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THE EFFECTS OF CO-TRIMOXAZOLE PROPHYLAXIS ON CD4 CELL-COUNT PROFILES IN HIV-POSITIVE PATIENTS STABILIZED ON ANTI-RETROVIRAL THERAPY

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A research report submitted to the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in Epidemiology (Biostatistics)

29 October 2019

I **Tshwaraganang Hope Modise** declare that this research report is my own work. It is being submitted for the degree of **Master of Science in Epidemiology (Biostatistics)** at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or at any other University.

29 October 2019 in Johannesburg, RSA

DEDICATION

I dedicate this work to God for the unconditional love he has given me in my life, and to my parents who have always inspired me and given me support. I thank God for my siblings who have always looked up to me and gave me their love and support throughout this journey.

ABSTRACT

Introduction Cotrimoxazole prophylaxis has been successfully used for many years in preventing opportunistic infections in patients living with HIV. There is lack of data from previous studies on the effects of CTX on CD4 cell count recovery. This study evaluated the effects of discontinuing CTX on CD4 profiles of HIV positive patients stabilized on ART in Uganda.

Methods The study used secondary data from the COSTOP double-blind placebo-controlled trial which ran in Uganda from 2011 to 2014 in Masaka and Entebbe. The study enrolled participants aged 18 years and above who were stabilized on ART. A linear regression model and mixed effects models were fitted to compare the CD4 cell count trajectories of participants randomized to receive placebo and participants randomized to continue CTX. The analysis included all participants with at least one CD4 cell count measure at follow up.

Results Participants who received placebo had a significantly higher final CD4 count [mean difference=37.32 cells/mm^3 (95% CI 31.44;43.20, p<0.001)] compared to those who continued CTX. Among the random intercept models, the best fitting model included a treatment arm by time interaction which showed that the increase in CD4 counts was significantly higher by 0.24 cells/mm^3 per week for participants randomized to placebo (95% CI 0.15;0.34, P<0.001). In the corresponding random slopes model the difference in slopes was reduced slightly to 0.21 cells/mm^3 per week for participants randomized to placebo (95 % CI 0.09;0.33, p<0.001).

Conclusions This study showed that discontinued CTX significantly increased the CD4 cell count, and continued CTX leads to slower CD4 recovery.

Keywords: **Cotrimoxazole, Ant-retroviral therapy, CD4 cell count.**

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LIST OF ABBREVIATIONS

HIV: Human Immunodeficiency Virus

AIDS: Acquired Immune Deficiency Syndrome

CD4: Cluster of Differentiation 4

PCP: Pneumocystis carinii pneumonia

AIDS: Acquired Immune Deficiency Syndrome

UNAIDS: Joint United Nations program on HIV and AIDS

CTX: Co-trimoxazole

ART: Antiretroviral Therapy

WHO: World Health Organization

DART: Development of Anti-Retroviral Therapy in Africa

HBAC: Home-Based AIDS Care Program

cART: Combination Antiretroviral Therapy

LIST OF FIGURES

CHAPTER ONE

FIGURE 1.1.1 WHO 2017 PREVALENCE OF HIV AND AIDS 1

CHAPTER Three

FIGURE 3.2.1 MEAN CD4 BOX PLOTS BY TREATMENT ARM. 23

FIGURE 3.2.2 MEAN CD4 CELL COUNT OVER TIME UP TO 24 MONTHS IN THE STUDY. 25

LIST OF TABLES

CHAPTER THREE

TABLE 3.1 STUDY POPULATION CHARACTERISTICS BY TREATMENT ARM. 20
TABLE 3.2 MEAN CD4 BY TREATMENT ARM, STRATIFIED BY SITE. 22
TABLE 3.3 MEAN CD4 BY TREATMENT ARM. 22
TABLE 3.4 LINEAR REGRESSION ANALYSIS OF FACTORS ASSOCIATED WITH FINAL CD4 23
TABLE 3.5 ADJUSTED ESTIMATE OF THE MEAN CD4 CELL COUNT 24
TABLE 3.6 MODEL 1 RANDOM INTERCEPT MODEL 25
TABLE 3.7 MODEL 2 RANDOM INTERCEPT MODEL INCLUDING INTERACTION 26
TABLE 3.8 MODEL 3 RANDOM SLOPE MODEL INCLUDING THE INTERACTION 28

