

HEMATOLOGICAL AND CLINICAL PROFILES OF HIV-INFECTED ADULTS INITIATING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN UGANDA

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Acronyms and Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ARC	AIDS Related Complex
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Azidothymidine
BMI	Body Mass Index
CI	Confidence Interval
CRF	Case Report Form
d4T	Stavudine
FDA	Food and Drug Administration
FTC	Emtricitabine
3TC	Lamivudine
HAART	Highly Active Antiretroviral Therapy
HDREC	Higher Degrees, Research and Ethics Committee
HIV	Human Immunodeficiency Virus
IDI	Infectious Diseases Institute
HSC	Hematopoietic Stem Cell
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MUJHU	Makerere University Johns Hopkins University
NIH	National Institutes of Health
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OI	Opportunistic Infection
PR	Prevalence Ratio
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RR	Risk Ratio
TNF α	Tumor Necrosis Factor alpha
WBC	White Blood Cell
WHO	World Health Organization

Abstract

Background

Cytopenias are the most common HIV-associated hematological abnormality; they become more prevalent as HIV progresses and are often fatal. Cytopenias have been associated with demographic, clinical and physiological factors. Data from resource-limited settings about the prevalence and correlates of cytopenia, the trend in hematological parameters while on treatment and the association of these changes with clinical outcomes are limited.

Methods

These were secondary analyses of hematological data that was collected from 400 HIV-infected subjects who were HAART-naïve or on HAART for ≤ 6 months and were enrolled into the Multivitamins, HAART and HIV/AIDS Trial in Uganda. Anemia was defined according to WHO guidelines while leucopenia and thrombocytopenia were defined using study-site-specific laboratory reference ranges for lack of generally accepted definitions. Univariate and bivariate analyses were done to describe the patient population and log-binomial regression was used to quantify the correlates of cytopenia. Multi-level mixed effects linear regression models were used to investigate trends in and factors associated with change in hemoglobin and other selected hematological parameters over time. The association between anemia, disease progression and/or death was explored using log-binomial regression models.

Results

At baseline, 65% of the 400 subjects had at least one form of cytopenia. Anemia occurred in 47.8%, leucopenia in 24.3%, thrombocytopenia in 8.3%, bicytopenia (any 2 forms of cytopenia) in 21.9% and only 2 had a pancytopenia (all the 3 forms of cytopenia). Cytopenia

was more prevalent in females, in subjects with lower CD4 cell count and those with lower body mass index (BMI).

During follow-up, there were significant increases in hemoglobin levels, the mean corpuscular volume (MCV), white blood cell counts and the platelet counts. These changes in hematological parameters were associated with CD4 cell count, sex, BMI and age. The mean corpuscular hemoglobin (MCH) also increased over time although the absolute values remained within the normal range (24-35 picograms) while the mean corpuscular hemoglobin concentration (MCHC) was more or less the same.

Conclusions

Cytopenias are a frequent complication in HIV-infected adults at initiation of HAART in Uganda. Identifying risk factors for these abnormalities and pre-HAART interventions may ameliorate these complications. Further studies are needed to confirm the causal pathway between HIV burden, hematological manifestations and clinical outcomes.

Keywords: Hematological abnormalities, HIV, Cytopenia, HAART, Uganda

1 Introduction and background

1.1 HIV infection and pathogenesis

HIV infection is transmitted through viral shedding at mucosal surface lesions in contact with infected body fluids such as blood, semen or vaginal secretions [1]. HIV entry into the host immune system is mainly through interactions between CD4 cells and the presence of chemokine coreceptors CCR5 and CXCR4[2, 3]. Other cells such as dendritic cells, monocytes and macrophages may be infected by HIV through CD4 and chemokine receptors[4]. However, viral uptake by some other cells may be independent of the presence of CD4 cells but rather mediated by other chemokines or viral proteins for example infection of astrocytes [5] and renal epithelial cells [6]. HIV transmission is initially followed by a rapid viral replication and activation of the immune system via leakage of the virus through the gastrointestinal tract mucosa into systemic circulation before reaching a viral set-point. This immune activation is characterized by the release of proinflammatory cytokines and chemokines, polyclonal B-cell activation and progressive CD4 depletion [7-9]. The chronic immune activation and inflammation may contribute to clinical progression [10, 11].

1.2 HIV-associated complications

HIV infection has been associated with a broad range of clinical complications and hematological abnormalities are among the most common. HIV infection influences all hematopoietic cell lines leading to a spectrum of hematological abnormalities the most important and the most common of these being abnormalities affecting the cellular elements in peripheral blood known as cytopenias [12]. Other hematological complications may involve the bone marrow as well as the coagulation pathways. Cytopenias may be caused by increased destruction or reduced production of white blood cells (leucopenia or neutropenia), red blood cells (anemia) and platelets (thrombocytopenia). The causes of hematological

abnormalities in HIV are multi-factorial with several contributing factors [7, 10, 13, 14] and their frequency increases with disease progression [15].

1.3 Normal hematopoiesis

Hematopoietic stem cells (HSCs) are rare self-renewing cells that reside in the bone marrow from which various types of blood cell lineages are produced. Hematopoiesis is the step-wise process by which HSCs generate multi-lineage progenitor cells that later commit to precursors of specific hematopoietic lineages and differentiate into mature blood cells: red blood cells, megakaryocytes, white blood cells and lymphoid cells [16, 17].

1.4 Pathogenetic mechanisms of cytopenias

HIV infection may affect processes that are important during the early stages of hematopoiesis or stem cell differentiation leading to a spectrum of morphological changes within the bone marrow microenvironment that are strongly associated with various forms and levels of peripheral blood cytopenias [18, 19]. These hematologic abnormalities caused by altered stem cell differentiation reach far beyond the loss of CD4⁺ cells and could be due to abnormal lineage specific expression of certain cellular genes such as cytokines interleukin 6 (IL-6) and granulocyte colony-stimulating factor that play a role in regulation of hematopoiesis [20]. HIV-1 induced hematopoietic suppression may be mediated by the HIV-1 encoded envelope glycoprotein gp120, the extracellular viral accessory protein Negative factor (Nef) and cellular proteins such as tumor necrosis factor alpha. Soluble Nef has been shown to induce a transcriptional factor PRAR γ in uninfected hematopoietic stem cells (HSCs) which in turn suppresses the expression of 2 other factors STAT5A and STAT5B that are necessary for proper functioning of HSCs [21]. HIV may directly infect the microvascular endothelial cells of the bone marrow and provide a continuous source of the virus [10]. This alters the cytokine levels thus affecting the stromal/progenitor cell microenvironment that

supports hematopoiesis. The release of viral proteins is thought to have cytotoxic effects on blood stem cells [22].

Comorbid opportunistic infections, neoplastic disorders, prolonged physiological stress, immune mediated factors and the cytotoxic effects of antiretroviral and antimicrobial therapy further confound the hematopoietic suppression making the causative role of HIV in vivo uncertain [13, 18]. The association of HIV-infection with hematological abnormalities is dependent on viral replication as the severity of these abnormalities increases with disease progression [21, 23]. Functional hematopoiesis may be restored or corrected by HAART [24, 25] however; prolonged use of HAART may on the other hand contribute to the persistent hematopoietic suppression. HIV-associated anemia in particular may occur through three basic mechanisms affecting the red blood cells (RBC):

Decreased RBC production resulting from infiltration of the bone marrow by infections including HIV itself, neoplasms, myelosuppressive drugs (including AZT), decreased production of endogenous erythropoietin or reduced response to erythropoietin and hypogonadism[4, 26-28].

Increased destruction of RBCs in the spleen or circulatory system by RBC autoantibodies, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, glucose-6-phosphate hydrogenase deficiency and medications [12, 17, 29, 30] and;

Ineffective RBC production mainly as a result of nutritional deficiencies of iron, folic acid and vitamin B12 may be due to malabsorption in the ileum, gastric infections or other gastric mucosa pathology. Adverse effects of ARVs such as anemia, neutropenia and thrombocytopenia have been observed in HIV positive individuals before and after the introduction of HAART and may persist even with the widespread use of these potent drugs [31].

1.5 Epidemiology of Cytopenia in the pre-HAART era

In a pre-HAART series of patients with AIDS, anemia was noted in 70% of patients, lymphopenia in 70%, neutropenia in 50%, and thrombocytopenia in 40% and the prevalence of these abnormalities was found to increase with severity of clinical disease [15, 31]. Anemia was found to be the most common hematologic abnormality associated with HIV infection affecting 60-80% of patients with AIDS, 50% of those with early stage disease and 18% of those who are asymptomatic [27, 32]. Several studies have since documented the presence of anemia as an independent risk factor for mortality among HIV infected individuals [32-38]. The incidence of the various cytopenias correlates with the degree of immunosuppression and isolated abnormalities could be indications of HIV infection itself. Costello observed that the bone marrow cellularity in the HIV-infected patient did not always correlate with peripheral blood findings [31]. However, further studies showed that these hematologic abnormalities both in peripheral blood and the bone marrow particularly pancytopenia and marrow cellularity indicated the bone marrow as a target organ of drugs, immune mechanisms, opportunistic infections and the HI virus itself [39, 40].

1.6 Epidemiology of Cytopenia in the era of HAART

Highly Active Anti-Retroviral Therapy (HAART) is the current standard of care for HIV-infected individuals even in resource-limited settings and it has proven clinical and immunological benefits. The scale up of HAART has altered the expected natural history of HIV infection by reducing the frequency of opportunistic infections, hematologic complications and other AIDS-related malignancies [41, 42]. However, in some individuals optimum treatment response is not achieved while in others these potent drugs lead to adverse effects. With the increasing availability of HAART some of these adverse effects may mimic manifestations of HIV itself.

The magnitude of hematological abnormalities has been described as varying by region or country [43], degree of suppression of the virus and expected survival of infected individuals [21, 23] as well as the availability and accessibility to the various classes of these potent antiretroviral drugs [44-47]. A thorough assessment of the patient's hematological profile is central to the clinician's informed choice of an appropriate antiretroviral regimen and overall patient care [41, 46, 48].

Anemia is still the most common cytopenia in the era of ART and was frequently associated with the use of zidovudine (ZDV) or azidothymidine (AZT) during the early 1980s when these two drugs were used in high doses as monotherapy for HIV infection [49]. In an early clinical trial of the efficacy of AZT in treatment of AIDS, it was found that there were significant reductions in the hemoglobin levels of patients receiving the drug compared to those receiving placebo. In addition, other cell lines including leucocytes and neutrophils also decreased while platelets increased in the majority of patients in the treatment arm compared to the placebo arm [50]. In general, patients with advanced disease were more likely to have hematologic toxic effects possibly due to reduced marrow reserves. In another study HAART status was not associated with anemia. Individuals who were prescribed zidovudine alone or HAART both experienced significant decreases in anemia prevalence. However, the prevalence of anemia was higher among those prescribed zidovudine with or without HAART [51]. Although the current use of relatively small doses of AZT in combination with other antiretrovirals has decreased the frequency of anemia, patients with advanced HIV disease on AZT at a dose of less than 500mg/day showed significantly decreased hemoglobin levels compared to those not taking the drug [52]. In a study documenting the potential for hematologic toxicity, Richman et al described the adverse reactions and AZT-related toxicities of a placebo trial in patients with AIDS and AIDS-related complex (ARC). Statistically significant reductions in hemoglobin values were observed in the AZT arm while

the mean corpuscular volume (MCV) increased progressively over time. White cell counts decreased in the AZT arm while the platelet numbers increased in many AZT recipients but remained the same in the placebo arm [50]. In a study of anemia in a rural HIV Ugandan cohort, Mugisha et al examined the prevalence at enrolment, incidence and associated risk factors prior to the introduction of HAART and found that the prevalence and incidence of anemia were higher in HIV-positive than HIV-negative individuals [53]. A more recent comparison of the baseline prevalence of cytopenias in treatment naïve patients at entry into a multicenter randomized clinical trial of HAART in diverse geographical settings showed that anemia was more common than neutropenia or thrombocytopenia. This study further demonstrated that the prevalence of hematological abnormalities in untreated individuals was independently related to geographical location, gender and chronic co-infections such as hepatitis B infection [43]. A prospective study of HIV-infected adults in Uganda showed that 15% of patients initiated on HAART had baseline anemia and of these 5% had severe baseline anemia. Subjects with baseline anemia (Hemoglobin \leq 9.5g/dl) experienced larger increases in median hemoglobin compared to the non-anemic patients and zidovudine was not associated with an increased risk of early severe anemia [54].

1.7 Cytopenias and clinical outcomes

The use of HAART in the treatment of HIV-infected patients has led to immune reconstitution, decreased risk of opportunistic infections and improved survival [55]. However, in some patients even when viral replication is controlled and adherence is good, the treatment response, often measured by immunological and hematological parameters, is sub-optimal and results in unfavorable outcomes.

Anemia is a non-AIDS defining HIV-related illness that has been associated with decreased survival [56-59]. The incidence of anemia is strongly associated with HIV disease

progression as measured by CD4 count [60]. Several studies have demonstrated anemia as an independent predictor of mortality although the mechanisms are unclear or indirect [32-34, 37, 61, 62]. Anemia in HIV infection has an insidious onset yet it can significantly affect the functional status of an individual; it causes fatigue, headaches, and other symptoms that may range from mild to disabling disease [43]. Kowalska et al. demonstrated that starting HAART was associated with an increase in hemoglobin levels but more importantly that current levels of hemoglobin are more predictive of disease progression than the hemoglobin measured at the time of starting HAART [48]. If treated, anemic HIV infected individuals who recover from anemia have better survival rates than those who do not recover [32, 34].

1.8 Management of cytopenia

Although data on the effect of anemia treatment on survival in HIV-infected individuals on HAART is limited, some interventions have been investigated. The use of appropriate interventions in the management of HIV-associated anemia is critical especially in non-emergency situations. An observational study demonstrated that transfusion of anemic HIV-infected individuals was associated with a three-fold excess mortality risk [51] while use of epoietin alfa (human erythropoietin produced in cell culture) was associated with improved survival in HIV [34]. A cross-sectional study of HIV-infected patients demonstrated that supplemental androgens had a potential role in the treatment of HIV-associated anemia and could be a cheaper alternative to erythropoietin therapy in addition to also treating the other effects of hypogonadism in these patients such as loss of lean body mass [63].

2 Rationale and Objectives

The prevalence of cytopenias is determined by a number of factors including but not limited to immunological and virological status thus the frequency of cytopenias increases with disease progression. In addition, the side effects of the drugs used to treat HIV itself notably

zidovudine as well as treatment or prophylaxis of co-infections with cotrimoxazole for instance have also been noted to have a cytotoxic effect on the bone marrow with or without clinical consequences [50, 64]. These factors may interact with each other and ultimately influence the clinical outcome of the patients. The potentially life-threatening impact of cytopenias in HIV infected individuals warrants the understanding of the multifaceted causation of these abnormalities, the direct role of HIV and the extent to which HAART ameliorates these hematologic disorders [65]. Hemoglobin has a strong relationship with AIDS-defining illnesses and death. The current literature is consistent in confirming that anemia is a strong and independent predictor of death in HIV-infected adults [32-34, 37, 48, 61, 62]. However, a gap still remains in characterizing the trend in hemoglobin levels during HIV disease [61] and particularly while patients are on therapy. The measurement of hemoglobin loss during HIV infection is not extensively reported yet its routine measurement is cheap. Studies have yielded conflicting findings on the effect of HAART (particularly AZT-based HAART) on hemoglobin levels; some have shown worsening while others did not demonstrate a significant association between AZT use and development of anemia [38, 46, 66, 67]. Previous studies on the impact of HAART on the change in hemoglobin levels have focused on single measurements of hemoglobin [32, 68-70] or used selected populations [14, 38, 67, 70, 71] or have had a short observation time. Therefore a systematic investigation of hematological profiles of HIV-infected patients on HAART, in relation to clinical/treatment outcomes is warranted.

2.1.1 General Objective:

The overall objective of this study was to examine the trend in hematological parameters over a period of 18 months and the association of these changes with the clinical outcomes in HIV positive adults initiating HAART.

2.1.2 Specific Objectives:

1. To determine the prevalence of and factors associated with cytopenias and other selected hematological abnormalities that is, mean cell volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in HIV-infected adults initiating HAART at baseline.
2. To examine changes in hemoglobin status and other selected hematological parameters and associated factors in HIV-infected adults initiating HAART from baseline over 18 months.
3. To examine the association of baseline anemia and other factors with HIV disease progression and with all-cause mortality, in adults initiating HAART up to 18 months of follow-up.

