SAGE Open Medicine
Volume 9: 1–12

DOI: 10.1177/20503121211020175
journals.sagepub.com/home/smo
SAGE

Hematological parameters abnormalities and associated factors in HIV-positive adults before and after highly active antiretroviral treatment in Goba Referral Hospital, southeast Ethiopia: A cross-sectional study

Negesso Duguma[1*], Girum Tesfaye Kiya[2*], Wondimagegn Adissu Maleko[2,3*] and Lealem Gedefaw Bimerew[2]

Abstract

Objectives: Hematological abnormalities of the major blood cell lines are frequently reported in patients with HIV-1 infection, in patients without antiretroviral therapy, and during the advanced stages of the disease. Chronic immune activation and inflammation results in the progressive depletion of CD4+ T-cells play a significant role in the clinical progression and pathogenesis of this infection. This study was aimed at assessing the prevalence of hematological abnormalities and their associated factors before and after the initiation of antiretroviral therapy in adults with HIV-1 infection in a referral hospital.

Methods: The study was conducted from 1 April to 30 June 2018, at Goba Referral Hospital. A total of 308 HIV-positive adults on treatment were enrolled during the study period. Socio-demographic and clinical data were collected using a structured questionnaire, with pre-highly active antiretroviral therapy data were extracted from medical records while post-treatment immuno-hematological measurements were done on blood samples collected at the time of enrollment.

Results: The prevalence of anemia, leukopenia, and thrombocytopenia before initiation of antiretroviral treatment was higher, although anemia and thrombocytopenia decreased correspondingly after initiation of treatment leukopenia increased by 4%. Mean values of immuno-hematological parameters before and after treatment initiation were significant ($p < 0.05$). CD4+ T-cell count <200 cells/μL was the only independent risk factor for anemia and leukopenia before highly active antiretroviral therapy, while stage IV disease, female sex, zidovudine, lamivudine, and nevirapine treatment, and intestinal parasite infection were predictors of anemia after treatment initiation.

Conclusion: The study revealed that hematological abnormalities are common in HIV infection, while the occurrence of abnormalities after highly active antiretroviral therapy initiation. Different risk factors are associated with hematological abnormalities at pre- and post-highly active antiretroviral therapy with regular monitoring of risk factors, adherence to the early initiation of highly active antiretroviral therapy, and conduct of further longitudinal studies are recommended.

Keywords

Hematological abnormalities, cytopenia, associated factors, HIV, adult, highly active antiretroviral therapy, southeast Ethiopia

Date received: 23 February 2021; accepted: 4 May 2021

Background

The global prevalence of human immunodeficiency virus (HIV) infection is estimated at over 76 million, with the infection contributing to more than 35 million deaths worldwide since its emergence.[1] By the end of 2018, about 37.9 million people[1] and 36.2 million adults and 1.8 million children age <15 years were living with HIV across the globe according to a 2019 report of UNAIDS, while the new

[1]Department of Medical Laboratory Sciences, Madda Walabu University, Goba, Ethiopia
[2]School of Medical Laboratory Sciences, Institute of Health, Jimma University, Jimma, Ethiopia
[3]Clinical Trial Unit, Jimma University, Jimma, Ethiopia

*These authors contributed equally to this work.

Corresponding author:
Wondimagegn Adissu Maleko, School of Medical Laboratory Sciences, Institute of Health, Jimma University, P.O.Box 378, Jimma, Ethiopia.
Emails: wondeade@gmail.com; wondimagegn.adissu@ju.edu.et

infection rate was showing a decline by an estimated 23% from 2010.[2] Besides, sub-Saharan Africa remains among the most affected regions by the pandemic, with more than 4.2% of individuals estimated to be living with HIV,[1] and the annual number of new infections in Ethiopia has shown a declining trend since 2002. Over the past two decades, HIV prevalence has decreased from 3.3% in 2000 to 0.9% in 2017, and AIDS-related deaths from 83,000 in 2000 to 15,600 in 2017. However, the number of HIV infections among adult Ethiopians was estimated at 722,248 in 2017, increasing by 3748 from 2016.[1,3]

Following transmission and cell entrance, HIV undergoes rapid intracellular replication and elicits activation of the host immune system, characterized by the release of pro-inflammatory cytokines and chemokine, polyclonal B-cell activation, and progressive depletion of CD4+ T-cells. The virus is also capable of attaching itself to CD4 negative blood cells such as erythrocytes, in the presence of complement receptor type 1 (CR1) also known as C3b/C4b receptor or CD35.[4,5] This chronic immune activation and inflammation contribute to the clinical progression and disease outcomes of infected individuals.[1,6] Although clinical outcomes of HIV infection are broad, a range of hematologic abnormalities especially cytopenias (anemia, leukopenia, neutropenia, and thrombocytopenia) are commonly identified as the first and frequent clinical complications of the infection irrespective of disease symptoms.[1,7–9]

Although a spectrum of hematological abnormalities such as peripheral and bone marrow cytopenia (anemia, leukopenia or neutropenia, and thrombocytopenia), and dysfunction of plasma coagulation pathways[1,9–13] is mainly caused by the virus itself in HIV-infected individuals, other factors and conditions such as OIs, immune mechanisms, associated malignancies, and antiretroviral (ARV) drugs have also been documented to have the potential to influence all hematopoietic cell lines through either abnormal cytokine expression and/or alteration of the bone marrow microenvironment.[8]

Studies suggest that cytopenias especially anemia1,[8,11,14–25] (prevalence ranging from 1.3% to 95% globally,[21] and 23.9% to 38.0% (31.0% pooled) in Ethiopia[19] before or at the start of highly active antiretroviral therapy (HAART)) would frequently occur in HIV-1 infected patients, mainly during advanced stages of the disease. The occurrence and magnitude of these hematologic abnormalities are likely dependent on the level of viral replication, associated with worsening of the clinical conditions in late-stage AIDS patients with high-level viremia. This would limit the use of antibacterial and antiviral drugs, suggesting the involvement of multiple pathogenic mechanisms.[8,25]

The cause of anemia in HIV infection is multifactorial,[18,22,26,27] and it was found to be dependent on the stage of HIV disease, age, sex, pregnancy status, and occurrence of OIs.[22] Yet, chronic diseases, OIs and parasitic infections, immune-related hematopoietic defects, decreased erythropoietin concentrations, and use of chemotherapeutic agents are among the conditions that are well-described as potential contributing factors for the development of HIV-related anemia.[22,26,27] Additional but uncommon and might be co-incidental mechanisms include micronutrient deficiencies and autoimmune destruction of red blood cells (RBCs).[20] Besides, the prevalence of anemia before and after HAART initiation differed significantly according to study reports conducted between 2008 and 2017 in various parts of Ethiopia, ranging from 11.4% to 56.2%, respectively.[11,17–19,25,27,28]

