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# HIV-Associated Anemia After 96 Weeks on Therapy: Determinants Across Age Ranges in Uganda and Zimbabwe

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## Abstract

Given the detrimental effects of HIV-associated anemia on morbidity, we determined factors associated with anemia after 96 weeks of antiretroviral therapy (ART) across age groups. An HIV-positive cohort ($n=3,580$) of children age 5–14, reproductive age adults 18–49, and older adults $\geq 50$ from two randomized trials in Uganda and Zimbabwe were evaluated from initiation of therapy through 96 weeks. We conducted logistic and multinomial regression to evaluate common and differential determinants for anemia at 96 weeks on therapy. Prior to initiation of ART, the prevalence of anemia (age 5–11 $<10.5$ g/dl, 12–14 $<11$ g/dl, adult females $<11$ g/dl, adult males $<12$ g/dl) was 43%, which decreased to 13% at week 96 ($p<0.001$). Older adults had a significantly higher likelihood of anemia compared to reproductive age adults (OR 2.60, 95% CI 1.44–4.70, $p=0.002$). Reproductive age females had a significantly higher odds of anemia compared to men at week 96 (OR 2.56, 95% CI 1.92–3.40, $p<0.001$), and particularly a greater odds for microcytic anemia compared to males in the same age group ($p=0.001$). Other common factors associated with anemia included low body mass index (BMI) and microcytosis; greater increases in CD4 count to week 96 were protective. Thus, while ART significantly reduced the prevalence of anemia at 96 weeks, 13% of the population continued to be anemic. Specific groups, such as reproductive age females and older adults, have a greater odds of anemia and may guide clinicians to pursue further evaluation and management.

## Introduction

Anemia is estimated to affect 1.62 billion people, or almost a quarter of the world's population.[1] It is a common complication of HIV infection, with etiologies including HIV infection itself, gastrointestinal and menstrual blood loss, opportunistic infections, neoplasms, medications, and malnutrition.[2–4] In sub-Saharan Africa, conditions including malaria, hookworm, tuberculosis, and hemoglobinopathies also contribute to anemia.[2] HIV-associated anemia has considerable consequences, including a worse quality of life and increased mortality, independent of markers such as CD4 or viral load.[2,5–7]

Antiretroviral therapy (ART) reduces the prevalence of HIV-related anemia.[8–11] However, studies suggest over 30% prevalence of anemia even while on ART, and it continues to predict mortality and poor quality of life.[2,4,8–10,12] The studies have been largely from developed countries and have focused on early determinants of anemia, primarily among individuals of reproductive age. With greater access to ART in sub-Saharan Africa, the HIV population has a wider age range and has been on therapy for several years. As HIV-infected individuals transition through life stages, it is important to understand the similarities and differences of risk factors for anemia. Utilizing two large pediatric and adult trials in Uganda and Zimbabwe, we investigated the prevalence and risk factors for HIV-related anemia after 96 weeks on ART, among children 5–14, reproductive age adults, and older adults greater than 50 years old.

## Materials and Methods

Anemia was evaluated among participants in the Delivery of Antiretroviral Therapy in Africa (DART) (ISRCTN 13968779) and Antiretroviral Research for Watoto (ARROW) (ISRCTN 24791884) trials in three sites in Uganda and one in Zimbabwe.[13,14] The primary aim of these trials was a randomized comparison of clinically driven monitoring (CDM)

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to laboratory with clinical monitoring (LCM) of ART. The primary efficacy outcome was new nonrecurrent WHO stage 4 clinical events and/or death; the primary toxicity outcome was serious adverse events (SAEs) in DART and Grade 3/4 AEs in ARROW. Neither trial found significant differences in toxicity outcomes between CDM and LCM,[13,14] so data from both randomized groups were pooled for this analysis.