3 Materials and Methods

3.1 Study design, setting and population

This study was nested in an on-going, larger clinical trial at the Infectious Diseases Institute (IDI) in Kampala, Uganda where 400 HIV-infected patients initiating HAART were randomized to either a multivitamin supplement (including vitamins B-complex, C and E) or a placebo, and followed for up to 18 months. Recruitment in the parent trial began in April 2010. All patients attending the IDI clinic who were eligible for HAART at the time the trial was recruiting were typically initiated on any one of these combinations of drugs as a first-line regimen:

- I. Two nucleoside reverse transcriptase inhibitors(NRTI) such as zidovudine (AZT) with lamivudine (3TC) and a non-nucleoside reverse transcriptase inhibitor(NNRTI) such as nevirapine or efavirenz

- II. A nucleotide reverse transcriptase inhibitor such as tenofovir with either lamivudine or emtricitabine (FTC) and a NNRTI.
- III. A triple nucleoside drug combination such as AZT, abacavir and 3TC

Brief description of the objectives, inclusion and exclusion criteria, methods and procedures of the parent trial

Objectives of the parent trial

The objective of the parent trial was to examine the efficacy of multivitamins in slowing disease progression among HIV-infected adults receiving HAART in Uganda using a randomized, double-blind, controlled study design [72]. The specific study objectives of the parent trial were:

- 1) To determine whether oral multivitamin supplements (including vitamins B-complex, C and E) given daily for 18 months will:
 - a) improve immune reconstitution (indicated by CD4 cell count);
 - b) improve weight gain and
 - c) improve quality of life.
- 2) To determine whether oral multivitamin supplements (including vitamins B-complex, C and E) given daily for 18 months will:
 - a) reduce risk of developing a new or recurrent disease progression event including all-cause death;
 - b) reduce the probability of changing drug therapy (indicated by switching from first- to second-line therapy), and

c) reduce the occurrence of adverse events associated with ART, indicated by peripheral neuropathy, severe anaemia, or diarrhea.

Recruitment in the trial began in April 2010 and follow-up of participants was completed in December 2013.

Inclusion and exclusion criteria of the parent trial

HIV-positive adults (≥ 18 years) registered at the Infectious Diseases Institute (IDI) were recruited into the parent trial if they:

- i) Were HAART-naïve and eligible to start HAART or had been taking HAART for not more than 6 months at trial start
- ii) Had a CD4 cell count done within 8 weeks prior to trial start
- iii) Resided within a 20 kilometer radius of IDI and had no intention of migrating within the next 18 months
- iv) Were able to provide informed consent to participate in the trial

Females were excluded if they were confirmed to be pregnant at trial start. Patients who were already attending special clinics within the IDI such as the TB clinic, the Sexual and Reproductive Health clinic as well as those who were already enrolled into another study were not recruited into the trial. However, once enrolled, subjects who needed referral to these clinics in the course of the trial were referred and were followed up until they completed trial procedures or left the trial (due to death, loss to follow up or withdrawal of consent to participate).

Methods and procedures of the parent trial

Prior to randomization and physical contact between the study staff and any patient, pre-eligibility screening was done by a designated study staff through conducting chart reviews of prospective HIV-infected patients ahead of their scheduled clinic visit to determine their potential eligibility for enrolment. The study staff then approached potential participants upon arrival at the clinic, informed them about the study and invited them for further eligibility confirmation and enrolment into the study. Upon obtaining written informed consent, the study participant was assigned a randomization number and study identification number which were used on all study documents. At each of the study visits, clinical and laboratory information was collected using standardized questionnaires and laboratory procedures. Blood specimens were taken from each participant at baseline and every 6 months to measure CD4 cell count and Complete Blood Count, malaria parasitemia and liver function. These specimens were analyzed by the Makerere University-John Hopkins University (MU-JHU) Core laboratory which is based at the study site (IDI) and is certified by the College of American Pathologists (CAP certificate number 7139001) and the results were provided to a designated study staff. Study participants were evaluated at baseline, and then at 3 months, 6 months, 12 months and at 18 months from the time of randomization into the study at which point they were terminated from the study. Ethical approval for the trial was obtained from the IDI Scientific Review Committee, the Higher Degrees, Research and Ethics Committee (HDREC) of the Makerere School of Public Health and the Uganda National Council of Science and Technology. All participants gave their written informed consent to participate in the trial.

3.2 Measurements and definitions

This was a secondary analysis of data on the Complete Blood Count (CBC, Beckman Coulter Act 5 Diff, Miami, Florida, USA), the CD4+ cell count (BD FACSCalibur System, Becton Dickson, San Jose, California, USA) and clinical information routinely collected at baseline

and at months 6, 12 and 18 as per parent trial protocol. Blood specimens were collected into EDTA vacutainers for both laboratory tests. The following laboratory parameters were evaluated: Hemoglobin, Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), total White Blood Cell count (WBC) and Platelet count. Cytopenia was defined as an abnormal hematological value of at least one of the 3 cell lines (white blood cells, platelets or hemoglobin) based on WHO guidelines where available or the reference ranges provided by the MU-JHU Core laboratory based at the study site. Cytopenias included anemia, leucopenia and thrombocytopenia. The prevalence of cytopenias was calculated as a proportion of patients with abnormal hematological values at baseline; the denominator being patients enrolled into the trial.

Anemia

Gender-specific definitions of anemia were used. Anemia was defined according to WHO guidelines as <12 g/dl for non-pregnant women and <13 g/dl for men. Anemia severity was graded as mild: 11-11.9 g/dl for women and 11-12.9 g/dl for men; moderate: 8-10.9 g/dl for both sexes; and < 8 g/dl as severe anemia for both sexes [73].

Other cytopenias

There are no generally accepted cut-offs for other cytopenias. A study in India used a cut off of total white blood cells < 4000 cells/ μ l to define leucopenia and platelet count <150 \times 10³ cells/ μ l to define thrombocytopenia in HIV-infected individuals [74]. A study conducted in European subjects evaluating the link between the burden of HIV-1 and hematological values in untreated subjects defined thrombocytopenia as <150 \times 10³ platelets/ μ l and leucopenia as <3000/ μ l [65]. Since most of the cut-off values from previous studies may not be comparable to our study population due to the different settings, we used the study site laboratory reference ranges (MUJHU Core laboratory) to define leucopenia and thrombocytopenia.

Other cytopenias were thus defined as follows:

Leucopenia if total white blood cell count $< 2.75 \times 10^9$ cells/litre, thrombocytopenia if platelet count $< 125 \times 10^9$ cells/litre for females and $< 156 \times 10^9$ cells/litre for males; bicytopenia if a subject had a combination of any 2 cytopenias and pancytopenia as having all three forms of cytopenia simultaneously.

Other selected hematological parameters

We defined the cut-off values for the following hematological parameters using the study site laboratory reference ranges as follows:

- a) Mean Corpuscular Volume (MCV) – 73 to 99 femtolitres(fl)
- b) Mean Corpuscular Hemoglobin (MCH) – 24 to 35picograms(pg)
- c) Mean Corpuscular Hemoglobin Concentration (MCHC) – 32 to 36 g/dl

3.3 Data management and statistical analyses

All case report forms (CRFs) were field edited by the Study Coordinator or designee and all inconsistencies resolved prior to faxing of the CRFs by a designated field staff. Data entry and management was done using Datafax which is a Food and Drug Administration (FDA) auditable, secure system where paper data forms are electronically captured and transmitted or faxed from remote sites to a National Institutes of Health (NIH) server. The Datafax management team reviewed all CRFs and queried any errors, invalid and/or missing values through regular quality assurance (QA) and quality control (QC) reports to the study team. The QC report, through the patient scheduling and tracking function of datafax, also identified overdue visits and missing CRF pages. The field staff corrected their respective data queries and all changes or corrections to the data fields were re-faxed and subsequently tracked by user, date and time.

Datafax can interface with a number of programming and statistical packages such as ORACLE, MYSQL, Stata and SAS. All data analyses for this study were done using Stata version 12.

3.3.1 Methods Objective 1:

We assessed the participants' baseline status using univariate analysis to describe the subjects' characteristics: continuous variables were summarized using means (if normally distributed) or medians (in case of non-parametric distributions) and categorical variables were summarized using frequencies and percentages. The prevalence of the various forms of cytopenia is reported as a percentage of subjects with cytopenia with the denominator being all subjects who were enrolled in the parent trial.

We used log-binomial regression models [75-77] to investigate the factors associated with cytopenias in general and with anemia, leucopenia and thrombocytopenia as individual outcome variables. Prevalence ratios (PR) and their 95% confidence intervals (95%CI) are reported. The independent factors investigated included demographic variables (e.g. sex, age, marital and employment status, highest level of education attained) and clinical/laboratory characteristics (e.g. CD4 cell count, body mass index, presence or history of oral candidiasis, presence or history of fever, etc.). Independent variables were considered to be significantly associated with the dependent variable if the respective p-value was below 0.05.

3.3.2 Methods Objective 2:

To examine the trend in hemoglobin status and other selected hematological parameters over the duration of follow-up, analyses of repeated measures taken over time were performed using a multi-level mixed effects linear model approach. We assessed the effect of time-dependent covariates on the individual slopes of hemoglobin, white blood cell count, platelet count and other selected hematological parameters over time. The multi-level mixed-effects

linear regression model with repeated measures is a generalization of the standard linear model that caters for correlation of data within an individual over time [78-81]. This approach was used to describe the change in hemoglobin and in each of the other selected hematological parameters over time as well as the factors associated with these changes.

Each selected hematological parameter was examined separately as the outcome variable of bivariate and multivariable models. Variables included in bivariate analyses were: visit number, sex, age, body mass index (BMI), CD4 cell count, HAART status at trial start (HAART naïve versus already on HAART), and duration on HAART at trial start. All variables available were examined in bivariate analyses and those variables with $p < 0.1$ or variables that were potentially important such as sex and age were initially included in the multivariable modeling but only retained in the final model if the multivariable $p < 0.05$. We also explored potential interactions between different covariates.

3.3.3 Methods Objective 3:

We examined the association between baseline anemia status and HIV disease progression on one hand and all-cause death on the other using log-binomial regression models. We used the baseline hemoglobin concentration as a measure of anemia status and investigated its association with disease progression and death from any cause in 3 separate models. Disease progression was defined as the first incident opportunistic infection (OI); in the first model we examined the risk of death and its association with baseline anemia status; in the second model we examined the risk of an incident OI and its association with baseline anemia and in the third model all-cause death and incident OIs were considered as a composite variable with a binary outcome and its association with baseline anemia was examined. The covariates included in all the 3 models were demographic characteristics (sex, age, marital and employment status, highest level of education attained) and clinical/laboratory characteristics

(cytopenia status that is presence or absence of any form of cytopenia, mean BMI, mean CD4 cell count, mean white blood cell count and mean platelet count). All variables were examined in bivariate analyses and those found to have a p-value < 0.1 or with biological plausibility (for example age and sex) were examined in the final model. Independent variables were considered to be significantly associated with disease progression or death if the respective p-value was below 0.05. Results from these models are presented as an estimate of the relative risk of death and/or incident OI, that is the risk ratio (RR) and its corresponding 95% confidence interval.

4 Results

4.1 Characteristics of study participants

Baseline hematological data were available for all 400 subjects enrolled in the parent trial of whom 277 (69.3%) were female. The mean age of the study population was 36 years (SD \pm 9.0) and the mean BMI was 23.8 (SD \pm 9.2) [Table 1].

Table 1: Baseline characteristics and values of the study population (N=400†)

Characteristic	Values	
Female, n (%)	277	(69.3)
Age, mean (\pm S.D) years	36	(\pm 9)
CD4 cell count, median (IQR) per μ l	142	(1-645)
On HAART, n (%)	200	(50)
Duration on HAART at enrollment, mean (\pm S.D) months	2.5	(\pm 1.6)
BMI†, mean (\pm S.D)	23.8	(\pm 9.2)
Married, n (%)	135	(33.8)
Employed, n (%)	345	(86.3)
Formal education (at least primary, n %)*	387	(97.0)
Hematological values, median (IQR)		
Hemoglobin, g/dl	12.3	(4.9-17.6)
White blood cell count (\times 10) ⁹ per litre	3.4	(1.2-9.9)
Platelets (\times 10) ⁹ per litre	244	(11-616)
Mean corpuscular volume, femtolitres (fl)	86	(58-126)
Red cell Distribution Width (RDW), %	13.6	(9.0-25.1)

†BMI data were missing for 3 participants due to missing weight at baseline

*One subject had education level as “Unknown”

SD = Standard Deviation
IQR = Inter Quartile Range

The median CD4 cell count was 142 cells/ μ l and 50% of the subjects had initiated antiretroviral therapy for not more than 6 months at the time of enrolment. The mean duration on HAART at the time of enrolment was 2.5 months (\pm 1.6 SD). The median hemoglobin concentration was 12.3 g/dl, the median white blood cell count was 3.4×10^9 cells per litre and the median platelet count was 244×10^9 cells per litre.

4.2 Prevalence of cytopenia at baseline

A total of 260 participants (65%) had at least one form of cytopenia. Anemia was the most common cytopenia occurring in 192 (47.8%) of the subjects followed by leucopenia occurring in 96 (24.3%), thrombocytopenia in 32 (8.3%), bicytopenia in 88 (21.9%) and only 2 subjects had a pancytopenia (Table 2).

Table 2: Distribution of cytopenias across the study population

Variables	n	Any Cytopenia†	Anemia†	Leucopenia†	Thrombocytopenia†
All participants	400	65.0	47.8	24.3	8.3
Demographic characteristics					
Gender					
Male	123	56.9	34.1	23.6	14.6
Female	277	68.6	53.8	24.5	5.4
Age					
18 to < 30	98	63.3	45.9	23.5	5.1
30 to < 35	80	70.0	50.0	26.3	11.3
35 to < 41	117	64.1	45.3	24.8	8.5
≥ 41	105	63.8	50.5	22.9	8.6
Education level					
None	12	66.7	33.3	41.7	8.3
Minimum Primary	206	65.5	48.5	21.8	8.7
Min. Secondary	141	66.7	50.4	25.5	8.5
Tertiary	40	57.5	40.0	27.5	5.0
Unknown‡	1	0.0	0.0	0.0	0.0
Employment status					
Unemployed	55	69.1	56.4	20.0	3.6
Employed	345	64.3	46.4	24.9	9.0
Marital status					
Not married	265	64.9	49.1	22.3	7.2
Married	135	65.2	45.2	28.1	10.4
Clinical characteristics					
CD4 count cells/μl					
<50	59	84.7	50.8	52.5	15.3
50 to <200	241	62.2	49.0	20.3	6.6
200 to < 350	88	59.1	43.2	15.9	9.1
≥ 350	12	66.7	41.7	25.0	0.0
BMI					
under weight	31	83.9	71.0	12.9	9.7
normal	261	66.7	50.2	24.1	8.8
over weight	105	54.3	34.3	25.7	6.7
Incomplete information*	3	100.0	66.7	100.0	0.0
HAART status at recruitment					
Not using HAART	200	68.0	47.0	26.0	12.0
AZT-based HAART	185	66.7	60.0	6.7	0.0
Non-AZT based HAART	15	61.6	47.6	23.8	4.9
Presence/history of oral candidiasis					
No	305	62.0	44.9	23.3	7.5
Yes	95	74.7	56.8	27.4	10.5
Presence/history of fever					
No	195	63.6	44.6	26.7	8.2
Yes	205	66.3	50.7	22.0	8.3

†The values presented other than “n” are percentages. †One participant had education level as “Unknown”.

*Three participants did not have baseline measurements of weight and so BMI could not be computed for these thus categorized as “Incomplete information”

Cytopenias were most prevalent in subjects aged between 30 and 35 years (70%) and in those with CD4 cell counts below 50 cells/μl (84.7%).

4.3 Factors associated with cytopenias at baseline

Factors found to be associated with any baseline cytopenia in multivariable regression were female sex; CD4 cell count and body mass index (Table 3).

