# Association of Pre-Antiretroviral Treatment Body Mass Index with $CD4^+$ T-lymphocyte Immune Reconstitution among HIV-Infected Adults and Adolescents Initiated on Antiretroviral Treatment: A Retrospective Longitudinal Study

**By**

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**Declaration**

I, Mosioma Philip Nyaribo, declare that this is my original work except where otherwise acknowledged and has not been submitted to either this or any other university for any academic award.

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**Dedication**

To my entire family and friends who have always been supportive, and to all those who helped me throughout the entire process of writing the proposal.

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**List of Acronyms**

| | |
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| AIDS | Acquired Immune Deficiency Syndrome |
| ANOVA | Analysis of Variance |
| ART | Antiretroviral Treatment |
| CCC | Comprehensive Care Clinic |
| EGPAF | Elizabeth Glazer Pediatric AIDS Foundation |
| HAART | Highly Active Antiretroviral Treatment |
| HIV | Human Immunodeficiency Virus |
| ICAP | International Centre for AIDS Care and treatment Programs |
| NASCOP | National AIDS and STI Control Program |
| PLHIV | People Living with HIV |
| PLOS | Public Library of Science |
| PSC | Patient Support Clinic |
| UNAIDS | United Nations AIDS programs in Kenya |
| USAID | United States Agency for International Development |
| WHO | World Health Organization |

**Abstract**

Human immunodeficiency virus (HIV) infection, causes acquired immune deficiency syndrome (AIDS) condition which is the world's leading pandemic that causes millions of deaths each year. HIV has no cure, infected patients rely on effective management with the use of antiretroviral treatment to ensure suppression of viral replication, increase of $CD4^+$ T-lymphocyte cells and increased time to disease progression, so that people living with HIV can enjoy health lives and reduce the risk of transmitting the virus to others.

$CD4^+$ count monitoring is an essential tool for initiating and monitoring of antiretroviral treatment and is greatly used in developing countries where Kenya is included. $CD4^+$ response is dependent on; environmental setting where treatment is being offered, individual and population characteristics that include; adherence, age, gender, baseline $CD4^+$ cell count, baseline viral load and the individuals Basal Metabolic Index (BMI) which is a measure of the patients nutritional status and is estimated as weight divided by height-squared ($kg/m^2$). This study attempts to provide a more recent, updated and clear association of these variables that independently predict patient's $CD4^+$ immune reconstitution.

This is a **retrospective longitudinal study** of ART-naïve, HIV-infected adults and adolescents initiated on standard first line Anti-Retroviral Treatment regimen and their $CD4^+$ response followed up for 18 months. **Study population** included adults and adolescents registered and initiated on standard first line Anti-Retroviral Treatment regimen, as part of routine comprehensive care program of the Kenyan Government in

conjunction with donor partners; USAID, ICAP and EGPAF at Masaba-North Sub-County PSC/CCC.

**Statistical Analysis** was done using multilevel mixed effect linear models in STATA to analyze the BMI categories CD4+ level intercept values and other variables coefficients and compare them to their reference groups to obtain P values. Repeated measures Analysis of variance was also used to determine if there a difference in CD4+ mean response between the four intervals of measurement. **Results** from the study indicate that BMI is an independent predictor of $CD4^+$ lymphocyte cells immune reconstitution for patients on antiretroviral treatment. While Age, Gender, and Number of ART interruptions were statistically significant when other variables were accounted for in the model over the 18 months of follow up. Whereas baseline BMI and WHO clinical stage were less statistically significant.

# Chapter 1.0 Introduction

## 1.1 Background of the study

HIV, the virus that causes AIDS continues to be the world's most serious health and development challenge. According to the World Health Organization (WHO) there were approximately 35 million people worldwide living with HIV/AIDS in the year 2014, of these 31.8 million were adults and 3.2 million were children (< 15 years old). The vast majority of people living with HIV are in low and middle income countries of the sub Saharan Africa region, with approximately 24.7 million people living with HIV/AIDS.

Immunological suppression and disturbance caused by HIV infection is responsible for the decline in $CD4^+$ T-lymphocyte cells count and increase in viral load which are predictive of both morbidity and mortality from AIDS related causes. The WHO statistics estimate that 1.5 million people in the World died in the year 2013 of AIDS related causes, of these 1.3million were adults and 190,000 were children. Even currently despite the advances in our scientific understanding of HIV its prevention and treatment, most people living with HIV or at risk of HIV do not have access to prevention, care and treatment and there is still no cure for the disease. However effective treatment with antiretroviral drugs can increase time to disease progression, suppress viral replication and increase $CD4^+$ T-lymphocyte cells, so that people with HIV can enjoy health lives and reduce the risk of transmitting the virus to others.

By 2014 WHO estimated 28.6 million people living with HIV (PLHIV) in the World were eligible for antiretroviral treatment (ART), of these 12.9 million were receiving ARTs of which 11.7 million were in low and middle income countries, 10.96 million were adults and 740,000 were children.

The decision to initiate ART in adults and adolescents relies on clinical and immunological assessment, WHO emphasizes the importance of using clinical parameters in deciding when to initiate ART, however it is recognized that the value of clinical staging in decision making of when to initiate and monitor ART is improved by additional information provided by baseline and subsequent (longitudinal) $CD4^+$ T-lymphocyte cell count. $CD4^+$ cell count criteria for ART initiation in adults and adolescents for low and middle income countries according to WHO 2013 guidelines of 200 – 350 cells/$mm^3$ is used and <200 cells/$mm^3$are also initiated on ART.

In spite of $CD4^+$ T-lymphocyte cell count responding rapidly to antiviral therapy and correlating with clinical outcome evaluation during therapy, the $CD4^+$ cell count shows different responses based on; type of setting were treatment is being delivered, individual and population characteristics that include; adherence, age, gender, baseline $CD4^+$ cell count, opportunistic infections i.e TB, baseline viral load and nutritional status of the patient - which is estimated by calculating the individuals Basal Metabolic Index (BMI) as weight divided by height-squared ($kg/m^2$).

Poor nutritional status at the start of ART has been identified as a predictor of mortality independent of immune status, while patients who gain weight in the early phase of treatment have improved prognosis. Thus nutritional support is becoming an integral part of ART programs in the sub-Saharan Africa and various supplement are now widely being used.

At present however there are few studies on BMI association with immune reconstitution among HIV infected adults initiating antiviral therapy in resource limited settings. And the influence of Age, Gender, Baseline $CD4^+$ cell count, WHO clinical staging, Number of ART interruptions, and TB status on the BMI association with $CD4^+$ T-lymphocyte immune reconstitution during the first 24 months of antiretroviral therapy. The difference in $CD4^+$ cell reconstitution over treatment follow up period, isn't well established either. This study will provide much of these information.

**1.2 HIV/AIDS Situation in Kenya**

Kenya being part of the countries in the sub Saharan Africa region had approximately 1.6 million people living with HIV/AIDS by 2014, 1.4 million adults and 190,000 children. Total number of deaths in the year 2013 of HIV/AIDS related causes was estimated at 58,000. PLHIV needing ART by 2014 in Kenya was estimated at 880,000 of these 656,359 had been initiated on ART; 596,228 were adults and 60,141 children, as per UNAIDS Kenya reports.

### 1.3 Problem Statement

Early research from the era before highly active antiretroviral therapy (HAART), revealed relationships between being overweight and obese and having higher CD4 cell counts, and lower viral load over time. Cross-sectional studies also found that higher body mass index (BMI) was associated with increased CD4 cell counts. However, longitudinal and retrospective data's have revealed contradicting results, with some reports noting non comparable immunological status between normal, overweight, and obese participants' BMI categories, whereas others have noted the highest CD4 counts among an overweight (but not normal, or obese) group, while still others have found that overweight and obese HIV-infected individuals have higher CD4 counts than normal or underweight peers.