TABLE OF CONTENTS

1 CHAPTER ONE: INTRODUCTION 1

1.1 Background 1

1.1.1 The HIV pandemic in Sub-Saharan Africa and Uganda 1

1.1.2 The Impact of ART 2

1.1.3 Role of Co-trimoxazole prophylaxis both Pre ART and post ART 3

1.2 Problem statement 4

1.3 Justification 4

1.4 Aim 4

1.5 Literature Review 5

1.5.1 The role of Co-trimoxazole and the Impact of ART in HIV patients 5

1.5.2 Modelling CD4 cell count and mixed effects models for longitudinal data. 8

2 CHAPTER 2: METHODOLOGY 11

2.1 Primary study 11

2.2 Present Study 13

2.2.1 Study design 13

2.2.2 Study site 13

2.2.3 Study population and sampling method 13

2.2.4 Study Variables 13

2.2.5 Data Management and analysis 14

2.2.6 Analysis for objective 1 14

2.2.7 Analysis for objective 2 15

2.2.8 Analysis for objective 3 17

2.2.9 Ethical consideration 18

3 CHAPTER 3: RESULTS 19

3.1 Baseline Characteristics of the patient population by treatment arm. 19

3.2 Objective 1 Results 21

3.3 Objective 2 Results 24

3.3.1 Random intercept model assessing the average difference in CD4 cell count. 25

3.3.2 Random intercept model including an interaction between time and treatment. 26

3.4 Objective 3 Results 27

3.4.1 Random slope model including interaction between time and treatment. 28

4 CHAPTER 4 DISCUSSION AND CONCLUSION 30

4.1 Discussion 30

4.2 Study strengths and limitations 31

4.3 Conclusion 32

4.4 Recommendations 32

5 REFERENCES 33

6 APPENDICES 36

6.1 Plagiarism declaration report 36

6.2 Ethics clearance certificate 37

1 CHAPTER ONE: INTRODUCTION

1.1 Background

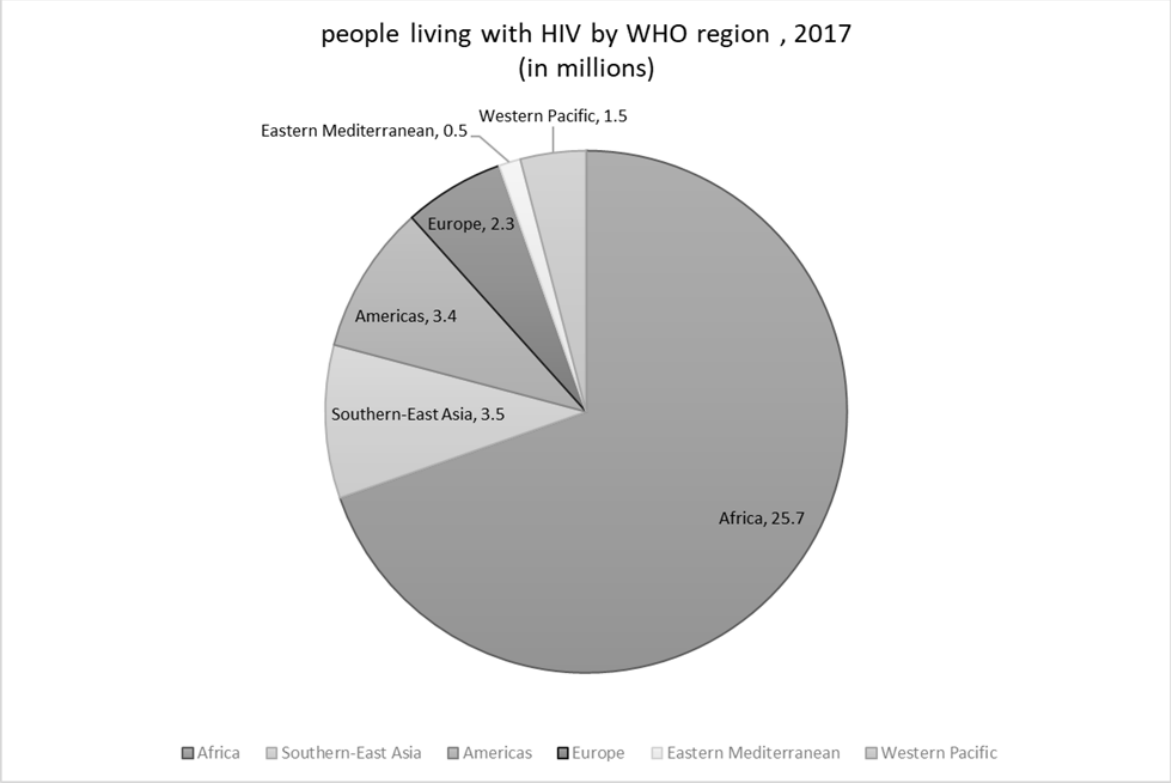
1.1.1 The HIV pandemic in Sub-Saharan Africa and Uganda

Human Immune Deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) have been a global public health challenge since early 1990s [1]. The virus causes AIDS by attacking the CD4 cells of the body [2].

WHO 2017 data report showed that there were about 36.9 million people living with HIV and AIDS worldwide [3]. Africa has the highest disease prevalence with 25.7 million people that live with HIV and AIDS [3].

The following graph data is from 2017 WHO report, showing the prevalence of HIV and AIDS in 2017 [3].

Figure 1.1.1 WHO 2017 prevalence of HIV and AIDS



The Uganda national population HIV impact assessment indicated that in 2016, approximately 1.3 million people were living with HIV [4] and approximately 28,000 people in Uganda died from AIDS related diseases in that year [5], which has reduced compared to approximately 60,000 deaths due to AIDS in 2010. The prevalence of HIV and AIDS in adults (15-49) was 6.5% in 2016 with 5,800 new infections in adults. The groups that are most affected by HIV are sex workers (with a prevalence of 34%), young girls and adolescent women, men who have sex with men (13%), people who inject drugs and people from Uganda's transient fishing communities [5]. The prevalence was higher in women (7.6%) than amongst men (4.7%) with the prevalence being higher in young women aged 15-24 years [5].

HIV infected patients are prone to opportunistic infections as the virus suppresses the immune system that helps the body fight against the infections [6]. Cotrimoxazole was primarily used as prophylaxis to prevent infections such as Pneumocystis carinni (PCP) and toxoplasmosis. The prophylaxis helped reduce the increased mortality and morbidity during the early years of HIV pandemic. [6]

Increased mortality and morbidity in HIV infected patients are caused by a number of opportunistic infections including tuberculosis. HIV infected patients are also more vulnerable to parasitic infections such as malaria [6]. Cotrimoxazole has shown great success in reducing mortality and morbidity caused by malaria [6].

1.1.2 The Impact of ART

Antiretroviral therapy (ART) has shown to be effective for treating HIV since 1996 [7]. The treatment is now widely used and is easily accessible and more affordable [7]. ART has proven to improve immune function, reduce mortality and decrease the risk of patients progressing to AIDS. ART also decreases the risk of transmission of the virus [7].

UNAIDS has a target to extend access to antiretroviral therapy to over 30 million people in 2020, an increase from 15.8 million in June 2015 [8]. In 2016 67% of people living with HIV in Uganda were receiving ART. The increased prevention and treatment services in the country resulted in a decline in the number of new HIV infections [4]. The increased access to treatment has helped suppress the viral load of 60% of people living with HIV in Uganda [4].

The global 90-90-90 treatment target was introduced to increase access to antiretroviral therapy [8]. In July 2017 about 20.9 million people globally had access to ART [8]. The UNAIDS report that was published in 2016 showed that 60% of the people living with HIV in Uganda had access to treatment [8], while 33% of adults and 53% of children were not yet on ART [5].

1.1.3 Role of Co-trimoxazole prophylaxis both Pre ART and post ART

Co-trimoxazole (CTX) prophylaxis is successful in preventing opportunistic infections in HIV-positive patients. The 2016 Uganda HIV and AIDS country progress report showed that approximately 966,513 people were on co-trimoxazole and 423,240 on pre -ART treatment [9].

Patients with CD4 cell count less than 200 cells/mm^3 are at a risk of developing pneumonia caused by Pneumocystis jiroveci and encephalitis caused by Toxoplasma gondii, and CTX is highly effective in preventing these infections [10, 11]. CTX is also effective in preventing other opportunistic infections [11, 12].

COSTOP was a non-inferiority trial which investigated whether it was safe to stop CTX among HIV positive patients who were stabilized on ART [10, 11]. Previous studies had limitations of small samples size, a short follow up period and were mostly observational studies and therefore more clinical trials are required [10, 11]. The World Health Organization (WHO) reported in 2006 that stopping co-trimoxazole had been shown to be safe among patients who had been stabilized on ART and who had shown immune recovery in well-resourced countries, however this was not the case for low-income and middle-income countries [13]. Evidence on whether it is safe to stop CTX prophylaxis in patients who are stabilized on ART in low-income and middle-income countries is lacking [13]. Discontinuing CTX may reduce the risk of haematological

toxicities associated with the use of CTX [10]. Stopping the routine intake of CTX has a potential to save costs in health settings of low-income countries [4].

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1.2 Problem statement

The benefits and risks of CTX prophylaxis are unknown in patients stabilized on ART, in whom the risk of opportunistic infections is much lower, so there is a need for randomized controlled trials that will help inform the decision as to whether it is safe to stop CTX among HIV infected patients who are taking ART and whose CD4 cell counts are stabilized above 250 cells/mm^3 [6]. There is a lack of data on the impact of discontinuing CTX prophylaxis on CD4 cell counts among patients that are stabilized on ART, due to lack of CD4 cell count monitoring. [13].

1.3 Justification

COSTOP was a randomized double-blind placebo controlled non-inferiority trial among HIV infected Ugandan adults stabilized on ART. [12]. This present study will address the gap in the literature by usingCOSTOP trial data to investigate the effect of discontinuing CTX on CD4 cell count in patients living with HIV.