Table 3: Factors associated with the presence of any Cytopenia at baseline

Results of uni- and multivariable log-binomial regression models

Covariate	n	% with cytopenia	Univariable (n=400)		Multivariable‡ (n=397*)	
			PR (95% CI)	p value	PR (95% CI)	p value
Sex						
Male	123	56.9	1		1	
Female	277	68.6	1.21 (1.01 to 1.43)	0.035	1.32 (1.11 to 1.57)	0.002
CD4 count cells/μl						
<50	59	84.7	1		1	
50 to < 200	241	62.2	0.73 (0.63 to 0.85)	<0.001	0.74 (0.63 to 0.86)	<0.001
200 to < 350	88	59.1	0.70 (0.57 to 0.86)	0.001	0.73 (0.59 to 0.90)	0.003
≥ 350	12	66.7	0.79 (0.52 to 1.19)	0.256	0.73 (0.48 to 1.11)	0.142
BMI						
under weight	31	83.9	1		1	
normal	261	66.7	0.79 (0.67 to 0.95)	0.011	0.82 (0.69 to 0.98)	0.028
over weight	105	54.3	0.65 (0.51 to 0.82)	<0.001	0.64 (0.51 to 0.81)	<0.001
Incomplete information*	3	100	-	-	-	-
Presence/history of oral candidiasis						
No	305	62.0	1		1	
Yes	95	74.7	1.21 (1.04 to 1.40)	0.012	1.08 (0.93 to 1.26)	0.300
HAART status at recruitment						
Not using HAART	200	68.0	1		1	
AZT-based HAART	185	61.6	0.91 (0.78 to 1.05)	0.193	0.98 (0.83 to 1.14)	0.774
Non-AZT based HAART	15	66.7	0.98 (0.68 to 1.42)	0.917	0.97 (0.67 to 1.40)	0.862
Age, years						
18 to < 30	98	63.3	1		1	
30 to < 35	103	70.0	1.11 (0.90 to 1.36)	0.341	1.15 (0.94 to 1.40)	0.184
35 to < 41	94	64.1	1.01 (0.83 to 1.24)	0.899	1.07 (0.89 to 1.30)	0.464
≥ 41	105	63.8	1.01 (0.82 to 1.24)	0.936	1.11 (0.90 to 1.37)	0.328

‡Multivariable model only includes the variables where data are shown because all others were lacking multivariable significance

*Three participants with missing BMI were not included into log-binomial regression model because the outcome did not vary in this stratum. Thus n=397 for the multivariable analysis.

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariable PR

The prevalence of any baseline cytopenia in females was 30% higher than in males (adjusted prevalence ratio (aPR) = 1.32; 95% confidence interval [CI]: 1.11-1.57). The aPR of having any form of cytopenia decreased with higher CD4 count; for CD4 count category 50 to < 200 cells/ μ l compared to CD4 count category < 50 cells/ μ l aPR=0.74 [95% CI: 0.63- 0.86, p< 0.001], and for CD4 count category 200 to <350 compared to CD4 count category < 50 cells/ μ l aPR=0.73 [95% CI: 0.59-0.90, p= 0.003]. Similarly the aPR of having any form of baseline cytopenia decreased with higher BMI; for normal compared to underweight BMI aPR=0.82 [95%CI: 0.69-0.98, p=0.045] and overweight compared to underweight BMI aPR=0.64 [95%CI: 0.51-0.81, p<0.001].

The factors found to have a multivariable association with baseline anemia were female sex and BMI (Table 4).

Table 4: Factors associated with the presence of anemia

Results of uni- and multivariable log-binomial regression models

Covariate	N=400		Univariable		Multivariable‡	
	n	% with anemia	PR (95% CI)	p value	PR (95% CI)	p value
Sex						
Male	123	34.1	1		1	
Female	277	53.8	1.58 (1.20 to 2.06)	0.001	1.76 (1.33 to 2.31)	<0.001
BMI						
under weight	31	71.0	1		1	
normal	261	50.2	0.71 (0.55 to 0.91)	0.033	0.75 (0.57 to 0.97)	0.031
over weight	105	34.3	0.48 (0.34 to 0.68)	0.001	0.46 (0.32 to 0.66)	<0.001
Incomplete information*	3	66.7	0.94 (0.41 to 2.16)	0.883	1.53 (0.64 to 3.65)	0.342
Presence/history of oral candidiasis						
No	305	44.6	1		1	
Yes	95	56.8	1.27 (1.02 to 1.57)	0.032	1.16 (0.94 to 1.44)	0.169
CD4 count cells/μl						
<50	59	50.8	1		1	
50 to <200	241	49.0	0.96 (0.73 to 1.28)	0.793	0.96 (0.73 to 1.27)	0.780
200 to <350	88	43.2	0.85 (0.60 to 1.20)	0.356	0.92 (0.66 to 1.29)	0.623
≥ 350	12	41.7	0.82 (0.40 to 1.67)	0.585	0.71 (0.37 to 1.37)	0.311
Age, years						
18 to < 30	98	45.9	1		1	
30 to < 35	80	50.0	1.09 (0.80 to 1.48)	0.587	1.19 (0.90 to 1.60)	0.225
35 to < 41	117	45.3	0.99 (0.74 to 1.32)	0.928	1.07 (0.81 to 1.41)	0.627
≥ 41	105	50.5	1.10 (0.83 to 1.46)	0.517	1.26 (0.96 to 1.67)	0.098
HAART status at recruitment						
Not using HAART	200	47.0	1		1	
AZT-based HAART	185	47.6	1.01 (0.82 to 1.25)	0.911	1.07 (0.87 to 1.31)	0.519
Non-AZT based HAART	15	60.0	1.28 (0.82 to 1.98)	0.275	1.09 (0.71 to 1.66)	0.705
Presence/history of fever						
No	195	44.6	1		1	
Yes	205	50.7	1.14 (0.92 to 1.89)	0.221	1.12 (0.91 to 1.37)	0.280

‡Multivariable model only includes the variables where data are shown because all others were lacking multivariable significance.

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariable PR

The adjusted prevalence of baseline anemia among females was 70% higher than that in males; aPR= 1.76 [95% CI: 1.33-2.31, p<0.001]. Anemia prevalence decreased with higher BMI; aPR of those in the normal BMI category compared to the underweight category was 0.75 [95% CI: 0.57-0.97, p=0.031] and for those in the overweight BMI category compared to the underweight category, aPR=0.46 [95% CI: 0.32-0.66, p< 0.001]. Similarly the prevalence of baseline anemia decreased with higher CD4 cell count category although these differences were not statistically significant: those in CD4 count category 50 to <200 cells/ μ l compared to CD4 count category < 50 cells/ μ l, unadjusted PR (uPR) =0.96, [95% CI: 0.73-1.28] and for those in CD4 count category 200 to <350, uPR= 0.85 [95% CI: 0.60-1.20]. The prevalence of baseline anemia in those with current or a previous history of oral candidiasis was 27% higher than in those with no prior history [uPR=1.27; 95% CI: 1.02-1.57, p=0.032]. Factors associated with the presence of baseline thrombocytopenia in multivariable regression were sex and whether a subject was on HAART at the start of the trial or not (Table 5).

Table 5: Factors associated with the presence of thrombocytopenia

Results of uni- and multivariable log-binomial regression

Covariate	N=400	Thrombo cytopenia (%)	Univariable		Multivariable	
			PR (95%CI)	P value	PR (95% CI)	P value
Sex	n					
Male	123	14.6	1		1	
Female	277	5.4	0.37 (0.19to 0.71)	0.003	0.38 (0.20 to 0.72)	0.003
HAART status at recruitment §						
Not on						
HAART	200	12.0	1		1	
On HAART	200	4.5	0.375 (0.18to 0.79)	0.009	0.38 (0.18 to 0.81)	0.012
CD4 count cells/µl						
<50	59	15.3	1		1	
50 to < 200	241	6.6	0.44(0.20 to 0.94)	0.033	0.60 (0.28to 1.29)	0.191
≥ 200 **	100	8.0	0.52(0.21 to 1.29)	0.158	0.89 (0.36 to 2.21)	0.807
BMI						
under weight	31	9.7	1		1	
normal	261	8.8	0.91(0.29 to 2.86)	0.873	0.98(0.33 to 2.94)	0.970
over weight	105	6.7	0.69(0.19 to 2.51)	0.572	0.99(0.28 to 3.49)	0.983
Incomplete information*	3	0.0	-	-	-	
Age, years						
18 to < 30	98	5.1	1		1	
30 to < 35	80	11.3	2.21(0.77 to 6.32)	0.141	1.75(0.61 to 5.01)	0.295
35 to < 41	117	8.5	1.68(0.59 to 4.73)	0.328	1.41(0.50 to 3.97)	0.512
≥ 41	105	8.6	1.68(0.58 to 4.84)	0.337	1.48(0.51 to 4.25)	0.470
Presence/history of oral candidiasis						
No	305	7.5	1		1	
Yes	95	10.5	1.40(0.69 to 2.83)	0.354	1.19(0.58 to 2.46)	0.630
Presence/history of fever						
No	195	8.2	1		1	
Yes	205	8.3	1.01(0.53 to 1.94)	0.975	1.03(0.54 to 1.93)	0.938

‡ Multivariable model only includes variables where data are shown because all others lacked significance

* Three participants with missing BMI could not be included into log-binomial regression since the outcome did not vary in this group

**Categories “200 to 350” and “≥350” were combined for this analysis because none of the participants with CD4 counts ≥350 had thrombocytopenia

§All subjects on HAART were categorized into one group for this analysis because none of the 15 subjects on AZT-based HAART had thrombocytopenia

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariable PR

Females had a 62% lower prevalence of baseline thrombocytopenia compared to males aPR=0.38; [95% CI: 0.20-0.72, p=0.003]. The prevalence of having thrombocytopenia among those on HAART compared to those who were not yet on HAART at the start of the trial was 62% lower aPR=0.38; [95% CI: 0.18- 0.81, p=0.012]. Patients in the CD4 count category 50 to <200 cells/ μ l had a 40% lower prevalence of baseline thrombocytopenia compared to those in CD4 count category <50 cells/ μ l (reference); aPR=0.60 [95% CI: 0.28 to 1.29, p=0.191] while those with CD4 count \geq 200 cells/ μ l had a 10% lower prevalence of baseline thrombocytopenia compared to the reference group aPR=0.89 [95%CI: 0.36 to 2.21, p=0.807].

The only factor associated with the presence of baseline leucopenia in multivariable regression was the CD4 count category (Table 6).

Table 6: Factors associated with presence of baseline leucopenia

Results of uni- and multivariable log-binomial regression models						
Covariate	n	% leucopenic	Univariable (n=400)		Multivariable‡ (n=397*)	
			PR (95% CI)	p value	PR (95% CI)	p value
CD4 count cells/µl						
<50	59	52.5	1		1	
50 to <200	241	20.3	0.39 (0.27 to 0.55)	<0.001	0.41 (0.29 to 0.59)	<0.001
200 to <350	88	15.9	0.30 (0.18 to 0.52)	<0.001	0.32 (0.19 to 0.55)	<0.001
≥ 350	12	25.0	0.48 (0.17 to 1.31)	0.149	0.64 (0.24 to 1.72)	0.377
HAART status at recruitment						
Not using HAART	200	26.0	1		1	
AZT-based HAART	185	23.8	0.26 (0.04 to 1.73)	0.162	0.32 (0.05 to 2.22)	0.25
Non-AZT based HAART	15	6.7	0.91 (0.65 to 1.30)	0.616	0.97 (0.68 to 1.39)	0.889
BMI*						
under weight	31	12.9	1		1	
normal	261	24.1	1.87 (0.73 to 4.79)	0.191	2.00 (0.80 to 5.01)	0.137
over weight	105	25.7	1.99 (0.75 to 5.26)	0.164	2.06 (0.80 to 5.30)	0.131
Incomplete information*	3	100	-	-	-	-
Sex						
Male	123	23.6	1		1	
Female	277	24.6	1.04 (0.71 to 1.52)	0.835	1.15 (0.80 to 1.64)	0.448
Age, years						
18 to < 30	98	23.5	1		1	
30 to < 35	80	26.3	1.12 (0.67 to 1.87)	0.669	1.01 (0.63 to 1.63)	0.971
35 to < 41	117	24.8	1.06 (0.66 to 1.70)	0.822	1.20 (0.77 to 1.88)	0.419
≥ 41	105	22.9	0.97 (0.59 to 1.61)	0.918	1.04 (0.65 to 1.67)	0.856
Presence/history of oral candidiasis						
No	305	23.3	1		1	
Yes	95	27.4	1.18 (0.80 to 1.73)	0.411	0.90 (0.62 to 1.31)	0.587
Presence/history of fever						
No	195	26.7	1		1	
Yes	205	22.0	0.82 (0.58 to 1.17)	0.272	0.77 (0.55 to 1.06)	0.106

* Three participants with missing BMI were not included into log-binomial regression model because the outcome did not vary in this stratum. Thus n=397 for the multivariable analysis.

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariable PR

The prevalence of baseline leucopenia in those with CD4 count of 50 to <200 cells/ μ l was 60 % lower compared to those with CD4 count <50 cells/ μ l; aPR=0.39 [95%CI: 0.27-0.55, p<0.001]. The prevalence of baseline leucopenia in those with CD4 count of 200 to <350 cells/ μ l was 70% lower than those with CD4 count <50 cells/ μ l; aPR= 0.307 [95%CI: 0.18-0.52, p <0.001]. Interestingly, the prevalence of baseline leucopenia in those with CD4 counts \geq 350 cells/ μ l was only 50% lower than in the reference group although this difference was not statistically significant.

4.4 Trends in hematological parameters following initiation of HAART

Of the 400 subjects enrolled in the trial, a total of 1517 laboratory observations were available for the analyses of the trends in hematological parameters. The reasons for the missing laboratory observations (n=83) included specimen not being collected at the consequent visit, death occurring before completion of all study procedures, clotted specimen, withdrawal from the trial and loss to follow-up.

The general trend in hemoglobin, selected Red Blood Cell (RBC) indices and other cell lines

We assessed trends over time in hemoglobin concentration and in the following RBC indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) from baseline through the follow-up time points.

The mean hemoglobin concentration of all 400 subjects at enrolment was 12.3 g/dl and increased over time reaching its maximum of 13.3 g/dl after 18 months of follow-up (Table 7). Of the 191 subjects who were anemic at baseline, 57.6% had mild anemia, 38.2% had moderate anemia and 4.2% had severe anemia. Furthermore, the majority of subjects (82%) had normocytic red blood cells (RBCs), 14% had microcytic RBCs and 4% had macrocytic RBCs.

Table 7: Hemoglobin Status and Other RBC Indices of HIV-Infected Adults while on HAART

Parameter	Time point in months (N)			
	0 (400)	6 (380)	12 (371)	18 (366)
Mean hemoglobin (g/dl)				
All subjects	12.3	12.9	13.2	13.3
Females	11.7	12.3	12.5	12.6
Males	13.6	14.4	14.8	14.8
Severity of anemia, n (%)				
All anemic	191 (47.8)	110 (28.9)	90 (24.3)	82 (22.3)
Mild	110 (27.5)	73 (19.2)	63 (17.0)	60 (16.4)
Moderate	73 (18.3)	32 (8.4)	26 (7.0)	19 (5.2)
Severe	8 (2.0)	5 (1.3)	1(0.3)	3 (0.8)
RBC Size, n (%)				
Normocytic	326 (82)	126(33)	121 (33)	119 (33)
Microcytic	57 (14)	246 (65)	242 (65)	239 (65)
Macrocytic	17 (4)	8 (2)	8 (2)	8 (2)
Other RBC Indices				
Mean MCV (fl)	87.5	101.7	102.8	102.9
Mean MCH (pg)	29.5	34.3	34.6	34.6
Mean MCHC (%)	33.6	33.7	33.6	33.5

The baseline mean hemoglobin concentration was higher for males (13.6 g/dl) than for females (11.7 g/dl) and this difference by sex remained over the duration of follow-up. The mean MCV at baseline was within the normal range (87.5 fl) but gradually increased over time to macrocytosis (mean MCV 102.9 fl) at 18 months. The mean MCH and the mean MCHC were within normal ranges from baseline throughout the follow-up duration.

Figures 1-3 show the trends in (1) mean hemoglobin concentration (2), mean WBC count and (3) mean platelet count over time in HIV-infected adults while on HAART.