Apart from cytopenia-associated anemia that is reviewed in sufficient detail in preceding paragraphs, leukopenia and thrombocytopenia are the second most frequent hematologic complications of HIV infection that have been reported by several studies. However, data related to these abnormalities' magnitude and their association with pertinent factors are limited[23] as well as for leukocytosis and thrombocytosis as part of hematological abnormality in HIV infection. Reports of different studies on the magnitude of leukopenia, neutropenia, and thrombocytopenia are as varied as the geographical location and the clinical stage of HIV infection, with prevalence ranging from 11.7% to 26.8% for leukopenia, 10% to 85% for neutropenia, and 7% to 21% for thrombocytopenia.[24,26,29–32] While neutropenia appears to result from the interplay of viral toxicity to hematopoietic cells, myelosuppressive drugs, secondary infections, and associated malignancies, the underlying pathologic mechanism of thrombocytopenia is decreased platelet (PLT) survival caused by increased peripheral PLT destruction mediated by the presence of antiplatelet antibodies and/or PLT-bound immune complexes, and impaired PLT production due to bone marrow suppression by myelosuppressive medications.[33–38]

Different predictors of hematological abnormality were reported among HIV-infected individuals before and after the initiation of HAART. Lower CD4+ T-cell counts, WHO clinical stage of disease, duration of HIV infection, HAART naïve or experienced, different HAART regimens, adherence, and duration, sex, concomitant intestinal parasite (IP) infections, and nutritional deficiencies were repeatedly reported as being the main predictors for the occurrences of those abnormalities.[1,8,11,14–26]

Early use of HAART improves the clinical, hematological, and immunological profiles of patients, delays the progression of the disease, and improves survival of HIV-positive individuals, this later contributes to reducing viral transmission.[11,39] Findings of previous studies in Ethiopia demonstrated significant differences in measured values of hematologic parameters (RBCs, white blood cell (WBC), PLT, hemoglobin (Hgb), hematocrit (Hct), mean cell volume, and corpuscular hemoglobin (MCV and MCH), mean corpuscular hemoglobin concentration (MCHC)), and immunologic parameter (CD4+ T-cell counts) observed between pre-HAART and on HAART individuals. Furthermore, findings of these studies documented a profound decrease in the occurrence of anemia (an increase in RBC, Hgb, and Hct), leukopenia, red cell distribution width (RDW), and thrombocytopenia associated with subsequent

increase in MCV, MCH, and CD4+ T-cell counts[11,17–19,25,29,30] among HIV cases adhering to ART, supporting that early initiation of HAART improves hematological and immunological parameters.[11,39–41]

Cognizant of the differences in clinical decisions as to when to start antiretroviral therapy (ART) and the recently diminishing attention given to the advantage of early initiation of ART to HIV-infected persons, recent attention has fallen in answering when to start ART, Ethiopia started implementing the "test and treat" policy for early initiation of ART in 2017 as recommended by WHO.[42] Studies reported in 2018 in Ethiopia after this implementation indicated that 77.2% thrombocytopenia and 72.8% anemia recovery rate have been documented after initiation of HAART by "test and treat" guideline.[18,43] However, several common serious adverse effects associated with ART, including azido thymidine class (AZT)-associated hematological abnormalities have been observed.[5]

It is important to monitor the change in all blood cell lines during HIV infection, for potential detection of the development of immuno-hematological abnormalities and make the necessary clinical interventions to avoid subsequent comorbidity.[44] Although several studies were conducted to describe the prevalence of hematological abnormalities (especially anemia) among HAART naïve and experienced HIV-positive individuals in different populations, the magnitude, change of hematological abnormalities, and associated factors before and after HAART initiation is not well documented and reported in Ethiopia. Therefore, this study aimed to determine abnormalities in hematological profiles and associated factors among adult HIV-positive individuals before and after initiation of HAART at Madda Walabu University Goba Referral Hospital, southwest Ethiopia.

Methods

Study setting

This facility-based cross-sectional study was conducted at Madda Walabu University Goba Referral Hospital, from 1 April to 30 June 2018. The hospital is located 445 km southeast of the capital Addis Ababa and has offered medical services as a teaching and referral hospital for 921,311 average individuals in the catchment area of southeast Ethiopia since 2014. The hospital provides HIV/AIDS interventions, including free diagnosis, treatment, and monitoring.[45] A total of 308 HIV-positive adults on HAART follow-up during the recruitment period were enrolled in this study. Sociodemographic and clinical data were collected using a structured questionnaire prepared and validated against literature reviews for the data collection. Hematological and immunological parameters (peripheral blood cells and CD4+ T-cell counts and white cell differentials), blood film for hemoparasites (mainly malaria), and stool examinations for IP detection and identifications were performed on blood and stool samples collected at the time of enrollment. All pre-HAART immuno-hematological and clinical data were collected retrospectively from patients' medical records for comparison. The total number of sample population 308 was determined using a single population proportion formula (number of participants or $n = Z_{\alpha/2}{}^2 \times p \times (1-p)/d^2$), as $Z_{\alpha/2}$ is the critical value of the normal distribution at $\alpha/2$ (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96), d is the margin of error of 5%, p is the sample proportion taken from a previous study[46] 29.7% of anemia prevalence,[29] and all participants were included in this study by implementing convenient sampling technique.

Inclusion and exclusion criteria

HIV-positive adults aged ⩾18 years, individuals on HAART treatment and follow-up during the study period, voluntarily gave written informed consent were included. Pregnant women, individuals on treatment for any hematological abnormalities during the previous 3 months, those taking vitamins and iron supplementation at the time of sampling, patients on HAART for less than 3 months, and individuals who had received a blood transfusion for the last 3 months were excluded.

Data collection, analysis, and interpretation

Data on socio-demographic, clinical characteristics, and past medical history of the study participants were collected using a validated structured questionnaire administered during an interview, and review of medical records (Attached as supplemental material).[1,17–19,30,47,48] All anthropometric measurements (values used to compute body mass index (BMI)) were taken twice and the mean value was used for analysis. Four milliliters (4 mL) of venous blood was collected through direct venipuncture using a BD Vacutainer® (BD Biosciences, NJ, USA) EDTA containing tubes. Hematological complete/full blood count (CBC/FBC): total and differential WBC count, RBC count, Hgb, Hct, MCV, MCH, MCHC, RDW, and PLT counts and immunological (CD4+ T-cell counts) parameters were assayed by CELL-DYN 1800® (Abbott Laboratories Diagnostics Division, USA), and BD FACSPresto™ (BD Biosciences, NJ, USA) machines, respectively.

Cytopenia is a reduction of any of the peripheral blood cell lines, resulting in leukopenia, anemia, neutropenia, and thrombocytopenia.[49] Anemia was defined as a Hgb value <130 g/L for adult males and <120 g/L for non-pregnant women. Severity was classified as mild (110–119 g/L for women and 110–129 g/L for men), moderate (80–109 g/L for both sexes), and severe (<80 g/L for both sexes).[50] Microcytosis was defined as MCV <80 fL and hypochromic as MCHC <310 g/L. Leukopenia is a total leukocyte/white cell count (TWBC) $<4 \times 10^9$/L and thrombocytopenia a PLT count $<150 \times 10^9$/L.[49] Both thick and thin blood films were prepared and stained using Giemsa stain for hemoparasite

detections and identification. A stool sample was also collected from each participant using a standard stool cup, sent to the medical parasitology laboratory, and examined using the direct wet mount for the detection of IPs. All data were checked for completeness, clarity, and consistency before any analysis.