The study will help answer whether CTX should be stopped or continued by showing its effects on the immune recovery of HIV infected patients.

1.4 Aim

The aim of this study is to compare the trajectory of CD4 cell counts between participants who were randomized to continue CTX prophylaxis and those who were randomized to discontinue CTX prophylaxis in the COSTOP trial which ran from January 2011 to July 2014.

Objectives of the study:

1. To compare the CD4 cell count at the end of the trial (2014) between HIV-infected patients on ART who were randomized to continue CTX prophylaxis and those who were randomized to placebo.
2. To compare the CD4 trajectories of patients on ART who were randomized to continued CTX prophylaxis and those who were randomized to placebo.
3. To investigate the variation of the CD4 cell count profiles within patients on CTX and within patients given placebo using random slope models.

1.5 Literature Review

1.5.1 The role of Co-trimoxazole and the Impact of ART in HIV patients

ART is given to HIV patients to prevent opportunistic infections and promote immune recovery [7]. It slows the progression of the virus by fighting against it. WHO guidelines in 2013 recommended that ART be given to all HIV patients with the CD4 cell count of $\leq$500 cells/mm^3. This was proven to be very effective from the START (Strategic timing of antiretroviral treatment) trial, which showed that early initiation of ART decreases AIDS defining illnesses by 57% and improves immune recovery of HIV patients [7].

CTX is a combination of trimethoprim and sulfamethoxazole, two drugs used to prevent the reproduction of bacteria [15,16]. The trimethoprim inhibits the production of the physiologically active tetrahydrofolic acid and sulfamethaxazole is an inhibitor of dihydrofolic acid [15]. It is a prophylaxis against opportunistic infections such as pneumonia, malaria and cerebral toxoplasma gondii [16]. It is widely available and can be purchased over the counter in many countries in Africa [15]. HIV-positive adults that have symptomatic HIV disease with a CD4 cell count less than 500 cells/mm³ are prescribed CTX [10]. Studies have shown that this treatment strategy has been associated with a 53% mortality reduction among HIV patients who have tuberculosis in Africa [17]. The prophylaxis however has side effects such as nausea, diarrhea and vomiting [17]. Other side effects reported occur in patients with renal dysfunction such as hyponatraemia and hyperkalaemia [17].

HIV infected patients that are co-infected with tuberculosis (TB) have a high rate of mortality and morbidity. A study that was conducted in South Africa has shown that CTX can reduce mortality and morbidity in patients that are co-infected with HIV and TB. CTX reduced mortality by 29% (95% CI 13-45 $p<0.001$) [18]. The highest death rate reduction from 21% to 12% was seen in adults between ages of 35- 44, with females having a greater reduction in mortality than males [18].

The World Health Organization guidelines have recommended CTX prophylaxis for all HIV-infected patients that have a low CD4 cell count and show symptoms of any infections or acute illnesses [15]. CTX prophylaxis has helped to reduce mortality in HIV infected patients regardless of their CD4 cell count, [10] and has reduced mortality by 25-46% in patients who are not on ART in Sub Saharan Africa [12]. CTX has also been widely used as an antimicrobial drug for treating malaria infections [17, 19].

HIV infected patients are prone to bacterial infections due to the disease attacking the CD4 cells that help the body fight against infections [17]. The CD4 cell count is monitored to help make appropriate decisions on health care and initiation of ART [17]. A systematic review showed that the rate of mortality and morbidity is lower in patients that are taking CTX after being stabilized on ART [19].

Clinical trials and observational studies conducted in high-income countries have shown that stopping CTX may be safe [20]. A study in Europe followed up a cohort of 325 adults for more than 13 months and there were no episodes of pneumocystis carinii pneumonia (PCP) after stopping CTX [20]. Further studies in the European cohort also showed no cases of toxoplasmosis after discontinuation of CTX [20].

A study in Malawi showed mortality risk reduction of 40.7% (p=0.0013) among patients who were taking ART and CTX, compared to patients that were only on ART [12]. CTX prophylaxis has helped decrease hospitalization and clinic visits and has shown the ability to improve the quality of life of patients living with HIV [12]. The study showed that there is significant difference between patients that receive ART and CTX and patients that are not receiving CTX (p=0.0020) [12].

The major limitation of this study is that it was a non-randomized study in which certain sites were giving CTX while other sites did not offer it. There is a possibility that the sites that were offering a higher level of care chose to offer CTX prophylaxis and such sites would be expected to show better outcomes even if they did not offer CTX prophylaxis [12]. Another limitation of this study was lack of data on the immune function that could have helped evaluate confounding effects of immunological characteristics of patients receiving CTX and those not receiving CTX [12].

In the 2015 Lancet HIV systematic review, WHO recommended that co-trimoxazole should be given with ART to patients that are in low income and middle-income areas that have a CD4 cell count of 350 cells/ mm^3 or lower. CTX prophylaxis is more strongly recommended in areas that have a high burden of disease [20].

An observational study conducted from data from the DART trial showed no benefits of CTX among patients that were on ART for 72 weeks and more [15]. Results from the study showed that ART helped to improve the immune recovery of HIV-infected patients and the additional benefits of CTX among patients that are taking ART were not clear [15].

A randomized controlled trial was conducted in Uganda in 2007. This study was nested in the 4 years home-based program AIDS care (HBAC) study [21]. The study investigated the effects of discontinuing CTX prophylaxis on malaria and diarrhea incidence. In the trial 384 participants were randomized to discontinue CTX and 452 participants were randomized to continue CTX

[21]. The study showed that patients who discontinued CTX have a high risk of getting malaria and diarrhea, with a total of 315 reported fever cases and of those 228(72%) arose among patients that were randomized to stop CTX.[21]. However, this study was stopped 4 months after initiation by the Data Safety and Monitoring Board due to high increased rate of malaria and diarrhea among patients randomized to discontinue CTX prophylaxis. Thus, the long-term effect of stopping CTX prophylaxis could not be determined [21]

Despite the evidence of the positive effects of CTX, its use has been limited in most countries due to poor health infrastructure, lack of patient monitoring and supply prior to ART [16].

1.5.2 Modelling CD4 cell count and mixed effects models for longitudinal data.

We propose to use mixed effects modelling in this study. These types of models have been used for modelling longitudinal CD4 count data in other settings. The CD4 is measured repeatedly to determine the health status of a person living with HIV [22]. There are two types of mixed effects models that are used in this study, the random intercept model and random slope model. Random effects models provide the individual or cluster-specific interpretation of the coefficients [22].

The general linear mixed model is given as:

$$y = X\beta + Zb + \varepsilon$$

where

y is the outcome Variable

X are the fixed effects variables

β coefficient vector of the fixed effects

Z design matix for the random effects

b vector of the random effects

ε is the residual error

Mixed effects models have been shown to be very successful in modelling longitudinal data and many studies have used these models to show the trend and changes in the CD4 cell count [23].

The following examples in the literature used different methods for modelling CD4 cell count. A study conducted at Jimma University Hospital in Ethiopia fitted a sequence of univariate linear mixed models to determine covariates that have an effect on the change of CD4 cell counts [22]. The final multivariate mixed model included all of the covariates that had a significant effect on the change in CD4 cell count from the univariate linear mixed effects models [22]. The final model included sex, time, functional status, educational level, patient status and the interaction between time and functional status [22].