Given the conflicting results from the studies, this current study was designed to explore a relationship between BMI at diagnosis and registration with disease progression (CD4+ cells count) among HAART initiating adults and adolescents, multiply adjusted for subjects; Age, Gender, Baseline CD4 T-cell count, WHO clinical staging, and Number of ART treatment interruptions retrospectively.

### 1.4 Purpose of the study

Is to establish and update knowledge on the relationship that exists between BMI of HIV positive patients at the time they are initiated on ART and the subsequent $CD4^+$ T – Lymphocyte immune reconstitution when on treatment.

### 1.5 Study Justification

Current scientific/Medical research has not been able to find a cure for HIV virus that causes AIDS, Worldwide millions of people die annually due to HIV/AIDS related causes. Effective antiretroviral treatment is the alternative used to; suppress viral replication rate, increase time to AIDS state and to increase the body's immunological defense mechanism; so that PLHIV can enjoy healthy lives and reduce the risk of transmitting HIV to others.

$CD4^+$ lymphocyte cell count is an important immunological response variable that helps medical practitioners in decision making during ART initiation and subsequent monitoring of the effectiveness of treatment in resource limited countries like Kenya. $CD4^+$ cell response to Anti-retroviral treatment has shown differences which haven't been well documented, based on the type of setting where treatment is being delivered, Individual and population characteristics that include; Adherence, Age, Gender, Baseline $CD4^+$, Baseline viral load, WHO clinical staging and nutritional status of the patients – which is estimated by calculating the individuals and populations BMI's. This study will provide updated information on the Association of pre-Anti-retroviral Treatment Body Mass Index with $CD4^+$ T-lymphocyte immune reconstitution among HIV-Infected Adults and adolescents initiated on Anti-Retroviral Treatment, while accounting for the individual and population characteristics.

## 1.6 Study Objectives

### 1.6.1 Broad Objective

To assess the relationship that exists between BMI of HIV positive patients at the time they are initiated on ART and the effects of WHO clinical staging, adherence, Baseline CD4 count, gender and age with the subsequent $CD4^+$ T – Lymphocyte immune reconstitution when they are on treatment.

### 1.6.2 Specific Objectives

1. To assess the effect of BMI at ART initiation as an independent predictor of $CD4^+$ T - lymphocyte immune reconstitution in HIV adult and adolescents on treatment.

2. To determine the impact of Gender, Age, WHO clinical staging, and Number of ART interruptions on $CD4^+$ T- lymphocyte immune reconstitution in HIV antiretroviral naive adult and adolescents initiated on Anti-Retroviral Treatment

3. To establish whether $CD4^+$ T- lymphocyte immune reconstitution in HIV antiretroviral naive adult and adolescents initiated on Anti-Retroviral Treatment differs with time on treatment; at initiation, 6 months, 12 months, and 18 months.

### 1.7 Research Question

Does $CD4^+$ T-lymphocyte immune reconstitution in HIV antiretroviral naïve adult and adolescents initiated on Anti-Retroviral Treatment (ART) differ with baseline Body Mass Index (BMI)?

### 1.8 Research Hypotheses

**Ho:** There's no difference in $CD4^+$ T-lymphocyte immune reconstitution in HIV antiretroviral naïve adult and adolescents initiated on Anti-Retroviral Treatment based on their baseline BMI categories for nutritional status BMI: < 18.5 (underweight), BMI 18.5 to 24.9 (normal weight), BMI 25 to 29.9 (overweight), and BMI > 30 (obese)

**Ha:** There is a difference in $CD4^+$ T- lymphocyte immune reconstitution in HIV antiretroviral naïve adult and adolescents initiated on Anti-Retroviral Treatment based on their baseline categories for nutritional status BMI: < 18.5 (underweight), BMI 18.5 to 24.9 (normal weight), BMI 25 to 29.9 (overweight), and BMI > 30 (obese).

# Chapter 2.0 Literature Review

## 2.1 General literature review

Chronic HIV infection is characterized by progressive loss of $CD4^+$ T cells. Early initiation of ART in the course of HIV/AIDS is associated with better survival and better long-term immune reconstitution and minimal chances of opportunistic infections. In 2009, the World Health Organization raised the recommended $CD4^+$ T cells threshold for ART initiation to 350 cells/$mm^3$; however, the extent of this recovery over time is difficult to predict, as it likely depends on multiple factors. Baseline $CD4^+$ T cell count remains the most relevant predictor of clinical progression and survival in subjects on antiretroviral therapy, but by itself it has been shown to inadequately account for the variability in ART-mediated immune restoration, and "on treatment" assessment of $CD4^+$ T cells retains a better prognostic value. Other factors positively associated with $CD4^+$ T cell immune reconstitution include; antiretroviral regimen and, in some studies, pre-ART viral load.

J.McMahon et al. (2010), accounts that in addition to viral and immunologic parameters, metabolic factors have been shown to be associated with disease progression, and are putative candidates to predict $CD4^+$ T cells recovery: advanced HIV infection (i.e., low CD4 counts) is associated with chronic inflammation and increased immune activation, with alteration of metabolic parameters associated with lipid metabolism and increased atherogenic risk (as assessed by increased carotid intima-media thickness) in subjects of both sexes. A number of studies have reported that subjects with advanced HIV infection have lower high density lipoprotein (HDL) cholesterol, higher low density lipoprotein

(LDL) cholesterol and triglycerides, and CD4 counts appear to directly correlate with HDL cholesterol. The existence of a relationship between metabolic markers, viremia and immune activation is also suggested by the observation that ART-mediated suppression of HIV replication results in a rapid normalization of a number of markers linked to cardiovascular risk, (Azzoni et al, 2011).

Malnutrition can lead to increased susceptibility to infection through suppression of immune defense mechanisms, and HIV infection can cause malnourishment through increased accompanying opportunistic infections, malabsorption, and anorexia. Thus Low body mass index (BMI) has been shown to independently predict immune reconstitution and mortality in HIV infected persons initiating ART. (Sicotte et al, 2014). On the other extreme side Obesity has been linked with numerous deleterious health outcomes, including hypertension, diabetes, heart disease, myocardial infarction, and stroke. In the general population, there also exists a relationship between obesity and development of infections. (R Sudfeld et al, 2013). However, the associations between underweight, normal weight, overweight, obesity and disease progression among HIV-infected populations' remains unclearly described.

**2.2 WHO HIV/AIDS clinical stage**

Clinical staging was developed by the WHO in 1990 and revised in 2007, it is based on clinical findings that guide the diagnosis evaluation and management of HIV/AIDS and it does not require a $CD4^+$ cell count. Patients greater than 15 years are assigned into one of four hierarchical clinical stages ranging from stage 1 (asymptomatic) to stage 4 (AIDS).

Patients are assigned to a particular stage when they demonstrate at least one clinical condition in that stage's criteria. However staging of PLHIV is not permanent fixture; a person who has been successfully treated for and recovered from opportunistic infection that placed them in higher WHO clinical stage may be downgraded to lower clinical stages if no other severe conditions are present.

**Stage 1.** Patients who are asymptomatic or have persistent generalized lymphadenopathy (lymphadenopathy of at least two sites [not including inguinal] for longer than 6 months) are categorized as being in stage 1, where they may remain for several years.

**Stage 2.** Even in early HIV infection, patients may demonstrate several clinical manifestations. Clinical findings included in stage 2 (mildly symptomatic stage) are unexplained weight loss of less than 10 percent of total body weight and recurrent respiratory infections (such as sinusitis, bronchitis, otitis media, and pharyngitis), as well as a range of dermatological conditions including herpes zoster flares, angular cheilitis, recurrent oral ulcerations, papular pruritic eruptions, seborrhoeic dermatitis, and fungal nail infections.