Quality assurance

The questionnaire was validated by review of literature and translated into the local language. A 3-day training was also given to all data collectors on the data collection tools, methodology, and maintaining quality to minimize technical and observer biases. Laboratory activities were performed in strict adherence to the manufacturer's instructions and laboratory standard operating procedures (SOPs). Reagents used were checked for their expiry date and prepared according to the manufacturer's instructions. Daily tri-level commercial control material (low, medium, and high value) was used for the hematology analyzer to check the accuracy and precision of all hematological parameters results, while all hematology result flags were subjected to manual differential and peripheral morphology examinations to confirm the result. Besides, instrument quality control and process controls were implemented for the FACSPresto™ machine. Instrument QC, which runs automatically, is a test that makes sure that the machine is operating properly every time it turns on, while process controls are based on liquid control materials that are processed like patient samples to monitor the performance of the system. Two independent laboratory technicians read all the blood films and stool wet mount slides, under the supervision of the principal investigator. Known malaria-positive blood films stained intermittently to validate the staining characteristics and technicians' performance.

Statistics

Data were entered into EpiData version 3.1 (EpiData Association, Odense, Denmark) and exported to SPSS version 20.0 (IBM® SPSS®, IBM Corp., Armonk, NY, USA) statistical software for analysis. Simple frequency summaries, binary and multiple logistic regression models, and paired *t*-tests were performed. Binary logistic regression analysis was performed for all potentially explanatory variables to investigate the relation between hematologic abnormalities with HAART, like WHO clinical stage, age, sex, type and duration of HAART regimen, IP, as well as the CD4+ T-cell count at pre-HAART and on HAART. Multivariate logistic regression analysis was performed for variables significantly associated with HAART after binary regression analysis by considering $p < 0.25$. All p-values < 0.05 were considered to be statistically significant. The regression analysis method was used as a method of checking the fitness of the model using a validation process of analysis, such as the goodness of fit of regression.

Ethical considerations

Ethical clearance and permission to conduct this study was obtained from Jimma University, Institute of Health Institutional Ethical Review Board and Clinical Director of Madda Walabu University Goba Referral Hospital, respectively. Specimens collected from the participants were analyzed only for the intended purpose of this study. Abnormal and positive results of study participants obtained from laboratory investigations were promptly reported to the treating physician. All clinical data and laboratory findings of the study participants were kept confidential and used for the sole purpose of this study.

Consent to participate

After the study aim, risks and benefits, and right to withdraw were described to participants, a written informed consent was obtained from each of them.

Results

Socio-demographic and clinical characteristics of study participants

A total of 308 HIV-positive adults (160 males and 148 females) were enrolled in this study, with 73.7% of them were within 30–49 years of age, while only 11.7% and 14.6% of them were within 18–29 years and ⩾50 years of age, respectively. Rural dwelling participants (82.1), individuals living with the country's low to medium monthly average income (76.6%), and peoples living in more or less large family size (2–10 individuals, 74.3%) account for a higher number of overall participants. Whereas 13.6% of participants unable to read and write, 64.6% completed elementary education, and 22.8% of individuals completed secondary and higher education. Most of the total individuals were married (47.7%), with 22.7% single, 14.3% divorced, and 15.3% of them were widowed.

Before the start of HAART medications 20.2% (26/129; 179 unrecorded) of participants had one or more OIs (candidiasis 8/129 (6.2%), herpes zoster 6/129 (4.7%), pneumonia 6/129 (4.7%), tuberculosis 3/129 (2.3%), and skin infections 3/129 (2.3%)) after review of retrospective medical records. This number significantly declined to 4.5% (14/308) after initiation of HAART, although all these OIs (candidiasis 7/308 (2.3%), herpes zoster 3/308 (1%), pneumonia 2/308 (0.6%), and tuberculosis 2/308 (0.6%)) were reported as previous infections. Diabetes mellitus and hypertension comorbidity was found in 6.5% and 5.2% of participants, respectively, while 32.8% (101/308) of individuals diagnosed with one or more IPs co-infections were

Table 1. Clinical characteristics of HIV-positive individuals before and after initiation of HAART at Madda Walabu University Goba Referral Hospital, southeast Ethiopia, 1 April–30 June 2018 ($n = 308$).

Variables		Before HAART		On HAART	
		n	%	*n*	%
CD4+ T-cells/μL[a]	<200	113	36.7	43	14.0
	200–500	125	40.6	107	34.7
	>500	70	22.7	158	51.3
BMI (kg/m^2)[a]	<18.5	152	49.4	50	16.2
	18.5–24.9	115	37.3	244	79.2
	⩾25	25	8.1	14	4.6
	BMI not recorded	16	5.2		
WHO clinical stage[a]	I	204	66.2	245	79.5
	II	70	22.7	35	11.4
	III	22	7.2	21	6.8
	IV	12	3.9	7	2.3
HAART regimen[a]	ZDV, 3TC, EFV	–	–	61	19.8
	ZDV, 3TC, NVR	–	–	160	52
	TDF, 3TC, EFV	–	–	37	12
	TDF, 3TC, NVP	–	–	48	15.6
	TDF, 3TC, LPV/r	–	–	2	0.6
HAART duration	3–12 months	–	–	9	2.9
	13–24 months	–	–	48	15.6
	25–36 months	–	–	61	19.8
	37–48 months	–	–	18	5.8
	49–60 months	–	–	119	38.6
	>60 months	–	–	53	17.3

[a]CD: cluster of differentiation; BMI: body mass index; HAART: highly active antiretroviral therapy; WHO: World Health Organization; ZDV: zidovudine;3TC: lamivudine; NVP: nevirapine; TDF: tenofovir; EFV: efavirenz; LPV/r: lopinavir/ritonavir.

detected after stool sample screening (*Ascaris lumbricoides* 42 (13.6%), *Hookworm species* 24 (7.8%), *Trichuris trichiura* 15 (4.9%), *Taenia species* 11 (3.6%), and *Strongyloides stercoralis* 9 (2.9%)). But only 5/308 (1.6%) participants were positive for hemoparasite infection after blood film screening (*Plasmodium vivax* 4/308 (1.3%) and *Plasmodium falciparum* 1/308 (0.3%)).

CD4+ T-cell counts of <200 cells/μL significantly found in patients before the start of HAART, while the total CD4+ T-cell increased after the start of ART medications. Similarly, many underweight patients recovered after the commencement of medication. More than half (52%) of patients were taking ZDV, 3TC, NVR regimen, and the majority of patients were taking ART medications for the last 49–60 months (Table 1).

Immuno-hematological parameters

Red blood cell counts and RDW were relatively higher before initiation of HAART, while total lymphocyte count (TLC), CD4+ T-cell, Hgb, Hct, MCV, MCH, and PLT counts were significantly increased after initiation of HAART ($p < 0.05$). There were significant mean value differences of these hematologic parameters before and after initiation of HAART ($p < 0.05$) (Table 2).

Hematologic abnormalities

Before initiation of HAART, the prevalence of anemia, leukopenia, neutropenia, lymphopenia, and thrombocytopenia was 31.8%, 18.2%, 15.6%, 2.9%, and 11.4%, respectively, while these cytopenias changed to 14.6%, 24%, 17.9%, 3.6%, and 4.5% after initiation of HAART. Normocytic normochromic anemia (53.1%) was common in patients before HAART initiation followed by microcytic hypochromic anemia (30.6%), whereas macrocytic normochromic anemia (64.4%) and normocytic normochromic anemias (16.3%) turn out to be prevalent after initiation of HAART (Table 3).

