Original Research

Cytopenias among ART-naive patients with advanced HIV disease on enrolment to care and treatment services at a tertiary hospital in Tanzania: A cross-sectional study

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Abstract

Background

HIV/AIDS causes high morbidity and mortality through both immunosuppression and complications not directly related to immunosuppression. Haematological abnormalities, including various cytopenias, occur commonly in HIV through immune and non-immune pathways. Though these complications could potentially cause serious clinical implications, published literature on the magnitude of this problem and its associated factors in Tanzania is scarce. This study aimed at determining the prevalence and risk factors of HIV-associated cytopenias among ART-naive patients enrolling for care and treatment services at Bugando Care and Treatment Centre (CTC) in Mwanza, Tanzania.

Methods

This was a cross-sectional clinic-based study done between March 2015 and February 2016, involving all antiretroviral therapy (ART)-naive adult HIV-positive patients enrolling for care and treatment services at Bugando CTC. Patients younger than 18 years and those with missing data were excluded. Data were analysed using Stata version 11 to determine the prevalence and risk factors of cytopenias.

Results

A total of 1205 ART-naive patients were included. Median age was 41 years (interquartile range [IQR] 32 to 48). Most participants were female (n = 789; 65.6%), with a female-to-male ratio of 2:1. The median baseline CD4 count was 200 cells/μL (IQR 113 to 439). About half (49%) of the study participants had baseline CD4 counts less than 200 cells/μL. Anaemia, leucopenia, and thrombocytopenia were found in 704 (58.4%), 285 (23.6%), and 174 (14.4%) participants, respectively, and these were strongly associated with advanced HIV infection.

Conclusions

The magnitude of cytopenias is high among ART-naive HIV-positive adults, and cytopenias are more marked with advanced HIV infection. Early diagnosis of HIV and timely initiation of ART could potentially reduce the number of people living with advanced HIV disease and its associated complications, including the cytopenias investigated in this study. Patients with cytopenias should undergo thorough screening for tuberculosis, which is an important and treatable correlate of cytopenia, in addition to close follow-up for any potential negative outcomes.

Introduction

HIV is still a global problem causing extensive morbidity and mortality worldwide, especially in sub-Saharan Africa, where more than 70% of all HIV cases occur.[1] HIV-1 is the most virulent form of HIV and is responsible for most of the global burden of HIV disease.[2]

Morbidity and mortality in HIV/AIDS are mainly attributed to opportunistic infections and AIDS-defining illnesses.[3] A considerable proportion of the ongoing efforts to reduce HIV/AIDS-related morbidity and mortality is devoted to scaling up access to HIV care and treatment services.[4] In 2014, about 28 million people living with HIV/AIDS were eligible for antiviral therapy (ART); of these, about 15.8 million people were estimated to be receiving ART.[1] The evidence accumulating on the effectiveness of ART suggests that the use of ART considerably improves survival of people living with HIV/AIDS by reducing the frequency of AIDS-related events.[5,6]

Despite these efforts and successeses, there is still a large burden of HIV-related morbidity and mortality, notably due to complications that are not secondary to immunosuppression.[7,8] Haematological complications are reported to be among the most common non-immunosuppressive complications of HIV. These frequently present as cytopenias, especially in ART-naive patients. These complications can have serious clinical consequences, including increased rate of AIDS progression and mortality from anaemia,[9] as well as increased risk of bacterial infection[10] and bleeding[11] from granulocytopenia and thrombocytopenia, respectively. However, published data on the magnitude of this problem and its associated factors are scarce in our setting. Therefore, the aim of this study was to determine the prevalence and risk factors of HIV-associated cytopenias among ART-naive adult HIV-positive patients enrolling for care and treatment services at Bugando Care and Treatment Centre in Mwanza, Tanzania.

Methods

Study design, setting, and population

This was a cross-sectional clinic-based study, carried out at Bugando Medical Centre (BMC), Care and Treatment Centre (CTC), between March 2015 and February 2016. BMC is a tertiary-level teaching hospital affiliated with the Catholic University of Health and Allied Sciences (CUHAS) and the Weill Bugando School of Medicine. It is located along the shores of Lake Victoria in Mwanza, Tanzania, and caters for the country's Lake and Western Zone. BMC has more than 900 beds and over 1000 employees. It currently serves a catchment population of around 16 million people from the Mwanza, Geita, Simiyu, Mara, Kagera, Shinyanga, Tabora, and Kigoma areas. It runs both teaching and treatment services, taking care of inpatients and outpatients. CTC activities are part of BMC's core and routine outpatient services. Currently, CTC serves more than 10,000 HIV-positive patients, either diagnosed within the centre or referred from one of the aforementioned catchment areas for expert management.

Table 1: General demographic, clinical, and laboratory characteristics of 1205 ART-naive HIV-positive adults

Characteristics	Frequency	Percentage	Median (IQR)
Demographic variables			
Age group (years)			
≥ 40 Years	611	50.7	-
< 40 Years	594	49.39	-
Median (IQR) age (years)	-	-	41 (32-48)
Sex			
Male	416	34.4	-
Female	789	65.6	-
WHO clinical stage of HIV disease			
Stage 1 & 2	697	57.8	-
Stage 3 & 4	508	42.2	-
Baseline CD4 count (cells/μL)			
< 200	594	49.3	-
200 to 350	260	21.6	-
351 to 500	97	8.1	-
> 500	254	21.1	-
Median (IQR) laboratory values			
CD4 count (cells/μL)	-	-	200 (113-439)
Haemoglobin concentration (g/dL)	-	-	11.3 (9.5-13.0)
White blood cell count ($x10^9$/L)	-	-	4.82 (3.6-6.2)
Platelet count ($x10^9$/L)	-	-	240 (182-309)