We sought to investigate anemia after 96 weeks on therapy, and therefore included DART and ARROW participants who were alive and in follow-up 96 weeks after ART initiation. Participants were ART naive prior to enrollment, except for past exposure to prevent mother-to-child transmission (PMTCT) of HIV. For the DART study, adults (18 years or older) were eligible if they had a CD4 count $<200$ cells/mm$^3$ and WHO stage 2 or greater. For the ARROW study, children 5–14 were eligible if they met WHO 2006 pediatric guidelines for ART (CD4 $<200$ cells/mm$^3$ and/or WHO stage 3 or 4 disease).[15] Younger children face unique factors for anemia including early childhood infections, PMTCT exposure, and birth complications that we were unable to compare across groups, and thus were excluded from the analysis. Exclusion criteria in both trials included signs of an acute infection, contraindications to take ART due to concomitant medications or abnormal laboratory values, or pregnancy/breastfeeding in women.

After enrollment, participants were initiated on ART. In 2003–2004, DART participants received zidovudine/lamivudine plus tenofovir, abacavir, or nevirapine, with 600 participants randomized to abacavir vs. nevirapine.[16] From 2007–2008, ARROW participants received one of two regimens: abacavir/lamivudine plus a nonnucleoside reverse transcriptase inhibitor (NNRTI, nevirapine or efavirenz) or a four-drug regimen of abacavir/lamivudine, zidovudine, and an NNRTI. After 36 weeks, ART regimens of those in the four-drug regimen were reduced to three drugs, removing either zidovudine or the NNRTI, as per the ARROW trial protocol.[14]

Follow-up included symptom assessment every 4–6 weeks and clinical examination and lymphocytic (CD4/CD8) and hematological testing every 12 weeks. Whereas results from LCM participants were given to clinicians, results for CDM participants were given upon request to inform clinical management (except for CD4 count, which was never returned) or if there was grade 4 toxicity that had not already been requested (protocol safety criteria).[13,14]

Anemia in this analysis was defined according to WHO guidelines, adjusted for African ethnicity.[17] For adults 15 years of age or older, anemia was defined as hemoglobin $<12.0$ g/dl in males and $<11.0$ g/dl in females. Children 5–11 years were considered anemic if they had a hemoglobin $<10.5$ g/dl, and for children 12–14 years, anemia was a hemoglobin $<11.0$ g/dl. Mild anemia was defined as 10.0–11.9 g/dl in male and 10.0–10.9 g/dl in female adults 15 years and older, 10–10.4 g/dl in children 5–11 years, and 10–10.9 g/dl in children 12–14 years. Moderate anemia was defined as 7.0–9.9 g/dl, and severe anemia was less than 7.0 g/dl, in all ages. Microcytosis in adults was defined as a mean corpuscular volume (MCV) less than 80 fl and macrocytosis if greater than 100 fl. For children ages 5–11 years, the normal MCV range was 70–92 fl, and for children 12–14 the MCV normal range was 76–102 fl. Microcytic, normocytic, or macrocytic anemia was defined as anemia with microcytosis, normocytosis, or macrocytosis, respectively. To assess nutritional status, low body mass index (BMI) was defined as a BMI $<18.5$ kg/m$^2$ for adults, whereas for children, it was defined as a BMI-for-age $Z$ score (UK) of $\leq 2$.

The cohort was stratified into three age groups for analysis: children ages 5–14 years, reproductive age adults 18–49 years, and older adults greater than 50 years. Wilcoxon signed-rank test and McNemar's test were used to compare pre-ART and week 96 variables. Therefore, by definition, analysis included those with follow-up at 96 weeks [639 of 667 (96%) in ARROW age $<14$, and 3,006 of 3,316 (91%) in DART]. Of these individuals, hemoglobin measurements were available for 635 (99%) in ARROW and 2,945 (98%) in DART. Simple and multiple logistic regression and multinomial logistic regression were used to identify risk factors for anemia at week 96 from pre-ART and week 96 variables. Variables were included in the multiple logistic models if the $p$-value was $<0.20$ on univariate testing. Heterogeneity of risk factors between age groups was evaluated by assessing the significance ($p<0.05$) of the interaction term between age group and the given risk factor. Hosmer–Lemeshow goodness of fit testing with 10 quantiles was used to evaluate the final model.