**Stage 3.** As disease progresses, additional clinical manifestations may appear. Those encompassed by the WHO clinical stage 3 (the moderately symptomatic stage) category are weight loss of greater than 10 percent of total body weight, prolonged (more than 1 month) unexplained diarrhea, pulmonary tuberculosis, and severe systemic bacterial infections including pneumonia, pyelonephritis, empyema, pyomyositis, meningitis, bone

and joint infections, and bacteremia. Mucocutaneous conditions, including recurrent oral candidiasis, oral hairy leukoplakia, and acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis, may also occur at this stage.

***Stage 4.*** The WHO clinical stage 4 (the severely symptomatic stage) designation includes all of the AIDS-defining illnesses. Clinical manifestations for stage 4 disease that allow presumptive diagnosis of AIDS to be made based on clinical findings alone are HIV wasting syndrome, *Pneumocystis pneumonia* (PCP), recurrent severe or radiological bacterial pneumonia, extrapulmonary tuberculosis, HIV encephalopathy, CNS toxoplasmosis, chronic (more than 1 month) or orolabial herpes simplex infection, esophageal candidiasis, and Kaposi's sarcoma. Other conditions that should arouse suspicion that a patient is in clinical stage include cytomegaloviral (CMV) infections (CMV retinitis or infection of organs other than the liver, spleen or lymph nodes), extrapulmonary cryptococcosis, disseminated endemic mycoses (e.g., coccidiomycosis, penicilliosis, histoplasmosis), cryptosporidiosis, isosporiasis, disseminated non-tuberculous mycobacteria infection, tracheal, bronchial or pulmonary candida infection, visceral herpes simplex infection, acquired HIV-associated rectal fistula, cerebral or B cell non-Hodgkin lymphoma, progressive multifocal leukoencephalopathy (PML), and HIV-associated cardiomyopathy or nephropathy.

## 2.3 Empirical Literature review

A study of Body Mass Index, immune status and virological control in HIV-infected men who have sex with men, by (Blashill et al, 2013) that analyzed $CD4^+$ count as the outcome variable, established a significant main effect for BMI category, $F(2, 318) = 3.7$, $p = 0.027$. To follow-up this significant main effect, Fisher's LSD analyses were conducted. Results revealed that overweight men ($M = 514$, $SE = 18$) possessed higher CD4 counts compared to normal weight men ($M = 451$, $SE = 18$), and neither group significantly differed from obese men ($M = 476$, $SE = 39$).

Given that the interaction between BMI category and time was non-significant, $F(4, 178) = 2.1$, $p = .09$, this finding indicates that the BMI category differences did not vary as a function of time. These procedures were repeated with the continuous BMI variable as the predictor. Results revealed a significant effect for BMI, $\gamma = 7.4$, $SE = 2.1$, 95% CI = [3.2, 11.5], $t(830) = 3.5$, $p < .0001$, indicating that a one unit increase in BMI was associated with a +7.4 change in $CD4^+$ count.

Crum-Cianflone et al. (2011), in the study Impact of weight on immune cell counts among HIV-infected persons; multiply adjusted longitudinal models for those diagnosed in the pre-HAART era (n = 397, mean follow-up of 6.5 years). Its post diagnosis mean decreases in the white blood cell count were less as the BMI category increased: -1,068, -590, -458, and -316 cells/$mm^3$ respectively ($P < 0.001$). Compared to normal-weight persons, those who were obese ($P < 0.04$) had smaller reductions in the white blood cell counts over time, with similar trends for those who were overweight ($P < 0.08$). Similar

findings were noted for the total lymphocyte count. Regarding the CD4/CD8 ratio, compared to normal-weight persons (- 0.10), those who were obese (-0.04; $P < 0.008$) had smaller reductions over time, while those who were underweight had larger reductions (- 0.27; $P < 0.001$).

The $CD4^+$ count and percentages had similar findings. Among patients diagnosed in the HAART era (n = 700; mean follow-up of 4.2 years), the mean post diagnosis changes in the white blood cell and total lymphocyte counts were significantly different over time by BMI category. The mean changes in CD4 counts over time were +5, +99, +111, and +71 cells/$mm^3$ respectively ($P < 0.001$). Consistent with the researcher's previous work (7), obese HIV infected persons, compared to those with normal weight, had significantly smaller increases in the CD4 count (+71 versus +99 cells/mm3; $P = 0.03$). HIV patients who were underweight, compared to those with normal weight, also had poorer CD4 increases over time (+5 versus +99 cells/mm3; $P = 0.007$). The change in CD4 percentage showed similar trends for obese versus normal weight persons (+3% versus +4%; $P < 0.001$) and underweight compared to normal-weight persons (+1% versus +4%; $P = 0.004$).

According to (Maman et al, 2012) in the study gender differences in immune reconstitution: a multi centric cohort analysis in sub-Saharan Africa that explored the association between gender and $CD4^+$ cells recovery among patients followed up for more than 9 months on ART. $CD4^+$ cells count increased regularly during the first 6 years after ART start, with a progressive slowing of immune reconstitution with time on ART.

Women experienced better reconstitution than men, after accounting for all the variables included in the model. After 1 year on ART, women had $CD4^+$ count 40 cells/$mm^3$ (95% CI 34–46) higher than men, with a yearly average increase of 20 $CD4^+$ cells/$mm^3$ (95% CI 16–23, $P < 0.0001$).

Patients with higher initial $CD4^+$ count reached higher $CD4^+$ levels after ART start. example 1 year after ART start, patients who initiated ART with CD4 count 100–150 cells/$mm^3$ had a $CD4^+$ count on average 107 cells/$mm^3$ higher than patients with initial count, 50 cells/$mm^3$ (95% CI 98–117). This difference increased with higher initial $CD4^+$ levels and was 284 cells/$mm^3$ (95% CI 272–296) for patients with initial $CD4^+$ of 250 cells/$mm^3$ or more compared to those with, 50 cells/$mm^3$ (LR test for trend, $P = 0.001$).

Age was also associated with immune reconstitution. Patients younger than 30 years old when they initiated ART had mean CD4 count 52 cells/mL (95% CI 42–62) higher than those aged more than 50 years, one year after ART start (LR test, P,0.001). Associations with initial BMI and WHO clinical stage were less strong and did not reach statistical significance when the other variables were accounted in the model. After 3 years of ART, women and men who had initial $CD4^+$ count 100–149 cells/$mm^3$ reached 475 cells/$mm^3$ (95% CI 468–482) and 388 cells/$mm^3$ (95% CI 379–397) respectively. After 6 years of ART, the same group of patients reached 608 cells/$mm^3$

(95% CI 594–621) and 467 cells/$mm^3$ (95% CI 449–484) respectively. After the first year of ART, the higher the initial CD4 cell count, the higher the level of $CD4^+$ count was achieved, and these differences continued over time.

In the study; Body Mass Index and $CD4^+$ T-Lymphocyte recovery in HIV infected men with viral suppression on Antiretroviral Therapy done by (Palermo et al, 2011) it established relationship between baseline BMI category and CD4 change from baseline; for the 357 subjects with 36 months follow-up, baseline BMI did predict change in $CD4^+$ T-lymphocyte count to month 36 (P = 0.005). In this model, after adjusting for baseline plasma HIV RNA, $CD4^+$ T-lymphocyte count, age, and race, relative to men with a normal BMI (18.5–25 kg/$m^2$), underweight men had $CD4^+$ increases that were 94 cells/$mm^3$ lower and overweight and obese men had increases that were 35 and 113 cells/$mm^3$ higher, respectively.