IQR = interquartile range; WHO = World Health Organization

All adult ART-naive HIV-positive patients at Bugando CTC were eligible for this study. Patients younger than 18 years and those with missing lab result details were excluded. A minimum sample size of 384 was calculated from the prevalence studies' formula by Leslie and Kish, assuming a cytopenia prevalence of 50% among ART-naive HIV-positive adults.[9,12] Consenting patients were sequentially enrolled until the sample size was reached. Pertinent patient data were recorded, including age, sex, opportunistic diseases present at the time of diagnosis, WHO clinical stage, baseline CD4 count, and most recent CD4 count. From the full blood count (FBC) the following values were recored: absolute red blood cell count (RBC), white blood cell count (WBC), platelet count (Plt), haemgolobin level (Hb), and red blood cell indices.

Blood sample collection and processing

Five millilitres of whole blood was collected by venepuncture in Vacutainer tubes containing EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for FBC and CD4 count estimation. The samples were sent to BMC main laboratory within 2 hours for analysis. The FBC values were determined using a CELL-DYN 1800 (Abbott Laboratories, Diagnostics Division, Santa Clara, CA, USA) automated blood analyser, and the absolute CD4 counts were determined using the BD FACSCount system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Statistical analysis

Data were analysed using EpiData version 3.1 (The EpiData Association, Odense, Denmark) and Stata version 11 (StataCorp, College Station, TX, USA). In this study haematological abnormalities were defined according to World Health Organization (WHO) haematological reference ranges and as used in other studies.[13-15] Anaemia was therefore defined as Hb < 12.0 for females and Hb < 13.0 g/dL for male subjects. Leucopenia was defined as an absolute WBC < 3.5×10^9/L, whereas thrombocytopenia was defined as an absolute Plt < 150×10^9/L. The cytopenias were further classified by degree of severity as used in other studies. Based on this, Hb of 10.0 to 12.0 g/dL (for females) or 10.0 to 13.0 g/dL (for males) was considered mild anaemia, while moderate anaemia was defined as Hb between 8.0 and 10.0 g/dL for both sexes, and Hb < 8.0 g/L was considered severe anaemia.[16,17] Mild, moderate, and severe thrombocytopenia were defined as Plt of 100 to 150 $\times 10^9$/L, 50 to 100 $\times 10^9$/L, and < 50×10^9/L, respectively.[18] Mild, moderate, and severe leucopenia were defined as an absolute WBC of 3.0 to 3.5 $\times 10^9$/L, 2.0 to 3.0 $\times 10^9$/L, and < 2.0×10^9/L, respectively.[19] Advanced HIV was defined as CD4 count < 200 cells/μL or presentation with a WHO stage 3 or 4 clinical event.[20] Continuous variables were expressed as medians with interquartile ranges (IQRs) and categorical variables were expressed as proportions and percentages. The effect of different risk factors on the odds of having HIV-associated cytopenias was investigated. A univariate logistic regression model followed by a multivariate logistic regression model was used to calculate odds ratios (ORs) and 95% confidence intervals to quantify the strength of association between HIV-associated cytopenias and their potential predictors. In all of our analyses, factors were considered significantly associated with the outcome variable when the P-value was ≤ 0.05.

Ethical considerations

Permission to conduct and publish the information from this study was obtained from the Joint CUHAS–BMC Research & Ethics Committee. Written informed consent was obtained from all study participants. To maintain confidentiality, patient identifiers were not recorded. The results from this study will be of benefit for both teaching purposes and optimisation of patient care in our setting.

Results

General demographic, clinical, and laboratory characteristics of study participants

In this study, a total of 1205 ART-naive patients were included, with a median age of 41 years (IQR 32-48). Most participants were female (n = 789; 65.6%), with a female-to-male ratio of 2:1. The majority (n = 697; 57.8%) were classified as having WHO clinical stage 1 or 2 disease, and the median baseline CD4 count was 200 cells/μL (IQR 113 to 439). About half (49%) of the study participants had baseline CD4 counts were less than 200 cells/μL, and 21% had CD4 counts above 500 cells/μL. The median baseline Hb was 11.3 g/dL (IQR 9.5 to 13.0), whereas the median WBC and Plt values were 4.82×10^9/L (IQR 3.6 to 6.2) and 240×10^9/L (IQR 182 to 309), respectively (Table 1a). Of the patients who presented with WHO clinical stage 1 or 2 HIV disease, most were diagnosed to have dermatological conditions, with pruritic papular eruption (PPE) being the most common diagnosis (n = 348; 28.9%), followed by herpetic eruptions (n = 172; 14.3%). On the other hand, among those who presented with WHO clinical stage 3 or 4 disease, tuberculosis (TB) was the commonest diagnosis (n = 425; 35.3%), followed by gastrointestinal candidiasis (n = 87; 7.2%), as summarised in Figure 1.