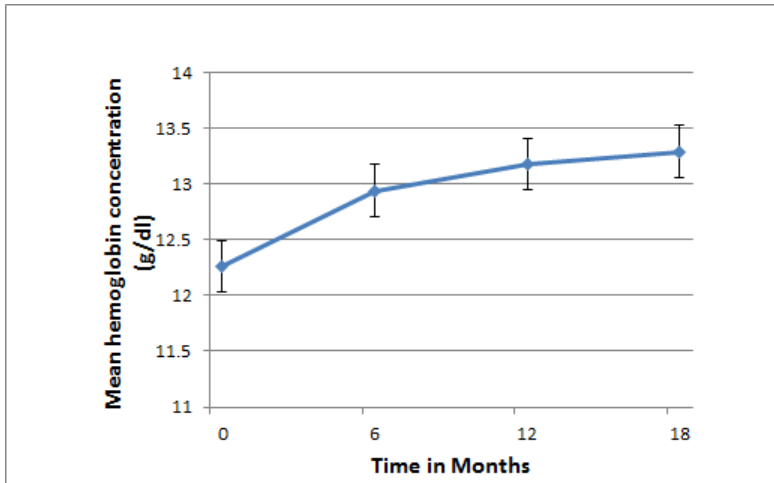


Figure 1: Trend in mean hemoglobin concentration over time. Data are means; bars are standard deviations

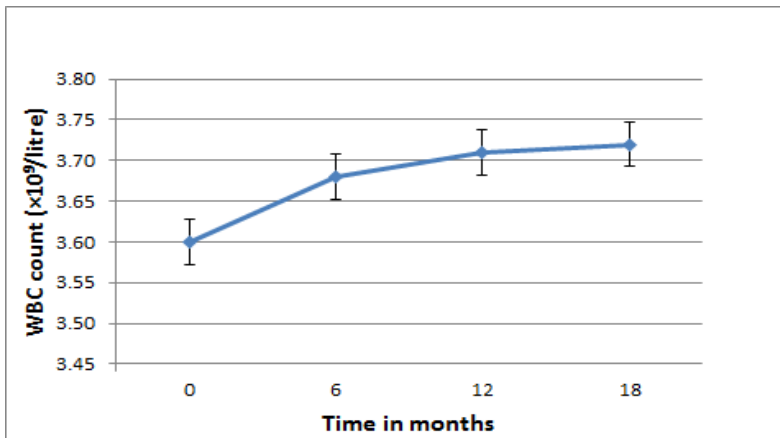


Figure 2: Trend in mean white blood cell count over time. Data are means; bars are standard deviations

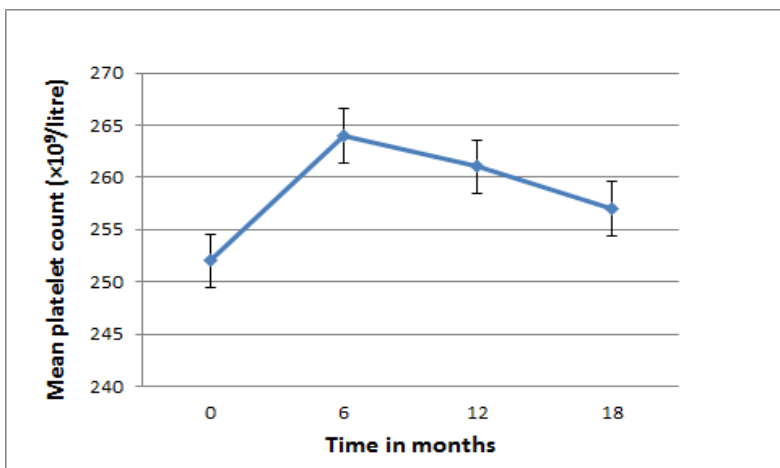


Figure 3: Trend in mean platelet count over time. Data are means; bars are standard deviations

Change in hemoglobin level over time

There was a statistically significant increase in hemoglobin level with each follow-up visit and hemoglobin levels were significantly higher at each visit compared to the baseline visit (Table 8).

Table 8: Change in hemoglobin in HIV-infected HAART-treated adults over 18 months

Results of bivariate and multivariable multilevel mixed effects linear regression models with Patient ID as random effect						
Covariate	n	Mean Hb (g/dl)	Bivariate coefficient	95% confidence interval	Multivariable coefficient	95% confidence interval
Visit number (Months)						
0	400	12.3	0	-	0	-
6	380	12.9	0.64	0.51 to 0.78 ^a	0.59	0.34 to 0.83 ^a
12	371	13.2	0.86	0.72 to 1.00 ^a	0.93	0.68 to 1.18 ^a
18	366	13.3	0.95	0.81 to 1.09 ^a	0.90	0.65 to 1.15 ^a
Sex						
Male	123	13.6	0	-	0	-
Female	277	11.7	-2.08	-2.36 to -1.80 ^a	-2.03	-2.34 to -1.71 ^a
BMI (kg/m ²)						
Underweight	22	11.3	0	-	0	-
Normal	264	12.2	1.19	0.77 to 1.62 ^a	0.86	0.48 to 1.25 ^a
Overweight	114	12.5	1.81	1.35 to 2.26 ^a	1.39	0.97 to 1.80 ^a
CD4 count (cells/ μ l)						
<50	59	12.1	0	-	0	-
50 to < 200	241	12.2	0.94	0.66 to 1.22 ^a	0.51	0.24 to 0.79
200 to < 350	88	12.6	1.43	1.14 to 1.73 ^a	0.67	0.37 to 0.97 ^a
\geq 350	12	11.9	1.74	1.41 to 2.06 ^a	0.81	0.47 to 1.15 ^a
Age (per year)	400	12.9	0.02	0.002 to 0.04 ^b	-0.001	-0.02 to 0.01 ^c
ART status at trial start						
ART naïve	200	12.3	0	-	0	-
On ART	200	12.2	0.12	-0.20 to 0.45 ^c	-0.11	-0.36 to 0.14 ^c
Duration on ART at trial start (Months)						
< 1 month	201	12.8	0	-	0	-
1-2 months	117	12.9	0.05	-0.32 to 0.43 ^c	-0.18	-0.46 to 0.11 ^c
\geq 3 months	82	13.1	0.26	-0.16 to 0.68 ^c	0.02	-0.30 to 0.35 ^c

a=p<0.001 b=0.001≤p≤0.05 c=p>0.05

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.05; all other independent variables (below the line) were added one at a time into the final model to obtain their respective multivariate coefficient

Hemoglobin levels significantly increased with higher body mass index (BMI), CD4 cell count category and age. However, hemoglobin levels for all visits compared to the baseline visit were significantly lower in females than in males by 2.03 g/dl [95% CI: -2.34 to -1.71] after controlling for visit number, BMI, CD4 cell count category and age. In the interaction analyses (data not shown in table) hemoglobin levels were lower by 0.45g/dl [95% CI: -0.74 to -0.16] and by 0.33 g/dl [95%CI:-0.62 to -0.04] in females than in males at 12 months and at 18 months respectively compared to the baseline visit.

Trend in the prevalence of anemia

The prevalence of anemia decreased from 47.8% at baseline to 28.9%, 24.3% and 22.3% at months 6, 12 and 18 respectively. Figure 2 shows the trend in the prevalence of anemia and its severity over time.

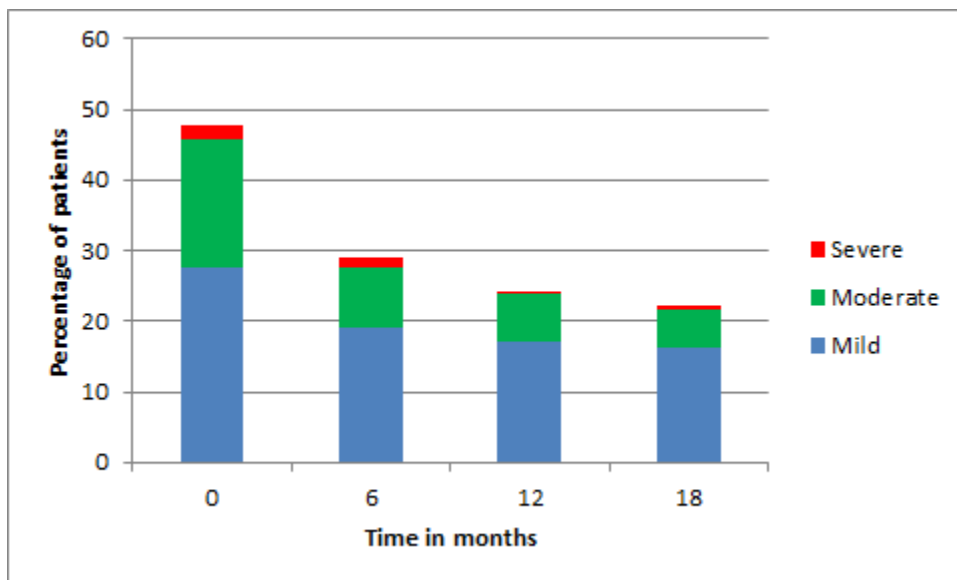


Figure 4: The trend in prevalence and severity of anemia over time

The prevalence of each grade of anemia that is mild, moderate and severe anemia reduced significantly over time (Table 7). The greatest reduction in anemia prevalence from baseline to 18 months was in subjects with moderate and severe grades of anemia by 72% and 59% respectively.

Change in Mean Cell Volume (MCV) over time

There was an increase in MCV with each follow-up visit compared to the baseline when adjusted for CD4 count, age and duration on ART at trial start (Table 9). However increments after the first follow-up visit at 6 months were small.

Table 9: Change in Mean Cell Volume (MCV) of HIV-infected HAART-treated adults

Results of bivariate and multivariable multilevel mixed effects linear regression models with Patient ID as random effect						
Covariate	N	Mean MCV (fl)	Bivariate Coefficient	95% confidence interval	Multivariable Coefficient	95% confidence interval
Visit number (Months)						
0	400	87.5	0	-	0	-
6	380	101.7	14.1	13.2 to 14.9 ^a	13.2	12.3 to 14.1 ^a
12	371	102.8	15.0	14.1 to 15.8 ^a	13.9	13.0 to 14.8 ^a
18	366	102.9	15.1	14.2 to 15.9 ^a	13.9	12.9 to 14.8 ^a
CD4 count (cells/μl)						
<50	59	84.4	0	-	0	-
50 to < 200	241	87.2	10.6	8.3 to 12.8 ^a	3.3	1.6 to 5.0 ^a
200 to < 350	88	90.0	18.1	15.7 to 20.5 ^a	4.6	2.7 to 6.6 ^a
≥ 350	12	89.7	22.1	19.5 to 24.7 ^a	5.3	3.1 to 7.5 ^a
Age (per year)	400	98.5	0.17	0.05 to 0.3 ^b	0.2	0.02 to 0.3 ^b
Duration on ART at trial start (months)						
<1 month	201	97.6	0	-	0	-
1-2 months	117	97.7	0.5	-2.1 to 3.1 ^c	-0.2	-2.8 to 2.3 ^c
≥3 months	82	101.8	4.4	1.5 to 7.4 ^b	3.8	0.9 to 6.7 ^b
BMI (kg/m²)						
Underweight	22	85.6	0	-	0	-
Normal	264	87.3	5.0	1.2 to 8.7 ^b	-0.2	-2.7 to 2.3 ^c
Overweight	114	88.2	9.7	5.8 to 13.7 ^a	-0.6	-3.3 to 2.1 ^c
ART Status at trial start						
ART naïve	200	83.2	0	-	0	-
On ART	200	91.7	2.1	-0.2 to 4.4 ^c	3.2	-18.7 to 25.1 ^c
Sex						
Male	123	88.8	0	-	0	-
Female	277	86.9	-0.6	-3.1 to 1.8 ^c	-0.4	-2.8 to 2.0 ^c

a=p<0.001

b= 0.001≤p≤0.05

c=p≥0.05

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.05; all other independent variables (below the line) were added one at a time into the final model to obtain their respective multivariate coefficient

Furthermore, in both models MCV increased with higher CD4 cell count category. This difference was statistically significant at 6 months after enrolment but remained significant at consequent visits only for those in the CD4 cell count category ≥350 cells/μl in the interaction analysis (data not shown).

For every one year increase in age, the MCV went up by 0.15 fl when adjusted for the visit number, CD4 cell count and duration on ART at trial start [95% CI: 0.02 to 0.3; p=0.019].

Change in mean corpuscular hemoglobin over time

The mean corpuscular hemoglobin (MCH) was significantly higher at each follow-up visit when compared to baseline, both in the bivariate and in the multivariable models (Table 10).

However, increments after the first follow-up visit at 6 months after enrollment were small.

Table 10: Change in Mean Corpuscular Hemoglobin (MCH) of HIV-infected HAART-treated adults

Results of bivariate and multivariable multilevel mixed effects linear regression models with Patient ID as random effect						
Covariate	N	Mean MCH	Bivariate Coefficient	95% confidence interval	Multivariable Coefficient	95% confidence interval
Visit number (Months)						
0	400	29.5	0	-	0	-
6	380	34.3	4.8	4.5 to 5.1 ^a	4.5	4.1 to 4.8 ^a
12	371	34.6	5.0	4.7 to 5.3 ^a	4.6	4.3 to 5.0 ^a
18	366	34.6	5.0	4.7 to 5.3 ^a	4.6	4.2 to 5.0 ^a
CD4 count (cells/μl)						
<50	59	28.6	0	-	0	-
50 to < 200	241	29.4	3.7	2.9 to 4.5 ^a	1.3	0.6 to 1.9 ^a
200 to < 350	88	30.3	6.2	5.4 to 7.1 ^a	1.7	1.0 to 2.4 ^a
≥ 350	12	30.4	7.6	6.6 to 8.5 ^a	1.9	1.1 to 2.7 ^a
Age (per year)	400	33.2	0.06	0.01 to 0.1 ^b	0.05	0.004 to 0.1 ^b
Duration on ART at trial start (Months)						
<1 month	201	32.9	0	-	0	-
1-2 months	117	32.9	0.1	-0.9 to 1.1 ^c	-0.1	-1.1 to 0.8 ^c
≥3 months	82	34.4	1.6	0.4 to 2.7 ^b	1.3	0.2 to 2.4 ^b
BMI (kg/m²)						
Underweight	22	28.8	0	-	0	-
Normal	264	29.5	1.8	0.4 to 3.1 ^b	-0.02	-1.0 to 0.9 ^c
Overweight	114	29.7	3.5	2.1 to 4.9 ^a	-0.003	-1.0 to 1.0 ^c
ART Status at trial start						
ART naïve	200	28.1	0	-	0	-
On ART	200	30.9	0.7	-0.1 to 1.5 ^c	0.5	-7.7 to 8.8 ^c
Sex						
Male	123	30.1	0	-	0	-
Female	277	29.3	-0.3	-1.3 to 0.6 ^c	-0.3	-1.2 to 0.6 ^c

a=p<0.001 b= 0.001≤p≤0.05 c=p>0.05

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.05; all other independent variables (below the line) were added one at a time into the final model to obtain their respective multivariate coefficient

The MCH increased with higher CD4 cell count category and this difference remained significant after adjusting for visit number, age and duration on ART at enrollment. In the

interaction analyses,(data not shown) the increase in MCH with higher CD4 count category remained statistically significant up to 18 months for those in the highest CD4 count category that is CD4 count ≥ 350 cells/ μ l.

Change in mean corpuscular hemoglobin concentration (MCHC) over time

Overall the mean corpuscular hemoglobin concentration (MCHC) did not change much over the whole duration of follow-up and none of the follow-up visits was significantly different from baseline. In the bivariate analyses, none of the examined variables showed a significant influence on MCHC thus we did not do multivariable modeling for this parameter (Table 11).

Table 11: Change in Mean Corpuscular Hemoglobin Concentration (MCHC) in HIV-infected Adults

Results of bivariate multi-level mixed effects linear regression models				
Covariate	N	Mean MCHC	Bivariate Coefficient	95% confidence interval
Visit number (Months)				
0	400	33.6	0	
6	380	33.7	0.1	-0.1 to 0.3 ^c
12	371	33.6	-0.04	-0.2 to 0.2 ^c
18	366	33.5	-0.1	-0.3 to 0.1 ^c
CD4 count (cells/μl)				
<50	59	33.8	0	
50 to < 200	241	33.6	0.1	-0.3 to 0.4 ^c
200 to < 350	88	33.6	-0.03	-0.4 to 0.3 ^c
≥ 350	12	33.8	-0.1	-0.5 to 0.2 ^c
Age (per year)	400	33.6	-0.002	-0.01 to 0.01 ^c
ART Status at trial start				
ART naïve	200	33.6	0	
On ART	200	33.6	-0.02	-0.2 to 0.2 ^c
Sex				
Male	123	33.7	0	
Female	277	33.6	-0.1	-0.3 to 0.1 ^c
Duration on ART at trial start (Months)				
<1 month	201	33.6	0	
1-2 months	117	33.5	-0.1	-0.3 to 0.2 ^c
≥3 months	82	33.7	0.1	-0.2 to 0.3 ^c
BMI (kg/m ²)				
Underweight	22	33.6	0	
Normal	264	33.6	0.1	-0.4 to 0.5 ^c
Overweight	114	33.6	0.01	-0.5 to 0.5 ^c

a=p<0.001 b= 0.001≤p≤0.05 c=p>0.05

Change in White Blood Cell (WBC) count over time

White blood cell (WBC) counts increased with consecutive follow-up visits compared to the baseline in the unadjusted model. However, after adjusting for sex and CD4 count category, the WBC counts decreased over time compared to the baseline and this difference was statistically significant (Table 12).