The authors used a square root transformation for the CD4 cell count. They fitted a linear mixed effects model that included random intercept and included quadratic time effects to predict the square root CD4 cell count [22].

A longitudinal study in northern Ethiopia was conducted to assess the trend of CD4 cell count [23]. In the study multivariate least squares linear regression models were fitted to assess the change of CD4 cell count [23]. A backward stepwise approach was used to exclude variables that were not significant [23] in the first multivariate model. A logistic regression model was also fitted to determine factors that affect immune recovery. This study also showed that immune recovery is more likely in patients with a high baseline CD4 cell count [23]. Results of the study showed that CD4 shows an increasing trend in the first 5 years of ART initiation and thereafter [23].

A study on modelling CD4 cell counts was conducted to estimate the possibly different effects of successive injections in repeated cycles of exogenous interleukin [24]. In this study different statistical models were used to determine the effect of different injections in a single cycle of exogenous interleukin comparing it to the effects of the injection in repeated cycles. This study

also looked at the efficacy of exogenous interleukin injections on the progression of CD4 cell counts [24].

The model included CD4+ T cell counts and the number of CD4+ T cell counts that expressed the ki67 proliferation marker. Log transformation was used, and the model used ordinary differential equations to describe the CD4 and Ki67 counts dynamics. A mixed effects model was fitted to determine the effects of a single injection cycle and the effects of three injection cycles on the proliferation of CD4 cell counts [24]. Using the cross-validation criterion the results showed that a model that had included the cycle effect was a best fit and showed that having two injection cycles was enough to improve the immune progression.

A linear mixed model was used to assess the change in CD4 cell count by HIV RNA level, including sex and age as the fixed effects, among patient initiating combination antiretroviral therapy (cART) in South Africa[25]. The study showed CD4 cell count slope average increase of 1.3 (95% confidence interval (CI): 1.1, 1.5) cells/mm3 per week between 6 and 48 months on cART, and also showed that a high HIV RNA value had a negative effect on the CD4 cell count slope [25]. The results only included CD4 cell count data from 6 months after the cART initiation as the author was only interested in long term effects of cART.

2 CHAPTER 2: METHODOLOGY

2.1 Primary study

Primary data was collected during the COSTOP trial. The trial was a double-blind placebo-controlled trial. The study was conducted in Uganda among HIV infected patients stabilized on ART [9, 10]. The objective of the study was to assess if discontinuation of CTX is not inferior, with respect to the incidence of pre-defined CTX- preventable events to the control regimen in which prophylaxis with CTX is continued and superior to continuing CTX prophylaxis with respect to reducing the incidence of haematological adverse events.

The study inclusion criteria were:

- HIV patients with documented CTX intake of 6 months or more
- Patients aged ≥18 years
- Documented ART intake of 6 months or more
- Patients who were clinically asymptomatic
- 2 CD4 cell counts measurements that were ≥ 250 cells/mm^3
- Patients who were able to attend the study clinics at 3-monthly intervals and in event of illness

The Exclusion criteria were:

- Patient with acute illnesses such as opportunistic infections or any co-morbidity
- Females in their first trimester of pregnancy
- Patients with known hypersensitivity to cotrimoxazole
- Patients who have grade 3/4 anemia, neutropenia or thrombocytopenia

Participants were assessed for eligibility. After enrolment, data were collected on socio-demographic and behavioral characteristics and on their medical history, including the WHO stage and use of ART. The participants were seen by a clinician monthly for the first 3 months and after every three months thereafter. Each participant had clinical history and physical examination, laboratory investigation of the CD4 cell count and haemotology, blood slides to test for malaria, adherence assessment and study drug prescription/refill at each visit. The adherence

was measured using a standard adherence questionnaire and pill count of the returned drug. All participants were provided with insecticide treated mosquito nets and were taught how to use them.

Participants in the study were randomized in a 1:1 ratio to continue or to stop CTX prophylaxis. The randomization schedule was provided by an independent MRC/UVRI statistician using random permuted blocks of variable size with separate randomization carried out in the four strata, which were defined by four possible combinations of study site and baseline CD4 cell count. Study clinicians had no knowledge of to which treatment arm a participant was randomized. The medication was identified by the corresponding trial number.

The study sample size was estimated using Schoenfeld's formula. It was calculated to show non-inferiority at 5 % significance level with 80% power under the given assumptions. The assumptions used were that the rate of CTX preventable events in the control arm would be 10 per 100 PYO, and that the rate of loss to follow up would be 4% per year. For a type I error of 0.05 (one-sided for non- inferiority), the upper limit of 95% confidence interval for the hazard ratio comparing the intervention arm with the control arm should be at most 1.25 in order to demonstrate non-inferiority. This led to a total of 2000 participants required for the trial, with 1000 participants in each arm. Under the assumptions, a total of 494 CTX preventable events were expected.

All the study participants were required to stop their current CTX and instead take either a 960 mg CTX tablet or a matching placebo tablet depending on the study arm to which they were randomized. The trial medication was dispensed every month for the first three months of the trial and every three months thereafter. Participants were requested to bring the medication packs at each visit. Participants for whom the CD4 cell count dropped to below 250 cells/ mm^3 or who developed illnesses that prevented further treatment with the trial medication were withdrawn from the study.

The CD4 cell count was measured at each visit, while haemotological events were assessed using laboratory tests that were carried out at the MRC laboratories. Severity of the measured laboratory parameters was graded using the division of AIDS toxicity grading table. The study

database was designed using Microsoft Access. The data was entered twice and validated before it was uploaded into the final study database.

Ethical approval for the study was granted by Uganda Virus Research Institute Research and Ethics Committee, the Uganda National Council for Science and Technology and the Ugandan Drug Regulatory Authority.

2.2 Present Study

2.2.1 Study design

This study is a secondary data analysis of data that was collected during the COSTOP randomized double blind placebo-controlled trial.

2.2.2 Study site

The study was conducted in Uganda in Entebbe and Masaka districts. Masaka is in South-Western Uganda. It is bordered by Sembabule, Mpigi, Rakai and Kalangala districts. Entebbe is the major town in Central Uganda and is 37 km away from the capital city of Uganda, Kampala.

2.2.3 Study population and sampling method

The study population consisted of HIV positive adults (18 years and above) who were stabilized on ART residing in Entebbe and Masaka towns in Uganda. All participants with at least one CD4 count measurement during the follow up period are included in the secondary data analysis.

2.2.4 Study Variables

The outcome variable and exposure and confounder variables are given below.

2.2.4.1 Outcome Variable

- CD4 cell count measurement

2.2.4.2 Exposure and confounder variable

1. Primary Exposure Variables

   Objective 1

   - Treatment arm

   Objective 2 and 3

   - Time since enrolment in to the trial
   - Treatment arm

2. Confounder Variables
   - Socio-demographic characteristics: age at baseline, sex, study center.
   - Clinical characteristics: duration on ART at baseline, baseline CD4 count.

