



## Red Cell Characteristics in Anaemic HIV Infected Patients

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### Authors' contributions

*This work was carried out in collaboration between all authors. Author AOT contributed to conceptualization, study design, literature searches, recruiting of patients and controls and paper write-up. Author OSO contributed to literature searches and review of manuscript. Author AOA performed study supervision and review of manuscript and author ASA contributed to study design, data analysis, study supervision and final review of manuscript. All authors read and approved the final version of the manuscript.*

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### ABSTRACT

**Background:** Anaemia is the most frequently encountered haematological complication in human immunodeficiency virus (HIV) infected patients and acquired immunodeficiency syndrome (AIDS) even in the era of highly active anti-retroviral therapy (HAART). Although HAART appears to be associated with a somewhat lower risk of anaemia, anaemia remains common in the HAART era. This study looks at the characteristics of red blood cells in anaemic HIV infected patients with

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emphasis on red cell indices.

**Materials and Methods:** A total of 60 patients aged 18-66 years were recruited, comprising of 40 HIV infected, treatment naïve individuals who were also anaemic patients and 20 individuals who were non anaemic patients.

Venous blood (5 mls) was collected by vacutainer from each patient who consented into an anti-coagulant K3 EDTA specimen bottle for haematological indices which included Haemoglobin (Hb), Packed cell volume (PCV), White blood cell (WBC) & differentials, Platelet count and red blood cell indices by automated counter, CD4+ cell count by semi-automated flow cytometer, and HIV viral load was quantitated using PCR- based diagnostic tests. Peripheral blood smear for morphology was done by routine manual methods and stained with Romanowsky stain.

**Results:** Majority (72.5%) have normocytic anaemia, 10.0% have microcytic anaemia and 17.5% have macrocytic anaemia. Majority (55.0%) of the HIV infected anaemic subjects have normocytic/normochromic red blood cells on morphology.

The mean WBC of HIV infected anaemic patients ( $7.20 \times 10^9/L \pm 4.93$ ) was significantly different from the mean WBC of HIV infected non-anaemic patients ( $5.66 \times 10^9/L \pm 1.74$ ) ( $p=0.008$ ).

The mean platelet count of HIV infected anaemic patients ( $277.27 \times 10^9/L \pm 126.76$ ) was significantly different from the mean platelet count of HIV infected non-anaemic patients ( $207.40 \times 10^9/L \pm 61.86$ ) ( $p=0.006$ ).

**Conclusion:** Anaemia in HIV infection was found to be normocytic normochromic anaemia.

*Keywords: Anaemia; red blood cells; Human Immunodeficiency Virus; acquired immuno-deficiency syndrome; highly active anti-retroviral therapy.*

## 1. INTRODUCTION

The haematopoietic stem cell is a target for the human immunodeficiency virus (HIV) [1], and anaemia, leukopenia, or thrombocytopenia, alone or in combination, invariably occurs during the course of HIV infection [2,3]. Although numerous morphological abnormalities have been identified in the bone marrow of HIV infected patients [4], the mechanisms for the reduction in the circulating formed elements of the blood are not fully understood. Many drugs used in the treatment of HIV and HIV-related disorders are thought to suppress formation of blood cells in the marrow [5]. Anaemia is the most common haematologic abnormality in HIV infection and its incidence increases with progression of the disease [2]. Anaemia is also a risk factor for death in patients with acquired immune deficiency syndrome (AIDS) [5].

The causes of anaemia in HIV infection includes increased destruction of red blood cells, a decrease in red cell survival, direct or indirect suppression of red cell production and micronutrient deficiencies (e.g. Iron, Vitamin B12, Folate) among many others. Histologically, bone marrow hypoproliferation and dysplasia are seen [2,3]. Dysplastic hematopoiesis is a common finding that accompanies HIV infection [6]. Hematopoietic dysplasia in such patients may result from medications, opportunistic infections,

or a direct effect of HIV on hematopoietic progenitors [7].

Definitions for anaemia normally use the level of haemoglobin (Hb) as a standard [8]. Anaemia is defined as the haemoglobin concentration below the accepted reference range for age, sex and race in a given population. This is normally in the range of  $16 \pm 2$  g/dL for men and  $14 \pm 2$  g/dL for women. The percentage of erythrocytes in relation to the total blood volume (haematocrit) normally ranges from  $47\% \pm 5\%$  for men and  $42\% \pm 5\%$  for women [8]. The mean corpuscular volume (MCV) is used as a pointer to distinguishing between the different types of anaemia, the normal range being 80- 95fL. Lower MCV (microcytic anaemia) is associated with iron deficiency and elevated MCV (macrocytosis) typically with vitamin B12 or folate deficiency. Although in the setting of HIV treatment macrocytosis has been linked to the use of Zidovudine (AZT) or Stavudine (d4T). Both AZT and d4T lead to macrocytosis but only AZT causes anaemia [9]. The reason for this difference is not clear. Bone marrow cell lineages are markedly more sensitive to AZT as compared with other nucleoside analogs and severe cytopenias is a common finding with use of AZT [5]. Supplementation with vitamin B12 or folate does not prevent AZT related macrocytosis, anaemia or neutropenia although a beneficial effect in certain subgroups of patients cannot be excluded [10].

Erythrocyte numbers are continuously adjusted to optimize oxygen carriage capacity by hormonal factors in the form of erythropoietin transmitted to the bone marrow from an oxygen sensor in the kidney. In the bone marrow, erythropoietin binds and activates specific receptors on the erythroid progenitor cells. These progenitor cells then develop into mature erythrocytes and are released into the circulation as reticulocytes, bone marrow examination may demonstrate pure red cell aplasia, erythroid maturation arrest, erythroid hypoplasia and megaloblastic erythropoiesis [11,12]. Bone marrow examination requires invasive techniques that may not be practicable for use in resource poor settings as a baseline investigation to characterize the type of anaemia in HIV infected individuals in order to give appropriate intervention.