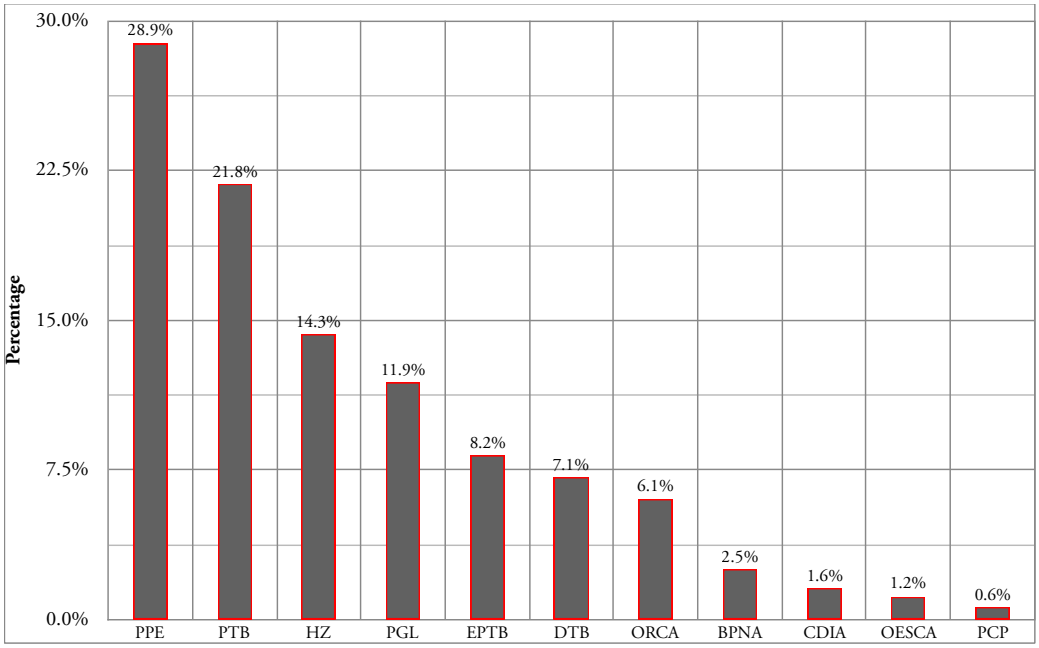

Figure 1: Opportunistic illness diagnoses among 1205 ART-naive HIV-positive adults

PPE = pruritic papular eruption; PTB = pulmonary tuberculosis; HZ = herpes zoster; PGL = persistent generalised lymphadenopathy; EPTB = extrapulmonary tuberculosis; ORCA = oral candidiasis; BPNA = recurrent bacterial pneumonia; CDIA = chronic diarrhoea; OESCA = oesophageal candidiasis; PCP = Pneumocystis jirovecii pneumonia

Table 2: Distribution of cytopenias by severity among 1205 ART-naive HIV-positive adults

Severity level	Anaemia	Leucopenia	Thrombocytopenia
Mild cytopenias			
Range	Hb (10.0 to 12.0♀ or 13.0♂ g/dL)	WBC (3.0 to 3.5 x10⁹/L)	Plt (100 to 150 x10⁹/L)
Frequency (%)	352 (29.2%)	133 (11.0%)	134 (11.1%)
Moderate cytopenias			
Range	Hb (8.0 to10.0 g/dL)	WBC (2.0 to 3.0 x10⁹/L)	Plt (50 to 100 x109/L)
Frequency (%)	204 (16.9)	137 (11.4%)	24 (2.0%)
Severe cytopenias			
Range	Hb (< 8.0g/dL)	WBC (< 2.0 x10⁹/L)	Plt (< 50 x10⁹/L)
Frequency (%)	148 (12.2%)	15 (1.2%)	16 (1.3%)

Hb = haemoglobin concentration (note slightly different ranges used for females♀ and males♂); Plt = platelet count; WBC = white blood cell count

The prevalence of cytopenias and their potential risk factors

A total of 704 (58.4%) of the study participants were found to have anaemia, whereas leucopenia and thrombocytopenia were found in 285 (23.7%) and 174 (14.4%) of the participants, respectively. Bicytopenia occurred in at least 88 (7.30%) of the participants, with anaemia and leucopenia being the most common combination (n = 193; 16.0%). Pancytopenia occurred in 61 (5.1%) of the studied patients (Figure 2). By severity, 148 (12.2%) of the study participants had severe anaemia, whereas severe leucopenia and thrombocytopenia were observed in 15 (1.2%) and 16 (1.3%) of the studied patients, respectively (Table 2). Anaemia commonly occurred among patients with WHO clinical stage 3 and 4 disease (48.3% and 33.5%, P < 0.001), and among those with baseline CD4 counts less than 200 cells/μL (OR = 1.3, P < 0.020). While tuberculosis was the commonest opportunistic condition, the odds of having anaemia were significantly associated with a pulmonary tuberculosis (PTB) diagnosis (OR = 1.5, P = 0.01) and disseminated tuberculosis (DTB) (OR = 2.7, P < 0.001). Using a multivariate regression model, anaemia was independently associated with WHO clinical stage 3 or 4 HIV disease (OR = 1.5, P = 0.016), CD4 counts < 200 cells/μL (OR = 1.3, P = 0.04), and DTB (OR = 2.1, P = 0.019), as summarised in Table 3.

Table 4 shows that leucopenia was strongly predicted by a female gender (OR = 1.5, P = 0.006) in addition to HIV WHO clinical stage 3 or 4 (OR = 1.8, P < 0.001), PTB (OR = 1.5, P = 0.016), DTB (OR = 2.0, P = 0.003) and baseline CD4 of less than 200 cells/μL (OR = 2.8, P <0.001). Using a multivariate model (see Table 4), leucopenia was independently associated with a female gender (OR = 1.5, P = 0.009), CD4 count less than 200/μL (OR = 2.8, P < 0.001), and DTB (OR = 1.8, P = 0.04).