The DART and ARROW studies received ethical approval from their respective institutions in Uganda, Zimbabwe, and the United Kingdom. This substudy analysis was also reviewed and approved by the Institutional Review Board of the Joint Clinical Research Centre (JCRC) and the Uganda National Council for Science and Technology (UNCST).

## Results

A total of 3,580 HIV-infected children and adults were included in the analysis, of whom 635 (18%) were children aged 5–14 years, 2,750 (77%) adults aged 18–49 years, and 195 (5%) adults older than 50 years. Characteristics at initiation of ART and week 96 are summarized in Table 1. CD4 count improved significantly in all age groups, from an overall median of 98 cells/mm$^3$ pre-ART to 271 cells/mm$^3$ at week 96 ($p<0.001$), as did nutritional status with an overall reduction in those subjects with low BMI from 17% to 5% ($p<0.001$). Prior to initiation of ART, 1,527 individuals (43%) had anemia, including 217 (34%) children, 1,228 (45%) reproductive age adults, and 82 (42%) older adults. Among those with anemia, 451 (30%) had microcytic anemia, 1,009 (66%) had normocytic anemia, and 67 (4%) had macrocytic anemia. At 96 weeks, there was a significant reduction in the prevalence of anemia to 482 cases (13%), including 44 children (7%), 407 (15%) reproductive age adults, and 31 (16%) older adults ($p<0.001$ for all groups and overall).

Among those with anemia at 96 weeks, 56 cases (12%) were microcytic, 250 (52%) were normocytic, and 174 (36%) were macrocytic. Severe anemia ($<7$ g/dl) was rare, with 2 cases pre-ART initiation and 11 cases at week 96 ($<1\%$). Iron use was minimal in this cohort, with 24 individuals ($<1\%$) taking iron supplements between weeks 94 and 98. Similarly, there were limited cases of malaria [clinical or blood slide confirmed, $n=28$ (1%)], acute febrile illness ($n=2$, $<1\%$), or WHO stage 3/4 events ($n=14$, $<1\%$) between weeks 94 and 98. WHO 3/4 events included HIV

Table 1. Comparison of Pre-Antiretroviral Therapy and Week 96 Characteristics (N = 3,580)