2.2.5 Data Management and analysis

Stata version 14 software was used for data analysis. Any CD4 cell count that was greater than 2000 cells/mm³ was set to missing (as the upper limit for the laboratory evaluation of CD4 cells is 2000). This analysis includes data from all the participants that took at least one dose of the medication and had at least one followed up visit. Participants with no follow up CD4 cell count were excluded.

2.2.6 Analysis for objective 1

To achieve objective 1 the change of the CD4 cell count was calculated as the difference between the last CD4 cell count measure and the first CD4 cell count measure.

The analysis was carried out to adjust for other factors that could affect the change in CD4 cell count. This was done by fitting the following linear regression model:

$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{4i} + \beta_5 x_{5i} + \beta_6 x_{6i} + \beta_7 x_{7i} + e_i$

Where:

y_i is the Final CD4 count for subject "i"

x_1 is the treatment arm for subject "i" (coded as 0 for CTX and 1 for PLC)

x_2 is the CD4 count at baseline (enrolment) for subject "i"

x_3 is the age at enrolment for subject "i"

x_4 is the sex of subject "i" (coded as 0 for male and 1 for female)

x_5 is the study site for subject "i" (coded as 0 for Entebbe and 1 for Masaka)

x_6 is the duration on ART at enrolment (in months) for subject "i"

x_7 is the time(in weeks) between the baseline (enrolment) and the final CD4 count

e_i is a random within subject error (residual)

The parameter of interest in this model is β_1 which measures the average difference in final CD4 counts between subjects randomised to PLC and those randomised to CTX. In addition to doing the analysis for CD4 counts the use of a square root transformation was investigated. The Breusch-Pagan / Cook-Weisberg test for heteroskedasticity was used as the criterion to choose between the transformed and untransformed CD4 cell count.

2.2.7 Analysis for objective 2

2.2.7.1 Model 1: Objective 2

The question we are answering here is whether on average CD4 counts differ between participants randomised to continue cotrimoxazole prophylaxis (CTX) and those randomised to stop CTX prophylaxis (PLC). First, graphical methods were used to show the mean CD4 count over time by treatment arm.

The covariates to adjust for in the analysis are baseline (enrolment) CD4 count, study site, age, sex and duration on treatment.

The model to be fitted can be written as follows:

$y_{ij} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 t_{ij} + u_{0i} + e_{ij}$

where

y_{ij} is the CD4 count at time t_{ij} for subject "i"

x_1 is the treatment arm for subject "i" (coded as 0 for CTX and 1 for PLC)

x_2 is the CD4 count at baseline (enrolment) for subject "i"

x_3 is the age at enrolment for subject "i"

x_4 is the sex of subject "i" (coded as 0 for male and 1 for female)

x_5 is the study site for subject "i" (coded as 0 for Entebbe and 1 for Masaka)

t_{ij} is the measure of weeks on the study for subject "i" on the jth occasion

u_{0i} is a random subject effect

e_{ij} is a random within subject error (residual)

The parameter of interest in this model is β_1 which measures the average difference in CD4 counts between subjects randomised to PLC and those randomised to CTX. The model has the advantage of simplicity and the disadvantage that the difference in average CD4 counts is assumed to be constant over time. Given the fact that at baseline there is no difference in the CTX status of participants (they were all on CTX prophylaxis at baseline) we might expect that initially the difference is close to zero and thereafter it increases with time. This is catered for in the next model.

2.2.7.2 Model 2: Objective 2

An alternative to model 1 would be to just add an interaction term between treatment arm and time to the model. This has the advantage that it is easy to compare to model 1, as model 1 will then be nested in model 2. In this case the question to be answered is whether there is a significant interaction between treatment arm and time, if so, the interaction term will measure the difference in the slopes of these two lines. As before we can adjust for baseline (enrolment) CD4 count, study site, age, sex and duration on treatment.

The model to be fitted can be written as follows:

$y_{ij} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 t_{ij} + \beta_7 x_1 * t_{ij} + u_{0i} + e_{ij}$

where

y_{ij} is the CD4 count at time t_{ij} for subject "i"

x_1 is the treatment arm for subject "i" (coded as 0 for CTX and 1 for PLC)

x_2 is the CD4 count at baseline (enrolment) for subject "i"

x_3 is the age at enrolment for subject "i"

x_4 is the sex of subject "i" (coded as 0 for male and 1 for female)

x_5 is the study site for subject "i" (coded as 0 for Entebbe and 1 for Masaka)

t_{ij} is the measure of weeks on the study for subject "i" on the jth occasion

u_{0i} is a random subject effect

e_{ij} is a random within subject error (residual)

In this case interest centers on the parameter β_7 which measures the average difference in the slopes between subjects randomized to PLC and those randomized to CTX.

2.2.8 Analysis for objective 3

In objective 3 we choose the best model fit from objective 2. The fixed component of this model is the same as model 2, however in the random component we added random slopes.

The model to be fitted can be written as follows:

$$y_{ij} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 t_{ij} + \beta_7 t_{ij*} x_1 + u_{1i*} t_{ij} + u_{i0} + e_{ij}$$

where

y_{ij} is the CD4 count at time t_{ij} for subject "i"

x_1 is the treatment arm for subject "i" (coded as 0 for CTX and 1 for PLC)

x_2 is the CD4 count at baseline (enrolment) for subject "i"

x_3 is the age at enrolment for subject "i"

x_4 is the sex of subject "i" (coded as 0 for male and 1 for female)

x_5 is the study site for subject "i" (coded as 0 for Entebbe and 1 for Masaka)

t_{ij} is the measure of weeks on the study for subject "i" on the jth occasion

u_{i0} is a random subject effector random intercept

u_{1i} is a random subject effect that allows the slopes of the CD4 time relationship to vary between individual participants - in this case a "random slope"

e_{ij} is a random within subject error (residual)

In this case like model 2 the interest centers on the parameter β_8 which measures the average difference in the slopes between subjects randomized to PLC and those randomized to CTX. This model adjusts for the variation on the random slopes.

2.2.9 Ethical consideration

Ethical approval was obtained from the University of the Witwatersrand (HREC) Human Research Ethics Committee (Medical).

3 CHAPTER 3: RESULTS

This analysis included all participants with at least one CD4 follow up measure, and 1.3% of participants who were excluded due to only having one CD4 count. Two CD4 counts, out of a total of 17,657 counts, were recorded as being above 2,000. These readings were considered as being in error since the assay used has an upper limit of 2,000, so these two counts were set to missing.

3.1 Baseline Characteristics of the patient population by treatment arm.

Table 3.1 shows baseline characteristics of the study participants. The study had more females than males (n=1610 (74%) versus n=586 (26%)). Slightly more participants were from Masaka than Entebbe (54% vs 46 %). Over half of the participants were over 40 years old and 10% were aged under 30 years. Just over half of the participants (52.3%) had a CD4 cell count between 250 and 499 at baseline. The overall mean nadir CD4 was about 150 cells/mm^3 with a large standard deviation of about 90 cells/mm^3 . At the beginning of the trial participants in the two arms had been on ART for a similar amount of time (mean 47months).

Table 3.1 Study population characteristics by treatment arm.