Similarly, among the 461 subjects with 24 months $CD4^+$ T-lymphocyte data, BMI predicted change in $CD4^+$ T-lymphocyte count (P = .03), with larger baseline BMI associated with greater CD4 increases. Among the 558 subjects with 12 months data, baseline BMI did not significantly predict change in $CD4^+$ T-lymphocyte count at week (P = .38). To explore the 12 months result, a post hoc analysis was conducted among the 327 subjects also in the 24 months analysis; in this 24 months analysis, BMI was significant (P = .02) and showed a similar positive association with change in $CD4^+$ T-lymphocyte count as seen at 24 months. Again, these models adjusted for baseline plasma HIV RNA, $CD4^+$ lymphocyte count, age, and race were significant.

Koethe et al. (2011) in the study; optimal Body Mass Index range associated with improved immune reconstitution among HIV-infected adults initiating Antiretroviral Therapy. Baseline BMI was associated with 12-month CD4 lymphocyte change after adjustment for age, race, PI usage, year of ART initiation, and baseline $CD4^+$ lymphocyte count and HIV-1 RNA level (P = 0.03). The relationship was nonlinear (P = 0.01), and diminished 12-month CD4 lymphocyte recovery was observed at the extremes of BMI for both sexes. To facilitate comparisons between hypothetical patients, values for the change in CD4 lymphocyte counts at the arbitrary BMI levels of 20, 25, 30, and 40 $kg/m^2$ were extracted; however, the measure of statistical association accompanying these data is based not on any specific BMI comparison but rather on the entire relationship across all BMI levels. The reference for all comparisons is a BMI of 25 $kg/m^2$. For example, a BMI of 20 $kg/m^2$, compared with the reference was associated with a reduced 12-month $CD4^+$ lymphocyte gain among both women (265 cells/ $mm^3$) and men (218 cells/ $mm^3$). Similarly, obese women and men with a BMI of 40 $kg/m^2$ had lower 12-month $CD4^+$ lymphocyte gains (212 and 217 cells/$mm^3$, respectively) compared with the reference. The interaction of sex and BMI did not appear to be an important determinant of $CD4^+$ lymphocyte change (P = 0.16 for the interaction term), but male sex was associated with lower CD4 recovery overall (P < 0.01).

Baseline plasma HIV-1 RNA level, nonwhite race, and year of ART initiation were also significantly associated with 12-month CD4 lymphocyte change (P= 0.05). When the model was further adjusted for other potential confounders in a sensitivity analysis, a longer duration from diagnosis of HIV infection to ART initiation was associated with a

lower 12-month CD4 lymphocyte gain (P = 0.03), but a history of an AIDS-defining event, injection drug use, or hepatitis C co infection was not associated with immune recovery (P = 0.23, 0.73, and 0.21, respectively; The relationship between BMI and CD4 lymphocyte change remained similar when these variables were included in the model

Association of Pre-Treatment Nutritional Status with Change in CD4 Count after Antiretroviral Therapy at 6, 12, and 24 Months in Rwandan Women; a study done by Kiefer et al. (2011), n = 537. The mean changes in CD4 count from pre ART initiation at 6, 12, and 24 months post initiation were 71 (+-107), 89 (+-109) and 153 (+-135) cells/mL, respectively. In univariate linear models, the only significant association (at $p < 0.05$) between any nutritional measure and pre-post ART change in CD4 count occurred at 6 months of follow up. The changes in CD4 count at 12 and 24 months were not significantly associated with any of the pre-ART nutritional measurements.

This study uses a retrospective cohort data of ART-naïve, HIV-infected adults and adolescents from a mixed up setting of rural and urban settlement, who were followed up for two years in a patient support clinic in Masaba-North sub-county of Nyamira county in Kenya. The study investigates the effect of pre ART BMI association with longitudinal immune reconstitution attained after 24 months of continuous ART use, by modelling CD4+ T cell reconstitution of the subjects observed.

# Chapter 3.0 Methodology

## 3.1 Study Design

A Retrospective longitudinal study of ART-naïve, HIV-infected adults and adolescents initiated on standard first line Anti-Retroviral Treatment regimen and followed up for 18 months. Data was retrieved from the pre-ART and ART registers containing HIV/AIDS patient's information, of the years 2011 to 2014.

## 3.2 Study Setting

A mixed up setting of both rural and urban settlements with a mix up of the social economic statuses, at Masaba-North Sub-County of Nyamira County. Located in Western region of Kenya it boarders; Bomet County to the East, Narok County to the south, Kisii County to the West, Homabay County to the North and Kericho County to the North East.

## 3.3 Study Population

Study population Included ART-naïve, HIV-infected adults and adolescents initiated on standard first line Anti-Retroviral Treatment regimen, as part of routine comprehensive care program of the Kenyan Government patient support clinic (PSC/CCC) in conjunction with donor partners; USAID, ICAP and EGPAF. Masaba-North Sub-County PSC offers health care services to approximately 1300, active pre-ART and ART initiated patients.

### 3.3.1 Study Population Inclusion Criteria

- HIV antiretroviral naive patients initiated on standard first line Anti-Retroviral Treatment regimen (combination of AZT + 3TC + NVP/EFV or TDF + 3TC + NVP/EFV or d4T + 3TC + EFV/NVP)
- Initiated through WHO clinical staging or $CD4^+$ cells/ $mm^3$ per the Kenyan ART monitoring Guideline
- A minimum follow up period of 18 months without loss to follow up or transfer to another clinic or death.
- Patients initiated on treatment at the same clinic and not transferred into the clinic.

### 3.3.2 Study population Exclusion criteria

- Those females who are pregnant or who get pregnant during follow up
- Those patients affected by (TB, Cryptococci meningitis) opportunistic infections. Because the infections overlap period cannot distinguished clearly between the $CD4^+$ measurement intervals.
- Children below the age of 15 years
- Development of drug resistance during follow up period

## 3.4 Sampling

### 3.4.1 Sample Size Calculation

A one sample situation formula for estimating a population proportion with specified absolute precision was used.

$$n = \frac{z^2 \times p\,(1-p)}{d^2}$$

(a) Anticipated population proportion $P$

(b) Confidence level 100(1-X) %

(c) Absolute precision required on either side of the proportion (in percentage points) $d$

A rough estimate of $P = 0.5$ which is the "safest" choice estimate of BMI for the population proportion will be used. Confidence levels of 95%, absolute precision of 5 percentage points $d = 0.05$

$$n = \frac{z^2 \times p\,(1-p}{d^2} \qquad n = \frac{1.96^2 * 0.5(0.5)}{0.05^2} \qquad n = \frac{0.9604}{0.0025}$$

n = 384 subjects

$$nf = \frac{n}{1 + (n/N)}$$

nf = the corrected sample size (when the population is less than 10,000).

N=the total number of target population

n=the calculated sample size from Fishers et al before correction for simple randomised sample (when the population is more than 10,000).

$$nf = \frac{384}{1 + (384/1300)} = 297$$

nf =297 subjects

### 3.4.2 Sampling technique

From all the patients who meet the inclusion and exclusion criteria a random sample of 297 patients was selected and used in the study.

### 3.5 Data collection

Data collection was undertaken by trained research assistants who collected the required information from the patient files. The data was entered in a database; edited, coded, classified and analyzed.

### 3.6 CD4 Analyzing Machine

CyFlow counter (CY-S-3022) flow cytometry system of Partec, equipped with a green (532nm) solid state laser and three optical parameters for the detection of side scatter (SSC), orange (FL2) and red (FL3) fluorescence signals will be used for $CD4^+$ counting.

### 3.6.1 Flow Cytometry

It is a technique for the analysis of cells or particles in aqueous suspension, cells or other biological particles pass in single cell stream through a measuring device. One or more lasers interrogate each particle, the system measure the degree and direction of scatter light; - indicators of the particles size, shape and structure. If particles have been stained with one or more fluorescent dyes known as fluorochromes; - the light source excites these dyes to provide additional biological information about each particle, such as specific surface markers. The unique advantage of Flow Cytometry is that they can

rapidly and quantitatively measure multiple simultaneous parameters on individual live cells.