| *Characteristic N (%) or median (IQR)* | *Age 5–14* (n = *635*) | | *Age 18–49* (n = *2750*) | | *Age 50+* (n = *195*) | | *Total* | |
|---|---|---|---|---|---|---|---|---|
| | *Pre-ART* | *Week 96* | *Pre-ART* | *Week 96* | *Pre-ART* | *Week 96* | *Pre-ART* | *Week 96* |
| Female | 218 (50) | | 1,815 (66) | | 101 (52) | | 2234 (62) | |
| BMI (kg/$m^2$) | 15.0 (14.1, 15.8) | 16.0 (15.2, 17.3)[a] | 21.2 (19.2, 23.5) | 23.5 (21.1, 26.4)[a] | 22.1 (19.8, 25.0) | 23.8 (21.2, 26.6)[a] | 20.5 (17.7, 23.1) | 22.5 (19.4, 25.7)[a] |
| Low BMI | 99 (16) | 13 (2)[a] | 469 (17) | 139 (5)[a] | 23 (12) | 9 (5)[b] | 591 (17) | 161 (5) |
| CD4 (cells/$mm^3$) | 251 (95, 392) | 630 (444, 916)[a] | 87 (32, 140) | 243 (170, 327)[a] | 96 (47, 147) | 231 (164, 327)[a] | 98 (38, 159) | 271 (182, 392)[a] |
| 350+ | 189 (30) | 530 (84) | | 558 (20) | | 38 (16) | 189 (5) | 1,126 (32) |
| 200–349 | 181 (29) | 60 (9) | | 1231 (45) | | 84 (43) | 181 (5) | 1,375 (39) |
| 100–199 | 105 (17) | 22 (3) | 1188 (43) | 688 (25) | 92 (47) | 54 (28) | 1,385 (39) | 764 (21) |
| 0–99 | 160 (25) | 21 (3) | 1562 (57) | 262 (10) | 103 (53) | 18 (9) | 1,825 (51) | 301 (8) |
| Hemoglobin (g/dl) | 11.1 (10.2, 11.9) | 12.2 (11.4, 12.9)[a] | 11.4 (10.3, 12.7) | 12.8 (11.8, 13.9)[a] | 11.6 (10.6, 12.7) | 12.9 (11.9, 13.9)[a] | 11.4 (10.3, 12.5) | 12.6 (11.7, 13.7)[a] |
| MCV (fl) | 80.0 (75.0, 83.8) | 89.1 (84.0, 94.7)[a] | 85.5 (80.0, 91.0) | 101.0 (94.0, 108.0)[a] | 86.4 (83.0, 92.0) | 103.0 (96.0, 110.0)[a] | 84.5 (79.0, 90.0) | 99.0 (91.0, 107.0)[a] |
| Microcytosis | 56 (9) | 10 (2) | 691 (25) | 122 (4) | 34 (17) | 8 (4) | 781 (22) | 140 (4) |
| Normocytosis | 565 (89) | 418 (66) | 1,931 (70) | 1,195 (44) | 148 (76) | 67 (34) | 2,644 (74) | 1,680 (47) |
| Macrocytosis | 14 (2) | 207 (33) | 128 (5) | 1,431 (52) | 13 (7) | 120 (62) | 155 (4) | 1,758 (49) |
| Anemic | 217 (34) | 44 (7)[a] | 1,228 (45) | 407 (15)[a] | 82 (42) | 31 (16)[a] | 1,527 (43) | 482 (13)[a] |
| Mild | 80 (13) | 23 (4) | 639 (23) | 249 (9) | 53 (27) | 22 (11) | 772 (22) | 294 (8) |
| Moderate | 137 (22) | 21 (3) | 587 (21) | 148 (5) | 29 (15) | 8 (4) | 753 (21) | 177 (5) |
| Severe | 0 | 0 | 2 (<1) | 10 (<1) | 0 | 1 (<1) | 2 (<1) | 11 (<1) |
| Zidovudine | 437 (69) | 210 (33)[a] | 2,750 (100) | 2,416 (88)[a] | 195 (100) | 168 (86)[a] | 3,382 (95) | 2,794 (78)[a] |
| Cotrimoxazole | 617 (97) | 590 (93)[b] | 1,743 (63) | 1,551 (56)[a] | 109 (56) | 110 (56) | 2,469 (69) | 2,251 (63)[a] |
| Multivitamins | 364 (57) | 399 (63)[a] | 0 | 45 (2)[a] | 0 | 3 (2) | 364 (10) | 447 (12)[a] |

[a]Difference between week 96 and pre-ART $p < 0.001$.
[b]Difference between week 96 and pre-ART $p = 0.001$.
Pairwise comparison using Wilcoxon signed-rank test and McNemar's test for categorical and continuous variables, respectively.
ART, antiretroviral therapy; BMI, body mass index; MCV, mean corpuscular volume.