Relationship between CD4+ T-cell counts and hematological abnormalities

Overall, the incidence of hematological abnormalities increased with a decrease in CD4+ T-cell counts. Patients demonstrating a CD4+ T-cell count of <200 cells/μL are more likely to develop anemia, leukopenia, and neutropenia before the start

Table 2. Immuno-hematological parameters of HIV-positive individuals before and after HAART initiation at Madda Walabu University Goba Referral Hospital, southeast Ethiopia, 1 April–30 June 2018 ($n = 308$).

Parameters	Before HAART (Mean ± SD)	On HAART (Mean ± SD)	*p*-value	*t*-value (95% CI*)
WBC ($\times 10^9$/L)	5.6 ± 2.6	5.7 ± 1.6	0.716	−1.21 (0.13–(−0.56))
TLC ($\times 10^9$/L)	1.9 ± 1.9	2.2 ± 0.9	**0.001***	2.86 (0.067–0.37)
ANC ($\times 10^9$/L)	3.1 ± 1.9	3.0 ± 1.9	0.481	−0.71 (−0.42–0.12)
RBC ($\times 10^{12}$/L)	4.2 ± 0.8	4.0 ± 0.8	**0.044***	−1.86 (−0.26–0.01)
Hgb (g/L)	122 ± 25	136 ± 28	**0.001***	7.08 (1.09–1.93)
Hct (L/L)	0.34 ± 0.068	0.38 ± 0.076	**0.001***	7.10 (2.99–5.29)
MCV (fL)	90.9 ± 11.9	94.2 ± 9.9	**0.001***	3.79 (1.57–4.96)
MCH (pg)	32.3 ± 5.0	33.3 ± 5.1	**0.011***	2.56 (0.23–1.78)
MCHC (g/L)	348 ± 25	350 ± 30	0.380	0.88 (−0.23–0.60)
RDW (CV %)	15.2 ± 1.4	14.3 ± 2.1	**0.001***	−5.99 (0.14–(−1.15))
PLT ($\times 10^9$/L)	242.4 ± 109.7	273.6 ± 117	**0.001***	3.47 (13.48–50.00)
CD4+ T-cells (cells/μL)	363.7 ± 275.6	520.2 ± 264.6	**0.001***	7.18 (113.74–199.25)

HAART: highly active antiretroviral therapy; CI: confidence interval; WBC: white blood cell; TLC: total lymphocyte count; ANC: absolute neutrophil count; RBC: red blood cell; Hgb: hemoglobin; Hct: hematocrit; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; fL: femtoliter; pg: pictograms; CV: coefficient of variation percentage; CD: cluster of differentiation.
*Level of significance ($p < 0.05$).

Table 3. Prevalence of hematological abnormalities of HIV-positive individual before and on HAART at Madda Walabu University Goba Referral Hospital, southeast Ethiopia, 1 April to 30 June 2018 ($n = 308$).

Abnormalities	Before HAART, *n* (%)	On HAART, *n* (%)
Anemia	98 (31.8)	45 (14.6)
Male	54 (55.1)	13 (28.9)
Female	44 (44.9)	32 (71.1)
Severe anemia	3 (3.1)	1 (2.2)
Moderate anemia	42 (42.9)	8 (17.8)
Mild anemia	53 (54.1)	36 (80)
Leukopenia	56 (18.2)	74 (24)
Male	26 (46.4)	34 (46.9)
Female	30 (53.6)	40 (54.1)
Neutropenia	48 (15.6)	55 (17.9)
Male	22 (45.8)	27 (49.1)
Female	26 (54.2)	28 (50.9)
Lymphopenia	9 (2.9)	11 (3.6)
Male	4 (44.4)	6 (54.5)
Female	5 (54.6)	5 (45.5)
Thrombocytopenia	35 (11.4)	14 (4.5)
Male	22 (62.9)	6 (42.9)
Female	13 (37.1)	8 (57.1)

HAART: highly active antiretroviral therapy.

of HAART medication, whereas these abnormalities significantly decreased after initiation of HAART. However, there was no significant association observed between hematological abnormalities and different categories of CD4+ T-cell counts in HIV-positive individuals on HAART ($p < 0.05$) (Table 4).

Factors associated with hematological abnormalities

HIV-positive individuals before HAART with CD4+ T-cell counts <200cells/μL were 4.2 times and 3.2 times more likely to develop anemia and leukopenia, respectively, than those who had CD4+ T-cell counts/μL >500 (Table 5). Besides, female HIV-positive individuals, those with IP infections, and WHO clinical stage IV patients had 2.6 ($p = 0.048$, adjusted odds ratio (AOR) = 2.6, 95% confidence interval (CI) = 1.3–4.8), 2.5 ($p = 0.048$, AOR = 2.5, 95% CI = 1.1–7.4), and 2.4 ($p = 0.038$, AOR = 2.4, 95% CI = 1.4–4.8) times more likely to develop anemia, respectively, than male individuals, without IP infections, and in other WHO clinical stages (I, II, and III). HAART taking HIV-positive individuals on ZDV, 3TC, and NVP therapy were 3.2 times more likely to develop anemia than individuals on other HAART regimens ($p = 0.001$, AOR = 3.2, 95% CI = 2.2–7.5). These factors were assessed for other hematological abnormalities (leukopenia, neutropenia, lymphopenia, and thrombocytopenia); however, no statistically significant associations were identified (Table 6).

Discussion

The prevalence of cytopenias in this study was anemia 31.8% versus 14.6%, leukopenia 18.2% versus 24%, neutropenia 15.6% versus 17.9%, lymphopenia 2.9% versus 3.6%, thrombocytopenia 11.4% versus 4.5%, and lower CD4+ T-cell counts 36.7% versus 14% before and after HAART initiation, respectively. Cytopenia as a result of anemia and thrombocytopenia was higher at pre-HAART, while leukopenia was

Table 4. Association of CD4+ T-cell counts with cytopenia in HIV-positive individuals at Madda Walabu University Goba Referral Hospital, southeast Ethiopia, 1 April to 30 June 2018 ($n = 308$).

Hematological abnormalities	CD4 counts (cells/μL) before HAART				Hematological abnormalities	CD4 counts (cells/μL) after HAART			
	<200	200–500	>500	*p*-value		<200	200–500	>500	*p*-value
Anemia	50 (47.2%)	35 (28%)	13 (16.9%)	**0.001***	Anemia	4 (9.3%)	19 (17.6%)	22 (13.9%)	0.783
Leukopenia	25 (23.6%)	28 (22.4%)	3 (3.9%)	**0.001***	Leukopenia	9 (20.9%)	19 (17.8%)	46 (29.3%)	0.176
Lymphopenia	2 (1.9%)	6 (4.8%)	1 (1.3%)	0.263	Lymphopenia	1 (2.3%)	3 (2.8%)	7 (4.5%)	0.785
Neutropenia	21 (19.8%)	24 (19.2%)	3 (3.9%)	**0.005***	Neutropenia	6 (14%)	18 (16.7%)	31 (19.7%)	0.274
Thrombocytopenia	15 (14.2%)	14 (11.2%)	6 (7.8%)	0.772	Thrombocytopenia	3 (7%)	4 (3.7%)	7 (4.5%)	0.346

CD: cluster of differentiation; HAART: highly active antiretroviral therapy.
*Level of significance ($p < 0.05$).