### 3.6.2 Principal of operation of the Flow Cytometer

A fluidic system uses air pressure regulation to ensure stable operation and consists of a sheath fluid line and a patient sample line feeding into the flow cell. As the sample enters the flow cell chamber, the outer, faster flowing sheath fluid hydro dynamically focuses this sheath fluid into a narrow core region within the jet and presents a single file of particles to the laser interrogation point. The laser beam focused onto the capillary causes scattering of light, providing information about different cellular characteristics. The intensity of the forward scattered light (FSC) correlates with the size of the cell. Cells with a high granularity create a stronger signal. The side scatter light (SSC) is emitted in an angle of $90^0$ and gives information about the granularity of the cell. Cells with a high granularity create a stronger signal because of the high refraction index of the granules.

## 3.7 Measuring Weight and Height/Length

Patients are explained to and requested to cooperate with the technicians in having their weight and height measured. Since the measurements are taken in a single facility there's no variance in the weighing and measuring machines used.

### 3.7.1 Measuring Weight

- Choose appropriate scale for the age of the client- adult weighing scale.
- Make sure the scale pointer is at zero and is on flat stable surface.

- Ensure that it is functioning well by weighing a known weight – yours!

- Ask the person to take off shoes, hat, and scarves so that he/she is wearing minimum clothing.

- Ask the person to stand straight on the centre of the balance platform (if the person cannot stand without help, take MUAC).

- Read the weight as soon as the indicator on the scale has stabilized.

- Record the weight to the nearest 0.1 kg.

- Make sure the weighing scale is calibrated to **zero** before each measurement is taken.

- Record the weight to the **nearest 100 grams**.

### 3.7.2 Measuring Height/Length

- Measure children who are 85 cm long or less (or under two years old) lying down. Measure taller children while they are standing.

- Make sure the client is barefoot and wearing no headgear.

- Make sure the client's shoulder blades, buttocks, and heels touch the vertical surface of the height/length board.

- Make sure the client's knees are fully straight and his/her hands are held down to the side.

- Make sure the client's neck is straight and his/her eyes look straight ahead.

- Place the headpiece of the height/length board firmly on the client's head.

- Read the measurement to the nearest 0.5 centimeter.

### 3.8 Statistical Analysis

Analysis was done using multilevel mixed effect linear models in STATA to analyze the BMI Categories CD4+ level intercept values and other model variables coefficients were compared to their reference categories to obtain P values. Repeated measures Analysis of variance was used to determine if there a difference in CD4+ mean response between the four intervals of measurement.

#### 3.8.1 Multilevel and Mixed-Effects Modeling

Mixed-effects modeling is basically regression analysis allowing two kinds of effects: *fixed effects,* meaning intercepts and slopes meant to describe the population as a whole, just as in ordinary regression; and also *random effects,* meaning intercepts and slopes that can vary across subgroups of the sample. The fixed-effects model assumes that the same intercept and slopes characterize all subjects in the analysis. A random effects model allows for each of the groups to have its own random intercept including a set of, *B* coefficients that describe all the groups, and also a random intercept *uo,* which varies from one group to the next… Mixed-modeling research often focuses on the fixed effects, with random effects included to represent heterogeneity in the data, but not of substantive interest.

The model is of the form:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk} \;\; ; \;\; k = 1, 2, .., n; i = 1, 2, .., a; \; j = 1, 2, .., b$$

Where

$\alpha_i$ is the random effect and $\beta_j$ is the fixed effect. $\gamma_{ij}$ is interaction term and it is a random effect.

Thus

$$\alpha_i \sim N(0,\sigma_\alpha{}^2) \;\; ; \quad \gamma_{ij} \sim N(0,\frac{(n-1)}{n}\sigma_{\alpha\beta}{}^2) \;\; ; \varepsilon_{ijk} \sim N(0,\sigma^2)$$

And $\beta_j{}'s$ have the constraint $\sum_{j=1}^{b}\beta_j = 0$

The random effects due to different levels of factor are independent as well as the interaction terms. Also the random components and error terms are mutually independent.

For this model we carry out the following tests:

$$(1)\ H_0 : \sigma_\alpha^2 = 0$$
$$(2)\ H_0 : \beta_1 = \beta_2 = .. = \beta_b = 0$$
$$(3)\ H_0 : \sigma_{\alpha\beta}^2 = 0$$

To carry out these tests;

$$E[\mathrm{MSFac1}] = \sigma^2 + bn\sigma_\alpha^2 + n\sigma_{\alpha\beta}^2$$
$$E[\mathrm{MSFac2}] = \sigma^2 + an\frac{\sum_{j=1}^{b}\beta_j^2}{b-1} + n\sigma_{\alpha\beta}^2$$
$$E[\mathrm{MSInt}] = \sigma^2 + n\sigma_{\alpha\beta}^2$$
$$E[\mathrm{MSE}] = \sigma^2$$

The test statistics are

$$F_1 = \frac{\mathrm{MSFac1}}{\mathrm{MSInt}} \sim F(a-1, ab(n-1))$$

$$F_2 = \frac{MSFac2}{MSInt} \sim F(b-1, (a-1)(b-1))$$

$$F_3 = \frac{MSInt}{MSE} \sim F((a-1)(b-1), ab(n-1))$$

### 3.8.2 Repeated measures Analysis of Variance (ANOVA)

Repeated measures ANOVA, is a test to detect any overall differences between related means. It is also known as ANOVA for correlated samples. This particular test requires one response variable which is continuous and one or more predictor variables which is categorical.

Advantage of repeated measures ANOVA designs is that; they provide good precision for comparing treatments since all sources of variability between subjects are excluded from experimental error. The subjects serve as their own control, hence only variation within subjects enters the experimental error.

#### *3.8.2.1 Assumptions for repeated measures ANOVA*

(i) Response measurements are normally distribute, this assumption is checked using normality tests such as quantile-quantile plots; histogram with normal curve among others.

(ii) Covariance between responses in each subject are equal. This concept is known as sphericity. It is an equivalent of assumption of homogeneity of variance for independent ANOVA.

This assumption is checked using Mauchly's Test for Sphericity, a non-significant result indicates the assumption has not been violated. If the assumption of sphericity is violated then the power of test is minimized and hence results may not be reliable.

### *3.8.2.2 Hypothesis for repeated measures ANOVA:*

As with any ANOVA, repeated measures ANOVA tests the equality of means

$$H_0 : \mu_1 = \mu_2 = .... = \mu_k \quad vs. \quad H_1 : \text{not all } \mu_i \text{'s are equal}$$

## 3.9 Ethical Considerations

Authority to conduct the study was sought from the University of Nairobi / Kenyatta National Hospital Ethics and Research committee. Permission was also sought from the coordinator HIV/AIDS Masaba North PSC to Access Data. The data collected from the patient registers was kept anonymous and confidential, patient reference identifiers were removed and anonymous coding numbers assigned.

## 3.10 Limitations of the study

The study being retrospective isn't able to account for the effect of opportunistic infections (TB, Cryptococci meningitis) since the infection periods were occurring at different times thus the infected patients were excluded. Other social variable could not be collected from the patient registers but all the relevant available data is obtained.