Table 2. Multiple Logistic Regression for Risk Factors of Anemia at Week 96

| | OR | 95% CI | p-value |
|---|---|---|---|
| Age group | | | |
| Child | 1.91 | 1.01, 3.62 | 0.05 |
| Reproductive age | 1.00 | | |
| Older adult | 2.60 | 1.44, 4.70 | 0.002 |
| Female | | | |
| Female and child | 0.89 | 0.46, 1.70 | 0.72 |
| Female and reproductive age | 2.40 | 1.82, 3.18 | < 0.001 |
| Female and older age | 0.66 | 0.29, 1.53 | 0.34 |
| Prior to initiation of ART | | | |
| Low baseline BMI | 0.96 | 0.71, 1.29 | 0.78 |
| CD4 (cells/mm$^3$) | | | |
| 350+ | 0.50 | 0.22, 1.16 | 0.11 |
| 200–349 | 0.79 | 0.36, 1.71 | 0.54 |
| 100–199 | 1.00 | | |
| 0–99 | 0.81 | 0.65, 1.01 | 0.06 |
| Anemia pre-ART | 3.30 | 2.65, 4.11 | < 0.001 |
| Microcytosis | 1.51 | 1.18, 1.92 | 0.001 |
| Week 96 | | | |
| Low BMI | 3.47 | 2.25, 5.34 | < 0.001 |
| CD4 Change (per 100 cells/mm$^3$) from baseline | 0.87 | 0.81, 0.94 | < 0.001 |
| Microcytosis | 2.76 | 1.84, 4.15 | < 0.001 |
| Zidovudine use[a] | 1.10 | 0.81, 150 | 0.52 |
| Cotrimoxazole Use[a] | 0.91 | 0.73, 1.14 | 0.42 |
| WHO stage 3/4 event[a] | 1.80 | 0.50, 6.51 | 0.37 |

[a]Defined if taken or occurred between weeks 94 and 98.
Hosmer–Lemeshow goodness of fit $p = 0.27$.

wasting, cryptococcosis, cryptosporidiosis, esophageal candidiasis, and extrapulmonary TB.

Univariate testing was conducted to suggest possible risk factors for anemia after 96 weeks on therapy (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/aid). Prior to initiation of ART, female gender, a low BMI, anemia at initiation, and microcytosis were associated with anemia, while higher CD4 counts protected against the development of anemia (Supplementary Table S1). Characteristics at week 96 associated with anemia included low BMI, microcytosis, zidovudine use, pregnancy, and having a WHO stage 3/4 event. Cotrimoxazole use and higher pre-ART CD4 were associated with lower odds of anemia.

In the multiple logistic model (Tables 2 and 3), older adults had greater than twice the odds of anemia compared to reproductive age individuals (OR 2.63, 95% CI 1.45–4.78, $p = 0.001$). There was significant heterogeneity of the impact of gender ($p < 0.001$), with females in the reproductive age group having an increased likelihood of anemia (OR versus males 2.56, 95% CI 1.92–3.40, $p < 0.001$), whereas odds were similar in males and females in the other groups. While low BMI at initiation of ART was not a significant risk factor, individuals with a low BMI at week 96 had over three times the likelihood of anemia (OR 3.47, 95% CI 2.25–5.34, $p < 0.001$). There appeared to be increased odds in adults compared to children, but heterogeneity was not statistically significant ($p = 0.42$). Greater CD4 increases from pre-ART baseline were associated with reduced likelihood of anemia (OR 0.87 per every 100 cells/mm$^3$ increase from week 0 to 96, 95% CI 0.81–0.94, $p < 0.001$), and the effect was greatest among reproductive age adults (heterogeneity $p = 0.002$). Zidovudine use did not have a significant effect overall, but odds of anemia appeared to be marginally increased among

Table 3. Multiple Logistic Regression by Age Group for Risk Factors of Anemia at Week 96