Thrombocytopenia was strongly predicted also by WHO clinical stage 3 or 4 disease (OR = 1.9, P < 0.001), baseline CD4 less than 200 cells/μL (OR = 1.6, P = 0.003), and DTB (OR = 2.5, P <0.001); all of these factors remained as independent risk factors for thrombocytopenia on a multivariate model, as summarised in Table 5.

Table 3: Univariate and multivariate analysis for factors associated with anaemia among 1205 ART-naive HIV-positive adults

Variable	Anaemia (Hb < 12.0♀ or 13.0♂ g/dL)		Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
	No (%)	Yes (%)				
Age group						
≥ 40 years	247 (49.3)	364 (51.7)	-	-	-	-
< 40 years	254 (50.7)	340 (48.3)	1.1 (0.9 to 1.4)	0.41	-	-
Sex						
Female	334 (66.7)	455 (64.6)	-	-	-	-
Male	167 (33.3)	249 (35.4)	0.9 (0.7 to 1.2)	0.46	-	-
WHO clinical HIV stage						
Stage 1 & 2	333 (66.5)	364 (51.7)	1.9 (1.5 to 2.3)	**< 0.001**	1.5 (1.1 to 2.2)	**0.016**
Stage 3 & 4	168 (33.5)	340 (48.3)	-	-	-	-
Baseline CD4 count (cells/μL)						
< 200	227 (45.3)	367 (52.2)	-	-	-	-
≥ 200	274 (54.7)	337 (47.8)	1.3 (1.0 to 1.7)	**0.020**	1.3 (1.0 to 1.6)	**0.040**
Opportunistic disease diagnosis						
Pruritic papular eruptions	158 (31.5)	190 (27.0)	0.8 (0.6 to 1.0)	0.10	-	-
Pulmonary tuberculosis	914 (18.2)	172 (24.4)	1.5 (1.1 to 1.9)	**0.010**	1.1 (0.7 to 1.7)	0.60
Herpes zoster	82 (16.4)	90 (12.8)	0.7 (0.5 to 1.0)	0.080	-	-
Persistent generalised lymphadenopathy	71 (14.2)	74 (10.5)	0.7 (0.5 to 1.0)	0.055	-	-
Extrapulmonary tuberculosis	33 (06.6)	66 (09.4)	1.5 (0.9 to 2.3)	0.084	-	-
Disseminated tuberculosis	19 (03.8)	67 (09.5)	2.7 (1.6 to 4.5)	**< 0.001**	2.1 (1.1 to 3.7)	**0.019**
Oral candidiasis	29 (05.8)	44 (06.3)	1.1 (0.7 to 1.8)	0.33	-	-
Bacterial pneumonia	8 (01.6)	22 (03.1)	2.0 (0.9to 4.5)	0.099	-	-

Hb = haemoglobin concentration; ♀ = female; ♂ = male; OR = odds ratio; CI = confidence interval; WHO = World Health Organization

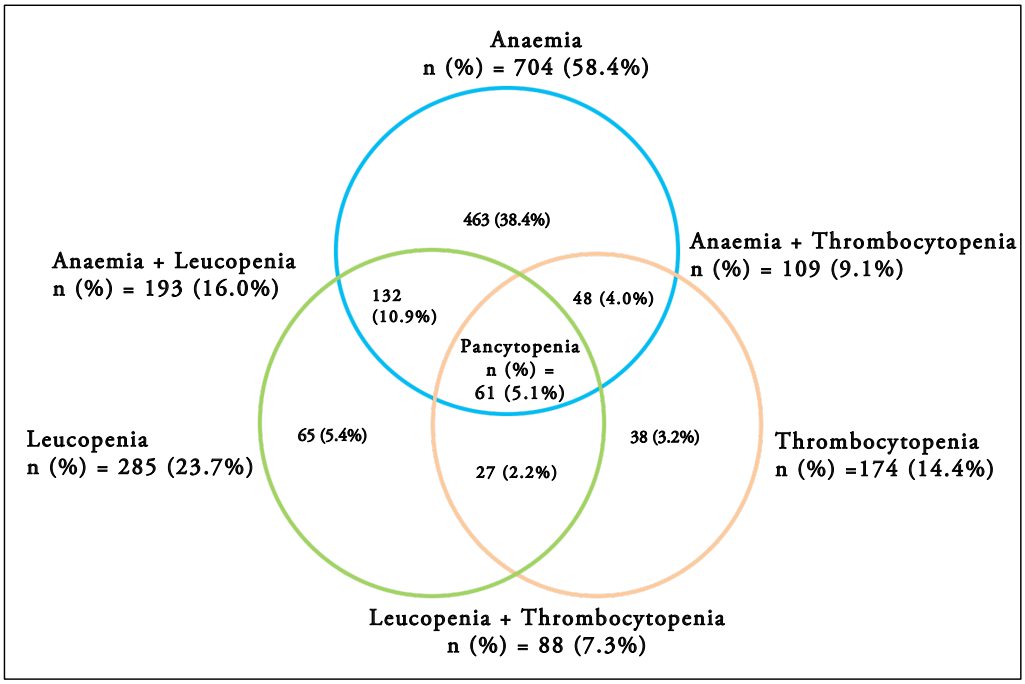

Figure 2: Distribution of cytopenias among 1205 ART-naive HIV-positive adults

Table 6 summarises the univariate and multivariate analyses for factors associated with multiple cytopenias. Patients who had multiple cytopenias were likely to be female (OR = 1.4, P = 0.05), in WHO clinical stage 3 or 4 (OR = 2.1, P < 0.001), with baseline CD4 counts less than 200 cells/μL (OR = 3.2, P < 0.001), and those diagnosed to have PTB (OR = 1.5, P = 0.015), DTB (OR=2.5, P < 0.001), and recurrent bacterial pneumonia (OR = 2.3, P = 0.04). On a multivariate logistic regression model, multiple cytopenias remained independently predicted by CD4 counts less than 200 cells/μL (OR = 3.1, P < 0.001), and DTB (OR = 2.5, P = 0.012). The strength of association of other factors with multiple cytopenias was not statistically significant.