Table 12: Change in white blood cell counts of HIV-infected HAART-treated adults over 18 months

Results of bivariate and multivariable multilevel mixed effects linear regression models with Patient ID as random effect						
Covariate	N	Mean White Blood Cell count (*10³/μl)	Bivariate Coefficient	95% confidence interval	Multivariable Coefficient	95% confidence interval
Visit number (Months)						
0	400	3.60	0	-	0	-
6	380	3.68	0.08	-0.05 to 0.20 ^c	-0.16	-0.29 to -0.03 ^b
12	371	3.71	0.13	0.004 to 0.25 ^b	-0.16	-0.29 to -0.03 ^b
18	366	3.72	0.14	0.02 to 0.26 ^b	-0.21	-0.34 to -0.01 ^b
Sex						
Male	123	3.89	0	-	0	-
Female	277	3.46	-0.24	-0.45 to -0.03 ^b	-0.31	-0.51 to -0.11 ^b
CD4 count (cells/μl)						
<50	59	2.98	0	-	0	-
50 to < 200	241	3.59	0.69	0.46 to 0.92 ^a	0.77	0.54 to 1.01 ^a
200 to < 350	88	3.99	0.87	0.63 to 1.10 ^a	1.03	0.78 to 1.29 ^a
≥ 350	12	3.77	1.29	1.03 to 1.55 ^a	1.52	1.22 to 1.81 ^a
ART Status at trial start						
ART naïve	200	3.45	0	-	0	-
On ART	200	3.74	0.17	-0.02 to 0.37 ^c	0.12	-0.07 to 0.31 ^c
Age (per year)	400	3.68	-0.002	-0.01 to 0.01 ^c	-0.01	-0.02 to 0.005 ^c
BMI (kg /m²)						
Underweight	22	3.74	0	-	0	-
Normal	264	3.58	-0.24	-0.58 to 0.11 ^c	-0.31	-0.64 to 0.02 ^c
Overweight	114	3.61	0.004	-0.35 to 0.36 ^c	-0.14	-0.49 to 0.21 ^c
Duration on ART at trial start (Months)						
<1 month	201	3.6	0	-	0	-
1-2 months	117	3.7	0.1	-0.1 to 0.3 ^c	0.07	-0.14 to 0.29 ^c
≥3 months	82	3.8	0.3	0.002 to 0.5 ^b	0.18	-0.06 to 0.43 ^c

a=p<0.001 b= 0.001≤p≤0.05 c=p>0.05

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.05; all other independent variables (below the line) were added one at a time into the final model to obtain their respective multivariate coefficient

WBC counts were lower in females compared to males by 0.31×10^3 cells/ μ l [95% CI: -0.51 to -0.11, $p=0.003$] when controlling for visit number and CD4 cell count category. However, in the interaction analysis WBC counts were higher in females than males by 0.49×10^3 cell/ μ l [95% CI: 0.23 to 0.75] 18 months after enrolment (data not shown).

Change in platelet count over time

Overall the platelet counts increased from baseline throughout the duration of follow-up after adjusting for sex, CD4 cell count category and BMI. The only decrease observed was at 18 months in the adjusted model however, the differences observed in this model were not significant (Table 13).

Table 13: Change in platelet counts of HIV-infected HAART-treated adults over 18 months

Results of bivariate and multivariable multilevel mixed effects linear regression models with Patient ID as random effect						
Covariate	N	Mean Platelet Count (*10³/μl)	Bivariate Coefficient	95% confidence interval	Multivariable Coefficient	95% confidence interval
Visit number (Months)						
0	400	252	0	-	0	-
6	380	264	11.9	4.5 to 19.3 ^b	6.1	-1.8 to 14.1 ^c
12	371	261	10.0	2.6 to 17.5 ^b	3.3	-5.1 to 11.6 ^c
18	366	257	6.7	-0.8 to 14.2 ^c	-1.1	-9.8 to 7.7 ^c
Sex						
Male	123	236	0	-	0	-
Female	277	259	37.2	22.9 to 51.6 ^a	36.3	21.7 to 50.9 ^a
CD4 cell count (cells/μl)						
< 50	59	215	0	-	0	-
50 to < 200	241	254	29.6	15.1 to 44.2 ^a	28.8	13.9 to 43.7 ^a
200 to < 350	88	264	37.0	21.9 to 52.1 ^a	36.4	20.0 to 52.9 ^a
≥ 350	12	296	44.1	27.4 to 60.9 ^a	43.0	24.2 to 61.9 ^a
BMI (kg/m²)						
Underweight	22	244	0	-	0	-
Normal	264	250	-25.0	-46.5 to 3.5 ^b	-29.0	-50.2 to -7.8 ^b
Overweight	114	257	-17.5	-40.2 to 5.2 ^c	-30.9	-53.6 to -8.1 ^b
ART status at enrolment						
ART naïve	200	225	0	-	0	-
On ART	200	279	18.7	5.1 to 32.2 ^b	20.2	7.1 to 33.4 ^b
Age (per year)	400	259	-0.4	-1.2 to 0.3 ^c	-0.2	-1.0 to 0.5 ^c
ART duration at trial start (Months)						
<1 month	201	250	0	-	0	-
1-2 months	117	272	21.6	5.9 to 37.4 ^b	23.8	8.6 to 39.0 ^b
≥3 months	82	259	12.0	-5.7 to 29.8 ^c	12.8	-4.4 to 30.0 ^c

a=p<0.001 b= 0.001≤p≤0.05 c=p>0.05

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.05; all other independent variables (below the line) were added one at a time into the final model to obtain their respective multivariate coefficient

Platelet counts for all visits were higher in females compared to males by 36.3×10^3 cells/μl [95% CI: 21.7 to 50.9, p<0.001] after controlling for CD4 count, visit number and BMI.

Similarly platelet counts increased with higher CD4 cell count category and in those who had

already been on HAART compared to those who were HAART-naïve at the time of enrolment [Coeff: 20.2; 95% CI: 7.1 to 33.4, p =0.003].

4.5 Association of baseline anemia and other factors with HIV disease progression and/or death in adults initiating HAART

HIV disease progression was defined by the first incident OI in a subject. A total of 17 deaths and 17 incident OIs occurred during the follow-up period. The causes of death were adjudicated based on available clinical, laboratory and vital status information. Tuberculosis (both suspected and confirmed) was the most common primary cause of death (n=5) followed by malignancy (n=2) including Kaposi's sarcoma and cervical cancer and cryptococcal meningitis (n=2). Other causes of death included pregnancy-related complications (n=1), toxoplasmosis (n=1) and deep vein thrombosis (n=1). The cause of death was unknown in 5 patients.

Association of baseline anemia and other factors with all-cause death

The multivariable factors found to be associated with death from any cause were the presence of baseline anemia and the mean CD4 cell count over the duration of follow-up (Table 14).

Table 14: Association of baseline anemia status and other factors with all-cause death in HIV-Infected Adults Initiating HAART

Covariate	n	% died	Bivariate Risk Ratio (95% CI)	Multivariate Risk Ratio (95% CI)
Baseline Anemia				
Absent	209	1.43	1	1
Present	191	7.85	5.47(1.61 to 18.6) ^b	5.38 (1.65 to 17.5) ^b
Mean CD4 count (cells/μl)			0.99 (0.98 to 0.99) ^a	0.99 (0.98 to 0.99) ^a
Age (per year)			1.01 (0.95 to 1.06) ^d	1.00 (0.96 to 1.06) ^d
Sex				
Male	123	3.3	1	1
Female	277	5.1	1.55(0.52 to 4.63) ^d	1.51 (0.54 to 4.21) ^d
Cytopenia status				
Absent	140	1.4	1	1
Present	260	6.2	4.31 (1.00 to 18.50) ^b	0.65 (0.06 to 7.45) ^d
Marital Status				
Not Married	265	4.2	1	1
Married	135	5.2	1.25 (0.49 to 3.15) ^d	1.35 (0.60 to 3.02) ^d
Employment Status				
Not Employed	55	1.8	1	1
Employed	345	4.9	2.71 (0.37 to 20.0) ^d	2.04 (0.32 to 13.2) ^d
Educational Level				
None	13	7.7	1	1
Primary	206	5.3	0.69 (1.00 to 4.98) ^d	0.27 (0.04 to 1.94) ^d
Secondary	141	2.8	0.37 (0.04 to 3.07) ^d	0.14 (0.02 to 1.06) ^c
Tertiary	40	5.0	0.65 (0.06 to 6.62) ^d	0.31 (0.03 to 2.88) ^d
Mean BMI			0.97 (0.84 to 1.13) ^d	1.01 (0.88 to 1.16) ^d
Mean WBC			1.05 (0.57 to 1.96) ^d	1.25 (0.81 to 1.93) ^d
Mean PLT			1.00 (0.99 to 1.01) ^d	1.00 (0.99 to 1.01) ^d

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariate RR

The mean values shown in the table are the mean of each specified independent variable per participant over the follow-up duration of 18 months.

a= $p \leq 0.001$ b= $0.001 < p \leq 0.05$ c= $0.05 < p \leq 0.1$ d= $p > 0.1$

There was a five-fold increased risk of death among those with anemia at baseline compared to those without baseline anemia after controlling for the mean CD4 cell count over the follow-up duration [adjusted RR (aRR) = 5.38; 95% CI: 1.65 to 17.5, $p=0.005$]. An increase in the mean CD4 cell count was associated with a decrease in the risk of death after adjusting

for baseline anemia status [aRR= 0.99; 95% CI: 0.98 to 0.99, p< 0.001]. Increase in age per year was not associated with risk of death after controlling for baseline anemia status and the mean CD4 cell count over the duration of follow-up [aRR 1.00 95% CI: 0.96 to 1.06]. Females had a 50% increased risk of death compared to males although this association was not significant [aRR= 1.51; 95% CI: 0.54 to 4.21]. Having any form of cytopenia compared to not having any form of cytopenia at baseline was associated with a four-fold increased risk of death [crude RR= 4.31; 95% CI: 1.01 to 18.50, p= 0.05]. However, after adjusting for baseline anemia status and the mean CD4 cell count, there was no association between the presence of any cytopenia and the risk of death.

Association of baseline anemia and other factors with incident OIs

The presence of baseline anemia was associated with a 16% increased risk of incident OIs however this association was not statistically significant after adjusting for the mean CD4 cell count, age and the mean body mass index over the duration of follow-up [aRR= 1.16; 95% CI: 0.47 to 2.84, p=0.750] (Table 15).

Table 15: Association of baseline anemia status and other factors with incident OIs in HIV-Infected Adults Initiating HAART

Covariate	n	% with OI	Bivariate Risk Ratio (95% CI)	Multivariate Risk Ratio (95% CI)
Baseline Anemia				
Absent	209	3.8	1	1
Present	191	4.7	1.23 (0.48 to 3.13) ^d	1.16 (0.47 to 2.84) ^d
Mean CD4 count (cells/ μ l)			0.99 (0.99 to 1.00) ^b	0.99 (0.99 to 1.00) ^b
Age (per year)			0.95 (0.91 to 1.00) ^c	0.97 (0.92 to 1.01) ^d
Mean BMI			0.87 (0.74 to 1.01) ^c	0.89 (0.75 to 1.05) ^d
Mean WBC			1.36 (0.90 to 2.06) ^d	1.42 (0.97 to 2.07) ^c
Sex				
Male	123	6.5	1	1
Female	277	3.3	0.50 (0.20 to 1.27) ^d	0.62 (0.22 to 1.72) ^d
Mean PLT			1.00 (1.00 to 1.01) ^d	1.00 (1.00 to 1.01) ^d
Cytopenia status				
Absent	140	4.3	1	1
Present	260	4.2	0.99 (0.37 to 2.62) ^d	0.52 (0.12 to 2.33) ^d
Marital Status				
Not Married	265	4.5	1	1
Married	135	3.7	0.82 (0.29 to 2.28) ^d	1.01 (0.35 to 3.00) ^d
Employment Status				
Not Employed	55	3.6	1	1
Employed	345	4.4	1.20 (0.28 to 5.09) ^d	1.34 (0.29 to 6.09) ^d
Educational Level				
Primary	219	3.7	1	1
Secondary	141	5.0	1.36 (0.50 to 3.67) ^d	1.41 (0.52 to 3.78) ^d
Tertiary	40	5.0	1.37 (0.30 to 6.22) ^d	1.33 (0.29 to 6.03) ^d

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariate RR

The mean values shown in the table are the mean of each specified independent variable per participant over the follow-up duration of 18 months.

a= $p \leq 0.001$ b= $0.001 < p \leq 0.05$ c= $0.05 < p \leq 0.1$ d= $p > 0.1$

The only multivariate factor found to be significantly associated with risk of developing an incident OI was the mean CD4 cell count. For every unit increase in the mean CD4 cell count, the risk of an incident OI decreased by 1% after adjusting for baseline anemia status, age and the mean BMI over the duration of follow-up [aRR= 0.99; 95% CI: 0.99 to 1.00,

p=0.008]. There was no association between the risk of an incident OI and the mean BMI over the duration of follow-up [aRR= 0.89; 95% CI: 0.75 to 1.05] or age [aRR= 0.97; 95% CI: 0.92 to 1.01].

Association of baseline anemia and other factors with all-cause death and incident OIs

When we considered death and incident OIs as a composite outcome, the presence of baseline anemia was associated with a 69% increase in risk of death or an incident OI [aRR= 1.69; 95% CI: 0.85 to 3.37] but this association was not statistically significant (Table 16).

Table 16: Association of baseline anemia and other factors with all-cause death and/or Incident OIs in HIV-infected Adults initiating HAART

Covariate	n	% died or had OI	Bivariate	Multivariate
			Risk Ratio (95% CI)	Risk Ratio (95% CI)
Baseline Anemia				
Absent	209	5.3	1	-
Present	191	8.9	1.69 (0.81 to 3.52) ^d	1.69 (0.85 to 3.37) ^d
Mean CD4 count (cells/ μ l)			0.99 (0.98 to 0.99) ^a	0.99 (0.98 to 0.99) ^a
Age (per year)			0.98 (0.94 to 1.02) ^d	0.97 (0.95 to 1.02) ^d
Sex				
Male	123	7.3	1	-
Female	277	6.9	0.94 (0.44 to 2.01) ^d	1.10 (0.52 to 2.33) ^d
Mean BMI			0.97 (0.87 to 1.08) ^d	1.00 (0.91 to 1.11) ^d
Mean PLT			1.00 (0.99 to 1.01) ^d	1.00 (0.99 to 1.01) ^d
Mean WBC			1.03 (0.68 to 1.57) ^d	1.23 (0.88 to 1.72) ^d
Cytopenia status				
Absent	140	5.7	1	-
Present	260	7.7	1.35 (0.61 to 2.98) ^d	0.56 (0.16 to 2.00) ^d
Marital Status				
Not Married	265	7.2	1	-
Married	135	6.7	0.93 (0.43 to 2.00) ^d	1.04 (0.51 to 2.11) ^d
Employment Status				
Not Employed	55	5.5	1	-
Employed	345	7.3	1.33 (0.41 to 4.26) ^d	0.97 (0.35 to 2.72) ^d
Educational Level				
None	13	7.7	1	-
Primary	206	7.3	0.95 (0.14 to 6.64) ^d	0.57 (0.08 to 4.24) ^d
Secondary	141	7.1	0.92 (0.13 to 6.66) ^d	0.57 (0.07 to 4.47) ^d
Tertiary	40	5.0	0.65 (0.06 to 6.62) ^d	0.41 (0.04 to 4.27) ^d

a= $p \leq 0.001$ b= $0.001 < p \leq 0.05$ c= $0.05 < p \leq 0.1$ d= $p > 0.1$

The solid line demarcates those independent variables that entered into the final multivariable model (above the line) at a p-value cut off of 0.1 at bivariate analyses; all other independent variables (below the line) were added one at a time into the multivariable model to obtain their respective multivariate RR

The mean values shown in the table are the mean of each specified independent variable per participant over the follow-up duration of 18 months.

The only multivariate factor that was significantly associated with death and incident OIs after adjusting for the baseline anemia status was the mean CD4 cell count. For every unit increase in the mean CD4 cell count over the duration of follow-up, the risk of death and incident OIs decreased by 1% [aRR= 0.99; 95% CI: 0.98 to 0.99, $p < 0.001$]. The risk of death and incident OIs decreased by 3% for every one year increase in age [aRR=0.97; 95% CI: 0.95 to 1.02] and increased by 10% in females compared to males [aRR= 1.10; 95% CI: 0.52 to 2.33] after adjusting for baseline anemia status and the mean CD4 cell count but none of these associations were significant. There was no difference in the risk of death and incident OIs with every unit increase in the mean BMI [aRR= 1.00; 95% CI: 0.91 to 1.11] or the mean platelet count [aRR=1.00; 95% CI: 0.99 to 1.01] over the duration of follow-up.