Table 5. Factors associated with anemia and leukopenia in HIV-positive individuals before HAART at Madda Walabu University Goba Referral Hospital, southeast Ethiopia, 1 April to 30 June 2018 ($n = 308$).

Parameters	Units	Anemia, *n* (%)	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
CD4+ T-cell count (cells/μL)	<200	50 (47.2)	3 (1.2–4.8)	**0.001***	4.2 (1.4–4.8)	**0.04***
	200–500	35 (28)	4.3 (1.14–4.6)	0.09	2.1 (2.3–6.3)	0.058
	>500	13 (16.9)	1.00		1.00	
Age (years)	18–29	10 (10.2)	1.0 (1.0–2.7)	0.21	0.4 (0.24–5.6)	0.28
	30–39	27 (27.6)	4.1 (3.1–5.7)	0.17	0.9 (0.71–2.7)	0.07
	40–49	48 (49)	1.2 (1.1–1.46)	0.05	2.7 (3.2–5.4)	0.82
	⩾50	13 (13.3)	1.00		1.00	
Parameters	Units	Leukopenia, *n* (%)	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
CD4+ T-cell count (cells/μL)	<200	25 (23.6)	0.4 (0.24–0.67)	**0.001***	3.2 (2.8–7.9)	**0.005***
	200–500	28 (22.4)	0.6 (0.26–1.67)	0.058	0.12 (0.16–1.67)	0.09
	>500	3 (3.9)	1.00		1.00	
Age (years)	18–29	10 (10.2)	1.1 (1–1.4)	0.03	0.1 (0.21–2.50)	0.064
	30–39	15 (15.3)	2.5 (1.65–3.95)	0.08	2.9 (0.21–2.40)	0.21
	40–49	12 (12.2)	3.0 (2.9–6.7)	0.24	1.8 (0.22–0.48)	0.53
	⩾50	19 (19.5)	1.00		1.00	

CD: cluster of differentiation; CI: confidence interval; COR: crude odd ratio; AOR: adjusted odd ratio.
1.00: reference group.
*Level of significance ($p < 0.05$).

higher after initiation of HAART. CD4+ T-cell counts <200 cells/μL was identified as a significant risk factor for cytopenia (anemia, leukopenia, and neutropenia) before HAART initiation, whereas HAART regimen (ZDV, 3TC, and NVP), WHO clinical stage IV, female sex, and concomitant IP infections predict the likelihood development of anemia after initiation of HAART.

Anemia prevalence before and after HAART initiation in this study is consistent with findings to other similar studies in Ethiopia[11,17–19,25,29,30] indicating that, even though anemia is still a public health problem, the decrease in the prevalence of anemia after HAART initiation is attributed to the positive effect of the treatment on the differentiation and survival of RBCs, decreases the viral load, and reduces the frequency of OIs.[51,52] Normocytic normochromic anemia was the most common type of anemia observed similar to other Ethiopia study reports,[29,53] with macrocytic normochromic (Macrocytosis) anemia found to be the second common type mainly after initiation therapy. This is concurrent with the findings of India[31] and Ethiopia.[16,54] This could be due to the effect of AZT on MCV as the majority of anemic patients were receiving zidovudine-based regimens, which is responsible for the development of macrocytosis associated with bone marrow toxicity.[55] Cytopenia especially anemia was significantly associated with WHO clinical stage IV disease, in patients taking HAART regimen of (ZDV, 3TC, and NVP), with female HIV-positive patients, and those patients with IP infection on HAART, similar to multiple study reports.[1,4,8,16,18,23,24,28,56]

Disease progression in HIV is related to OIs and the development of anemia. The common cause of severe anemia in HIV is atypical mycobacterial infection.[27] Parvovirus B19 infection can also induce anemia as the virus selectively infects erythrocyte precursor resulting in pure red cell aplasia.[57] Kaposi's sarcoma, lymphoma, or gastrointestinal

Table 6. Factors associated with predictors of anemia in HIV-positive individuals on HAART at Madda Walabu University Goba Referral Hospital, southeast Ethiopia, 1 April to 30 June 2018 ($n = 308$).

Variables	Occurrence	Anemia, *n* (%)	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Intestinal parasites	Yes	35 (77.8%)	10.4 (9.3–15.4)	0.022*	2.5 (1.1–7.4)	**0.048***
	No	10 (22.2%)	1.00		1.00	
Sex	Female	32 (71.1%)	3.1 (1.8–3.9)	0.04*	2.6 (1.3–4.8)	0.048*
	Male	13 (28.9%)	1.00		1.00	
WHO clinical stages	I	2 (4.4%)	1.00		1.00	
	II	31 (68.9%)	3.0 (2.05–4.7)	0.02	0.2 (0.4–.3)	0.1
	III	5 (11.1%)	3.8 (2.2–3.9)	0.007	0.4 (0.02–1.2)	0.5
	IV	7 (15.6%)	5.6 (4.1–7.8)	0.029*	2.4 (1.4–4.8)	**0.038***
HAART regimen	ZDV, 3TC, EFV	3 (6.7%)	19.3 (15.3–23.7)	0.5		
	ZDV, 3TC, NVP	23 (51.1%)	5.6 (3.6–8.2)	0.045*	3.2 (2.2–7.5)	**0.001***
	TDF,3TC, EFV	8 (17.8%)	3.6 (0.02–1.3)	0.9		
	TDF,3TC, NVP	11 (24.4%)	3.4 (0.1–2.2)	0.85		
	TDF,3TC, LPV/r		1.00		1.00	

CI: confidence interval; WHO: World Health Organization; HAART: highly active antiretroviral therapy; COR: crude odd ratio; AOR: adjusted odd ratio; 1.00: reference group; ZDV: zidovudine; 3TC: lamivudine; NVP: nevirapine; TDF: tenofovir; EFV: efavirenz; LPV/r: lopinavir/ritonavir.
*Level of significance ($p < 0.05$).

tract bleeding, or parasitic infections can also result in iron deficiency as a result of chronic blood loss. HIV enteropathy, bacterial, and other viral intestinal infections may also alter nutrient absorption through impaired intestinal uptake, leading to micronutrient deficiency.[58,59] Besides, IP infestations cause mucosal membrane damage leading to gastrointestinal bleeding, and malnutrition caused by parasite consumption of digested food. HIV may not directly affect bone marrow hematopoietic progenitor pool but indirectly alter cellular and cytokine microenvironment and/or through the interaction of the virus or viral products with the cell surface resulting in defective hematopoietic functions.[58] Hematological toxicity and myelosuppression are also frequent side effects of ARTs and other chemotherapeutic agents. Zidovudine treatment is associated with bone marrow suppression and increases the risk of anemia development.[58,59] The increased prevalence of anemia in females could be attributed to menstrual blood loss and potential drains on iron stores that occur with pregnancy and delivery.[58,60]

Although the occurrence of leukopenia was found relatively lower at pre-HAART but was found to be the most commonly reported abnormality consistent with multiple study reports.[9,23,30,31,48] On the contrary to other hematological abnormalities, the prevalence of leukopenia, neutropenia, and lymphopenia showed an increasing pattern after initiation of HAART was in agreement with other reports,[1,14,30,31] but lower than studies conducted in Cameroon and Gondar University.[8,29] This abnormality could have resulted from the use of a zidovudine-containing HAART regimen, which can cause leukopenia by suppression of bone marrow production and cytotoxicity of T-cells eventually decreasing the survival of T-cells.[61] Whereas neutropenia and lymphopenia before and after initiation of HAART showed some degree of discrepancy from other studies, which might be due to the difference in the clinical condition of the patient, study population, sample size, and variation in study design attributable to the use of the same patient in this study.