## Chapter 4.0 Analysis and Results

**Table 1. Summary statistics of the observations**

| | Obs | Mean | Std. Dev. | Min | Max |
|---|---|---|---|---|---|
| Id | 1188 | 149 | 85.7721 | 1 | 297 |
| months | 1188 | 9 | 6.71103 | 0 | 18 |
| Age | 1188 | 37.9259 | 8.98344 | 17 | 67 |
| Gender | 1188 | 1.72727 | 0.44555 | 1 | 2 |
| weight | 1188 | 59.9815 | 11.2714 | 27 | 124 |
| Height | 1188 | 162.983 | 7.22322 | 146 | 185 |
| BMI | 1188 | 22.6049 | 3.97269 | 13.44 | 45.55 |
| WHOstaging | 1184 | 1.84122 | 0.83769 | 1 | 4 |
| ARTinterups | 1188 | 0.83838 | 0.64705 | 0 | 3 |
| counts | 1180 | 382.607 | 223.153 | 2 | 1559 |
| BMI_group | 1188 | 2.13468 | 0.63222 | 1 | 4 |

Table 1; data of 297 study subjects was analyzed in the study, four repeated measures were obtained from each subject giving a total of 1188 observations (324 (27.27%) for male, 864 (72.73%) for female). Minimum amount of $CD4^{+}$ estimated in the study was 2 and a maximum of 1559. The lowest weight of recorded on the study was 27kgs and 124kgs at the highest. The lowest age of patients in the study was 17 years and the

highest was 67 years. A minimum estimated BMI from the cohort was 13.44 and a maximum of 45.55.

**Table 2. Gender with BMI category tabulation**

| | **categorical BMI** | | | | |
|---|---|---|---|---|---|
| **Gender** | 1 | 2 | 3 | 4 | **Total** |
| M | 28 | 272 | 24 | 0 | 324 |
| | 2.36 | 22.9 | 2.02 | 0 | 27.27 |
| F | 100 | 540 | 184 | 40 | 864 |
| | 8.42 | 45.45 | 15.49 | 3.37 | 72.73 |
| **Total** | 128 | 812 | 208 | 40 | 1,188 |
| | 10.77 | 68.35 | 17.51 | 3.37 | 100 |

BMI categories; 10.77% (2.36% male 8.42female) of the patients in the sample were underweight, 68.35% (22.90% male 44.45% female) normal weight, 17.51% (2.02 male 15.49 female) overweight, 3.37% (0.00% males 3.37% females) were obese (Table 2).

**Table 3. Gender with ART interruptions tabulation**

| gender | No: of ART interruptions | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | **Total** |
| M | 80 | 196 | 44 | 4 | 324 |
| | 6.73 | 16.5 | 3.7 | 0.34 | 27.27 |
| F | 268 | 500 | 88 | 8 | 864 |
| | 22.56 | 42.09 | 7.41 | 0.67 | 72.73 |
| **Total** | 348 | 696 | 132 | 12 | 1,188 |
| | 29.29 | 58.5 | 11.11 | 1.01 | 100 |

Proportion of patients and their respective number of ART interruptions was as follows; 3 = 1.01%, 2 = 11.11%, 1 = 58.59%, 0 = 29.29% (Table. 3).

**Table 4. Gender with WHO clinical stage tabulation**

| **gender** | **WHO clinical stage** | | | | **Total** |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| M | 104 | 96 | 116 | 8 | 324 |
| | 8.78 | 8.11 | 9.8 | 0.68 | 27.36 |
| F | 396 | 300 | 148 | 16 | 860 |
| | 33.45 | 25.34 | 12.5 | 1.35 | 72.64 |
| **Total** | 500 | 396 | 264 | 24 | 1,184 |
| | 42.23 | 33.45 | 22.3 | 2.03 | 100 |

From the data 42.23% of the patients were classified in WHO clinical stage 1, 33.45% in stage 2, 22.30 in stage 3, and 2.03% in stage 4 (Table 4).

**Table 5. Overall fixed effect linear regression**

| counts | Coef. | Std. Err. | t | P>t | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| months | | | | | | |
| 6 | 61.51014 | 12.00253 | 5.12 | 0.000 | 37.95309 | 85.06718 |
| 12 | 96.22956 | 12.03311 | 8 | 0.000 | 72.6125 | 119.8466 |
| 18 | 125.0868 | 12.0884 | 10.35 | 0.000 | 101.3612 | 148.8124 |
| Age | 0 | (omitted) | | | | |
| 2.Gender | 0 | (omitted) | | | | |
| WHOstaging | | | | | | |
| 2 | 0 | (omitted) | | | | |
| 3 | 0 | (omitted) | | | | |
| 4 | 0 | (omitted) | | | | |
| ARTinterups | | | | | | |
| 1 | 0 | (omitted) | | | | |
| 2 | 0 | (omitted) | | | | |
| 3 | 0 | (omitted) | | | | |
| BMI_group | | | | | | |
| 2 | 0 | (omitted) | | | | |
| 3 | 0 | (omitted) | | | | |
| 4 | 0 | (omitted) | | | | |
| _cons | 312.661 | 8.491825 | 36.82 | 0.000 | 295.9943 | 329.3277 |

Table 5, is the general fixed effect linear regression output of 297 subjects, mean baseline $CD4^+$ estimate from the study when all predicting variables are fixed at a constant is 312.6 (95% C.I; 295.9 - 329.3) with an averagely estimated increase of 61.5 (95% C.I; 37.9 – 81.5) at 6 months, 96.2 (95% C.I; 72.6 – 119.8) at 12 months, 125.1 (95% C.I; 101.3 – 148.8) at 18 months.

**Table 6. Fixed effect linear regression for Underweight BMI category (1)**

| counts | Coef. | Std. Err. | t | P>t | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| months | | | | | | |
| 6 | 122.9688 | 33.18255 | 3.71 | 0.000 | 57.04582 | 188.8917 |
| 12 | 160.9199 | 33.58156 | 4.79 | 0.000 | 94.20424 | 227.6355 |
| 18 | 203.1851 | 33.95554 | 5.98 | 0.000 | 135.7265 | 270.6437 |
| Age | 0 | (omitted) | | | | |
| Gender | 0 | (omitted) | | | | |
| WHOstaging | 0 | (omitted) | | | | |
| ARTinterups | 0 | (omitted) | | | | |
| _cons | 252.9435 | 23.51636 | 10.76 | 0.000 | 206.2241 | 299.6628 |

Mean baseline $CD4^+$ estimate for underweight category when all predicting variables are fixed at a constant is 252.9 (95% C.I; 206.2 - 299.6) with an averagely estimated increase of 122.9 (95% C.I; 57.0 – 188.9) at 6 months, 160.9 (95% C.I; 94.2 – 227.9) at 12 months, 203.2 (95% C.I; 135.7 – 270.6) at 18 months (Table 6).

**Table 7. Fixed effect linear regression for normal weight BMI category (2)**

| counts | Coef. | Std. Err. | t | P>t | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| months | | | | | | |
| 6 | 61.34653 | 14.19238 | 4.32 | 0.000 | 33.47357 | 89.2195 |
| 12 | 100.4942 | 14.21883 | 7.07 | 0.000 | 72.56925 | 128.4191 |
| 18 | 130.9334 | 14.29055 | 9.16 | 0.000 | 102.8677 | 158.9992 |
| Age | 0 | (omitted) | | | | |
| Gender | 0 | (omitted) | | | | |
| WHOstaging | 0 | (omitted) | | | | |
| ARTinterups | 0 | (omitted) | | | | |
| _cons | 298.5689 | 10.04015 | 29.74 | 0.000 | 278.8507 | 318.2872 |

Mean baseline $CD4^{+}$ estimate for normal weight category when all predicting variables are fixed at a constant is 298.6 (95% C.I; 278.9 - 318.3) with an averagely estimated increase of 61.3 (95% C.I; 33.5 – 89.2), 100.5 (95% C.I; 72.6 – 128.4), 130.9 (95% C.I; 102.9 – 158.9) at 6 months, 12 months, and 18 months respectively (Table 7).