Table 4: Univariate and multivariate analysis for factors associated with leucopenia among 1205 ART-naive HIV-positive adults

Variable	Leucopenia (WBC < 3.5 x 10⁹/L)		Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
	No (%)	Yes (%)				
Age group						
≥ 40 years	467 (50.8)	144 (50.5)	-	-	-	-
< 40 years	453 (49.2)	141 (49.5)	1.0 (0.7 to 1.3)	0.95	-	-
Sex						
Female	336 (36.6)	78 (27.4)	-	-	-	-
Male	582 (63.4)	207 (72.6)	1.5 (1.1 to 2.0)	0.006	1.5 (1.1 to 2.0)	**0.009**
WHO clinical HIV stage						
Stage 1 & 2	563 (61.2)	134 (47.1)	1.8 (1.3 to 2.3)	**< 0.001**	1.3 (0.9 to 2.0)	0.17
Stage 3 & 4	357 (38.8)	151 (52.9)	-	-	-	-
Baseline CD4 count (cells/μL)						
< 200	399 (43.4)	195 (68.4)	-	-	-	-
≥ 200	521 (56.6)	90 (31.6)	2.8 (2.1 to 3.7)	**< 0.001**	2.8 (2.1 to 3.7)	**< 0.001**
Opportunistic disease diagnosis						
Pruritic papular eruptions	271 (29.5)	76 (26.6)	0.9 (0.6 to 1.2)	0.36	-	-
Pulmonary tuberculosis	186 (20.2)	77 (27.0)	1.5 (1.1 to 2.0)	**0.016**	1.3 (0.8 to 2.0)	0.29
Herpes zoster	146 (15.9)	26 (09.1)	0.5 (0.3 to 0.8)	**0.005**	-	-
Persistent generalised lymphadenopathy	119 (12.9)	26 (09.1)	-	-	-	-
Extrapulmonary tuberculosis	77 (8.4)	22 (07.7)	0.9 (0.6 to 1.5)	0.73	-	-
Disseminated tuberculosis	54 (5.9)	32 (11.2)	2.0 (1.2 to 3.2)	**0.003**	1.8 (1.0 to 3.2)	**0.042**
Oral candidiasis	48 (5.2)	25 (08.8)	1.7 (0.8 to 3.8)	0.17	-	-
Bacterial pneumonia	20 (2.2)	10 (03.5)	1.6 (0.8 to 3.5)	0.21	-	-

WBC = white blood cell count; OR = odds ratio; CI = confidence interval; WHO = World Health Organization

Discussion

The objective of this study was to determine the prevalence and risk factors of HIV-associated cytopenias. Anaemia was found to be the most frequent HIV-associated cytopenia, occurring in 704 (58.4%) of the study participants, followed by leucopenia and thrombocytopenia, which were found in 285 (23.7%) and 174 (14.4%) of the participants, respectively. In previous studies, anaemia has been reported in more than 60% of patients.[9,14] Compared to our study, lower anaemia prevalences have been reported in India (22%),[21] Ethiopia (35%),[22] Uganda (47.8%),[23] and Nigeria (57.5%).[24] On the other hand, our study reports a lower anaemia prevalance than several other studies that investigated anaemia prevalence among ART-naive patients. In a study from India, by Byomakesh et al., anaemia was found in 65.5% of the study participants,[14] while a prior study in rural Tanzania by Johannessen et al. in 2011 reported a prevalence of 77.4% among ART-naive HIV-positive adults.[25] Daka et al. reported an anaemia prevalence of 86.5% among ART-naive HIV positive patients in Ethiopia.[26]

Among a number of studies that have reported on HIV-associated cytopenias involving other cell lines, results have been consistently comparable to our findings. Leucopenia is also common in ART-naive patients, with a prevalence that ranges between 10% and 44%.[12,27] A study from northern Ethiopia indicated that 16.6% of ART-naive patients had leucopenia, while 9.0% had thrombocytopenia.[28] In Uganda, leucopenia and thrombocytopenia were reported in 24.3% and 8.3% of the study participants, respectively.[23] Akinsegun et al., in a 2010 study, reported leucopenia and thrombocytopenia in 26.8% and 16.1% of ART-naive patients, respectively, in a Nigerian population.[29] Earlier studies indicated that platelet counts of less than 150 were more common among ART-naive patients, with prevalence figures ranging between 6% and 30% in most studies.[12,27,30]

Evidence from available studies has shown that patients with anaemia have reduced survival time and accelerated HIV disease progression. For instance, an earlier study in developed countries by Mocroft et al. indicated that, at 12 months, HIV patients with mild anaemia had a significantly lower survival rate than those without anaemia (84.1% vs. 96.9%), and that prognosis and survival time was even worse among severely anemic patients.[31] Similarly, in the USA, Sullivan and colleagues found that survival rate was considerably reduced among anaemic HIV patients, with a relative risk of death of 148%.[8] Another study in Tanzania by O'Brien et al. showed that anaemia is an independent risk factor for mortality and disease progression among HIV-positive women.[32] In this study patients with anaemia had increased risk of mortality, with a hazard ratio (HR) of 2.06 for mild anaemia, 2.7 for moderate anaemia, and 3.19 for severe anaemia.

