] * time9[i , j] + bspline[10] * time10[i , j]
                }
                b[i , 1:2] ~ dmnorm(meanb[], tau[ , ])
                basecd4.imp[i] <- cut(base.cd4[i])
                baselogvl.imp[i] <- cut(base.logvl[i])
                base.cd4[i] ~ dnorm(mu1[i], tau1.int)
                mu1[i] <- beta1[1] + beta1[2] * sex.f[i] + beta1[3] * age.count[i]
                base.logvl[i] ~ dnorm(mu2[i], tau2.int)
                mu2[i] <- beta2[1] + beta2[2] * sex.f[i] + beta2[3] * age.count[i]
                sex.f[i] ~ dbern(phi[i])
                phi[i] <- max(1.0E-5, min(0.99999, phitemp[i]))
                logit(phitemp[i]) <- beta3[1] + beta3[2] * age.count[i] + beta3[3] *
baselogvl[i] + beta3[4] * basecd4[i]
                baselogvl[i] ~ dnorm(mua[i], taua.int)
                mua[i] <- betaa[1] + betaa[2] * sex.f[i] + betaa[3] * age.count[i]
                basecd4[i] ~ dnorm(mub[i], taub.int)
                mub[i] <- betab[1] + betab[2] * sex.f[i] + betab[3] * age.count[i]
        }
        sigmau0 ~ dunif(0, 100)
        sigmau1 ~ dunif(0, 100)
        corrb ~ dunif(-1, 1)
        sigmaU[1 , 1] <- pow(sigmau0, 2)
        sigmaU[2 , 2] <- pow(sigmau1, 2)
        sigmaU[1 , 2] <- sigmau0 * sigmau1 * cor
        sigmaU[2 , 1] <- sigmaU[1 , 2]
        tau[1:2 , 1:2] <- inverse(sigmaU[ , ])
        tau1.int <- pow(sigma1.imp,  -2)
        sigma1.imp ~ dunif(10, 100)
        tau2.int <- pow(sigma2.imp,  -2)
```

```
        sigma2.imp ~ dunif(10, 100)
        taua.int <- pow(sigmab.imp,  -2)
        sigmaa.imp ~ dunif(10, 100)
        taub.int <- pow(sigmab.imp,  -2)
        sigmab.imp ~ dunif(10, 100)
        for( k in 1 : 5 ) {
                beta[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta1[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                beta2[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 4 ) {
                beta3[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betaa[k] ~ dnorm(0, 0.001)
        }
        for( k in 1 : 3 ) {
                betab[k] ~ dnorm(0, 0.001)
        }
        for( d in 1 : 10 ) {
                bspline[d] ~ dnorm(0, 1.0E-6)
        }
}
```