The prevalence of thrombocytopenia before HAART initiation was greater than that of HAART taking individuals (11.4% vs 4.5%), possibly due to the rapid increase in PLT count induced by AZT,[62,63] and the association of infection-induced cytopenia to the effect of early hematopoietic progenitor cells functions and bone marrow suppression.[35,64] This is consistent with studies conducted in Uganda, Nigeria, Tanzania, and Ethiopia[23,29,30,48,65] but lower than studies conducted in India and Cameroon.[8,31] These observed differences might be due to platelet immune destruction by the spleen related to malaria and other blood parasite endemicity, the clinical condition of the patient, the use of different study populations, and the ineffective platelet production.

CD4+ T-cell counts <200 cells/μL were found to be associated with the major hematological abnormality (cytopenia) that might be in association with higher plasma viral load and reported mainly as independently associated with an increased risk of developing anemia characterized by peripheral immunosuppression and a significant decrease in bone marrow hematopoietic activity.[44] This is in keeping with studies conducted in India, Cameroon, and Ethiopia.[8,17–20,55,56,62,63,66–68] This progressive depletion in CD4+ T-lymphocytes is the fundamental finding during disease advancement, eventually leading to marked immunosuppression and development of AIDS, causing immunological deterioration of infected individuals before initiation of treatment, but could also be due to the direct and indirect effect of the virus, the toxicity of the drugs, and OIs.[25,53]

Cytopenia recovery rate in this study was found to be 54% for anemia, 60.5% for thrombocytopenia, and 59.3% for previously lowered CD4+ T-cell count which improved

after initiation of HAART. HAART initiation was found an effective treatment in reverting hematological abnormalities (especially cytopenias) in patients without treatment alike to other study reports. Besides the profound recovery of CD4+ T-cells, total WBCs, neutrophils, lymphocytes, PLTs, and Hgb values increased after treatment initiation, except the RBCs. Consistent findings were reported in Ethiopia and elsewhere, supporting the timely detection of HIV infection and early initiation of HAART significantly improve potential hematological abnormalities (cytopenias)[9,11,17–19,25,29,30–38] showing implementation of "test and treat" pave clinical benefits for patients for whom HAART was initiated early.19,42,43,69 The decrease in or diminished expected increment in RBC count after treatment initiation is explained by the cause of medication-induced anemia, as ZDV is mainly associated with myelotoxicity possibly by inhibiting erythroid precursor cells in the bone marrow leading to decreased RBC production manifesting in anemia.[44,70,71]

Limitations of the study

Data on viral load were incomplete, irregular, with many misses which were not included in this study to compare hematological abnormalities with. The causes of hematological abnormalities in HIV infection are multifactorial. Thus, this study's inability to identify all causes of hematological abnormalities will not permit the cause and effect of cytopenias in HIV-infected individuals evaluated. Other hematologic abnormalities, such as coagulation profiles and hemostatic abnormalities also not assessed in this study due to reagent availability. The questionnaires were not pilot-tested/pre-tested earlier to actual data collection later to the preparation and validation.

Conclusion

There was a decline in the prevalence of anemia and thrombocytopenia accompanied by a significant increase in CD4+ T-cells after the initiation of HAART. The study revealed also that the most common hematological abnormalities were anemia, leukopenia, and thrombocytopenia. Normocytic normochromic anemia was common before HAART, while macrocytic normochromic anemia was common after initiation of HAART, and mild anemia was the commonest type of anemia in the study. There were statistically significant differences in the mean values of RBC, Hgb, Hct, MCV, MCH, RDW, TLC, PLT, and CD4+ T-cell counts before and after HAART initiation HIV-positive individuals.

The risk factors for hematological abnormalities were different in HIV-positive individuals before HAART than in individuals on HAART. A CD4+ T-cell count <200 cells/μL was only the risk factor of anemia, leukopenia, and neutropenia in HIV-positive individuals before HAART. Anemia, leukopenia, and neutropenia decreased as CD4+ cell count increased and no statistically significant association was found between CD4+ cell counts and hematological abnormalities in HIV-positive individuals on HAART. Female sex, WHO clinical stage IV disease, HAART regimen (ZDV, 3TC, and NVP), and IP infestation had a statistically significant association with anemia in HIV-positive individuals on HAART. Therefore, routine monitoring of hematological parameters and risk factors for any hematological abnormalities including periodic screening for IP is recommended to take appropriate clinical interventions. Early initiation of ART is beneficial in reducing the magnitude of hematological abnormalities in this population. Further longitudinal studies with long-term follow-up are needed to explore the causes of hematological abnormalities.

Acknowledgements

The authors express their deepest gratitude to Jimma University and the School of Medical Laboratory Sciences for supporting this study, volunteering study subjects who took their time to give us all the relevant information and clinical specimens, and the staff at the ART clinic of Goba Referral Hospital for collecting the data. Their utmost gratitude also goes to Hillary Johnstone (Dr), her family, and Zewudineh Sahlemariam (Mr) for proofreading the writing of this article.

Confidentiality

All data and laboratory findings from the study participants were kept confidential and used for the sole purpose of this study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from the Jimma University, Institute of Health Institutional Ethical Review Board (REF: IHRPGD/136/2018, 22 March 2018), and the Madda Walabu University Goba Referral Hospital clinical director.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the Jimma University for data collection, analysis, and resource; no any authors received special or specific funding for this study.

Informed consent

After the study aim, risks and benefits, and right to withdraw were described to participants, written informed consent was received from each of them. Abnormal and positive results of study participants were promptly reported to the treating physician.

ORCID iDs

Wondimagegn Adissu Maleko https://orcid.org/0000-0003-1624-3114

Lealem Gedefaw Bimerew https://orcid.org/0000-0002-6660-4435

Supplemental material

Supplemental material for this article is available online.