Despite the high frequency of cytopenias in other cell lines, clinical implications and specific treatments are not well described. Available studies from developed countries

Table 5: Univariate and multivariate analysis for factors associated with thrombocytopenia among 1205 ART-naive HIV-positive adults

Variable	Thrombocytopenia (Plt < 1.5 x 10^9/L)		Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
	No (%)	Yes (%)				
Age group						
≥ 40 years	530 (51.4)	081 (46.6)	-	-	-	-
< 40 years	501 (48.6)	093 (53.4)	0.8 (0.6 to 1.1)	0.24	-	-
Sex						
Female	672 (65.2)	117 (67.2)	-	-	-	-
Male	359 (34.8)	57(32.8)	1.1 (0.8 to 1.5)	0.60	-	-
WHO clinical HIV stage						
Stage 1 & 2	619 (60.1)	78 (44.8)	1.9 (1.3 to 2.6)	**< 0.001**	1.6 (1.1 to 2.2)	**0.010**
Stage 3 & 4	412 (39.9)	96 (55.2)	-	-	-	-
Baseline CD4 count (cells/µL)						
< 200	490 (47.5)	104 (59.8)	-	-	-	-
≥ 200	541 (52.5)	70 (40.2)	1.6 (1.2 to 2.3)	**0.003**	1.6 (1.1 to 2.2)	**0.008**
Opportunistic disease diagnosis						
Pruritic papular eruptions	313 (30.4)	34 (19.5)	0.6 (0.4-0.8)	**0.004**	-	-
Pulmonary tuberculosis	216 (21.0)	47 (27.0)	1.4 (1.0 to 2.0)	0.074	-	-
Herpes zoster	151 (14.7)	21 (12.1)	0.8 (0.5 to 1.3)	0.37	-	-
Persistent generalised lymphadenopathy	126 (12.2)	19 (10.9)	0.9 (0.5 to 1.5)	0.63	-	-
Extrapulmonary tuberculosis	94 (9.1)	5 (2.9)	0.3 (0.1 to 0.7)	**0.009**	-	-
Disseminated tuberculosis	62 (0 6.0)	24 (13.8)	2.5 (1.5 to 4.1)	**< 0.001**	1.9 (1.1 to 3.2)	**0.022**
Oral candidiasis	59 (05.7)	14 (8.1)	1.4 (0.8 to 2.6)	0.24	-	-
Bacterial pneumonia	22 (2.1)3	8 (4.6)	2.2 (1.0 to 5.0)	0.060	-	-

Plt = platelet count; OR = odds ratio; CI = confidence interval; WHO = World Health Organization