Variable	Cotrimoxazole	Placebo	Total
	N=1089(50%)	**N=1089(50%)**	**N=2178(100%)**
Sex (n, %)			
Male	286(26.3%)	282(25.9%)	568(26.0%)
Female	803(73.7%)	807(74.1%)	1610(74.0%)
Site (n, %)			
Entebbe	501(46.1%)	499(45.8%)	1000(45.9%)
Masaka	588(54.0%)	590(54.2%)	1178(54.1%)
CD4 strata (n, %)			
250–499 cells/mm^3	570(52.3%)	568(52.2%)	1138(52.3%)
500 or more cells/mm^3	519(47.7%)	521(47.8%)	1040(47.8%)
Age Group in years (n, %)			
<30	85(8.4%)	83(8.1%)	168(8.3%)
31-40	360(35.7%)	396(38.7%)	756(37.2%)
>40	564 (55.9%)	544(53.2%)	1108(54.5%)
CD4nadir (mean,sd)			
	154.75(96.1)	147(87.4)	151.1(91.9)
ART duration at baseline (in months) (mean, sd)			
	47.88(25.1)	47.19(25.3)	47.53(25.2)

3.2 Objective 1 Results

In this analysis we compared the final CD4 cell counts ((at the end of the trial) between the two study arms, for this objective 1 this analysis included 2148 individuals. Figure 2 shows the box plots of these by study arm. This analysis showed that at the end of the trial participants that discontinued CTX had a higher CD4 cell count than participants that continued CTX.

Table 3.2 shows the mean CD4 by treatment arm stratified by site and Table 3 shows the overall mean CD4 by treatment arm. The baseline mean CD4 cell count is similar in the two treatment arms. There is a difference in the final CD4 cell count between the two treatment arms, patients that had discontinued CTX have a higher CD4 at the end of the trial compared to patients that continued CTX.

A linear regression model was fitted to evaluate the mean difference in the final CD4 counts between arms adjusting for baseline CD4, duration on ART at enrollment, site, gender and age of the participants. Table 3.4 shows that participants in the placebo arm had a significantly higher mean final CD4 count [mean difference= 37.32 cells/mm^3 (95% CI 31.44;43.20, p<0.001)] compared to those in the CTX arm after adjusting for baseline CD4 and other covariates.

Participants in Masaka had a significantly lower final CD4 cell count [mean difference= -15.65(-21.63;-9.68, p<0.001)] than participants in Entebbe. Females had a significantly higher final CD4 counts [mean difference=47.59 (95% CI 40.71;54.47, p<0.001)] than males. Weeks on study and age had no significant effect on the final CD4.

Table 3.2 Mean CD4 by treatment arm, stratified by site.

Over	Mean	Std. Err.	[95% Conf. interval]	
CD4_at_baseline				
Ctx Masaka	538.84	3.88	531.24	546.44
Placebo Masaka	539.16	4.19	530.95	547.36
Ctx Entebbe	495.50	3.40	488.84	502.16
Placebo Entebbe	495.69	3.31	489.21	502.17
Cd4_at_endline				
Ctx Masaka	547.18	3.82	539.69	554.67
Placebo Masaka	570.92	4.06	562.96	578.87
Ctx Entebbe	523.32	3.74	516.00	530.65
Placebo Entebbe	574.96	3.93	567.25	582.67

Table 3.3 Mean CD4 by treatment arm.

	Mean	Std. Err.	[95% Conf. Interval]
CD4 at baseline			
CTX	517.31	2.59	(512.22;522.39)
Placebo	517.56	2.69	(512.29;522.82)
Final CD4			
CTX	536.33	2.68	(530.08;540.58)
Placebo	572.93	2.83	(567.39;578.46)

Figure 3.2.1 Mean CD4 box plots by treatment arm.

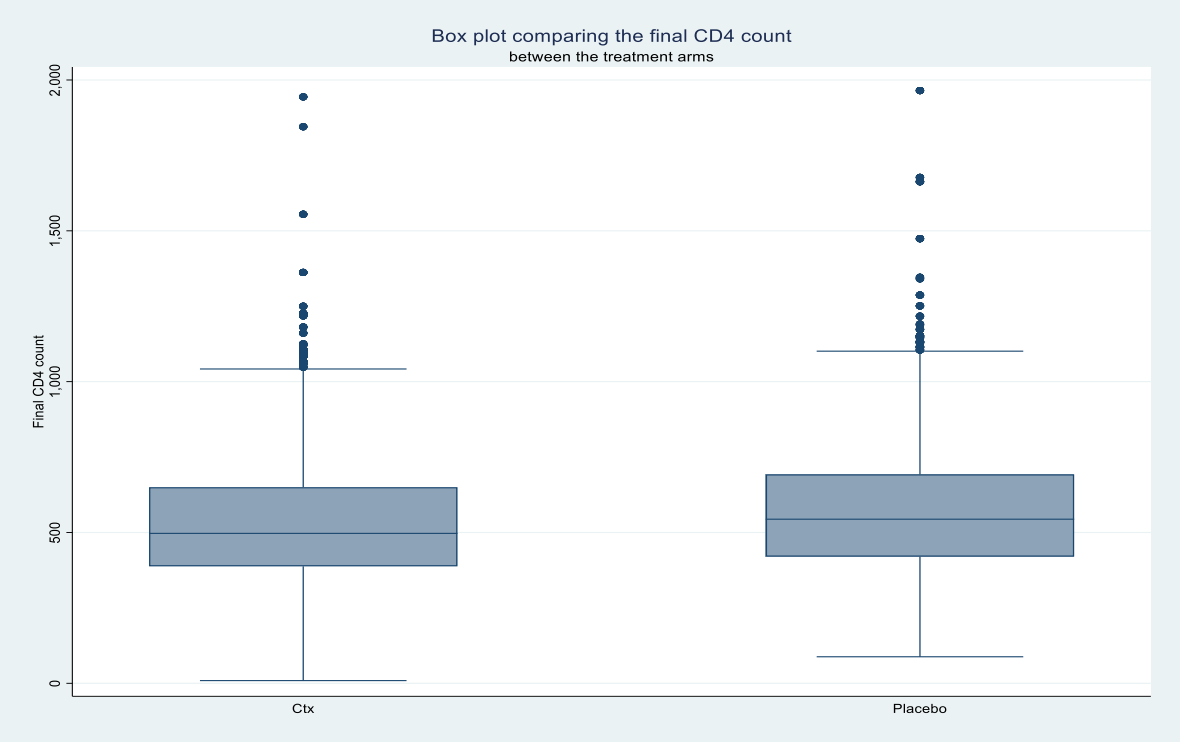


Table 3.4 Linear regression analysis of factors associated with Final CD4

Parameter	**Coefficient [mean Final CD4 change]**	**P-Value**	**95% Confidence Interval. lower**	**Upper**
Treatment arm (Placebo vs CTX)	37.32	p<0.001	31.44	43.20
CD4_at_baseline	-0.16	0.40	-0.54	0.22
Age_in_years	0.67	p<0.001	0.65	0.68
Sex (Females vs Males)	47.59	p<0.001	40.71	54.47
Site (Masaka vs Entebbe)	-15.65	p<0.001	-21.63	-9.68
ART_duration	-0.22	p<0.001	-0.35	-0.10
weeks	-0.02	0.63	-0.09	0.05
_cons	181.59	p<0.001	162.75	200.42

Table 3.5 shows that the adjusted estimate of the final CD4 count of the group that were randomized to continue CTX is significantly higher compared to those that were randomized to continue CTX.