**Table 8. Fixed effect linear regression for overweight BMI category (3)**

| counts | Coef. | Std. Err. | t | P>t | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| months | | | | | | |
| 6 | 38.78846 | 31.91614 | 1.22 | 0.226 | -24.2648 | 101.8417 |
| 12 | 55.96154 | 31.91614 | 1.75 | 0.082 | -7.09167 | 119.0147 |
| 18 | 82.55769 | 31.91614 | 2.59 | 0.011 | 19.50448 | 145.6109 |
| Age | 0 | (omitted) | | | | |
| Gender | 0 | (omitted) | | | | |
| WHOstaging | 0 | (omitted) | | | | |
| ARTinterups | 0 | (omitted) | | | | |
| _cons | 373.9231 | 22.56812 | 16.57 | 0.000 | 329.3377 | 418.5084 |

Mean baseline $CD4^{+}$ estimate for overweight category, fixed effect linear regression model is 373.9 (95% C.I; 329.3 - 418.5) with an averagely estimated increase of 38.8 (95% C.I; -24.3 – 101.8), 55.9 (95% C.I; -7.1 – 119.0), 82.6 (95% C.I; 19.5 – 145.6) at 6 months, 12 months, and 18 months respectively (Table 8).

**Table 9. Fixed effect linear regression for obese BMI category (4)**

| counts | Coef. | Std. Err. | t | P>t | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| months | | | | | | |
| 6 | -13.7 | 67.84951 | -0.2 | 0.841 | -152.916 | 125.5157 |
| 12 | 16.3 | 67.84951 | 0.24 | 0.812 | -122.916 | 155.5157 |
| 18 | -11.5 | 67.84951 | -0.17 | 0.867 | -150.716 | 127.7157 |
| Age | 0 | (omitted) | | | | |
| Gender | 0 | (omitted) | | | | |
| WHOstaging | 0 | (omitted) | | | | |
| ARTinterups | 0 | (omitted) | | | | |
| _cons | 466.3 | 47.97685 | 9.72 | 0 | 367.8596 | 564.7404 |

Mean baseline $CD4^{+}$ estimate for obese category when all predicting variables are fixed at a constant is 466.3 (95% C.I; 367.9 – 564.7) with an averagely estimated change of -13.7 (95% C.I; -152.9 – 125.5), 16.3 (95% C.I; -122.9 – 155.5), -11.5 (95% C.I; -150.7 – 127.7) at 6 months, 12 months, and 18 months respectively (Table 9).

**Table 10. Overall ML random effects linear regression**

| counts | Coef. | Std. Err. | z | P>z | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| months | | | | | | |
| 6 | 61.51014 | 11.98111 | 5.13 | 0.000 | 38.02759 | 84.99268 |
| 12 | 96.44177 | 12.00831 | 8.03 | 0.000 | 72.90591 | 119.9776 |
| 18 | 125.4597 | 12.06034 | 10.4 | 0.000 | 101.8218 | 149.0975 |
| Age | -2.25341 | 1.107186 | -2.04 | 0.042 | -4.42346 | -0.08337 |
| 2.Gender | 30.79989 | 22.72355 | 1.36 | 0.175 | -13.7375 | 75.33723 |
| WHOstaging | | | | | | |
| 2 | -53.2017 | 22.29842 | -2.39 | 0.017 | -96.9058 | -9.49757 |
| 3 | 2.924144 | 27.08323 | 0.11 | 0.914 | -50.158 | 56.00631 |
| 4 | -82.4053 | 68.55709 | -1.2 | 0.229 | -216.775 | 51.96409 |
| ARTinterups | | | | | | |
| 1 | -124.36 | 22.65495 | -5.49 | 0.000 | -168.763 | -79.9575 |
| 2 | -129.651 | 35.22449 | -3.68 | 0.000 | -198.689 | -60.6119 |
| 3 | -132.56 | 96.63621 | -1.37 | 0.17 | -321.964 | 56.84318 |
| BMI_group | | | | | | |
| 2 | -14.2429 | 31.39537 | -0.45 | 0.65 | -75.7767 | 47.29084 |
| 3 | 24.85462 | 37.35373 | 0.67 | 0.506 | -48.3573 | 98.06659 |
| 4 | -9.00871 | 60.63397 | -0.15 | 0.882 | -127.849 | 109.8317 |
| _cons | 488.7423 | 60.76948 | 8.04 | 0.000 | 369.6363 | 607.8482 |

Overall Maximum Likelihood random effects linear regression output of 297 subjects, mean baseline $CD4^{+}$ estimate from the study when all predicting variables are changing with time during follow up is 488.7 (95% C.I; 369.6 – 607.8) with an average change over time for every variable as follows; age = -2.25 (95% C.I; -4.42 – -0.08), gender = 30.8 (95% C.I; -13.7 – 75.3), WHO clinical staging in reference to stage one; stage 2 = -53.2 (95% C.I; -96.0 – -9.5), stage 3 = 2.9 (95% C.I; -50.0 – 56.0), stage 4 = -82.4 (95% C.I; -216.7 – 51.9).

Number of ART interruptions in reference to no interruption; 1 = -124.4 (95% C.I; -168.8 – -79.9), 2 = -129.7 (95% C.I; -198.7 – -60.6), 3 = -132.6 (95% C.I; -321.9 – 56.8). BMI categories in reference to underweight; normal weight (2) = -14.2 (95% C.I; -75.8– -47.3), over weight (3) = 24.8 (95% C.I; -48.3 – 98.0), obese (4) = -9.0 (95% C.I; -127.9 – 109.8).

**Table 11. ANOVA**

| | analysis of variance | | | | |
|---|---|---|---|---|---|
| Source | SS | df | MS | F | Prob > F |
| Between groups | 2619168 | 3 | 873055.9 | 18.3 | 0.000 |
| Within groups | 56091808 | 1176 | 47697.12 | | |
| Total | 58710976 | 1179 | 49797.27 | | |

One way ANOVA output F (3, 1179) = 18.3, p = 0.000.

| | Bartlets Test for equal variance | | |
|---|---|---|---|
| Means | 0 | 6 | 12 |
| 6 | 61.3098<br>0.004 | | |
| 12 | 97.2895<br>0 | 35.9798<br>0.272 | |
| 18 | 126.31<br>0 | 65.0003<br>0.002 | 29.0205<br>0.648 |

Comparison of mean CD4 levels by months of measurement.

## Chapter 5.0 Discussion and conclusion

This longitudinal study aimed to examine the progress of $CD4^+$ lymphocyte immune reconstitution predicted by baseline BMI among a cohort of patients initiated on antiretroviral treatment. The results indicate an overall starting mean $CD4^+$ count of 312.6 (95% C.I; 295.9 - 329.3) Prob F < 0.0001, with an average estimated increase of 61.5 (95% C.I; 37.9 – 81.5) at 6 months, 96.2 (95% C.I; 72.6 – 119.8) at 12 months, 125.1 (95% C.I; 101.3 – 148.8) at 18 months.

BMI categories at ART initiation predicted $CD4^+$ lymphocyte immune reconstitution as follows: For the patients in the underweight category their baseline mean $CD4^+$ count was 252.9 (95% C.I; 206.2 - 299.6), with an average estimated increase of 122.9 (95% C.I; 57.0 – 188.9) at 6 months, 160.9 (95% C.I; 94.2 – 227.9) at 12 months, 203.2 (95% C.I; 135.7 – 270.6) at 18 months. For the normal weight category of patients, their baseline mean $CD4^+$ count increased compared to the underweight category but with a reduced rate of increment over the 6, 12, 18 months measuring intervals; 298.6 (95% C.I; 278.9 – 318.3), with an average estimated increase of 61.3 (95% C.I; 33.5 – 89.2), 100.5 (95% C.I; 72.6 – 128.4), 130.9 (95% C.I; 102.9 – 158.9) at 6 months, 12 months, and 18 months respectively.