| | Children | | | Reproductive age | | | Older adults | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | p-value | OR | 95% CI | p-value | OR | 95% CI | p-value | p value[a] |
| Female | 0.81 | 0.42, 1.54 | 0.51 | 2.56 | 1.92, 3.40 | < 0.001 | 0.74 | 0.30, 1.79 | 0.50 | < 0.001 |
| Prior to initiation of ART | | | | | | | | | | |
| Low baseline BMI | 0.61 | 0.22, 1.70 | 0.35 | 1.03 | 0.74, 1.43 | 0.85 | 0.94 | 0.26, 3.40 | 0.93 | 0.47 |
| CD4 (cells/mm$^3$) | | | | | | | | | | 0.89 |
| 350+ | 0.55 | 0.20, 1.50 | 0.24 | | | | | | | |
| 200–349 | 0.81 | 0.31, 2.11 | 0.67 | | | | | | | |
| 100–199 | 1.00 | | | 1.00 | | | 1.00 | | | |
| 0–99 | 1.07 | 0.41, 2.77 | 0.90 | 0.80 | 0.63, 1.02 | 0.07 | 1.03 | 0.43, 2.45 | 0.95 | |
| Anemia pre-ART | 1.57 | 0.81, 3.06 | 0.18 | 3.64 | 2.85, 4.64 | < 0.001 | 3.68 | 1.46, 9.29 | 0.01 | 0.13 |
| Microcytosis | 2.29 | 0.94, 5.57 | 0.07 | 1.47 | 1.13, 1.90 | 0.004 | 1.41 | 0.44, 4.56 | 0.56 | 0.55 |
| Week 96 | | | | | | | | | | |
| Low BMI | 1.15 | 0.13, 10.6 | 0.90 | 3.39 | 2.14, 5.36 | < 0.001 | 5.84 | 0.95, 36.08 | 0.06 | 0.42 |
| CD4 change (per 100 cells/mm$^3$) from baseline | 0.95 | 0.90, 1.00 | 0.05 | 0.76 | 0.68, 0.84 | < 0.001 | 0.94 | 0.64, 1.37 | 0.73 | 0.002 |
| Microcytosis | 4.04 | 0.79, 20.53 | 0.09 | 2.77 | 1.79, 4.28 | < 0.001 | 1.40 | 0.18, 11.06 | 0.75 | 0.54 |
| Zidovudine use[b] | 1.91 | 1.00–3.63 | 0.05 | 0.87 | 0.62, 1.23 | 0.43 | 3.34 | 0.62, 18.24 | 0.16 | 0.06 |
| Cotrimoxazole Use[b] | 0.95 | 0.28, 3.28 | 0.94 | 0.90 | 0.71, 1.14 | 0.39 | 0.63 | 0.26, 1.53 | 0.31 | 0.88 |

[a]Heterogeneity $p$-value evaluating the interaction between the risk factor and age group.
[b]Defined if taken or occurred between weeks 94 and 98.
Hosmer–Lemeshow goodness of fit (children $p = 0.60$, reproductive $p = 0.20$, older $p = 0.62$).

Table 4. Multinomial Logistic Regression on the Effects of Gender and Age on Anemia Subtype

| | *Microcytic anemia (56 cases)* | | | *Normocytic anemia (250 cases)* | | | *Macrocytic anemia (174 cases)* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *OR*[a] | *95% CI* | p-*value* | *OR* | *95% CI* | p-*value* | *OR* | *95% CI* | p-*value* |
| Child female vs. male | 0.44 | 0.04, 4.96 | 0.51 | 0.85 | 0.41, 1.77 | 0.67 | 0.57 | 0.13, 2.42 | 0.45 |
| Reproductive age female vs. male | 33.27 | 4.54, 243.72 | 0.001 | 3.25 | 2.22, 4.77 | < 0.001 | 1.79 | 1.22, 2.64 | 0.003 |
| Older female vs. male[b] | | | | 0.76 | 0.18, 3.10 | 0.70 | 0.51 | 0.17, 1.48 | 0.22 |

[a]Adjusted for CD4 category and low BMI at week 96.
[b]Only two older women (0 men) had microcytic anemia at week 96.

children ($p = 0.05$). There was no effect of cotrimoxazole on anemia overall or in any subgroup ($p = 0.42$).