References

1. Wisaksana R, de Mast Q, Alisjahbana B, et al. Inverse relationship of serum hepcidin levels with CD4 cell counts in HIV-infected patients selected from an Indonesian prospective cohort study. *PLoS ONE* 2013; 8(11): e79904.
2. The global HIV/AIDS epidemic data and trends, https://www.hiv.gov/hiv-basics/overview/data-and-trends/global-statistics
3. Kibret GD, Ferede A, Leshargie CT, et al. Trends and spatial distributions of HIV prevalence in Ethiopia. *Infect Dis Poverty* 2019; 8(1): 90.
4. Horakova E, Gasser O, Sadallah S, et al. Complement mediates the binding of HIV to erythrocytes. *J Immunol* 2004; 173(6): 4236–4241.
5. Gudina EK, Teklu AM, Berhan A, et al. Magnitude of antiretroviral drug toxicity in adult HIV patients in Ethiopia: a cohort study at seven teaching hospitals. *Ethiop J Health Sci* 2017; 27(Suppl. 1): 39–52.
6. Merino KM, Allers C, Didier ES, et al. Role of monocyte/macrophages during HIV/SIV infection in adult and pediatric acquired immune deficiency syndrome. *Front Immunol* 2017; 8: 1693.
7. Thulasi RR, Manimaran D, Hemanathan G, et al. Hematological abnormalities in HIV infected individuals in correlation to CD4 counts and ART status. *Asian J Med Sci* 2016; 7(4): 14–18.
8. Wankah PN, Tagny CT and Mbanya DN. Profile of blood cell abnormalities among antiretroviral therapy naïve HIV patients attending the Yaounde University Teaching Hospital, Cameroon. *BMC Hematol* 2014; 14(1): 15.
9. Fan L, Li C and Zhao H. Prevalence and risk factors of cytopenia in HIV-infected patients before and after the initiation of HAART. *Biomed Res Int* 2020; 2020: 3132589.
10. Kibirige CN, Menendez FA, Zhang H, et al. Late-emerging strains of HIV induce T-cell homeostasis failure by promoting bystander cell death and immune exhaustion in naïve CD4 and all CD8 T-cells. *Med Hypotheses* 2014; 83(1): 69–73.
11. Daka D, Lelissa D and Amsalu A. Prevalence of anaemia before and after the initiation of antiretroviral therapy at ART centre of Hawassa University Referral Hospital, Hawassa, south Ethiopia. *Sch J Med* 2013; 3(1): 1–6.
12. Kyeyune R. *Hematological and clinical profiles of HIV-infected adults initiating highly active antiretroviral therapy (HAART) in Uganda*. Doctoral Dissertation, https://edoc.ub.uni-muenchen.de/20148/1/Kyeyune_Rachel.pdf
13. Kyeyune R, Saathoff E, Ezeamama A, et al. Hematological profiles of HIV-infected adults initiating highly active antiretroviral therapy (HAART) in Uganda. *AIDS Res Hum Retrovir* 2014; 30(S1): A216.
14. Ferede G and Wondimeneh Y. Prevalence and related factors of anemia in HAART-naïve HIV positive patients at Gondar University Hospital, northwest Ethiopia. *BMC Hematol* 2013; 13(1): 8.
15. Fiseha T, Tamir Z, Seid A, et al. Prevalence of anemia in renal insufficiency among HIV infected patients initiating ART at a hospital in northeast Ethiopia. *BMC Hematol* 2017; 17: 1.
16. Woldeamanuel GG and Wondimu DH. Prevalence of anemia before and after initiation of antiretroviral therapy among HIV infected patients at Black Lion Specialized Hospital, Addis Ababa, Ethiopia: a cross-sectional study. *BMC Hematol* 2018; 18: 7.
17. Tesfaye Z and Enawgaw B. Prevalence of anemia before and after initiation of highly active antiretroviral therapy among HIV positive patients in northwest Ethiopia: a retrospective study. *BMC Res Note* 2014; 7: 745.
18. Gedefaw L, Yemane T, Sahlemariam Z, et al. Anemia and risk factors in HAART naïve and HAART experienced HIV positive persons in southwest Ethiopia: a comparative study. *PLoS ONE* 2013; 8(8): e72202.
19. Negesse A, Getaneh T, Temesgen H, et al. Prevalence of anemia and its associated factors in human immuno deficiency virus infected adult individuals in Ethiopia. A systematic review and meta-analysis. *BMC Hematol* 2018; 18: 32.
20. Kosalaraksa P, Bunupuradah T, Vonthanak S, et al. Prevalence of anemia and underlying iron status in naïve antiretroviral therapy HIV-infected children with moderate immune suppression. *AIDS Res Hum Retrovir* 2012; 28(12): 1679–1686.
21. Belperio PS and Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. *Am J Med* 2004; 116(Suppl. 7A): 27S–43S.
22. Shet A, Arumugam K, Rajagopalan N, et al. The prevalence and etiology of anemia among HIV-infected children in India. *Eur J Pediatr* 2012; 171(3): 531–540.
23. Kyeyune R, Saathoff E, Ezeamama AE, et al. Prevalence and correlates of cytopenias in HIV-infected adults initiating highly active antiretroviral therapy in Uganda. *BMC Infect Dis* 2014; 14: 496.
24. Assefa M, Abegaz WE, Shewamare A, et al. Prevalence and correlates of anemia among HIV infected patients on highly active anti-retroviral therapy at Zewditu Memorial Hospital, Ethiopia. *BMC Hematol* 2015; 15: 6.
25. Dikshit B, Wanchu A, Sachdeva RK, et al. Profile of hematological abnormalities of Indian HIV infected individuals. *BMC Hematol* 2009; 9: 5.
26. Kreuzer KA and Rockstroh JK. Pathogenesis and pathophysiology of anemia in HIV infection. *Ann Hematol* 1997; 75(5–6): 179–187.
27. Bain BJ. Pathogenesis and pathophysiology of anemia in HIV infection. *Curr Opin Hematol* 1999; 6(2): 89–93.
28. Adane A, Desta K, Bezabih A, et al. HIV-associated anaemia before and after initiation of antiretroviral therapy at Art Centre of Minilik II Hospital, Addis Ababa, Ethiopia. *Ethiop Med J* 2012; 50(1): 13–21.
29. Enawgaw B, Alem M, Addis Z, et al. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, northwest Ethiopia: a comparative cross-sectional study. *BMC Hematol* 2014; 14(1): 8.
30. Akinbami A, Oshinaike O, Adeyemo T, et al. Hematologic abnormalities in treatment-naïve HIV patients. *Infect Dis Res Treat* 2010; 3: S6033.
31. Kathuria S, Bagga PK and Malhotra S. Hematological manifestations in HIV infected patients and correlation with CD4 counts and antiretroviral therapy. *J Contemp Med Res* 2016; 3(12): 3495–3498.