The study estimated an increased baseline mean $CD4^+$ count for the overweight category compared to underweight and normal weight categories but with a further reduced rate of increment over the 6, 12, 18 months measuring intervals; 373.9 (95% C.I; 329.3 - 418.5),

with an average estimated change of 38.8 (95% C.I; -24.3 – 101.8), 55.9 (95% C.I; -7.1 – 119.0), 82.6 (95% C.I; 19.5 – 145.6) at 6 months, 12 months, and 18 months respectively. The study estimated a high baseline mean $CD4^+$ count for obese category compared to underweight, normal weight and overweight categories but with very low and a negative rate of change over the 6, 12, 18 months measuring intervals; 466.3 (95% C.I; 367.9 – 564.7) Prob > F = 0.9693, with an average estimated change of -13.7 (95% C.I; -152.9 – 125.5), 16.3 (95% C.I; -122.9 – 155.5), -11.5 (95% C.I; -150.7 – 127.7) at 6 months, 12 months, and 18 months respectively.

**Conclusion for specific objective 1;** Rate of $CD4^+$ count change for underweight, normal weight and overweight categories is significant with (Prob F < 0.0001, Prob F < 0.0001, Prob > F = 0.00746) respectively, rate of change for obese category was not significant (Prob > F = 0.9693).

This study results concurred with the study done by Kiefer et al. (2011), n = 537. The mean changes in CD4 count from pre ART initiation at 6, 12, and 24 months post initiation were 71 (+-107), 89 (+-109) and 153 (+-135) cells/mL, respectively [13]. And that done by (Palermo et al, 2011) which established relationship between baseline BMI category and CD4 change from baseline; for the 357 subjects with 36 months follow-up, baseline BMI did predict change in $CD4^+$ T-lymphocyte count to month 36 (P = 0.005). In this model, after adjusting for baseline plasma HIV RNA, $CD4^+$ T-lymphocyte count, age, and race, relative to men with a normal BMI (18.5–25 $kg/m^2$), underweight men had $CD4^+$ increases that were 94 $cells/mm^3$ lower and overweight and obese men had

increases that were 35 and 113 cells/$mm^3$ higher, respectively. It disagrees with Crum-Cianflone et al. (2011), study which established a post diagnosis mean decreases in the white blood cell count as the BMI category increased: -1,068, -590, -458, and -316 cells/$mm^3$ respectively ($P < 0.001$). Compared to normal-weight persons, those who were obese ($P < 0.04$) had smaller reductions in the white blood cell counts over time, with similar trends for those who were overweight ($P < 0.08$) [17].

We fitted an overall maximum likelihood random effects linear regression model that accounted for the effects of; gender, age, WHO clinical stage and number of ART interruptions on $CD4^+$ T-lymphocyte cells count over time. The results indicate an overall baseline mean $CD4^+$ count of 488.7 (95% C.I; 369.6 – 607.8) Prob chi2 < 0.0001, with an average change over time for every variable as follows: For every 1 year increase in age $CD4^+$ count decreases by -2.25 (95% C.I; -4.42 – -0.08) P>|z| = 0.042. Females experienced a better increase in $CD4^+$ count compared to males = 30.8 (95% C.I; -13.7 – 75.3) P>|z| = 0.175, the result agree with the study by Maman et al, (2012) which established that after 1 year on ART, women had $CD4^+$ count 40 cells/$mm^3$ (95% CI 34–46) higher than men [14].

Considering WHO clinical stage 1 as reference; patients in stage 2 had an average $CD4^+$ count decrease with time of -53.2 (95% C.I; -96.0 – -9.5) P>|z| = 0.017 (significant), in stage 3 patients had an average change of 2.9 (95% C.I; -50.0 – 56.0) P>|z| = 0.914 (not significant), and those in stage 4 had an average decrease of -82.4 (95% C.I; -216.7 – 51.9) P>|z| = 0.229 (non-significant).

For the number of ART interruptions, selecting no interruptions (0) as the reference category; subjects with 1 interruption had an average $CD4^+$ count decrease of -124.4 (95% C.I; -168.8 – -9.9) P |z| < 0.0001 (highly significant), those who had 2 interruptions their average decrease.

Was -129.7 (95% C.I; -198.7 – -60.6) P |z| < 0.0001 (highly significant), and those who had 3 interruptions = -132.6 (95% C.I; -321.9 – 56.8) P>|z| = 0.170 (non-significant).

For BMI categories underweight as the reference category; subjects in normal weight had an average $CD4^+$ count change of -14.2 (95% C.I; -75.8– -47.3) P>|z| = 0.650 (not significant), for the overweight they had an average change of 24.8 (95% C.I; -48.3 – 98.0) P>|z| = 0.506 (not significant), and the obese subjects had an average $CD4^+$ count change of -9.0 (95% C.I; -127.9 – 109.8) P>|z| = 0.882 (not significant).

**Specific objective 2 conclusion;** The results of this study indicate that; Age, Gender, and Number of ART interruptions were statistically significant when other variables were accounted for in the model over the 18 months of follow up. While baseline BMI and WHO clinical stage were less statistically significant when other variables were accounted for in the model. Becoming consistent with the study by (Blashill et al, 2013) which found out that the interaction between BMI category and time was non-significant, F (4, 178) = 2.1, p = .09 [1]. And those of the study by (Maman et al, 2012) which concluded that Associations with initial BMI and WHO clinical stage were less strong

and did not reach statistical significance when the other variables were accounted in the model after 3 years of ART [14].

**Specific objective 3 conclusion;** The study established statistically significant mean differences between the $CD4^+$ count intervals F (3, 1179) = 18.3, $p < 0.0001$. A six months interval mean change was statistically significant between baseline (0) – 6 months p = 0.004, but for 6 – 12 months p = 0.0272 and 12 – 18 months p = 0.648 the result was not significant. For >6 months interval (0 – 12, 0 – 18, 6 – 18) mean $CD4^+$ count change was significant ($p< 0.0001$). In summary the study established that BMI is an independent predictor of $CD4^+$ lymphocyte cells immune reconstitution for patients on antiretroviral treatment.

### 5.1 Recommendation

Basal Metabolic Index (BMI) which is a measured as determinant of the patients nutritional status and Since it is a predictor of improved CD4 count, there needs to be put more effort in improving the BMI of PLHIV.

More studies can be done to clearly discuss the association of WHO clinical stage and number of ART interruptions as independent predictors of immune reconstitution, because the resulted in low non significance when all variables were accounted for in random effects model.

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## Appendix i: Data analysis commands (stata)

```
reshape long countm, i(Id) j(count)
recode count (0=0) (6=1) (12=2) (18=3) (24=4), gen (months_group)
describe Id months Age Gender weight Height BMI WHOstaging ARTinterups counts
summarize months Age Gender weight Height BMI WHOstaging ARTinterups counts
BMI_group
tab Gender BMI_group, cell
tab Gender ARTinterups, cel
tab Gender WHOstaging, cel
tab WHOstaging BMI_group, cel
xtset Id months_group
xtset Id months
xtreg counts months Age Gender WHOstaging ARTinterups BMI_group, fe
xtreg counts months_group Age Gender WHOstaging ARTinterups BMI_group, fe
xtreg counts months Age Gender WHOstaging ARTinterups if BMI_group==1, fe
xtreg counts months Age Gender WHOstaging ARTinterups if BMI_group==2, fe
xtreg counts months Age Gender WHOstaging ARTinterups if BMI_group==3, fe
xtreg counts months Age Gender WHOstaging ARTinterups if BMI_group==4, fe
xtreg counts months_group Age Gender WHOstaging ARTinterups BMI_group,mle
anova counts Id months, repeated(months)
```

## Appendix ii: Request to access letter

## Appendix iii. KNH – UON ERC approval letter