Given the interaction between gender and age group, we explored the impact of age and gender on subtypes of anemia at week 96 (Table 4). Multinomial logistic regression found that compared to males in the same age strata, reproductive age females had significantly higher odds of microcytic anemia (OR 33.27, 95% CI 4.54–243.72, $p = 0.001$), adjusted for low BMI and CD4. They also had a significantly greater odds of both normocytic ($p < 0.001$) and macrocytic ($p = 0.003$) anemia compared to reproductive age males. There were no other significant differences between males and females on subtype of anemia among children or older adults. In a separate multivariable logistic regression analysis among women of reproductive age, we also found that current pregnancy was associated with a significant 2.5 times greater odds of anemia at week 96 (data not shown).

## Discussion

HIV-associated anemia is a multifactorial disease that continues to complicate care despite greater access to therapy. By exploring the burden and risk factors for HIV-associated anemia after 96 weeks on therapy, we evaluated the similarities and differences across different life stages to illuminate potential relationships that can guide evaluation and management of HIV-positive patients in resource-limited settings similar to Uganda and Zimbabwe.

Prior to starting therapy, there was a high prevalence of anemia at 43%. This is consistent with other studies in sub-Saharan Africa that demonstrate a pre-ART prevalence of anemia of 63–77.4%.[8,11] This reflects low-grade anemia, with only two cases of severe anemia at initiation. After 96 weeks on ART, the prevalence significantly decreased to 13%, and all age groups had increases in hemoglobin. This is similar to HIV-negative individuals in related settings; a study in Rwanda found that HIV-negative women had an anemia prevalence of 8%.[18] Given there are an estimated 767,292 individuals currently on therapy in Uganda and Zimbabwe, 13% still represents almost 100,000 individuals and a continued burden among this population.[19]

In evaluating the possible factors associated with anemia, there were few acute clinical events that occurred around week 96, including malaria, WHO stage 3 or 4 events, or acute febrile illness. Older individuals had over twice the odds of anemia compared to reproductive age individuals, adjusted for extent of HIV infection, nutritional status, and therapy. In sub-Saharan Africa, there are an estimated 3 million individuals greater than 50 years old living with HIV/AIDS.[20] HIV infection and/or the effects of therapy may accelerate the onset of comorbidities associated with aging.[21] Regardless of HIV infection, older individuals also have an increased risk of anemia, as a consequence of other comorbidities, increased inflammation, and reduced erythropoietic function.[22] In our sample, older adults represented only 5% of the sample, and thus definitive conclusions are limited. However, with a rising number of HIV-infected individuals entering into older ages, it is important to understand that they face a greater likelihood of anemia while on therapy that may guide further management by practitioners.

HIV infection can promote malnutrition via opportunistic infections and chronic inflammation, while malnutrition can dysregulate immune function.[23] We found that independent of HIV disease extent, low BMI was associated with over three times the odds of anemia at week 96, especially among adults. While there was no significant effect within the pediatric subgroup, the power to detect effects within the different subgroups was limited. Moreover, microcytosis prior to ART initiation and at week 96 was associated with anemia among children, and may reflect a more child-specific mechanism of malnutrition through iron deficiency. Several studies show a high burden of iron deficiency anemia in HIV-infected children, with benefits after supplementation.[24–26]

The association of hemoglobin, CD4, and progression of HIV infection is well established.[10,11,27,28] Consistent with these findings, we found lower odds of anemia in those with higher increases in CD4 counts from pre-ART baseline. Surprisingly, we found that those with pre-ART CD4 less than 100 cells/mm$^3$ also had a marginal level of protection. This could be due to a survival bias; those who had a low CD4 count and survived to 96 weeks may have had a good response to ART, whereas those with low CD4 count who died in the first 96 weeks were not included in our analysis by design. This is supported by another substudy from the DART and ARROW trials, which found mortality rates of 3.3% of children and 5.4% of adults in the first year after initiation of therapy, and demonstrated that low hemoglobin levels were an independent predictor of mortality early after initiation of therapy.[29] Hemoglobin has been suggested as a marker of virological failure,[30,31] and thus patients with anemia may need to be closely evaluated for signs of treatment failure.