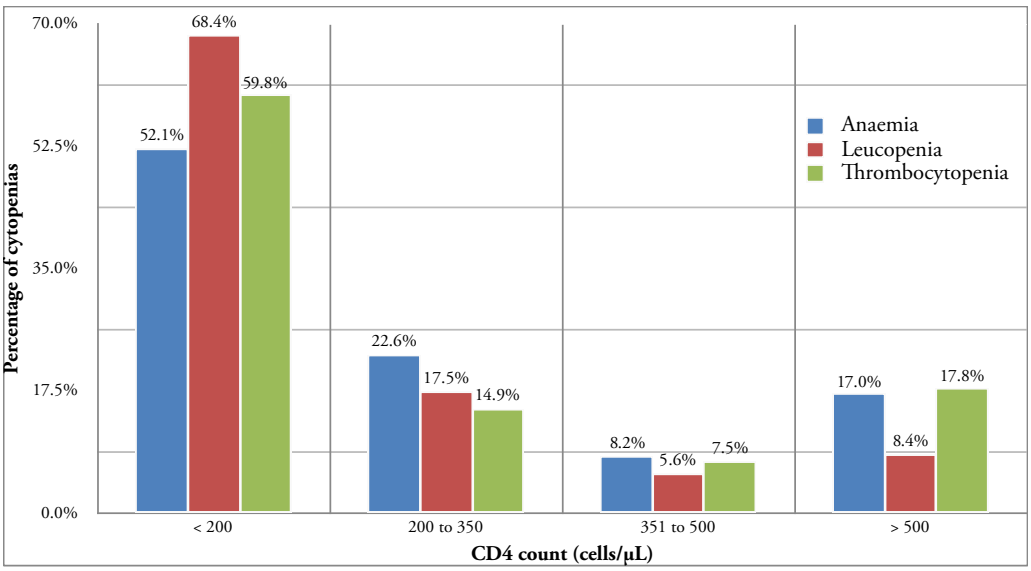

Figure 3: Cytopenia type by CD4 count among 1205 ART-naive HIV-positive patients

indicate that a reduction in absolute white blood cell count has been associated with increased risk of severe bacterial infection and hospitalisation,[10] and that patients with thrombocytopenia have a high potential for bleeding.[11] However, with a lack of comparable studies from Africa, these findings are difficult to generalise to the African region. Initiation of ART has generally been regarded as the standard management for HIV/AIDS patients. In addition to the restoration of immunological function, ART has also been shown to improve HIV-related cytopenias, especially anaemia.[25,33,34] A study by Johannessen et al. recorded a reduction of anaemia from 77.4% among ART-naive patients to 38.2% after 12 months of ART.[25] A study in Nigeria demonstrated a reduction in anaemia prevalence from 69.17% to 51.15% after ART.[35] Another study in Nigeria, by Denue et al., also showed a significant improvement of haematological parameters with ART: the prevalence of anaemia was reduced from 57.5% pre-ART to 24.3% post-ART, while leucopenia and thrombocytopenia were reduced from 6.1% and 9.6% to 1.7% and 1.2%, respectively.[24] An important observation from available studies is that even with this great effect of ART on HIV-associated cytopenias, the reversal of these complications is usually incomplete. This suggests that the mechanisms underlying HIV-associated cytopenias are complex and can only be reversed partially with the use of ART. Among other mechanisms, studies suggest reduced marrow activity in the production of different cell lines, which can be due to HIV itself, opportunistic infections, or cytokine-mediated changes,[36,37] with a tendency for the effects of these mechanisms to increase in magnitude with worsening HIV disease. For example, regarding anaemia, studies illustrate that among asymptomatic HIV-positive patients, anaemia occurs in 8% to 18% of patients, as compared to 20% to 50% of patients with intermediate disease progression, and 70% to 75% of patients with advanced HIV.[38,39] In an Ethiopian study, where 29.7%, 16.6%, and 9% of ART-naive patients had anaemia, leucopenia, and thrombocytopenia, respectively, it was indicated that at CD4 counts greater than 350 cells/μL, only 18.8%, 15.3%, and 3.5% of these patients had anaemia, leucopenia, and thrombocytopenia respectively. Comparatively, at CD4 counts less than 200 cells/μL, it was indicated that there was a much higher rate of cytopenias with anaemia, leucopenia, and thrombocytopenia being reported in 34.5%, 43.1%, and 15.5% of the ART-naive patients with cytopenias.[28] Another study in Nigeria by Akinsegun et al., studying haematological abnormalities in ART-naive patients, demonstrated that at CD4 counts above 500 cells/μL, anaemia occurred in only 5.26% of the study participants, while leucopenia and thrombocytopenia were found in 10.5% and 13.15% of the study participants, respectively. Conversely, at a CD4 count of less than 200 cells/μL, anaemia was present in between 50% and 71% of study participants, whereas leucopenia occurred in 55% to 75% of participants, and thrombocytopenia in 15% to 53.3%.[29] These observations are also supported by our finding that among those patients with anaemia, 8.2%, 22.6%, and 52.1% had mild, moderate, and severe immunosuppression. Additionally, in the current study it was noted that at CD4 counts above 500 cells/μL, only 17.0%, 8.4%, and 17.8% of patients had anaemia, leucopenia, and thrombocytopenia, respectively, compared to 52.1%, 68.4%, and 59.8% at CD4 counts less than 200 cells/μL (Figure 3).

In our study, patients with WHO clinical stage 3 or 4 disease were 1.8 times more likely to have anaemia and leucocytopenia and 1.9 times more likely to have thrombocytopenia. Additionally they were 2.1 and 2.7 times more likely to have bicytopenia and pancytopenia than patients with WHO clinical stage 1 or 2 disease. Patients with baseline CD4 counts less than 200 cells/μL were 1.4 to 2.8 times more likely to have anaemia, thrombocytopenia, and leucopenia.

Table 6: Univariate and multivariate analysis for factors associated with multiple cytopenias among 1205 ART-naive HIV-positive adults

Variable	Multicytopenia		Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
	No (%)	Yes (%)				
Age group						
≥ 40 years	507 (50.1)	104 (53.9)	-	-	-	-
< 40 years	505 (49.9)	089 (46.1)	1.2 (0.8 to 1.6)	0.34	-	-
Sex						
Female	651 (64.3)	138 (71.5)	-	-	-	-
Male	361 (35.7)	55 (28.5)	1.4 (1.0 to 1.9)	**0.050**	1.4 (1.0 to 2.0)	0.067
WHO clinical HIV stage						
Stage 1 & 2	615 (60.8)	82 (42.5)	2.1 (1.5 to 2.9)	**< 0.001**	1.3 (0.8 to 2.2)	0.24
Stage 3 & 4	397 (39.2)	111 (57.5)	-	-	-	-
Baseline CD4 count (cells/μL)						
< 200	455 (45.0)	139 (72.0)	-	-	-	-
≥ 200	557 (55.0)	54 (28.0)	3.2 (2.2 to 4.4)	**< 0.001**	3.1 (2.2 to 4.4)	**< 0.001**
Opportunistic disease diagnosis						
Pruritic papular eruptions	299 (29.6)	48 (24.9)	0.8 (0.6 to 1.1)	0.19	-	-
Pulmonary tuberculosis	208 (20.6)	55 (28.5)	1.5 (1.1 to 2.2)	**0.015**	1.4 (0.8 to 2.4)	0.19
Herpes zoster	157 (15.5)	15 (7.8)	0.5 (0.3 to 0.8)	**0.006**	-	-
Persistent generalised lymphadenopathy	125 (12.4)	20 (10.4)	0.8 (0.5 to 1.4)	0.44	-	-
Extrapulmonary tuberculosis	86 (8.5)	13 (6.7)	0.8 (0.4 to 1.4)	0.42	-	-
Disseminated tuberculosis	60 (5.9)	26 (13.5)	2.5 (1.5 to 4.0)	**< 0.001**	2.3 (1.2 to 4.3)	**0.012**
Oral candidiasis	58 (05.7)	15 (07.8)	1.4 (0.8 to 2.5)	0.28	-	-
Bacterial pneumonia	21 (02.1)	9 (4.7)	2.3 (1.0 to 5.1)	0.040	2.1 (0.8 to 5.0)	0.11