5 Discussion

5.1 *Prevalence of cytopenia at the time of initiating HAART*

The overall prevalence of cytopenia in this study was high at 65% (260/400), with anemia occurring in 48%, leucopenia in 24% and thrombocytopenia in 8%. We evaluated the types and magnitude of cytopenias in HIV-infected adults at initiation of HAART in an urban cohort in Uganda and found a higher prevalence of all 3 forms of cytopenia in this population compared to two other studies in HIV-infected individuals prior to initiation of HAART; one in South Korea and another, a multicenter study in nine countries: Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, the USA and Zimbabwe. The baseline prevalence of anemia was 3% in the Korean study and 11.9% in the multicenter study; neutropenia occurred in 10% and 14.3 % and thrombocytopenia in 2.4% and 7.2% respectively [43, 82]. Apart from the different settings of these studies, these differences could be due to different inclusion/exclusion criteria used in these studies for example, subjects with severe forms of anemia were

excluded from the Korean and multicenter studies thus under-estimating the true prevalence or due to different cut-off values used to define the cytopenias.

Anemia was the most prevalent cytopenia in this study at 48% and this is consistent with a systematic review of studies that documented prevalence rates of 35 to 65% in individuals in the US and Europe before or at the start of HAART [33, 83] and 18 to 77% in Africa and Asia [66, 74, 84]. Furthermore these findings are consistent with pre-HAART [15, 32] and HAART studies [49, 50] that have shown anemia to be the most common cytopenia. Our data show a higher prevalence of anemia compared to 2 relatively large cohorts: the Therapeutics Research, Education and AIDS Training in Asia (TREAT Asia) HIV Observational Database (TAHOD) from the Asia-Pacific region [85] and the Development of Antiretroviral Therapy (DART) trial in Uganda and Zimbabwe [86]. Anemia was reported in 18.2% (170/932) of those who were tested at baseline in the TAHOD cohort and in 12% of the 3,314 participants randomized in the DART trial at baseline. The difference in the baseline prevalence of anemia between our study population and the TAHOD cohort could be due to different ethnicities with the TAHOD cohort being of Asia-Pacific origin and predominantly Chinese as well as gender distribution differences between the 2 populations: the TAHOD cohort is predominantly male (72%) while our study population was predominantly female (65%) thus leading to different gender-based risks for anemia in both populations. Our data originate from an urban cohort in Uganda and further show a higher prevalence of anemia compared to a rural cohort in Uganda [53]. The prevalence of anemia in the rural cohort was 18.9% compared to 48% in our study even though lower hemoglobin cut-off values were used to define anemia in the rural cohort and CD4 counts were comparable across the two populations. In our study anemia was

defined as <12.0g/dl in females vs. <11.0 g/dl in the rural cohort while in males it was defined as <13.0 g/dl vs. <12.0g/dl respectively. These differences could be due to differences in modifiable factors which may vary between rural and urban populations such as dietary factors, alcohol and tobacco use etc., although we could not confirm this hypothesis.

5.2 *Correlates of cytopenia in HIV-infected individuals initiating HAART*

In our study female sex, lower CD4 cell counts and lower body mass index (BMI) were independent predictors of having a cytopenia at baseline. The causative role of HIV *in vivo* in altering the bone marrow microenvironment thus inhibiting hematopoiesis and directly resulting in cytopenia is uncertain [13, 18]. Our findings however are consistent with other studies that have shown that females are more likely to have anemia than males [43, 53, 84] and that have demonstrated an association between low CD4 cell count and the presence or development of anemia [67, 83], neutropenia [64, 87] and thrombocytopenia [87]. The gender-specific differences in cytopenia prevalence rates particularly with regard to anemia may be due to additional demands in women of reproductive age during menstruation and pregnancy-related events. The highest rates of cytopenia occurred in subjects with advanced HIV (CD4 count < 200 cells/ μ l). Cytopenias occur more frequently with HIV progression and/or as viral replication persists and patients may present with multiple cytopenias [15, 21, 88]. Our findings are comparable with those from other studies in developing countries that have shown an increase in the prevalence of various forms of cytopenia with advanced HIV-disease [64, 74, 84, 89]. The prevalence of anemia in this study was 49% in subjects with CD4 counts below 200 (n=300) while for subjects with higher CD4 counts (n=100), it was 43%. Similarly subjects with CD4 counts below 200 had a higher

prevalence of leucopenia than those with higher CD4 counts (27% versus 17% respectively). Finally the prevalence of thrombocytopenia was similar for both CD4 categories (8.3% versus 8% respectively). However, these differences by CD4 category <200 or ≥ 200 were statistically significant only for leucopenia prevalence. We demonstrated that among other factors such as BMI and age, hemoglobin levels significantly increased with high CD4 cell count. This is consistent with other studies that have shown an association between leucopenia and low CD4 counts with anemia [14, 46, 67]. The association between low CD4 cell count and anemia or leucopenia may be due to the dysregulatory effect of HIV on the function of early hematopoietic progenitor cells through the viral accessory protein Negative factor [21].

The main factors associated with having baseline anemia in this study were female sex and low BMI. Similar risk factors for anemia were demonstrated by the PEARLS study, a multicenter comparative analysis of pre-treatment hematological abnormalities in HIV-infected individuals [43] and the DART trial [86]. Women constitute a growing at-risk subpopulation for HIV infection and the prevalence of anemia in HIV-infected women has been evaluated in 2 urban cohorts in the US [90]. In the HER study, the authors compared the prevalence and cumulative incidence of anemia in 797 HIV-positive women and 389 HIV-negative women and found that the prevalence was higher by 13% in the HIV-positive cohort than the HIV-negative cohort [38]. The Women's Interagency HIV Study (WIHS) evaluated anemia in HIV-infected women and found a prevalence of 37% in the HIV-positive women and 17% in the HIV-negative women [67]. Interestingly, even subjects with a normal BMI had a higher prevalence of anemia [PR=0.43; 95%CI: 0.19-0.99, p=0.048] than those who were overweight [PR=0.17; 95%CI: 0.07-0.41, p<0.001]. The association of anemia with

low BMI is possibly due to nutrient deficiencies of iron, folate and vitamin B12 and to chronic malnutrition [84]. Consequently improvement of social and nutritional conditions may reduce anemia prevalence and incidence in resource-limited settings [89]. We did not exclude malaria as well as hereditary causes of anemia in this population such as thalassemias and sickle-cell disease as possible causes of anemia in our study.

The main factor associated with having baseline leucopenia in this study was low CD4 cell count suggesting that the stage of HIV infection may contribute to baseline leucopenia. The most clinically relevant subtype of leucopenia is neutropenia. The PEARLS study also found that neutropenia was associated with low CD4 cell count among other factors such as the platelet count, hemoglobin, sex, ethnicity and geographical location [43]. Neutropenia in HIV-infection may result from decreased production of granulocyte colony-stimulating growth factor (G-CSF) and autoimmunity. The presence of neutropenia in HIV-infection predisposes to infections such as bacteremia and effective HAART may reduce the incidence of neutropenia and thus of bacterial infections [91].

Baseline thrombocytopenia was the least frequent of all the forms of cytopenia and was associated with sex and HAART status at enrolment. Females were less likely to have thrombocytopenia compared to males and those with HAART experience were less likely to have thrombocytopenia than those who were HAART naïve. These findings are similar to the PEARLS study which demonstrated a lower prevalence of thrombocytopenia compared to neutropenia and anemia and that males were more likely to have thrombocytopenia in addition to other risk factors such chronic hepatitis B infection and neutropenia [43].

5.3 Trends in hematological parameters in HIV-infected adults on HAART

We found that the use of HAART was associated with an increase in hemoglobin levels during one and a half years of follow-up and that this hemoglobin response was associated with BMI, CD4 cell count and age. Our data demonstrate a hemoglobin response that is consistent with other studies that have shown that the use of HAART for at least 6 months is associated with an increase in hemoglobin and a decreased likelihood of anemia [14, 33, 35, 92, 93]. In 2 urban cohort studies, one that examined the impact of HAART on anemia in HIV-infected women (the Human Immunodeficiency Virus Epidemiological Research (HER) study) [94] and another that documented the risk factors and natural history of HIV-infection in injection drug users (the AIDS Linked to Intravenous Experiences (ALIVE) study) [14], Semba et al demonstrated reductions in anemia after one year of HAART of approximately 32% and 17% respectively in these highly-selected urban populations. Our study extends these observations to show a 50% reduction in anemia after one year of HAART in a more heterogeneous, urban population with fewer HAART regimen options compared to these 2 cohorts. There are several mechanisms which may account for the improvement of anemia following HAART initiation. Anemia of chronic disease accounts for a large proportion of anemia during HIV infection as a result of the chronic inflammation during HIV infection and the myriad of opportunistic infections[14]. Anemia of chronic disease is defined as anemia occurring in association with infectious diseases, neoplasms and inflammatory conditions presenting with hypoferrremia (iron deficiency) in the presence of adequate storage iron and the absence of any other known cause of anemia [7, 95]. Its pathogenesis is not well understood but is thought to result from three processes: shortened erythrocyte survival, failure of bone

marrow erythropoiesis to compensate the increased demand during infection and inflammation and reduced erythropoietin production, a hormone that is essential for the survival, proliferation and differentiation of erythroid progenitor cells [96, 97] . HAART has demonstrated the potential to reduce the occurrence of several opportunistic infections [91, 98-101]. HAART also appears to reverse the cytokine dysregulation observed during HIV infection and to control monocyte activation thus reducing the expression of certain cytokines like tumor necrosis factor (TNF) alpha which may play a role in the suppression of erythropoiesis [102-104]. Another mechanism by which HAART may improve anemia is through the reduction of chronic diarrhea and enteric infections such as microsporidiosis and cryptosporidiosis [98, 100] thus improving absorption and metabolism of micronutrients that are essential for erythropoiesis such as vitamin A, vitamin B12, folate and iron [14, 98, 105]. In this present study, most patients (92%) received AZT-based HAART and yet demonstrated a consistent hemoglobin increase throughout the follow-up period. A study comparing the incidence of anemia in AZT-naïve patients initiating AZT-containing HAART and those initiating non-AZT based HAART, Curkendall found that patients on AZT-based HAART had a greater risk of developing new anemia or worsening anemia compared to those on non-AZT based HAART [93]. In contrast, Kiragga et al demonstrated that the majority of patients initiating AZT-containing HAART experienced an improvement in hemoglobin suggesting that baseline severe anemia should not be the only determining criterion to avoid use of AZT in patients initiating HAART in resource-poor settings [54]. In a study undertaken to document the correlates of anemia in an urban HIV-infected population in the US as well as to investigate the relationship between testosterone levels and prevalence of anemia, it was found that there was no significant association between current zidovudine use and anemia [63]. Furthermore,

in comparison to patients without anemia in this US cohort, anemic patients were more likely to have more advanced HIV disease, lower recent and nadir CD4 cell counts and higher recent and peak viral loads. Effective use of HAART irrespective of whether zidovudine is part of the regimen is associated with improvement in hemoglobin levels [46].

There was an overall reduction in the prevalence of all grades of anemia observed in our study but not a complete correction to normal hemoglobin levels after one and a half years on HAART. It is possible that other contributing factors to the persistence of anemia despite HAART in this setting are due to micronutrient deficiencies e.g iron and vitamin deficiencies. However, Semba et al also found that 25% of the women in the HER study were still anemic after one year on HAART [38]. Studies have shown that even though the introduction of HAART has led to significant reductions in the prevalence of severe anemia, mild to moderate anemia persists in some individuals [14, 46, 65, 94]. In a large prospective study of HIV-infected adults attending the Infectious Diseases Institute clinic at the time when HAART was being rolled out in Uganda, HAART initiation led to increased hemoglobin levels for the majority of patients but 3.5% developed early severe anemia within 6 months of HAART initiation [54]. Kiragga et al attributed this to concurrent tuberculosis (TB), a low MCV and baseline severe anemia. The persistence of mild to moderate forms of anemia or hemoglobin levels between 10 and 14g/dl is clinically significant because it has been associated with decreased quality of life measured by functional status and fatigue [46, 106, 107].

An elevation of the MCV was observed in our study throughout the follow-up period from a mean of 87.5 femtolitres (fl) at the time of recruitment into the study to a mean of 102.9 fl after 18 months. Macrocytosis (MCV >100fl) is estimated to be about 2% in

the general population [108] and has been described in HIV-infected populations in both zidovudine-exposed and zidovudine-naïve patients. Macrocytosis is thought to result from the use of thymidine analogues that is AZT and d4T but also with the use of lamivudine [9, 11, 109, 110]. Changes in the MCV specifically macrocytosis in HIV-positive patients taking nucleoside reverse transcriptase inhibitors (NRTIs) are used as surrogate markers for adherence to HAART [111]. Furthermore, about 40% of patients who develop macrocytosis have co-morbid anemia [108]. In our study, 12 out of the 17 patients (70%) with macrocytosis at baseline were anemic.

The mean white blood cell (WBC) and platelet counts in the present study at the start and throughout the follow-up period were generally higher than those observed by Servais et al. in HIV-infected patients from Luxemburg and Belgium who were treatment naïve (Luxemburg sub-population) and then observed for one year while on HAART (Belgium sub-population) [65]. WBC counts in our study rose from a mean of 3600 to 3720 cells/ μ l compared to 2260 to 3600 cells/ μ l in the 2 European sub-populations. Interestingly the platelet recovery in our cohort was not sustained throughout the follow-up period; the mean platelet count rose from 252,000 to 264,000 cells/ μ l at 6 months and then gradually decreased to 257,000 cells/ μ l at 18 months. This is in contrast to the findings from the longitudinal study by Servais et al where platelets steadily and significantly increased from 110,000 to <180,000 cells/ μ l at 24 months.

These data suggest that HAART may induce changes in clinically relevant hematological parameters leading to the correction of some abnormalities such as anemia and potential worsening of other parameters like the MCV [82, 94], the MCHC and in some populations such as this cohort, the platelet count.

5.4 Association of baseline anemia and other factors with HIV-disease progression and/or death in adults initiating HAART

We found that baseline anemia and the mean CD4 cell count over the duration of follow-up were the main factors associated with the risk of disease progression and/or death in this population independent of age, sex and body mass index among other factors. Our findings are similar to those of a cohort of HIV-infected women in Tanzania in whom O'Brien et al. showed an association of anemia with disease progression and both all-cause mortality and AIDS-related mortality [62]. In our study baseline anemia was associated with a 5-fold increased risk of death while every unit increase in the mean CD4 cell count was associated with a 1% decrease in the risk of death. In contrast, O'Brien et al. did not find a significant association between baseline hemoglobin and time to death or disease progression possibly because only one measure of hemoglobin may not predict long-term risk of disease progression and furthermore, a single measure of hemoglobin is more strongly associated with disease progression in advanced HIV than in early stage disease as in their study population of asymptomatic women [62]. These data are however consistent with several other studies in developed countries that have demonstrated that hemoglobin level and/or anemia are strong independent predictors of morbidity and mortality in HIV-infected individuals [32-34, 37, 48, 61, 62] as well as progression to AIDS/opportunistic infections [71, 112]. The EuroSIDA study for example, is a prospective study of HIV-infected patients in 70 centres across Europe, Argentina and Israel that have provided data on consecutive patients seen in the outpatient clinic and enrolled into 5 cohorts at various time points (that is from 1994 to 2001) [33, 61, 113, 114]. Lundgren et al showed that despite the fact that the mechanism underlying why hemoglobin was a strong prognostic marker was not known, mild and severe anemia were independently

associated with clinical disease progression and that the relative hazard of death increased markedly for HIV-infected anemic patients enrolled in the EuroSIDA study compared to those without anemia [61]. The evidence from our study is consistent with the literature that has shown an association between a) HIV-related anemia and various factors such as female sex, CD4 cell counts below 200 cells/ μ l, ethnicity and zidovudine use [32, 33, 61, 67] and b) the presence of anemia and decreased survival in these patients despite the differences in patient population, hemoglobin cut-offs used to define anemia and available HAART regimens [22, 33, 34, 37, 61, 67, 93, 115-117]. We have further shown that initiation of HAART was associated with recovery from anemia and this is consistent with the findings from other studies [14, 66, 94]. In developed countries, recombinant human erythropoietin (epoietin alfa) is recommended for the treatment of HIV-associated anemia as a routine practice if correctable causes of anemia have been ruled out [118-120]. Behler et al further showed a negative association between anemia and the use of supplemental androgens and hypothesized that supplemental androgens stimulated erythropoietin production thus improving hemoglobin levels [63]. In these settings the use of epoietin alfa has been associated with improved survival [34] and quality of life [121, 122] however, because of its cost it is neither routinely available nor a feasible public health intervention in resource-limited settings such as Uganda.

5.5 *Strengths and limitations of the study*

This study provides a comparative analysis of the prevalence estimates and correlates of all forms of cytopenia in HIV-infected adults at initiation of HAART in Uganda. It further demonstrates the trend in hematological parameters in HAART-treated individuals in a resource-limited setting and thus suggests these parameters as surrogate markers for monitoring treatment response in these settings.

This study had the limitation of observational studies that these data do not confirm a causal pathway between hematological abnormalities and clinical outcomes with HAART. Furthermore, the statistical power of these analyses, being nested within a larger randomized clinical trial of multivitamin supplementation, were bound by the parent study and any losses to follow-up.

We did not adjust for the intervention that is multivitamin supplementation versus placebo because the intervention data were not accessible at the time of these analyses due to logistical and trial-related reasons. However, we do not think that this would have affected our results since patients were randomly allocated to either treatment arm.

The various causes of cytopenias and especially anemia could not be assessed. Bone marrow biopsies were not done as part of the parent study protocol therefore exclusion of low cell counts due to disorders of production could not be confirmed.

The assessment of red blood cell indices was incomplete without film reports, ferritin and transferrin measurements thus we were not able to differentiate causes of anemia due to problems of iron storage and transportation.

6 Conclusion

The prevalence of baseline cytopenias was high at 65% and in particular 48% of our study population was anemic. Our findings have shown that initiation of HAART improves hemoglobin levels and corrects anemia even when AZT-based HAART is used as a first-line regimen in resource-limited settings. Other studies have shown that the use of protease inhibitors (PIs) in first-line regimens may provide added benefits such as less hematologic toxicity and reverse HIV-induced inhibition of hematopoiesis [65]. However, the feasibility of PI-based HAART at a public health level especially in

resource-limited settings needs further investigation in terms of cost-effectiveness and future options for long-term therapy. Other selected hematological parameters may have various responses to HAART with some improving such as the white blood cell count and others potentially worsening such as the platelet count. The only significant predictors of cytopenias at the initiation of HAART are sex, body mass index and CD4 cell count. Thus screening for clinically relevant hematological parameters prior to initiation of HAART as well as during HAART must be prioritized and interventions should take into consideration gender-based risks, nutritional interventions and other contributing factors to immune suppression. Finally, despite a 50% reduction in anemia prevalence in our study, more than one fifth of patients did not reach normal hemoglobin levels while on HAART. This means that additional interventions need to be identified in the subset of patients in whom hematological abnormalities persist with HAART because these patients are at risk for disease progression and death particularly the anemic, wasted patient with persistently low or declining CD4 cell counts. In summary, these findings have implications for screening for cytopenias at entry into HIV care, the choice of an optimal initial HAART regimen and monitoring of these parameters while on HAART so as to intervene where the abnormalities may be potentially reversible. Randomized controlled trials of HAART are needed to confirm the causal pathway between HIV burden, hematological abnormalities and clinical outcomes.

References

1. Shattock, R.J. and J.P. Moore, *Inhibiting sexual transmission of HIV-1 infection*. Nat Rev Microbiol, 2003. **1**(1): p. 25-34.
2. Grossman, Z., et al., *Pathogenesis of HIV infection: what the virus spares is as important as what it destroys*. Nat Med, 2006. **12**(3): p. 289-295.
3. Berger, E.A., P.M. Murphy, and J.M. Farber, *Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease*. Annual review of immunology, 1999. **17**: p. 657-700.
4. Hambleton, J. *Hematologic Complications of HIV*. Oncology, 1996.
5. Liu, Y., et al., *CD4-Independent Infection of Astrocytes by Human Immunodeficiency Virus Type 1: Requirement for the Human Mannose Receptor* Journal of Virology, 2004: p. 4120-4133.
6. Chen, P., et al., *Virological Synapses Allow HIV-1 Uptake and Gene Expression in Renal Tubular Epithelial Cells*. J Am Soc Nephro, 2011. **22**: p. 496-507.
7. Semba, R.D., *Pathogenesis of anemia during human immunodeficiency virus infection*. Journal of investigative medicine, 2001. **49**(3): p. 225.
8. Hunt, P., et al., *Relationship between T Cell Activation and CD4 T Cell Count in HIV-Seropositive Individuals with Undetectable Plasma HIV RNA Levels in the Absence of Therapy*. The Journal of Infectious Diseases, 2007. **197**: p. 126-33.
9. Eyer-Silva, W.A., et al., *Macrocytosis in Patients on Stavudine*. Scandinavian journal of infectious diseases, 2001. **33**(3): p. 239-240.
10. Folks, T., et al., *Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow*. Science, 1988. **242**(4880): p. 919-22.
11. Genn é, D., et al., *Causes of Macrocytosis in HIV-infected Patients not Treated with Zidovudine*. Journal of Infection, 2000. **40**(2): p. 160-163.
12. Coyle, T.E., *Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome*. Med Clin North Am, 1997. **81**(2): p. 449-70.
13. Moses, A., J. Nelson, and G.C. Bagby, *The Influence of Human Immunodeficiency Virus-1 on Hematopoiesis*. Blood, 1998. **91**(5): p. 1479-1495.
14. Semba, R.D., N. Shah, and D. Vlahov, *Improvement of Anemia Among HIV-Infected Injection Drug Users Receiving Highly Active Antiretroviral Therapy*. JAIDS Journal of Acquired Immune Deficiency Syndromes, 2001. **26**(4): p. 315-319.
15. Zon, L.I., C. Arkin, and J.E. Groopman, *Haematologic manifestations of the human immune deficiency virus (HIV)*. British Journal of Haematology, 1987. **66**(2): p. 251-256.
16. Blanche, P., et al., *Toxoplasmosis-Associated Hemophagocytic Syndrome in a Patient with AIDS: Diagnosis by the Polymerase Chain Reaction*. Clinical Infectious Diseases, 1994. **19**(5): p. 989-990.
17. Sasadeusz, J., *Reactive haemophagocytic syndrome in human immunodeficiency virus infection*. The Journal of infection, 1990. **20**(1): p. 65.
18. Koka, P.S. and S.T. Reddy, *Cytopenias in HIV Infection: Mechanisms and Alleviation of Hematopoietic Inhibition*. Current HIV Research, 2004. **2**(3): p. 275-282.
19. Burstein, Y., et al., *Alterations in Human Fetal Hematopoiesis Are Associated with Maternal HIV Infection*. Pediatr Res, 1992. **32**(2): p. 155-159.
20. Fauci, A.S., *Host factors and the pathogenesis of HIV-induced disease*. Nature, 1996. **384**(6609): p. 529-534.
21. Kirchhoff, F., *Is Nef the elusive cause of HIV-associated hematopoietic dysfunction?* Journal of Clinical Investigation, 2008. **118**(5): p. 1622-1625.

22. Kulkosky, J., A. Laptev, and S. Shetty, *Human immunodeficiency virus type 1 Vpr alters bone marrow cell function*. *Blood*, 1999. **93**(6): p. 1906-15.
23. Prost, S., et al., *Human and simian immunodeficiency viruses deregulate early hematopoiesis through a Nef/PPAR γ /STAT5 signaling pathway in macaques*. *The Journal of Clinical Investigation*, 2008. **118**(5): p. 1765-1775.
24. Isgro, A., et al., *Recovery of Hematopoietic Activity in Bone Marrow from Human Immunodeficiency Virus Type 1-Infected Patients during Highly Active Antiretroviral Therapy*. *AIDS Research and Human Retroviruses*, 2000. **16**(15): p. 1471-1479.
25. Baillou, C., et al., *Highly active antiretroviral therapy corrects hematopoiesis in HIV-1 infected patients: interest for peripheral blood stem cell-based gene therapy*. *AIDS*, 2003. **17**(4): p. 563-574.
26. Sipsas, N.V., et al., *Circulating Autoantibodies to Erythropoietin Are Associated with Human Immunodeficiency Virus Type 1—Related Anemia*. *Journal of Infectious Diseases*, 1999. **180**(6): p. 2044-2047.
27. Spivak, J., et al., *Serum Immunoreactive Erythropoietin in HIV-Infected Patients*. *JAMA*, 1989. **261**(21): p. 3104-3107.
28. Dobs, A.S., *Androgen therapy in AIDS wasting*. *Baillière's Clinical Endocrinology and Metabolism*, 1998. **12**(3): p. 379-390.
29. McGinniss, M.H., et al., *Red cell autoantibodies in patients with acquired immune deficiency syndrome*. *Transfusion*, 1986. **26**(5): p. 405-409.
30. Rule, S., C. Reed, and C. Costello, *FATAL HAEMOPHAGOCYTIC SYNDROMES IN HIV-ANTIBODY POSITIVE PATIENT*. *British Journal of Haematology*, 1991. **79**(1): p. 127-127.
31. Costello, C., *Haematological abnormalities in human immunodeficiency virus (HIV) disease*. *Journal of Clinical Pathology*, 1988. **41**(7): p. 711-715.
32. Sullivan, P., et al., *Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: results from the multistate adult and adolescent spectrum of HIV disease surveillance project*. *Blood*, 1998. **91**: p. 301 - 8.
33. Mocroft, A., et al., *Anemia is an independent predictive marker for clinical prognosis in HIV infected patients from across Europe. EuroSIDA study group*. *AIDS*, 1999. **28**: p. 943 - 50.
34. Moore, R.D., J.C. Keruly, and R.E. Chaisson, *Anemia and Survival in HIV Infection*. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 1998. **19**(1): p. 29-33.
35. Berhane, K., et al., *Impact of Highly Active Antiretroviral Therapy on Anemia and Relationship Between Anemia and Survival in a Large Cohort of HIV-Infected Women: Women's Interagency HIV Study*. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 2004. **37**(2): p. 1245-1252.
36. Lundgren, J.D., et al., *A Clinically Prognostic Scoring System for Patients Receiving Highly Active Antiretroviral Therapy: Results from the EuroSIDA Study*. *Journal of Infectious Diseases*, 2002. **185**(2): p. 178-187.
37. Moore, R.D., *Human Immunodeficiency Virus Infection, Anemia, and Survival*. *Clinical Infectious Diseases*, 1999. **29**(1): p. 44-49.
38. Semba, R.D., et al., *Prevalence and Cumulative Incidence of and Risk Factors for Anemia in a Multicenter Cohort Study of Human Immunodeficiency Virus—Infected and —Uninfected Women*. *Clinical Infectious Diseases*, 2002. **34**(2): p. 260-266.
39. Spivak, J.L., B.S. Bender, and T.C. Quinn, *Hematologic abnormalities in the acquired immune deficiency syndrome*. *The American journal of medicine*, 1984. **77**(2): p. 224-228.
40. Treacy, M., et al., *Peripheral blood and bone marrow abnormalities in patients with HIV related disease*. *British Journal of Haematology*, 1987. **65**(3): p. 289-294.
41. Huang, S.S., et al., *Reversal of Human Immunodeficiency Virus Type 1-Associated Hematosuppression by Effective Antiretroviral Therapy*. *Clinical Infectious Diseases*, 2000. **30**(3): p. 504-510.

42. Wolf, T., et al., *Changing incidence and prognostic factors of survival in AIDS-related non-Hodgkin's lymphoma in the era of highly active antiretroviral therapy (HAART)*. *Leukemia & Lymphoma*, 2005. **46**(2): p. 207-215.
43. Firnhaber, C., et al., *Comparisons of anemia, thrombocytopenia, and neutropenia at initiation of HIV antiretroviral therapy in Africa, Asia, and the Americas*. *International Journal of Infectious Diseases*, 2010. **14**(12): p. e1088-e1092.
44. Carbonara, S., et al., *Response of Severe HIV-Associated Thrombocytopenia to Highly Active Antiretroviral Therapy Including Protease Inhibitors*. *Journal of Infection*, 2001. **42**(4): p. 251-256.
45. Pottage, J.C., et al., *Treatment of human immunodeficiency virus—related thrombocytopenia with zidovudine*. *JAMA*, 1988. **260**(20): p. 3045-3048.
46. Moore, R.D. and D. Forney, *Anemia in HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy*. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 2002. **29**(1): p. 54-57.
47. Sullivan, P.S., D.L. Hanson, and J.T. Brooks, *Impact on Hemoglobin of Starting Combination Antiretroviral Therapy With or Without Zidovudine in Anemic HIV-Infected Patients*. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 2008. **48**(2): p. 163-168
10.1097/QAI.0b013e3181685714.
48. Kowalska JD, e.a., *Current Hemoglobin Levels are More Predictive of Disease Progression Than Hemoglobin Measured at Baseline for Patients Receiving AntiRetroviral Therapy for HIV type 1 Infection*. *AIDS Research and Human Retroviruses*, 2007. **23**(10).
49. Sloand, E., *Hematologic complications of HIV infection*. *AIDS Rev*, 2005. **7**(4): p. 187-96.
50. Richman, D.D., et al., *The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex*. *New England Journal of Medicine*, 1987. **317**(4): p. 192-197.
51. Buskin, S.E. and P.S. Sullivan, *Anemia and its treatment and outcomes in persons infected with human immunodeficiency virus*. *Transfusion*, 2004. **44**(6): p. 826-832.
52. Koch, M.A., et al., *Toxic Effects of Zidovudine in Asymptomatic Human Immunodeficiency Virus-Infected Individuals With CD4<R>+</R> Cell Counts of 0.50x10<R>9</R>/L or Less: Detailed and Updated Results From Protocol 019 of the AIDS Clinical Trials Group*. *Arch Intern Med*, 1992. **152**(11): p. 2286-2292.
53. Mugisha, J.O., et al., *Anaemia in a rural Ugandan HIV cohort: prevalence at enrolment, incidence, diagnosis and associated factors*. *Tropical Medicine & International Health*, 2008. **13**(6): p. 788-794.
54. Kiragga, *Baseline severe anemia should not preclude use of zidovudine in antiretroviral-eligible patients in resource-limited settings*. *J Int AIDS Soc*, 2010. **13**(42).
55. Valdez, H., et al., *Changing Spectrum of Mortality Due to Human Immunodeficiency Virus: Analysis of 260 Deaths during 1995–1999*. *Clinical Infectious Diseases*, 2001. **32**(10): p. 1487-1493.
56. Apolonio, E.G., et al., *Prognostic Factors in Human Immunodeficiency Virus-Positive Patients with a CD4⁺ Lymphocyte Count <50/μL*. *The Journal of Infectious Diseases*, 1995. **171**(4): p. 829-836.
57. Rabeneck, L., et al., *Predicting outcomes in HIV-infected veterans: II. survival after AIDS*. *Journal of Clinical Epidemiology*, 1997. **50**(11): p. 1241-1248.
58. Spino, C., *Predictors of Survival in HIV Infected Persons with 50 or Fewer CD4 cells/mm³*. *JAIDS*, 1997. **5**(15): p. 346-355.
59. Turner, B.J., L. Markson, and F. Taroni, *Estimation of survival after AIDS diagnosis: CD4 T lymphocyte count versus clinical severity*. *Journal of Clinical Epidemiology*, 1996. **49**(1): p. 59-65.
60. Obirikorang, *Blood hemoglobin measurement as a predictive indicator for HIV/AIDS progression in resource-limited setting*. *J Biomed Sci*, 2009. **16**(1).

61. Lundgren, J.D. and A. Mocroft, *Anemia and Survival in Human Immunodeficiency Virus*. Clinical Infectious Diseases, 2003. **37**(Supplement 4): p. S297-S303.
62. O'Brien, M.E., et al., *Anemia Is an Independent Predictor of Mortality and Immunologic Progression of Disease Among Women With HIV in Tanzania*. JAIDS Journal of Acquired Immune Deficiency Syndromes, 2005. **40**(2): p. 219-225.
63. Behler C, S.S., et al, *Anemia and HIV in the Antiretroviral Era: Potential Significance of Testosterone*. AIDS Research and Human Retroviruses, 2005. **21**(3): p. 200-206.
64. Toure, S., et al., *Incidence of neutropenia in HIV-infected African adults receiving co-trimoxazole prophylaxis: a 6-year cohort study in Abidjan, Côte d'Ivoire*. Transactions of The Royal Society of Tropical Medicine and Hygiene, 2006. **100**(8): p. 785-790.
65. Servais, J., et al., *HIV-Associated Hematological Disorders Are Correlated With Plasma Viral Load and Improve Under Highly Active Antiretroviral Therapy*. Journal of Acquired Immune Deficiency Syndromes, 2001. **28**(3): p. 221-225.
66. Johannessen, *Antiretroviral treatment reverses HIV-associated anemia in rural Tanzania*. BMC Infect Dis, 2011. **11**(190).
67. Levine, A.M., et al., *Prevalence and Correlates of Anemia in a Large Cohort of HIV-Infected Women: Women's Interagency HIV Study*. JAIDS Journal of Acquired Immune Deficiency Syndromes, 2001. **26**(1): p. 28-35.
68. Saah, *Factors Influencing Survival After AIDS: Report from the Multicenter AIDS Cohort Study (MACS)*. Journal of acquired immune deficiency syndromes (1988), 1994. **7**(3): p. 287.
69. Creagh-Kirk, T., et al., *Survival experience among patients with aids receiving zidovudine: Follow-up of patients in a compassionate plea program*. JAMA, 1988. **260**(20): p. 3009-3015.
70. Swanson, C.E. and D. Cooper, *Factors influencing outcome of treatment with zidovudine of patients with AIDS in Australia. The Australian Zidovudine Study Group*. AIDS, 1990. **4**(8): p. 49-57.
71. Morfeldt-Manson, L., et al., *Clinical signs and laboratory markers in predicting progression to AIDS in HIV-1 infected patients*. Scandinavian journal of infectious diseases, 1991. **23**(4): p. 443-9.
72. Guwatudde, D., *Multivitamin supplementation in HIV infected adults initiating antiretroviral therapy in Uganda: the protocol for a randomized double blinded placebo controlled efficacy trial*. BMC Infect Dis, 2012. **12**(304).
73. World Health Organisation, *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*, V.a.M.N.I.S. Department of Nutrition for Health and Development, Editor 2011, World Health Organization: Geneva.
74. Dikshit, B., et al., *Profile of hematological abnormalities of Indian HIV infected individuals*. BMC Blood Disorders, 2009. **9**(1): p. 5.
75. Hardin, J.W. and M.A. Cleves, *Generalized linear models: Extensions to the binomial family*. Stata Technical Bulletin, 1999. **50**: p. 21-25.
76. McCullagh, P. and J.A. Nelder, *Generalized Linear Models*. 2nd ed1989, London: Chapman&Hall/CRC.
77. Wacholder, S., *Binomial regression in GLIM: Estimating risk ratios and risk differences*. American Journal of Epidemiology, 1986. **123**: p. 174-184.
78. Dempster, A.P., N.M. Laird, and D.B. Rubin, *Maximum likelihood from incomplete data via the EM algorithm*. Journal of the Royal Statistical Society, 1977. **39**: p. 1-38.
79. Laird, N.M. and J.H. Ware, *Random-effects models for longitudinal data*. Biometrics, 1982. **38**: p. 963-974.
80. Thompson Jr., W.A., *The problem of negative estimates of variance components*. Annals of Mathematical Statistics, 1962. **33**: p. 273-289.
81. Goldstein, H., *Efficient statistical modelling of longitudinal data*. Annals of Human Biology, 1986. **13**: p. 129-141.

82. Choi, S.Y., *Hematological manifestations of human immunodeficiency virus infection and the effect of highly active anti-retroviral therapy on cytopenia*. Korean Journal of Hematology, 2011. **46**(4): p. 253-257.
83. Harris, R.J., et al., *Prognostic importance of anaemia in HIV type-1-infected patients starting antiretroviral therapy: collaborative analysis of prospective cohort studies*. Antiviral therapy, 2007. **13**(8): p. 959-967.
84. Subbaraman, R., et al., *Factors associated with anaemia in HIV-infected individuals in southern India*. International Journal of STD & AIDS, 2009. **20**(7): p. 489-492.
85. Zhou, J., et al., *The TREAT Asia HIV Observational Database: Baseline and Retrospective Data*. Journal of acquired immune deficiency syndromes (1999), 2005. **38**(2): p. 174-179.
86. Team, D.T., *Prevalence, incidence and predictors of severe anaemia with zidovudine-containing regimens in African adults with HIV infection within the DART trial*. Antiviral therapy, 2006. **11**(6): p. 741.
87. Marks, K.C., Robin; Bussel, James; Talal, Andrew; Glesby, Marshall, *Risk Factors for Thrombocytopenia in HIV-Infected Persons in the Era of Potent Antiretroviral Therapy*. Journal of Acquired Immune Deficiency Syndromes, 2009. **52**(5): p. 595-599.
88. Spivak, J.L., S.E. Selonick, and T.C. Quinn, *Acquired Immune Deficiency Syndrome and Pancytopenia*. JAMA, 1983. **250**(22): p. 3084-3087.
89. De Santis, G.C., et al., *Hematological abnormalities in HIV-infected patients*. International Journal of Infectious Diseases, 2011. **15**(12): p. e808-e811.
90. Belperio, P.S. and D.C. Rhew, *Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature*. The American journal of medicine, 2004. **116**(7, Supplement 1): p. 27-43.
91. Tumbarello, M., et al., *HIV-Associated Bacteremia: How It Has Changed in the Highly Active Antiretroviral Therapy (HAART) Era*. JAIDS Journal of Acquired Immune Deficiency Syndromes, 2000. **23**(2): p. 145-151.
92. Pornprasert, S., et al., *Evolution of Hematological Parameters in HIV-1-Infected Patients With and Without Thalassemia Carriages During Highly Active Antiretroviral Therapy*. HIV Clinical Trials, 2009. **10**(2): p. 88-93.
93. Curkendall, S.M., et al., *Incidence of anaemia among HIV-infected patients treated with highly active antiretroviral therapy*. HIV Medicine, 2007. **8**(8): p. 483-490.
94. Semba, R., *Highly Active AntiRetroviral Therapy Associated with Improved Anemia among HIV-infected Women*. AIDS Patient Care and STDs, 2001. **15**(9): p. 473-480.
95. Means, R.T., Jr. and S.B. Krantz, *Progress in understanding the pathogenesis of the anemia of chronic disease [see comments]*. Blood, 1992. **80**(7): p. 1639-1647.
96. Spivak, J.L., *The blood in systemic disorders*. The Lancet, 2000. **355**(9216): p. 1707-1712.
97. Cartwright, G.E., *The anemia of chronic disorders*. Seminars in hematology, 1966. **3**(4): p. 351.
98. Bini, E.J. and J. Cohen, *Impact of protease inhibitors on the outcome of human immunodeficiency virus-infected patients with chronic diarrhea*. Am J Gastroenterol, 1999. **94**(12): p. 3553-3559.
99. Maschke, M., et al., *Incidence and prevalence of neurological disorders associated with HIV since the introduction of highly active antiretroviral therapy (HAART)*. Journal of Neurology, Neurosurgery & Psychiatry, 2000. **69**(3): p. 376-380.
100. Maggi, P., *Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1*. European journal of clinical microbiology & infectious diseases, 2000. **19**(3): p. 213.
101. Moore, R.D. and R.E. Chaisson, *Natural history of HIV infection in the era of combination antiretroviral therapy*. AIDS, 1999. **13**(14): p. 1933-1942.

102. Murphy, M., *Effects of recombinant tumor necrosis factor, lymphotoxin, and immune interferon on proliferation and differentiation of enriched hematopoietic precursor cells.* Experimental hematology, 1988. **16**(2): p. 131.
103. Kreuzer, K.A., *Inadequate erythropoietin response to anaemia in HIV patients: relationship to serum levels of tumour necrosis factor-alpha, interleukin-6 and their soluble receptors.* British Journal of Haematology, 1997. **96**(2): p. 235.
104. Amirayan-Chevillard, N., et al., *Impact of highly active anti-retroviral therapy (HAART) on cytokine production and monocyte subsets in HIV-infected patients.* Clinical and Experimental Immunology, 2000. **120**(1): p. 107-112.
105. Tang, A.M., et al., *Improved Antioxidant Status Among HIV-Infected Injecting Drug Users on Potent Antiretroviral Therapy.* JAIDS Journal of Acquired Immune Deficiency Syndromes, 2000. **23**(4): p. 321-326.
106. Breitbart, W., et al., *Fatigue in Ambulatory AIDS Patients.* Journal of Pain and Symptom Management, 1998. **15**(3): p. 159-167.
107. Groopman, J.E., *Fatigue in cancer and HIV/AIDS.* Oncology, 1998. **12**(3): p. 335-44.
108. Horstman, A., S. Serck, and R. Go, *Macrocytosis associated with monoclonal gammopathy.* Eur J Hematol, 2005. **75**(2): p. 146-9.
109. Romanelli, F., K. Empey, and C. Pomeroy, *Macrocytosis as an Indicator of Medication (Zidovudine) Adherence in Patients with HIV Infection.* AIDS Patient Care and STDs, 2002. **16**(9): p. 405-411.
110. Khawcharoenporn, T., et al., *Lamivudine-associated macrocytosis in HIV-infected patients.* International Journal of STD & AIDS, 2007. **18**(1): p. 39-40.
111. Steele, R., et al., *Mean cell volume (MCV) changes in HIV-positive patients taking nucleoside reverse transcriptase inhibitors (NRTIs): a surrogate marker for adherence.* International Journal of STD & AIDS, 2002. **13**(11): p. 748-54.
112. Moore, R.D., et al., *Long-term safety and efficacy of zidovudine in patients with advanced human immunodeficiency virus disease.* Archives of Internal Medicine, 1991. **151**(5): p. 981-986.
113. Lundgren, J.D., *Regional differences in use of antiretroviral agents and primary prophylaxis in 3122 European HIV-infected patients. EuroSIDA Study Group.* Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology (formerly Journal of Acquired Immune Deficiency Syndromes) (now JAIDS Journal of Acquired Immune Deficiency Syndromes), 1997. **16**(3): p. 153.
114. Phillips, A.N., *Survival in 2367 zidovudine-treated patients according to use of other nucleoside analogue drugs. The EuroSIDA Study Group.* Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology (formerly Journal of Acquired Immune Deficiency Syndromes) (now JAIDS Journal of Acquired Immune Deficiency Syndromes), 1998. **17**(3): p. 239.
115. Sathe, S., et al., *Severe anemia is an important negative predictor for survival with disseminated Mycobacterium avium-intracellulare in acquired immunodeficiency syndrome.* Am Rev Respir Dis, 1990. **142**: p. 1306-12.
116. Steinberg, *Predictors of Outcome in AIDS Patients Receiving Zidovudine.* Journal of acquired immune deficiency syndromes (1988), 1989. **2**(3): p. 229.
117. Volberding, P., *Consensus statement: Anemia in HIV infection—current trends, treatment options, and practice strategies.* Clinical Therapeutics, 2000. **22**(9): p. 1004-1020.
118. Grossman, H., et al. *Once-weekly epoetin alfa dosing is as effective as three times-weekly dosing in increasing hemoglobin levels and is associated with improved quality of life in anemic HIV-infected patients.* in *Proceedings of the XIV International AIDS Conference (Barcelona).* 2003. Stockholm: International AIDS Society.

119. Saag, M., et al. *Once-weekly epoetin alfa increases hemoglobin and improves quality of life in anemic HIV+ patients.* in *Proceedings of the 39th Annual Meeting of the Infectious Diseases Society of America (San Francisco)*. 2001. Alexandria,VA: Infectious Diseases Society of America.
120. Volberding, P.A., et al., *Anemia in HIV Infection: Clinical Impact and Evidence-Based Management Strategies.* *Clinical Infectious Diseases*, 2004. **38**(10): p. 1454-1463.
121. Phair, J.P., et al., *Recombinant human erythropoietin treatment: Investigational new drug protocol for the anemia of the acquired immunodeficiency syndrome: overall results.* *Archives of Internal Medicine*, 1993. **153**(23): p. 2669-2675.
122. Revicki, D., et al., *Recombinant human erythropoietin and health-related quality of life of AIDS patients with anemia.* *JAIDS*, 1994. **7**(5): p. 474-84.

CURRICULUM VITAE

RESEARCH EXPERIENCE

March 2010-April 2014: *Trial Coordinator, the Multivitamins, HAART and HIV/AIDS Trial in Uganda.* Key responsibilities/achievements:

- Developed Standard Operating Procedures and Critical revision of the data collection tools and Manual Of Operation
- Implemented the study protocol according to GCP-ICH guidelines
- Coordinated the recruitment of the targeted 400 participants as well as follow-up and trial close out procedures.
- Regular progress reporting
- Oversaw trial data cleaning, data quality control and management.
- Staff recruitment and training
- Management of the trial's logistics and other administrative tasks.

Oct 2007-Feb 2010: *Research Medical Officer, the International HIV-Associated Opportunistic Pneumonias (IHOP) Study.* Key responsibilities/achievements:

- Screening, consenting and enrolment of study participants
- Supervision of the laboratory team to ensure proper collection of study specimen
- Prompt reporting of all results to the ward teams, initiation of TB treatment and making appropriate referrals
- Pioneered and developed follow-up protocols; coordinated the follow-up of study participants
- Progress reporting to the lead investigators on the vital status of study participants and the retention rates

July 2006-July 2007: *Intern Doctor, Mulago National Referral Hospital*

Part-time Research Assistant on a study assessing the efficacy of rapid diagnostic tests (RDTs) for the detection of *Plasmodium falciparum* malaria in adults presenting to the accidents and emergency unit of Mulago National Referral hospital. I was primarily responsible for screening and enrolment of participants, supervising the laboratory technician in specimen collection, communicating the results to the participants, prescribing antimalarials for those with clinical symptoms and/or positive RDTs and updating the principal investigator on the study progress.

FORMAL TRAINING

Oct 2010-present: Doctoral Program in International Health (Center for International Health, Ludwig-Maximilians University, Munich, Germany)

March 2014: Post Graduate Diploma in Project Planning and Management (Uganda Management Institute)

2001-2006: Bachelor of Medicine and Bachelor of Surgery (Makerere College of Health Sciences)

Relevant additional training successfully completed:

- Investigator Training Program by Pfizer, South Africa Team (Kampala, April 2014)
- Data management & data analysis in Stata (Kampala, June 2013)
- Survival Analysis for Epidemiologists Course (Kampala, July-August 2012)
- GCP Training (Kampala, May 2012) and Online refresher course by-CITI (Kampala, 2013)
- Infection and Immunity (Mbeya, Tanzania July 2011)
- Applied Biostatistics (Kampala, July 2011)
- Training in HIV/AIDS Care and Management of Anti-Retroviral Therapy (August 2007)

LIST OF PUBLICATIONS

1. **Kyeyune R**, Saathoff E, Ezeamama AE, Loescher T, Fawzi W, Guwatudde D. Prevalence and Correlates of Cytopenias in HIV-infected Adults Initiating Highly Active Anti-Retroviral Therapy in Uganda; *BMC Infectious Diseases* 2014,14:496
2. Guwatudde D, Ezeamama AE, Bagenda D, **Kyeyune R**, Wamani H, Mugusi H, Spiegelman D, Wang M, Manabe YC, Fawzi WW. Multivitamin Supplementation in HIV-infected adults initiating antiretroviral therapy in Uganda: the protocol for a randomized double blinded placebo controlled efficacy trial; *BMC Infectious Diseases* 2012, 12:304
3. Paudel D, Abera M, **Kyeyune R**, Solis-soto M, Lohani A, Wandiga S, Nji A and Guenter Forschl, Inequalities in Health: Efforts, Realities and Way Forward; *World Medical and Health Policy* 2012 July 4(2):1-5
4. **Kyeyune R**, den Boon S, Cattamanchi A, Davis JL, Worodria W, Yoo SD and Huang L. Causes of early mortality in HIV-infected TB suspects in an East African referral hospital; *J Acquir Immune Defic Syndr.* 2010 December; 55(4):446-450
5. Yoo SD, Worodria W, Davis JL, Cattamanchi A, den Boon S, **Kyeyune R**, Kitembo H and Huang L. The prevalence and clinical course of HIV-associated pulmonary cryptococcosis in Uganda; *J Acquir Immune Defic Syndr.* 2010 July; 54(3):269-274
6. Davis JL, Worodria W, Kitembo H, Metcalfe JZ, Cattamanchi A, Kawooya M, **Kyeyune R**, den Boon S, Powell K, Okello R, Yoo SD and Huang L. Clinical and radiographic factors do not accurately diagnose smear-negative tuberculosis in HIV-infected inpatients in Uganda: a cross-sectional study; *PLoS ONE* 2010 March;5(3)e9859
7. Cattamanchi A, Davis JL, Worodria W, Kitembo H, den Boon S, Yoo SD, Matovu J, Kiidha J, Nankya F, **Kyeyune R**, Byanyima P, Andama A, Osmond P, Hopewell P and Huang L. Sensitivity and specificity of fluorescence microscopy for diagnosing pulmonary tuberculosis in a high HIV prevalence setting; *Int J Tuberc Lung Dis* 2009 September;13(9): 1130-1136
8. Cattamanchi A, Davis JL, Worodria W, Yoo SD, Matovu J, Kiidha J, Nankya F, **Kyeyune R**, Andama A, Joloba M, Osmond D, Hopewell P and Huang L. Poor

performance of universal sample processing method for diagnosis of pulmonary tuberculosis by smear microscopy and culture in Uganda; *Journal of Clinical Microbiology* 2008, 46(10):3325

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