Table 3.5 Adjusted estimate of the mean CD4 cell count

	Margin	Std. Err.	[95% Conf.	Interval]
Treatment				
CTX	535.61	2.07	531.55	539.67
Placebo	572.65	2.09	568.55	576.75

The Cook-Weisberg test for homogeneity of variance showed overwhelming evidence of non-constant variance so the use of a square root transformation was investigated. The square root transformed CD4 cell count still showed overwhelming evidence of non-constant variance, so it was decided to use the untransformed CD4 count in analysis for ease of interpretation.

3.3 Objective 2 Results

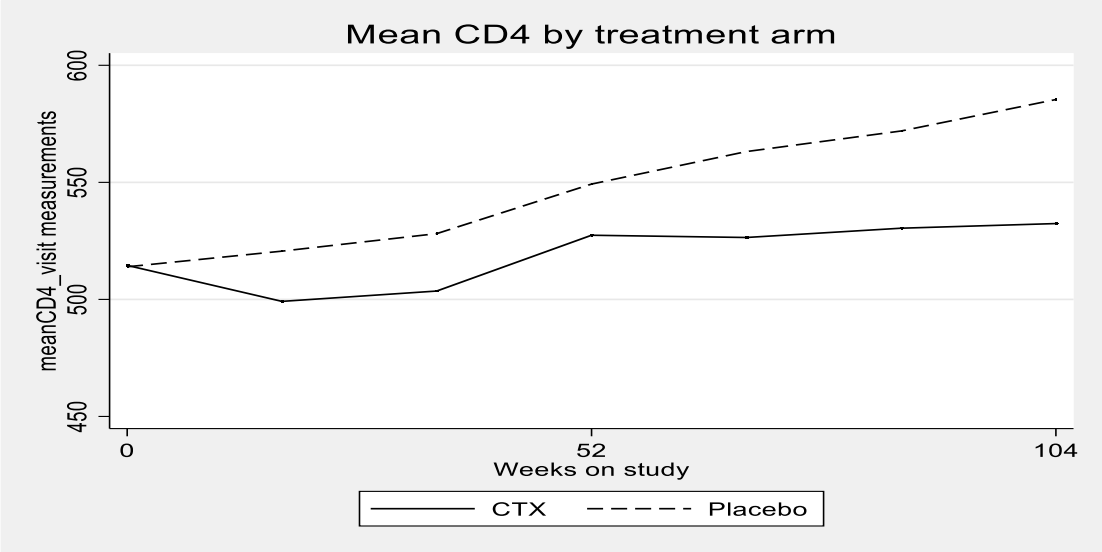
For objective two the question we answered is whether the mean profile of CD4 counts differs between the participants in the treatment arms. Graphical representation was used, and thereafter linear mixed effects models were fitted with CD4 cell count as the outcome variable adjusting for baseline (enrolment) CD4 count, study site, age in years and sex. In this analysis we look at models where each participant has its own intercept. The first model included the time in weeks of each CD4 cell count measure and the random intercept (CTX number).

The second model that we fitted was similar to the first model but in addition, it included the interaction between study treatment and time (in weeks). This analysis included 2148 individuals (12,402 observations).

Figure 3.2 below shows the mean CD4 cell count of the participants randomized to take CTX and participants randomized to take placebo by month since randomization. This graph shows that there is a difference between the mean CD4 cell count of the participants randomized to take

CTX and participants randomized to discontinue CTX. Patients that were randomized to discontinue CTX had a higher CD4 cell count compared to patients randomized to continue CTX.

Figure 3.2.2 Mean CD4 cell count over time up to 24 months in the study.



3.3.1 Random intercept model assessing the average difference in CD4 cell count.

Table 3.6 Model 1 random intercept model

Parameter	**Coefficient**	**P-Value**	**95% Confidence Interval**	
	[mean CD4 change]		lower	upper
Treatment arm (Placebo vs CTX)	23.99	p<0.001	15.95	32.02
CD4_at_baseline	0.73	p<0.001	0.71	0.75
Age_in_years	-0.16	0.52	-0.66	0.34
Sex (Female vs Males)	31.39	p<0.001	22.01	40.77
Site (Masaka vs Entebbe)	-3.74	0.37	-11.87	4.39
Weeks on study	0.41	p<0.001	0.36	0.45
_cons	98.59	p<0.001	73.47	123.71
Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
Individual participant intercept SD	82.59	1.64	79.44	85.86
Residual SD	106.51	0.76	105.04	108.00

In this model we looked at whether on average CD4 cell counts differs between participants randomized to continue cotrimoxazole prophylaxis (CTX) and those randomized to stop CTX prophylaxis (PLC). Table 3.6 shows that on average CD4 cell count is significantly higher for participants randomized to take placebo by 23.99 cells/mm^3 (95% CI 15.95;32.02 p<0.001) than participants randomized to continue CTX.

The other covariates that showed to have a significant effect on the CD4 cell count were time on the study (in weeks) with a significant increase of 0.41 cells/mm^3(95% CI 0.36;0.45 p<0.001),) per week, and CD4 cell count is significantly higher in females by 31.39 cells/mm^3 (95% CI 22.01;40.77 p<0.001) than males. Age and site had no effect on CD4 recovery.

3.3.2 Random intercept model including an interaction between time and treatment.

The results of adding an interaction between treatment arm and time in weeks are shown in Table 3.7.

Table 3.7 Model 2 random intercept model including interaction

Parameter	**Coefficient**	**P-value**	**95% Confidence Interval**	
	[mean CD4 change]		lower	Upper
Treatment arm (Placebo vs CTX)	8.78	0.09	-1.21	18.76
CD4_at_baseline	0.73	p<0.001	0.71	0.75
Age_in_years	-0.16	0.54	-0.66	0.34
Sex (Female vs Males)	31.33	p<0.001	21.96	40.71
Site (Masaka vs Entebbe)	-3.73	0.37	-11.85	4.39
Weeks on study	0.29	p<0.001	0.22	0.35
Interaction between Treatment arm and weeks on the study	0.24	p<0.001	0.15	0.34
_cons	105.89	p<0.001	80.63	131.16
Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
Individual participant intercept SD	82.56	1.64	79.41	85.83
Residual SD	106.38	0.76	104.91	107.88

This model included the interaction between treatment arm and time on the study (in weeks). From table 3.7 the treatment arm by time on the study is significant, with the average increase in CD4 cell counts being higher by 0.24 cells/mm^3 per week (95% CI 0.15;0.34 p<0.001) for participants who are randomized to take placebo. For participants randomized to continue with CTX prophylaxis the increase is 0.29 cells/mm^3 (95% CI 0.22;0.35 p<0.001) per week, so for participants randomized to take placebo it is 0.29+0.24 = 0.53 cells/mm^3 per week.

CD4 cell count is significantly higher in females by 31.33 cells/mm^3 (95% CI 21.96;40.71p<0.001) than in males. Other factors included in the model namely age, and site had no significant effect on CD4 cell count.