OR = odds ratio; CI = confidence interval; WHO = World Health Organization

HIV-associated cytopenias represent an ongoing challenge because of the variety of potential aetiologies, which are in most cases difficult to adequately investigate and treat. For example, anaemia is the commonest form of HIV-associated cytopenia and has frequently been associated with several factors that may either cause reduced bone marrow activity, chronic blood loss, or peripheral red blood cell destruction.[40] Reduced bone marrow activity frequently follows (1) marrow infiltrative diseases like opportunistic infections and malignances,[41] (2) inadequate marrow stimulation (for example, from reduced erythropoietin production),[42] (3) bone marrow suppression (from medications like zidovudine [AZT] and cotrimoxazole [CTX] or from the suppressive effects of soluble proteins from HIV and cytokines),[43,44] (4) nutritional deficiencies with reduced raw material supply (iron, folate, and vitamin B12),[45,46] among others. In HIV patients, chronic blood loss is a potential cause of anaemia—commonly from gastrointestinal lesions like Kaposi's sarcoma, oral and oesophageal candidiasis, cytomegalovirus, oesophagitis, or colitis, and from hookworm infestations.[40] Haemolytic anaemia in HIV-positive patients usually follows autoimmune reaction (autoimmune haemolytic anaemia, AIHA), and though less frequent, patients with AIHA may potentially react to blood transfusion when this cause of anaemia is not ruled out.[47]

Thrombocytopenia in HIV patients has been shown to be a frequent complication among cirrhotic patients with associated portal hypertension, where it occurs in more than 50% of this subgroup of patients.[48,49] Reduced production of thrombopoetin, splenic sequestration, and platelet autoantibodies developing following hepatitis C infection, among others, form a list of possible mechanisms for thrombocytopenia among cirrhotic patients.[50,51] A number of drugs have also been implicated to cause drug-induced thrombocytopenia (DITP), including trimethoprim, quinine, nonsteroidal antiinflammatory drugs (NSAIDs), and anticonvulsants.[52,53] Furthermore, thrombocytopenia has been reported in more than 30% of patients with sepsis. In these patients, thrombocytopenia is usually associated with disseminated intravascular coagulation (DIC), with increased platelet destruction.[54] The thrombocytopenia in sepsis results partly from an associated nonimmune platelet destruction and bone marrow suppression with severity of thrombocytopenia correlating closely to severity of sepsis.[55] HIV-associated thrombotic thrombocytopenic purpura (TTP) is an important cause of thrombocytopenia, first described in 1987.[56] HIV is presumed to precipitate TTP through direct endothelial damage, which in turn causes local generation of thrombin and consumption of metaloprotease,[57] and it commonly occurs in patients with advanced HIV disease.[58] TTP is usually characterised by microvascular platelet deposition and thrombus formation in selected organs with eventually thrombocytopenia, fever and haemolytic anaemia, neurological and renal dysfunction, among others.[59]

In HIV patients, the causes of leucopenia include (1) reduced bone marrow activity from infiltrative conditions, marrow toxicity, and nutritional deficiency; (2) increased apoptosis; (3) HIV infection itself; (4) tuberculous marrow infiltrate; and (5) neoplastic infiltrates.[60-62] On the other hand, most drugs which are currently useful in HIV, such as CTX, AZT, and gancyclovir are myelotoxic,[63] and they can potentially cause neutropenia. HIV antibodies against gp120 form another mechanism which leads to suppression of marrow progenitors.[64] Leucopenia could also follow nutritional deficiency of Vitamin B12,[62] and a shortened neutrophil lifespan from accelerated apoptosis in HIV.[65]

With regards to opportunistic diseases present at the time of diagnosis in our study, TB was the most common diagnosis, and it was strongly associated with all forms of cytopenia, especially the disseminated form of TB, which was as an independent risk factor for cytopenias. This supports the understanding that TB is one of the marrow infiltrative diseases that can potentially lead to reduced production of all cell lines. These findings are in agreement with several other studies. A prior study from Muhimbili had shown that patients with anaemia were 3 times more likely to have TB than patients without anaemia.[66] Studies from elsewhere have also indicated that the prevalence of TB among anaemic HIV patients is high especially among patients with severe anaemia. A study from South Africa by Kerkhoff et al., assessing the prevalence of TB among HIV anaemic patients, indicated that TB was diagnosed in only 8.8% of non-anemic HIV patients, compared to 16.5%, 26.0%, and 40.0% among those with mild, moderate, and severe anaemia, respectively.[67] In an earlier study from the USA studying 76 bone marrow samples among patients with cytopenias, 55 samples (72%) were positive for TB.[68] Additionally, a study from India indicated that TB was a common bone marrow infection among patients with anaemia, leucopenia, and thrombocytopenia.[69] These findings are clinically important, especially in resource-limited settings where HIV and TB burden are highest, such that TB represents an important reversible cause of cytopenia and should routinely be suspected among HIV patients presenting with cytopenia.

Study limitations

This study had a number of limitations. This was a single centre, and it was a hospital-based study, therefore the findings may not necessarily be generalisable. Since this was a cross-sectional study, there was no follow up, and hence a longitudinal study is recommended to answer questions related to outcomes.

Conclusions

HIV-associated cytopenias are common and tend to increase in magnitude and severity with worsening HIV disease. These findings also suggest that efforts to diagnose HIV at earlier stages might serve as an important strategy in the reduction of most cytopenias in this subgroup of patients. Earlier diagnosis may lead to earlier ART initiation and improved outcomes. Furthermore, patients with advanced HIV should be given special attention, as they are more likely to be affected by cytopenias, which may only partially resolve with ART. Tuberculosis, in particular, should be ruled out often, as it is a treatable cause of the cytopenias commonly seen in HIV-positive patients.

Competing interests

The authors declare that they have no conflicts of interest.

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