32. Saha D, Kini JR and Subramaniam R. A study of the hematological profile of human immunodeficiency virus positive patients in coastal south Indian region. *J Med Sci* 2015; 35(5): 190.
33. Koefoed K and Ditzel HJ. Identification of talin head domain as an immunodominant epitope of the antiplatelet antibody response in patients with HIV-1-associated thrombocytopenia. *Blood* 2004; 104(13): 4054–4062.
34. Metcalf Pate KA, Lyons CE, Dorsey JL, et al. Platelet activation and platelet-monocyte aggregate formation contribute to decreased platelet count during acute simian immunodeficiency virus infection in pig-tailed macaques. *J Infect Dis* 2013; 208(6): 874–883.
35. Evatt BL. HIV Infection and thrombocytopenia. *Curr Hematol Rep* 2005; 4(2): 149–153.
36. Barboni G, Candi M, Bayon M, et al. Prevalencia de trombocitopenia en niños con HIV/sida (Prevalence of thrombocytopenia in HIV infected children). *Medicina* 2010; 70(5): 421–426.
37. Kouri YH, Borkowsky W, Nardi M, et al. Human megakaryocytes have a CD4 molecule capable of binding human immunodeficiency virus-1. *Blood* 1993; 81(10): 2664–2670.
38. Shi X, Sims MD, Hanna MM, et al. Neutropenia during HIV infection: adverse consequences and remedies. *Int Rev Immunol* 2014; 33(6): 511–536.
39. Insight Start Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. *New Engl J Med* 2015; 373(9): 795–807.
40. Kitahata MM, Gange SJ, Abraham AG, et al. Effect of early versus deferred antiretroviral therapy for HIV on survival. *New Engl J Med* 2009; 360(18): 1815–1826.
41. Voirin N, Routy JP, Smith D, et al. Effect of early initiation of highly active antiretroviral therapy on CD4 cell count and HIV-RNA viral load trends within 24 months of the onset of acute retroviral syndrome. *HIV Med* 2008; 9(6): 440–444.
42. World Health Organization. Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy. *World Health Organization*, July 2017, https://apps.who.int/iris/bitstream/handle/10665/255885/WHO-HIV-2017.18-eng.pdf;sequence=1
43. Woldeamanuel GG and Wondimu DH. Prevalence of thrombocytopenia before and after initiation of HAART among HIV infected patients at Black Lion Specialized Hospital, Addis Ababa, Ethiopia: a cross sectional study. *BMC Hematol* 2018; 18: 9.
44. Marchionatti A and Parisi MM. Anemia and thrombocytopenia in people living with HIV/AIDS: a narrative literature review. *Int Health* 2021; 13(2): 98–109.
45. Mamo A, Mama M, Solomon D, et al. Treatment outcomes and predictors among tuberculosis patients at Madda Walabu University Goba Referral Hospital, southeast Ethiopia. *Infect Drug Resist* 2021; 13: 4763–4771.
46. Daniel WW. *Biostatistics: a foundation for analysis in the health sciences*. 7th ed. New York: John Wiley & Sons, 1999.
47. Teklemariam Z, Mitiku H and Mesfin F. Prevalence of anemia and nutritional status among HIV-positive children receiving antiretroviral therapy in Harar, eastern Ethiopia. *HIV AIDS* 2015; 7: 191–196.
48. Gunda DW, Godfrey KG, Kilonzo SB, et al. Cytopenias among ART-naïve patients with advanced HIV disease on enrolment to care and treatment services at a tertiary hospital in Tanzania: a cross-sectional study. *Malawi Med J* 2017; 29(1): 43–52.
49. Hoffbrand AV, Catavsky C, Tuddenham EGD, et al. *Postgraduate haematology*. 6th ed. New York: John Wiley & Sons, 2011, p. 985.
50. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity, https://www.who.int/vmnis/indicators/haemoglobin/en/
51. Fokouo JV, Vokwely JE, Noubiap JJ, et al. Effect of HIV infection and highly active antiretroviral therapy on hearing function: a prospective case-control study from Cameroon. *JAMA Otolaryngol Head Neck Surg* 2015; 141(5): 436–441.
52. Denue BA, Kida IM, Hammagabdo A, et al. Prevalence of anemia and immunological markers in HIV-infected patients on highly active antiretroviral therapy in northeastern Nigeria. *Infect Dis Res Treat* 2013; 6: 25–33.
53. Alamdo AG, Fiseha T, Tesfay A, et al. Anemia and its associated risk factors at the time of antiretroviral therapy initiation in public health facilities of Arba Minch Town, southern Ethiopia. *Health* 2015; 7(12): 1657.
54. Deressa T, Damtie D, Workineh M, et al. Anemia and thrombocytopenia in the cohort of HIV-infected adults in northwest Ethiopia: a facility-based cross-sectional study. *EJIFCC* 2018; 29(1): 36–47.
55. Steele RH, Keogh GL, Quin J, et al. Mean cell volume (MCV) changes in HIV-positive patients taking nucleoside reverse transcriptase inhibitors (NRTIs): a surrogate marker for adherence. *Int J STD AIDS* 2002; 13(11): 748–754.
56. Jam S, Ramezani A, Sabzvari D, et al. A cross-sectional study of anemia in human immunodeficiency virus-infected patients in Iran. *Arch Iran Med* 2009; 12(2): 145–150.
57. Naides SJ, Howard EJ, Swack NS, et al. Parvovirus B19 infection in human immunodeficiency virus type 1-infected persons failing or intolerant to zidovudine therapy. *J Infect Dis* 1993; 168: 101–105.
58. Evans RH and Scadden DT. Haematological aspects of HIV infection. *Baillieres Best Pract Res Clin Haematol* 2000; 13(2): 215–230.
59. Volberding PA, Levine AM, Dieterich D, et al. Anemia in HIV infection: clinical impact and evidence-based management strategies. *Clin Infect Dis* 2004; 38(10): 1454–1463.
60. Curkendall SM, Richardson JT, Emons MF, et al. Incidence of anaemia among HIV-infected patients treated with highly active antiretroviral therapy. *HIV Med* 2007; 8(8): 483–490.
61. Servais J, Nkoghe D, Schmit JC, et al. HIV-associated hematologic disorders are correlated with plasma viral load and improve under highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001; 28(3): 221–225.
62. Murphy MF, Metcalfe P, Waters AH, et al. Incidence and mechanism of neutropenia and thrombocytopenia in patients with human immunodeficiency virus infection. *Br J Haematol* 1987; 66(3): 337–340.
63. Zon LI, Arkin C and Groopman JE. Haematologic manifestations of the human immune deficiency virus (HIV). *Br J Haematol* 1987; 66(2): 251–256.
64. Kirchhoff F and Silvestri G. Is Nef the elusive cause of HIV-associated hematopoietic dysfunction? *J Clin Invest* 2008; 118(5): 1622–1625.
65. Debasu M, Menon MK, Belayneh Y, et al. Anti-retroviral treatment related haematological disorders among HIV-

infected children attending HIV clinic at Yekatit 12 Hospital, Addis Ababa, Ethiopia. *Int Blood Res Rev* 2015; 4(2): 1–8.
66. Attili SV, Singh VP, Rai M, et al. Hematological profile of HIV patients in relation to immune status—a hospital-based cohort from Varanasi, north India. *Turk J Haematol* 2008; 25(1): 13–19.
67. Santiago-Rodríguez EJ, Mayor AM, Fernández-Santos DM, et al. Profile of HIV-infected hispanics with pancytopenia. *Int J Environ Res Public Health* 2015; 13(1): ijerph13010038.
68. Dhurve SA and Dhurve AS. Bone marrow abnormalities in HIV disease. *Mediterr J Hematol Infect Dis* 2013; 5(1): e2013033.
69. Federal Ministry of Health. National guidelines for comprehensive HIV prevention, care and treatment. *Federal Ministry of Health, Addis Ababa, Ethiopia*, 2017, https://www.humanitarianresponse.info/sites/www.humanitarianresponse.info/files/documents/files/national_comprehensive_hiv_care_guideline_2018-endorsed.pdf
70. Agarwal D, Chakravarty J, Chaube L, et al. High incidence of zidovudine induced anaemia in HIV infected patients in eastern India. *Indian J Med Res* 2010; 132: 386–389.
71. Berhane Y, Haile D and Tolessa T. Anemia in HIV/AIDS Patients on antiretroviral treatment at Ayder Specialized Hospital, Mekele, Ethiopia: a case-control study. *J Blood Med* 2020; 11: 379–387.