While zidovudine-based regimens are associated with an overall increase in hemoglobin levels,[28,32] macrocytic anemia is a well-known complication.[8,33] This is reflected in the large increase in prevalence of macrocytosis, from 4% to 49% overall. Although we did not find an overall effect of zidovudine use, children on zidovudine had a marginally increased association with anemia. However, it is important to note that children did not have episodes of severe anemia,[14] and therefore any impact of zidovudine is restricted to low-grade anemia. In addition, recent WHO treatment guidelines do not recommend zidovudine in first line therapy for adults, and thus future studies may demonstrate a further reduction in anemia at 96 weeks of therapy with newer regimens.[34]

In comparing the association of anemia across age groups, we found a large and specific effect among women of reproductive age. Several cohorts have found HIV-infected women have a greater risk of anemia compared to both HIV-negative women and HIV-positive men, and anemia is an independent predictor of mortality.[7,10,35–38] The cause of this increased risk is likely due to menstruation and pregnancy, associated with blood loss and greater micronutrient requirements including iron.[39–41] Consistent with this, we found that reproductive age women, but not females in the other age groups, had a significantly increased association of microcytic anemia compared to males. Women also had increased odds of normocytic anemia, which may reflect blood loss or a combination effect of microcytic and macrocytic anemia from zidovudine and/or cotrimoxazole use. Increased odds of macrocytic anemia in this age range may be due to differences in adherence,[42] but warrants further investigation. As HIV-infected girls enter into adulthood, they enter a specific period where they have a significantly greater likelihood of anemia and may benefit from micronutrient supplementation.[43] At the same time, there is evidence that HIV-infected women enter menopause at an earlier age,[44] when these effects would be reduced and other factors of older age may take precedence as a basis for anemia.

There are several advantages to our study in contributing to our understanding of HIV-associated anemia. Studies on this topic have been conducted largely in developed countries, or have been in adults or pregnant women. In our analysis, we have included a large sample from sub-Saharan Africa, encompassing a wide age range to allow for stratification and comparison of groups. The period of 96 weeks was chosen primarily because past studies have focused on the first year on therapy. However, several studies have shown that there may be initial effects from therapy or continued effects from HIV infection.[5,8,29] We sought to describe factors associated with anemia at a later time point to reflect a population in a resource-limited setting that had survived and had been on therapy for a prolonged period.

At the same time, there were limitations to our analysis that suggest areas for further exploration. We would have benefited from greater etiological testing, including red blood cell indices, iron studies, assessment for helminthic infection diagnosis, and evaluation of hemoglobinopathies. We also did not have HIV-negative cases that would have allowed us to compare the 13% prevalence of anemia to the background population. While the cohort we created deliberately included those who were alive at week 96, this excluded those who died prior to this period; results are therefore generalizable primarily to a population that responds to ART and does not experience early mortality.

The cohort included individuals who had advanced disease; although recent guidelines have expanded to those with CD4 $\leq 500$ cell/mm$^3$,[34] experience in high-income countries suggests that late presenters will continue to contribute a large minority.[45] Though severe anemia may have been a more specific outcome, this represented only a small percentage of our sample and power was too low to attempt this. While we benefited from data from two large randomized trials conducted by similar trials groups, there are limitations in pooled analysis, including differences in the period of evaluation, staff, and therapy given between the two studies. However, both trials recruited adults and children from identical centers in Uganda and Zimbabwe, so underlying populations should have been similar. While there may be differences between geographic sites, inclusion criteria and overall care were standardized among all centers.

While ART improves anemia status in HIV-infected individuals in resource-limited settings, there are other factors that contribute to anemia across age groups. As HIV-infected individuals age, greater investigation of anemia status may be required during specific periods, including reproductive age females and adults as they enter older age.

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### Author Disclosure Statement

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