Potential nonlinearity of the effect of time on CD4 counts was investigated by adding a quadratic term (weeks^2) (95% CI -0.0016;0.0017 p=0.98) to the model, as well as its interaction with treatment arm (95% CI -0.0028;0.0019 p=0.71). This was found to be non-significant.

3.4 Objective 3 Results

In this analysis the model fitted had the same fixed effects as model 2, however in addition the model included random slope corresponding to the time (in weeks). This model was chosen based on the likelihood ratio test that showed that model 2 is a better fit compared to model 1. Similar to objective 2, the analysis included 2148 individuals (12402 observations).

3.4.1 Random slope model including interaction between time and treatment.

Table 3.8 Model 3 random slope model including the interaction

Parameter	**Coefficient**	**P-value**	**95% Confidence Interval**	**Interval]**
	[mean CD4 change]		lower	upper
Treatment arm (Placebo vs CTX)	10.41	0.01	2.87	17.96
CD4_at_baseline	0.78	p<0.001	0.77	0.80
Age_in_years	-0.23	0.29	-0.66	0.19
Sex (Female vs Males)	17.74	p<0.001	9.71	25.77
Site (Masaka vs Entebbe)	5.28	0.14	-1.67	12.23
Weeks on study	0.34	p<0.001	0.26	0.42
Interaction between Treatment arm and weeks on the study				
	0.21	p<0.001	0.09	0.33
_cons	85.53	p<0.001	64.14	106.92
Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
Individual participant				
Slope SD	0.81	0.04	0.75	0.89
intercept SD	39.03	2.91	33.71	45.17
correlation(weeks,_cons)	0.74	0.14	0.35	0.91
Residual SD	100.31	0.78	98.80	101.86

Table 3.8 shows that on average the CD4 cell count of participants randomized to take placebo increases by 0.21 cells/mm^3 per week (95 % CI 0.09;0.33 p<0.001) more than for participants randomized to continue with CTX prophylaxis. For participants randomized to continue CTX prophylaxis the CD4 count increases by 0.34 cells/mm^3 per week (95 % CI 0.26;0.42 p<0.001), so for participants randomized to placebo the Cd4 cell count increases by 0.34+0.21=0.55 cells/mm^3 per week.

CD4 cell count is significantly higher in females by 18 cells/mm^3 (95% CI 10;26 p<0.001) than males. Other factors included in the model namely age and site had no significant effect on CD4 cell count.

Model 3 gives a significantly better fit (p<0.001) than model 2

4 CHAPTER 4 DISCUSSION AND CONCLUSION

4.1 Discussion

This study evaluated the effects of CTX prophylaxis on CD4 trajectories of participants who were randomized to discontinue CTX and participants who were randomized to continue CTX stabilized on ART in Uganda. We found that there is a significant difference on the final CD4 cell count between participants who continued CTX and those who discontinued CTX. Our results agree with the main COSTOP study regarding positive effect that stopping CTX has CD4 cell count progression [10]. Results from the COSTOP have shown that discontinuing CTX leads to increased malaria infections 362 (276 PLC, 86 CTX) participants experienced at least one episode of confirmed clinical malaria ($P<0.0001$) and other CTX preventable infections and continuing CTX has shown to increase hematological adverse events (neutropenia) 551 participants (318 CTX; 233 PLC) [10]. Even though CTX has shown to reduce risks of bacterial infections, this study has shown that continuing CTX leads to slower CD4 cell count recovery Participants who had a faster CD4 recovery showed to have a high number of individuals who experienced malaria episodes. The observed difference in CD4 count trajectories between the two trial arms is small and may not be clinically important.

WHO CTX guidelines have recommended that CTX prophylaxis may be discontinued in patients stabilized on ART who show CD4 cell count recovery [13]. It also recommended that CTX prophylaxis may be continued in resource limited countries that have no CD4 cell count monitoring and high malaria prevalence [13]. This study in agreement with the WHO guidelines has shown that stopping CTX prophylaxis increases immune recovery in patients who are stabilized in CTX

Random intercept and random slope models were fitted to compare the CD4 count trajectories between patients randomized to stop CTX and patients randomized to continue CTX. Two different random intercept models were fitted to compare the CD4 trajectories and from the two models, best fitting model is the model including interaction between treatment arm and time on the study (in weeks). This model is similar to the model used in the study that was conducted in Ethiopia that used mixed effects models to determine covariates that influence the change in the

CD4 cell count [22]. The model used in that study included an interaction between time and exposure variable (functional status) and used root transformed CD4 [22]. However, in our model the CD4 cell count is not transformed as the transformed CD4 did not achieve constant variance.

We found that the random slopes model gave a better fit when modelling the CD4 cell count trajectory than the random intercept model . From the random slopes model, we found that there is a difference between the two treatment arms in the CD4 cell count trajectories. Participants who were randomized to discontinue CTX have a higher CD4 cell count increase compared to participants that were randomized to continue CTX, our results agree with the main COSTOP study regarding positive effect that stopping CTX has CD4 cell count progression [10].

In addition, we also found that sex has an effect on CD4 cell count recovery. Females have a higher CD4 cell count increase compared to males. This is the same as results from previous studies that have shown that sex has a significant effect on CD4 cell count progression [23, 24].

4.2 Study strengths and limitations

The strengths of this study are a long follow up time and a large sample size. The study included all the participants with at least one CD4 cell count follow up measurement, which ensured no missing data for the analysis and the main study was a double blinded randomized controlled trail which minimized the selection bias. The limitations of the study was loss to follow-up which resulted in a different number of participants being analysed at different time points, Another limitation was that participants could access CTX as over the counter medication and thus some participants randomized to placebo might have continued to take CTX A minor limitation of the study was 1.3% of participants were excluded from the analysis, this is a small percentage of the participants. It would have also been useful in the analysis of CD4 cell counts to adjust for whether or not the patient had viral load suppression-however the limitation in this trial is that viral loads were not measured.

4.3 Conclusion

The study findings show that CD4 cell count increased over time in both study groups. . Although CTX is known to be effective in preventing opportunistic infections, this study also showed that continued CTX is associated with a slower increase in CD4 cell count and the observed difference was statistically significant-however it is still quite a small difference and may not be clinically important.

4.4 Recommendations

CD4 cell count monitoring is recommended in resource limited settings that have a high prevalence of bacterial infections and still provide CTX to prevent bacterial infections. More clinical studies are needed that will assess the progress of the viral load suppression between the two study arms.

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6 APPENDICES

6.1 Plagiarism declaration report

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I _**Tshwaraganang Hope Modise**____ (Student number: **1254550**_____) am a student

registered for the degree of _**MSc in Epidemiology(Biostatistics)**_ in the academic year **2019**____.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature:  _____ Date:**2019/10/29** _____

6.2 Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Mlles TH Modise and BAS Nonyane

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180351

NAME: (Principal Investigator)	Mlles TH Modise and BAS Nonyane
DEPARTMENT:	School of Public Health Medical School University
PROJECT TITLE:	The effects of Co-trimoxazole prophylaxis on CD4 cell-count profiles in HIV-positive patients stabilized on anti-retroviral therapy
DATE CONSIDERED:	06/04/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor J Levin
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/06/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **March** and will therefore be due in the month of **March** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES