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Predictors of Anemia Among HIV Patients in Uganda

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Predictors of Anemia among HIV Patients in Uganda

A Thesis Presented

By

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DEDICATION

For Mummy and Daddy

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ABSTRACT

PREDICTORS OF ANEMIA AMONG HIV PATIENTS IN UGANDA

MAY 2017

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HIV-related anemia is associated with increased risk of death. Prior studies suggest an inverse relationship between CD4 cell count and anemia, and a positive relationship between HIV-clinical stage and anemia. However, none have assessed the role of antiretroviral therapy (ART) treatment combinations in these relationships. Therefore, we conducted a cross-sectional study to evaluate the relationship between CD4 cell count, HIV clinical stage and anemia among 4803 Ugandan HIV-patients, and assessed the role of ART treatment combinations. We included HIV patients (> 15 years) receiving ART therapy combinations between 2010- 2015. We evaluated CD4 cell count and anemia using blood tests, and HIV-clinical staging was based upon the World Health Organization HIV-clinical staging system. Information on ART treatment combinations was obtained from patients' medical records. Multinomial logistic regression was used to model the relationship between CD4 cell count, HIV clinical stages and anemia. We performed a sensitivity analysis to examine the role of ART treatment combinations. The odds of being severely anemic were highest among those classified in the low CD4 cell count category (<200cells/μL), and those at WHO stage IV. Odds Ratios were 3.7 (95% CI; 1.48-9.26) and 3.2 (95% CI; 1.75-5.70), respectively. Stratification by ART treatment combinations

(TDF-based versus ADZ-based combination treatment) indicated an increase in the odds of being anemic with increase in HIV-clinical stage (stage II, OR: 1.99, 95% CI; 1.44-2.78; stage III, OR:3.17, 95% CI; 2.21-4.54, & stage IV, OR; 4.42, 95% CI; 2.68-7.30), for individuals receiving TDF-based treatment only. Results suggest that HIV-patients with a low CD4 cell count and, those in advanced HIV-clinical stages should consider regular hemoglobin follow-up to identify and treat anemia at its earliest stages.

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CHAPTER 1

INTRODUCTION

A. Human Immunodeficiency Virus

The Human Immunodeficiency Virus (HIV) destroys CD4 cells, gradually damaging the immune system.[1] CD4 cells are a type of white blood cells that signal activation of the body's immune response upon virus detection. A higher CD4 cell count is indicative of a strong immune system.[2] Without treatment, HIV–infected individuals typically progress through four clinical stages of infection, namely; primary HIV-infection; clinically asymptomatic stage; symptomatic HIV infection; and Acquired Immunodeficiency Syndrome (AIDS).[3] These stages are typically referred to as HIV clinical stages I, II, III and IV.

HIV clinical stage I. Primary HIV Infection (HIV clinical stage I) lasts a few months after infection and is often characterized with presence of Persistent Generalized Lymphadenopathy (PGL), that is, the enlargement of lymph nodes, in at least 2 body sites for a period of 6 months. During this stage, there is a large amount of HIV in the peripheral blood and the immune system initiates production of HIV antibodies.

HIV clinical stage II. Clinical asymptomatic stage (HIV clinical stage II) lasts for an average of ten years, and is devoid of major symptoms; however, HIV is active in the lymph nodes.

HIV clinical stage III. HIV clinical stage III is also known as the symptomatic HIV Infection stage, and results from mild damage to the immune system marked by presence of HIV- related bacterial infections such as pneumonia. HIV clinical stage III develops due

to; 1) damaged lymph nodes; 2) increased CD4 cell destruction; and 3) failure of the immune system to replace destroyed CD4 cells.

HIV clinical stage IV. HIV clinical stage IV is often referred to as AIDS, and results from severe damage to the immune system. At present, confirmation of AIDS diagnosis occurs when an HIV infected individual develops one or more HIV-related opportunistic infections such as cryptococci meningitis and HIV- related cancers such as Kaposi's sarcoma.

Prevalence of HIV in Uganda

In 2015, an estimated 1.5 million Ugandans were living with HIV. HIV prevalence among adults, aged 15 to 49, was estimated to be 7.1% (6.6% - 7.7%). The number of deaths due to AIDS was estimated to be 28,000 individuals.

The number of new HIV infection cases increased by 21% between 2005 and 2013.[4] However, there was an observed decrease of new cases between 2013 and 2015. Specifically, the number of new HIV infection cases reduced from 140,000 in 2013 to 83,000 in 2015.[5] The reduction in new infections has been attributed to the increased use of antiretroviral therapy (ART) treatments.[6] ART refers to combinations of antiretroviral drugs that suppress HIV viral replication and prevent progression of the disease.

B. HIV- related Anemia

Anemia is a condition that occurs when hemoglobin levels in a person's blood drop below normal. Hemoglobin is a protein molecule in the red blood cells that carries oxygen throughout the body.[7] Normal hemoglobin levels are defined as 13 grams (g) or higher per deciliter (dL) of blood.[8] The decrease in hemoglobin levels is due to body conditions that negatively influence the number of red blood cells present in the body. In HIV-patients,

conditions that are likely to influence red blood cell quantity are amount of CD4 cell count and the level of HIV clinical stage (presence or absence of HIV-related clinical manifestations).[9]

Prevalence of Anemia in HIV- infected individuals in Uganda

In different clinical settings, the prevalence of HIV- related anemia among HIV-infected individuals receiving ART varies greatly.[11] Prevalence rates range from 65% to 95% in sub-Saharan Africa[12–14] and 20% to 60% in developed countries.[10,15] This is due to differences in the prevalence of anemia risk factors; such as endemic malnutrition, helminthes infections and a different spectrum of opportunistic infections.[11]

Established Risk Factors. Known HIV-related anemia risk factors include gender and type of ART medication Women, as compared to men, are more likely to develop anemia due to blood loss during menstruation, pregnancy and delivery.[17–19] Anemia in HIV-infected individuals also results from the direct effect of antiretroviral agents, particularly zidovudine, on the hematopoietic process in the body.[20] Hematopoietic is the process through which blood or blood cells are formed in the body.

Sequelae of HIV-related Anemia. Anemia diagnosis in HIV-infected individuals has been associated with reduced quality of life,[21] quick progression of HIV,[14,22] decreased survival time,[23] and increased risk for death.[15]

C. Epidemiological Background

Recent literature suggests an association between CD4 cell count, HIV- clinical stage and HIV –related anemia. [24–27] In particular, epidemiological research suggests a inverse association between CD4 cell count and anemia, [24–27] and a positive association between HIV- clinical stages and anemia.

Epidemiology of the association between CD4 cell count and anemia

Between 2009 and 2016, three studies examined the relationship between CD4 cell count and HIV-related anemia in low–resource settings.[25–28] All three studies were cross-sectional; of these, two were single site clinic studies performed in Tanzania[25] and India[26]. The third study was conducted in rural China[28] using national data.

Study findings indicate that individuals with low CD4 cell count have increased odds of being anemic. Odds ratios were 2.27 with 95% confidence Interval (CI) 1.33 – 3.88), for study participants in Tanzania, 1.21 (95% CI; 1.07 – 1.3) for China and 5.0 (95% CI; 4.0 – 6.3) for Ethiopia.

Although all three study findings indicate an inverse association between CD4 cell count and HIV-related anemia, none of the studies controlled for ART treatment in the analysis. Combination ART treatment has been found to be positively associated with CD4 cell count and inversely associated with anemia.[29,30] Failure to adjust for combination ART treatment may have underestimated the association between CD4 cell count and anemia.

Epidemiology of the association between HIV-clinical stage and anemia

One of the three studies described above assessed HIV-clinical stage as an exposure. This study was conducted in Tanzania, [25] and compared HIV-clinical stage classified into two categories (HIV clinical stage I and II versus HIV-clinical stage III and IV).

Findings show that individuals classified in HIV-clinical stage III and HIV clinical stage IV were more likely to be anemic compared to those classified at HIV-clinical stage II and HIV-clinical stage I. Odds Ratios were 5.3 (95% CI; 3.15 – 8.9).

However, the relationship between HIV-clinical staging and anemia was not adjusted for ART treatment. Because ART treatment is positively associated with HIV-clinical stage and negatively associated with anemia, it is likely that this association was underestimated.

Therefore, we conducted a cross-sectional study among Ugandan HIV-infected adults (N = 4803) to examine the relationship between CD4 cell count, HIV-clinical stage, and anemia and adjusting for ART treatment.

CHAPTER 2

STUDY DESIGN AND METHODS

A. Study Design

We used a cross-sectional design to examined the association between CD4 cell count, HIV- clinical stage and anemia among HIV patients in Uganda. Data were electronically captured by the Ministry of Health national surveillance system between 2010 and 2015. The Ministry of Health developed the system in 2010. The aim of the system is to promote the use of locally generated health data to facilitate evidence-based decision making on planning and resource allocation within the country's healthcare sector.

B. Study Population

The study population comprised of 5276 HIV patients older than 15 years who received ART between 2010 and 2015 at three regional referral hospitals (RRH): 1) Hoima RRH in mid-western Uganda; 2) Fort-Portal RRH in western Uganda; and 3) Mubende RRH in central Uganda.

Eligibility

We excluded 1) those younger than 15 years of age (n= 287), and 2) women who were pregnant (n = 186). The final sample number used in the analysis is 4803 (Figure 1). Pregnant women were excluded from the study due to differences in physiological mechanisms[29] and clinical definitions of anemia.

C. Study Methods

Assessment of CD4 cell count

We assessed CD4 cell count as a categorical variable. Categories for CD4 cell count were: Low (< 200 cells per microliter (cells/μL); Moderate (200 - ≤ 500 cells/μL); and High (> 500 cells/μL).

Laboratory Procedure. To obtain CD4 cell counts, we performed complete blood count tests at the central public health laboratory located at Makerere University. Blood was drawn using venipuncture and kept at ambient temperatures. The laboratory center used tritest reagents to determine CD4 cell count.

Assessment of HIV Clinical Stage

HIV clinical staging was based upon the World Health Organization (WHO) clinical staging system. The WHO clinical staging system is widely used in resource-limited countries, particularly in the sub-Saharan African Region. [30, 31] To accommodate facilities with no or limited access to laboratory, the WHO HIV clinical staging system established standardized clinical guidelines for direct medical decision making, based on the patient's clinical manifestations.

Individuals are classified in WHO stage I if they have Persistent Generalized Lymphadenopathy PGL in at least two sites of the body for longer than 6 months.

HIV infected individuals are classified into WHO Stage II when clinical outcomes include: 1) unexplained weight loss of less than 10 percent of total body weight; 2) recurrent respiratory infections (such as sinusitis, bronchitis, otitis media, and pharyngitis), and 3) range of dermatological conditions including herpes zoster flares, angular cheilitis, and recurrent oral ulcerations.

WHO stage III signs and symptoms include: 1) weight loss of greater than 10 percent of total body weight; 2) prolonged (more than 1 month) unexplained diarrhea; 3) pulmonary tuberculosis; and 4) severe systemic bacterial infections including pyomyositis, meningitis, bone and joint infections, and bacteremia.

WHO stage IV includes all of the above illnesses, and presence of HIV wasting syndrome, Pneumocystis pneumonia (PCP), recurrent severe or radiological bacterial pneumonia, extra-pulmonary tuberculosis, HIV encephalopathy, CNS toxoplasmosis, chronic herpes simplex infection, esophageal candidiasis, and kaposi's sarcoma. Other diagnostic conditions include cytomegalovirus infections, extra pulmonary cryptococcosis, disseminated non-tuberculous mycobacteria infection, tracheal, bronchial or pulmonary candida infection etc.

In this study categories for HIV clinical stages were defined as WHO stage I, WHO stage II, WHO stage III and IV.

Assessment of Anemia

We assessed anemia using blood hemoglobin levels according to WHO hemoglobin cut-off points. Per WHO guidelines, non-anemic patients were described as those with > 12 g/dl for non-pregnant women and >13 g/dl for men. Anemia severity was categorized as mild anemia (10 - < 13/12 g/dL), moderate anemia (8 - < 10 g/dL), and severe anemia (< 8 g/dL) for both sexes.[8]

Laboratory Procedures. All patients participated in a standardized laboratory assessment consisting of hematological tests. Hematological indices including, hemoglobin concentration, and red blood cell indices (mean corpuscular volume and mean corpuscular hemoglobin), were determined using an automated hematology analyzer - COULTER®

Ac·T™ 5diff CP (Cap Pierce). We categorized hemoglobin concentration according to WHO criteria.

Covariates.

We collected data on age, gender, ART, presence or absence of tuberculosis, and duration on ART medication from the system. Covariates were selected based on inclusion in prior literature [24–27] and their potential for confounding as demonstrated in previous findings.

Statistical Analysis

Using cross-tabulation, we assessed the unadjusted relationship between CD4 cell count and anemia, and HIV-clinical stage and anemia. We used chi-square tests to calculate p-values for categorical variables, and ANOVA for continuous variables. We used multinomial logistic regression to model the relationship between CD4 cell count, HIV–clinical stages and anemia. We used a change-in-estimate approach to evaluate confounding. Potential confounders included age, gender, length and type of ART treatment, and Tuberculosis status. Covariates that caused a 10% change in coefficients for either CD4 cell count or HIV-clinical stage were adjusted for in the final model.

Stratification Analysis. We assessed anemia dichotomously (anemic vs non-anemic) and stratified study participants based on type of ART treatment received, those with missing data on type of ART treatment were excluded. Therefore, study participants were classified as receiving either a AZT- or TDF- based treatment combination (n = 4457). We calculated adjusted odds ratios (OR) and 95% confidence intervals to determine the strength of associations between the CD4 cell count, HIV clinical stage and anemia.

CHAPTER 3

RESULTS

Characteristics of the Study Population.

A total of 4803 HIV positive individuals (1668 males and 3135 females), receiving ART treatment therapy combinations between 2010-2015, were used to examine the relationship between CD4 cell count, HIV clinical stage and anemia. The mean age and standard deviation (mean ± S.D) of the study participants was 38.9 ± 10.5 years, with a range of 15 to 94 years. The median CD4 count was 220.0 cells/μL (IQR: 27 – 295). Thirty-four percent of the study participants were classified at WHO stage 1, 36% at WHO stage II, 24% WHO stage III, and 6.1% as WHO stage IV. Approximately 68% of the study participants reported receiving HIV treatment for a duration of 3 years or less, only 3% reported tuberculosis medication use. Sixty percent of the study participants were using AZT-based treatment combination (Table 2).

Prevalence of Anemia

Anemia was present in 28.8% of the study participants. Prevalence of mild, moderate and severe anemia were 20.6%, 4.5% and 3.7%, respectively. Gender was significantly associated with anemia (*P*-value < 0.001) with gender-specific prevalence rates of 30% (female) and 23.2% (males). CD4 cell count significantly decreased with increase in anemia severity levels (*P*- value < 0.001); that is, the mean ± SD CD4 cell count for study participants with no anemia, mild, moderate and severe anemia were 236.6 ± 210.2, 194.2 ± 145, 143.1 ± 135.2 and 147.1 ± 142.2, respectively. Prevalence of anemia differed significantly by WHO clinical stage; for example, study participants with mild anemia comprised of 30% of individuals categorized at WHO stage 1, 42% at WHO stage

II, 23% at WHO clinical stage III, and 5.6% at WHO clinical stage IV. Within the *severe anemia* category, about 18% were at WHO stage I, 30% at WHO stage II, 39% at WHO stage III & 13% at WHO stage IV (Table 2).

ART treatment type was significantly associated with anemia. Almost half of the study participants with *severe anemia* (48%) reported using a TDF-based treatment, and 62% of those categorized as having *mild anemia* reported using AZT-based treatment. (Table 2)

The mean CD4 cell count decreased with increase in anemia severity; for instance, individuals with no anemia had a mean CD4 cell count of 236.6 (201.2), and those with mild anemia had a mean CD4 cell count of 194 (145). Anemia was most prevalent among individuals at WHO stage II (Table 2).

CD4 cell count and Anemia

Mild anemia. Compared to individuals with high CD4 cell count, those with moderate CD4 cell count (>200 - ≤ 500 cells/uL) were 94% more likely to be mildly anemic (OR, 1.94, 95% CI: 1.28 - 2.93), and those with a low CD4 cell count had a 2-fold increase in odds of being mildly anemic (OR, 3.12, 95% CI: 2.18 – 4.47) (Table 2).

Moderate anemia. Compared to study participants with high CD4 cell count, those with moderate CD4 cell count had a 3-fold increase in odds of having moderate anemia (OR, 3.21, 95% CI: 1.60 – 3.65). Those with a low CD4 cell count had a 6-fold increase in odds of having moderate anemia (OR, 6.10, 95% CI: 2.21 – 16.78).

Severe anemia. Compared to those with a high CD4 cell count, a moderate CD4 cell count was insignificantly associated with increased odds of having severe anemia (OR: 1.92;

95% CI: 0.752 – 4.89), and those with a low CD4 cell count had a 3-fold increase in odds of having severe anemia (OR, 3.70, 95% CI: 1.48 – 9.26).

HIV clinical stages and Anemia

Mild anemia. Using study participants at WHO stage I as a reference group, those at WHO clinical stage II were 29% (OR: 1.29, 95% CI: 1.08 – 1.54) more likely to have mild anemia. WHO clinical stage III and IV were not statistically significantly associated with having mild anemia, the odds ratios are 0.93 (95% CI: 0.76 – 1.16) and 1.22 (95% CI: 0.88 – 1.71) respectively.

Moderate anemia. Compared to study participants in WHO stage I, those at WHO clinical stage 1I had increased odds of having moderate anemia (OR, 1.29, 95% CI: 0.94 – 2.07). Those at WHO clinical stage III and WHO stage IV had a 2-fold increase of having moderate anemia with odds ratios of 2.28 (95% CI: 1.53 – 3.38) and 2.16 (95% CI: 1.23 – 3.79), respectively.

Severe anemia. Compared to study participants in WHO stage I, those at WHO clinical stage II were 62% more likely to have severe anemia (OR, 1.62, 95% CI: 1.03 – 2.55), and those at WHO clinical stage III were at a 2-fold increase of having severe anemia (OR, 2.72, 95% CI: 1.73 – 4.29). Those at WHO clinical stage IV had a 3-fold increase of having severe anemia (OR, 3.16, 95% 1.75 – 5.70).

We examined the joint effect of CD4 cell count and HV on anemia(yes/no) and found that individuals with a lower CD4 cell count and at higher HIV clinical stages had increased odds of having anemia compared to those with a high CD4 cell count and at lower HIV-clinical stages (Figure 2).

Sensitivity Analysis

When we stratified the study participants per treatment received, we found that 64% of the study participants were receiving AZT-based treatment, and had relatively lower CD4 cell counts with a mean of 192 (138). On average, those receiving the AZT-based treatment were older (40yrs. ± 10.4) than those receiving TDF-based treatment (36yrs. ± 10). Most of the study participants receiving TDF-based treatment were at HIV clinical stage 1 (44%), while most of those receiving AZT-based treatment were at HIV clinical stage II (Table 3).

Results from the stratified analysis indicate a statistically significant trend of increasing anemia with increasing HIV clinical stage among individuals receiving TDF-based treatment only. Compared to those classified at WHO stage I, those at WHO stage II were 99% more likely to be anemic (OR, 1.99; 95% CI: 1.44 – 2.77), those at WHO stage III had a 3-fold increase of being anemic (OR, 3.17; 95% CI: 2.21 – 4.53), and those at WHO stage IV had a 4-fold increase (OR; 4.42; 95% CI: 2.68 – 7.29) compared to those receiving AZT-based regimen within the same stage.

CHAPTER 4

DISCUSSION

A. Primary Findings

In this study population, prevalence of anemia was 28.8%. Females had a significantly higher prevalence of anemia compared to males.

CD4 cell count. We found that HIV-infected patients with low CD4 cell count (< 200 cells/µl) had a significant increase in the odds of being anemic, compared to those with high CD4 cell count (> 500 cells/µl).

HIV clinical staging. In comparison to the WHO stage 1, study results suggest that patients at advanced HIV clinical stages (WHO stage IV) had increased odds of 1.22, 2.16 and 3.16 of having mild, moderate and severe anemia, respectively. This is in line with the study research hypothesis.

Joint effect of the two exposures. When we examined the joint effect of CD4 cell count and HIV clinical stage on anemia and found similar trends of the association. That is, those with lower CD4 cell counts and at higher HIV clinical stages had increased odds of having anemia compared to those with higher counts, at lower clinical stages.

The findings suggest that CD4 cell count may be predictive of early anemia onset (mild or moderate anemia), and HIV clinical stages may be more predictive of severe anemia. Additionally, study findings suggest that CD4 cell count is inversely associated with anemia, and HIV clinical stages are positively associated with anemia, which is in line with the research hypothesis.

Our study findings are consistent with results of Gunda et al. and Yin et al. who reported that anemia was more prevalent among patients in the most advanced stages of

HIV. And also, consistent with the results of Levine *et al.* and Volberding *et al.* who reported that more severe levels of anemia are found among HIV- infected individuals exhibiting low CD4 cell counts.

We found that for individuals receiving TDF-based treatment, the odds of being anemic increased with an increase in HIV clinical stage. Absence of a similar association among those receiving AZT-based treatment may be due to survivor bias. It is quite possible that individuals receiving this treatment are deceased, and therefore were not present to be selected for inclusion in the study. Possible explanations for increased risk for death in the *AZT-based treatment* subgroup are; 1) AZT-based treatments are recommended for women at childbearing age to prevent mother-to-child HIV transmission and, 2) one of AZT's known health side effects is anemia. Given that the female gender and AZT are established risk factors for anemia, the observed findings may be explained by differences in the characteristics of individuals receiving a particular treatment (AZT versus TDF). Therefore, interpretation of the finding that there is an increase in the odds of being anemic with increase in HIV clinical stage within the TDF-based treatment subgroup only, should be precautious and further investigated prospectively.

To our knowledge, this is the first study to assess the relationship between CD4 cell count, HIV clinical stage and anemia, and control for ART treatment combinations. This approach provides more precise measures compared to those shown in prior research and adds to the existing literature on the association between CD4 cell count, HIV clinical stage and anemia among HIV –infected adults.

B. Physiological Mechanisms

CD4 cell count. The physiologic association between CD4 cells and anemia among HIV-infected individuals can be explained through two basic mechanisms; 1) reduced red blood cell production, and 2) increased red blood cell destruction. First, CD4 cell destruction caused by HIV infection leads to a disruption in the levels of natural chemicals and normal functioning of the bone marrow, which in turn, reduces production of red blood cells and therefore may lead to anemia. Secondly, CD4 cell destruction leads to a faulty immune system where groups of disorders produce autoantibodies that attack the body's own red blood cells, thus increasing red blood cell destruction, which may lead to anemia.

HIV clinical staging. Blood loss and infection of the bone marrow may contribute to the physiologic relation between HIV-clinical stage and anemia. Neoplastic diseases in HIV infected individuals such as Kaposi's sarcoma, atypical mycobacteria, Hodgkin's lymphoma and non-Hodgkin's Lymphoma infect the bone marrow directly, or lead to gastrointestinal blood loss that in turn reduces the number of red blood cells in the body which may lead to anemia.

C. Study Strengths and Limitations

Non-Differential Misclassification of CD4 Cell Count

We obtained CD4 cell counts for all study participants using complete blood count tests performed at the central public health laboratory at Makerere University. Complete blood count test is standard for establishment of CD4 cell count.[32] Therefore, non-differential misclassification of CD4 cell count is unlikely to occur. If present, we would expect an underestimation of the relationship between CD4 cell count and anemia with bias towards the null.

Non-Differential Misclassification of HIV-Clinical Stage

For this study, HIV–clinical staging is based upon WHO HIV-clinical staging system. WHO HIV-clinical staging is widely used in low-resource settings.[33,31] However, the system relies on clinical manifestations of HIV, rather than laboratory confirmatory tests. Therefore, non-differential misclassification of exposure (HIV clinical stage) is highly likely. Bias due to non-differential misclassification will lead underestimation of the true OR with bias towards the null.

Selection bias

Selection bias would occur if anemic patients with low CD4 cell count and those at advanced stages of HIV were more likely to participate in the study. This would occur if such patients were aware of the study hypothesis, and were more inclined to participate in a study on this topic. Because participants were randomly selected from the ministry of health surveillance system (eliminating any form of self-selection into the study), selection bias is unlikely to occur.

Information Bias

Information bias could occur if one's anemia status influenced collection of information on exposure variables (CD4 cell count and HIV- clinical stage). It is highly unlikely that information bias occurred in this study because data from all participants (regardless of exposure status) was collected through routine medical visits, and captured by the ministry of health surveillance system, from which it was extracted for this study.

Non-Differential Misclassification of Anemia

Hemoglobin levels were obtained through blood tests performed at the central public health laboratory at Makerere University. Anemia definition was per WHO criteria

for hemoglobin concentration in the blood. Although hemoglobin concentration in blood is acceptable for anemia diagnosis,[34] it is considered insufficient.[35] More recently, it's been argued that evaluation of hemoglobin levels jointly with reticulocyte count, bilirubin and mean corpuscular volume is better for anemia diagnosis.[36] Therefore, non-differential misclassification of anemia is possible, causing an underestimation of the study results with a bias towards the null value. The magnitude of this effect may be moderate as hemoglobin concentrations are generally accepted standard for anemia diagnosis for HIV-infected individuals.[35]

Confounding

We did not have data on iron intake which is a potential confounder for the relationship between CD4 cell count and anemia. Due to iron's independent inverse relationship with both CD4 cell count and anemia, bias due to confounding is likely and may result into an overestimation of the study results. However, the impact of such bias on the study results is expected to be minor because nutritional habits (the major source of iron) may not vary greatly among individuals in this region.

Temporal sequence

Due to the cross-sectional nature of the study, information on the exposures (CD4 cell count and HIV–clinical stage) and outcome of interest (anemia) were collected at the same point in time. Therefore, we cannot ascertain whether the exposures led to anemia, or alternatively, if anemia influenced the exposures. This is particularly true for HIV clinical stage. For example, it is more likely that patients were categorized into HIV clinical stage III&IV because they exhibited anemia symptoms.

On the contrary, it is unlikely that anemia would cause a reduction in CD4 cell count considering the uniqueness of the study population (HIV-infected individuals). The biological mechanism through which the HIV-virus operates in the body may describe a causal pathway between CD4 cell and anemia. When the body is infected with HIV, the virus directly destroys CD4 cells. CD4 cell destruction results into a fragile immune system that adversely affects red blood cell count, likely leading to anemia.[39] Therefore, among HIV-infected individuals, it is highly unlikely that a low CD4 cell count may result from anemic conditions, and because no prior literature suggests such an association, we expect the impact of this bias to be minor.

For this cross-sectional study, a proper temporal sequence needed to ascertain causality between HIV –clinical stage and anemia will not be firmly established.

Survivor Bias

Survivor bias would occur if HIV- patients with very low CD4 cell count were more likely to die from severe anemic conditions, and therefore could not be included in the study sample. Similarly, if those with high HIV-clinical stages III&IV were more likely to die because of severe anemia. It is possible that our study results were underestimated due to this bias. The odds ratio of severely anemic patients (OR: 3.7) is lower than that of moderately anemic patients (OR: 6.10), this was not as expected and is likely due to survivor bias.

D. Conclusion

In HIV- infected individuals, a low CD4 cell count and advanced clinical stages are associated with anemia. Although our analyses cannot show whether this relationship is causal, our findings are consistent with prior studies that have assessed this relationship.

Consideration should be given to evaluating CD4 cell count, HIV clinical stage and anemia in a prospectively to address the issue of issue of survivor bias. From a clinical standpoint, the results suggest that patients with low CD4 cell count and those in advanced HIV clinical stages, should have regular hemoglobin follow up to identify anemia at its earliest stages.

Figure 1. Exclusion Criteria

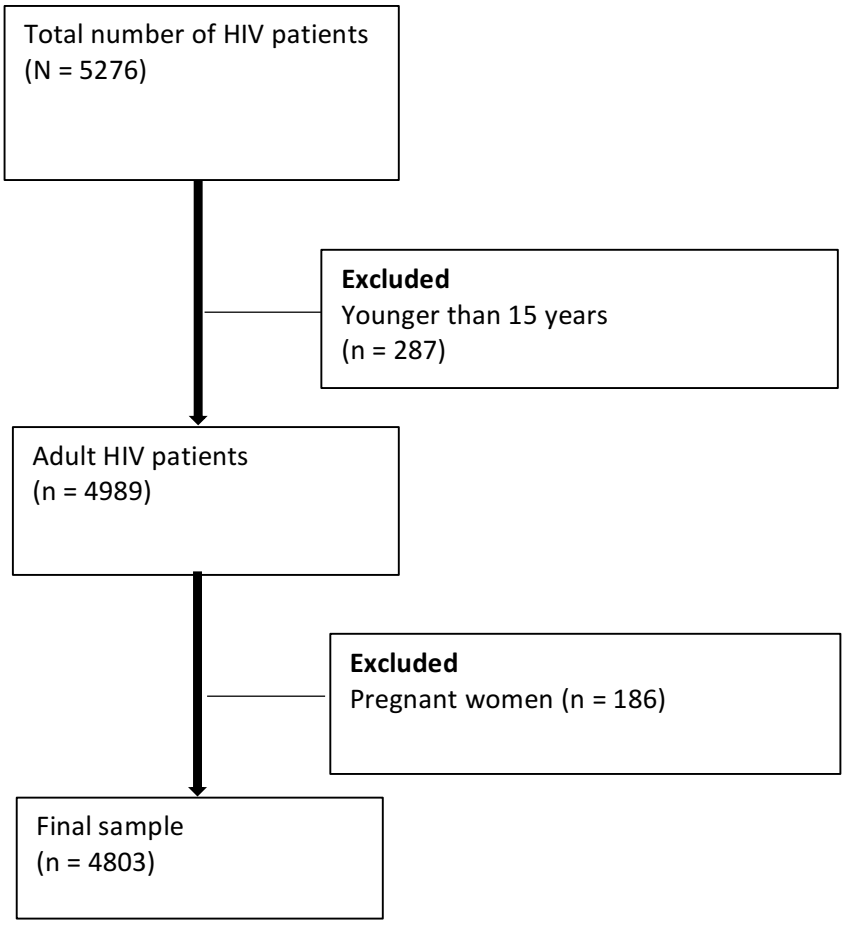


Table 1. Distribution of covariates per Anemia status among Ugandan HIV patients 2010-2015

Characteristics		Total N = 4803	%	Anemia status: No anemia 3471 (72.3%)	Mild anemia 941 (20.6%)	Moderate anemia 215 (4.5%)	Severe anemia 176 (3.7%)	p- value [a]
CD4 cell count *mean (SD)*		220 (188.9)		236.6 (201.2)	194.2 (145.0)	143.1(135.2)	147.1(142.2)	< 0.01
Age *mean (SD)*		38.9 (10.5)		38.7 (10.6)	39.5 (10.5)	39.9 (9.1)	39.7 (9.5)	< 0.01
HIV clinical stage	WHO stage I	1648	34.3%	1283 (35.9%)	287 (30.5%)	46 (21.4%)	32 (18.2%)	< 0.01
	WHO stage II	1727	35.9%	1211 (34.9%)	398 (42.3%)	65 (30.2%)	53 (30.1%)	
	WHO stage III	1133	23.6%	784 (22.6)	197 (20.9%)	83 (38.6%)	69 (39.2%)	
	WHO stage IV	295	6.1%	193 (5.6%)	59 (6.2%)	21 (9.8%)	22 (12.5%)	
Duration on HIV treatment	Less than 1 year	1262	32.9%	981 (38.0%)	180 (19.8%)	50 (25.2%)	51 (34.7%)	< 0.01
	Between 1 & 3 yea	1341	35.0%	803 (31.1%)	388 (42.6%)	86 (43.5%)	64 (43.5%)	
	Between 3 & 5 yea	688	17.9%	406 (15.7%)	223 (24.5%)	40 (20.2%)	19 (12.9%)	
	More than 5 years	545	14.2%	390 (15.1%)	120 (13.2%)	22 (11.1%)	13 (8.8%)	
Gender	Female	3135	65.3%	2190 (63.1%)	697 (74.1%)	147 (68.4%)	101 (57.4%)	< 0.01
	Male	1668	34.7%	1281 (36.9%)	244 (25.9%)	68 (31.6%)	75 (42.6%)	
Tuberculosis status	Tuberculosis	165	3.4%	119 (3.4%)	22 (2.3%)	11 (5.1%)	13 (7.4%)	< 0.01
	No Tuberculosis	4638	95.6%	3352 (96.6%)	919 (97.7%)	204 (94.9%)	163 (92.6%)	
ART combinations	AZT-3TC-NVP	2308	48.1%	1600 (46.1%)	591 (62.8%)	67 (31.2%)	50 (28.4%)	< 0.01
	AZT-3TC-EFV	560	11.7%	372 (10.7%)	151 (16.1%)	23 (10.7%)	14 (8.0%)	
	TDF-3TC-EFV	1589	33.1%	1262 (36.4%)	135 (14.4%)	106 (49.3%)	86 (48.9%)	
	Unknown	346	7.2%	237 (6.8%	64 (6.8%)	19 (8.8%)	26 (14.8%)	

[a] p-value derived from chi-square tests for categorical variables and ANOVA for continuous variables.
Abbreviations: AZT, Azido-thymidine; TDF, Tenofovir disoproxil

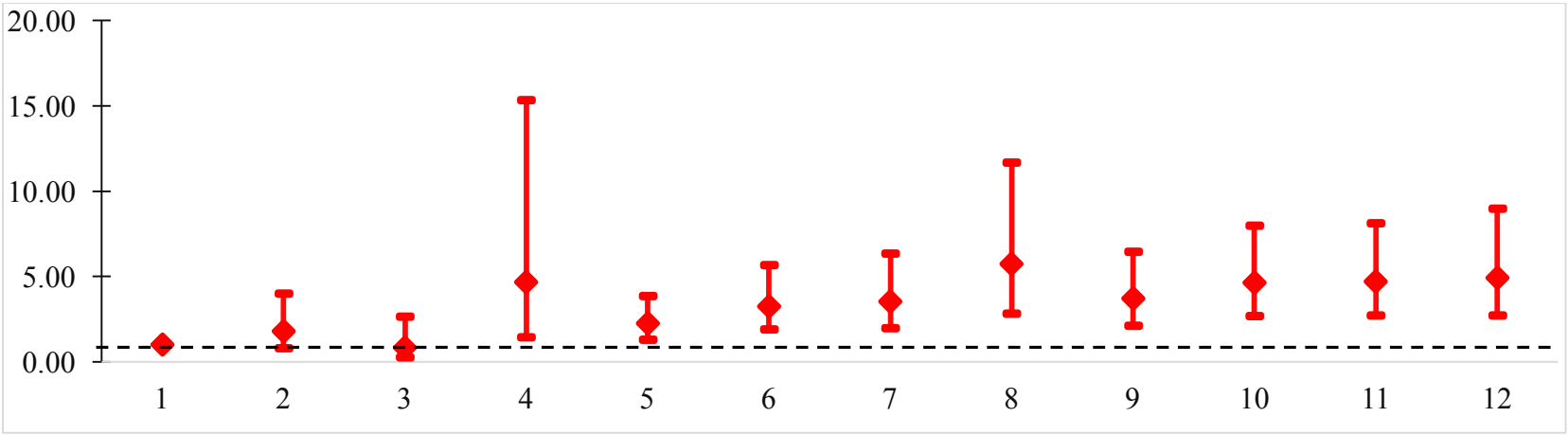
Table 2. Adjusted Odds Ratios and 95% confidence intervals for CD4 cell count, HIV clinical stage, and Anemia in HIV patients 2010-2015

		Mild anemia vs No Anemia (n = 941)		Moderate anemia vs No Anemia (n = 215)		Severe anemia vs No Anemia (n = 176)	
		OR	95% CI	OR	95% CI	OR	95% CI
CD4 cell counts							
High (> *500 cells/μL*)	37	1.00	referent	1.00	referent	1.00	referent
Moderate (*200 - ≤ 500 cells/μL*)	510	1.94	1.28 - 2.93	3.21	1.15 - 8.93	1.92	0.75- 4.89
Low (< *200 cells/μL*)	785	2.41	1.60 - 3.65	6.10	2.22- 16.77	3.70	1.48 - 9.26
p-trend			< 0.001		< 0.001		< 0.001
WHO HIV - clinical staging							
WHO stage I	365	1.00	referent	1.00	referent	1.00	referent
WHO stage II	516	1.29	1.08 - 1.54	1.39	0.94 - 2.07	1.62	1.03 - 2.55
WHO stage III	349	0.93	0.76 - 1.16	2.28	1.53 - 3.38	2.72	1.73 - 4.29
WHO stage IV	102	1.22	0.88 - 1.71	2.16	1.23 - 3.79	3.16	1.75 - 5.70
p-trend			< 0.001		< 0.001		< 0.001

Model adjusted for age, gender and antiretroviral therapy combinations

Abbreviations: CI, Confidence Interval; WHO, World Health Organization

Figure 2. Odds Ratios and 95% Confidence Intervals of the joint effect of CD4 cell count and Anemia (yes/no)



[1]Figure

1. High CD4 cell count and WHO Stage I ***(n = 163)***
2. High CD4 cell count and WHO Stage II ***(n =77)***
3. High CD4 cell count and WHO Stage III ***(n = 46)***
4. High CD4 cell count and WHO Stage IV ***(n = 16)***
5. Moderate CD4 cell count and WHO Stage 1 (***n = 947)***
6. Moderate CD4 cell count and WHO Stage II ***(n = 751)***
7. Moderate CD4 cell count and WHO Stage III ***(n = 311)***
8. Moderate CD4 cell count and WHO Stage IV ***(n = 73)***
9. low CD4 cell count and WHO Stage I ***(n =538)***
10. low CD4 cell count and WHO Stage II ***(n = 899)***
11. low CD4 cell count and WHO Stage III **(n = 776)**
12. low CD4 cell count and WHO Stage IV ***(n = 206)***

[1] Study participants were classified into 12 groups as shown above.

Table 3. Distribution of characteristics of study participants for each treatment combination

Characteristics	**TDF - based treatment**	**AZT - based treatment**
Number of study participants	1589	2868
Age *mean (SD)*	36.7 (10)	40.2 (10.4)
CD4 cell count *mean (SD)*	286 (246.9)	191.68 (137.7)
Duration on ART treatment (yrs.)	1.7 (0.8)	2.3 (1.02)
Gender (Female)	65%	64%
HIV clinical stage		
WHO stage I	700 (44%)	870 (30%)
WHO stage II	484 (30%)	1126 (40%)
WHO stage III	309 (19%)	709 (25%)
WHO stage IV	96 (6%)	163 (9%)

Table 4. Odds Ratios and 95% Confidence Intervals for HIV clinical stage and Anemia (yes/no) in HIV patients 2010-2015 stratified by ART treatment therapy.

			Adjusted*	
	non-cases	anemia cases	OR	95% CI
AZT-based treatment (n = 2868)				
WHO stage I	614	256	1.00	referent
WHO stage II	747	379	1.23	1.02 - 1.50
WHO stage III	498	211	1.04	0.83 - 1.30
WHO stage IV	113	50	1.09	0.76 - 1.58
TDF - based treatment (n = 1589)				
WHO stage I	621	79	1.00	referent
WHO stage II	380	104	1.99	1.44 - 2.78
WHO stage III	204	105	3.17	2.21 - 4.54
WHO stage IV	57	39	4.42	2.68 - 7.30

Abbreviations: AZT, Azido-thymidine; TDF, Tenofovir disoproxil

*Adjusted for Gender and CD4 cell count

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