This study aimed to determine the types of anaemia in treatment naïve HIV infected patients using red blood cells characteristics, especially the red cell indices. Hence, providing a simple, less invasive and easy way of characterising the types of anaemia in HIV infected individuals in resource poor settings for prompt intervention.

## 2. MATERIALS AND METHODS

This was a cross sectional study carried out on a total of 60 subjects comprising of 40 HIV infected treatment naïve subjects with anaemia of Hb level <10 g/dL (study group) and 20 HIV infected non-anaemic subjects (control group). The study was approved by the Ethical committee of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria and was conducted according to Helsinki Declaration on the conduct of research on human subjects. All patients who gave written informed consent were recruited from the HIV clinic of the Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos, Nigeria.

Inclusion criteria were patients infected with HIV and were anaemic with Hb level <10 g/dL, treatment naïve patients and HIV infected control patients with Hb level >10 g/dL.

HIV infected females who were pregnant were excluded.

### 2.1 Procedure

Participants filled out questionnaires which had relevant information including age, sex, HIV risk

factors, History of Post- exposure prophylaxis, Previous ARV experience, other medical conditions.

Venous blood (5 mls) was drawn from each participant by vacutainer into anti-coagulant K3 EDTA specimen bottle for haematological indices which included Haemoglobin (Hb), Packed cell volume (PCV), White blood cell (WBC) & differentials, Platelet count and red cell indices by automated counter, CD4+ cell count by semi-automated flow cytometer (Partec flow cytometer), and HIV viral load was quantitated using PCR- based diagnostic tests by Roche amplicon.

Peripheral blood film morphology was done by routine manual methods and stained with Romanowsky stain.

### 2.2 Statistical Analysis

Data was analysed using computer statistical software packages: SPSS for windows version 16.0, Microsoft excel 2007 and Epi Info version 3.5.1 2008 as applicable.

Data was expressed as means and standard deviation (standard error of sample) and associations between measured variables was tested using chi-squared and students t-test, and correlations were made.

The statistical significance was set at  $p < 0.05$ .

## 3. RESULTS

The ages of the patients ranged between 18 and 66 years. The study group consisted 20 males and 40 females. The mean age of male HIV infected anaemic patients ( $45.15 \pm 5.63$ ,  $n=13$ ) was significantly higher than that of female HIV infected anaemic patients ( $34.16 \pm 7.21$ ,  $n=25$ )  $p=0.005$ . Table 1

In the HIV infected populations that were anaemic 8 (57.1%) of 14 patients in age group 40-49 years were males and 14 (87.5%) of 16 patients in age group 30-39 years were females while in HIV infected populations that were non-anaemic 3 (75.0%) of 4 patients in age group  $\geq 50$  years were males and 6 (85.7%) of 7 patients in age group 30-39 years were females. HIV infected males tended to be older than female HIV infected patients. There was significant difference in the ages of the anaemic HIV infected males and females ( $p=0.005$ ) Table 1.

Out of the 40 anaemic HIV infected patients 29 (72.5%) have normocytic anaemia (MCV 74-94 fl), 4 (10.0%) have microcytic anaemia (MCV <74 fl) and 7 (17.5%) have macrocytic anaemia (MCV >94 fl). The MCH <25 pg was found in 21 (52.5%) (hypochromia), MCH 25-31pg (normochromia) was seen in 18 (45.0%) and MCH >31 pg (hyperchromia) in 1 (2.5%). The MCHC <30 g/dL was found in 15 (37.5%) and MCHC of 30-35 pg found in 25 (62.5%). Using count statistics, 14 (48.28%) out of 29 that have MCV within normal limits (normocytic anaemia) also have MCH within normal range (normochromic anaemia); 20 (68.97%) out of the 29 who have MCV within normal limits also have MCHC within normal limits as shown in Table 2.

Twenty two (55.0%) of the HIV infected anaemic patients have normocytic/normochromic red cells on morphology, 6 (15.0%) have microcytic/hypochromic red cells as shown in Table 3.

The mean CD4+ cell count of HIV infected non-anaemic patients (530.55 cells/ $\mu$ L $\pm$ 423.35) was significantly higher than that of HIV infected anaemic patients (188.18 cells/ $\mu$ L $\pm$ 157.09) ( $p=0.0009$ ). Likewise the mean viral load of HIV infected anaemic patients (455244.89 copies/ $\mu$ L $\pm$ 651540.89) was significantly higher than that of HIV infected non-anaemic patients (48575.25 copies/ $\mu$ L $\pm$ 113531.29) ( $p=0.0005$ ).

**Table 1. Distribution of age and sex of patients by category**

Subjects category	Age group (years)	Sex of subjects (Frequency (%))		Total	Test	P value
		Male	Female			
<b>HIV/Anaemic</b>						
	<20	0(0)	1(100)	1(100)	$\chi^2=14.99$	0.005
	20-29	0(0)	4(100)	4(100)		
	30-39	2(12.5)	14(87.5)	16(100)		
	40-49	8(57.1)	6(42.9)	14(100)		
	$\geq 50$	3(100)	0(0)	3(100)		
n=38		13(34.2)	25(65.8)	38(100)		
Mean age		45.15 $\pm$ 5.63	34.16 $\pm$ 7.21		t=5.17	0.000
<b>HIV/Non-anaemic</b>						
	<20	0(0)	1(100)	1(100)	$\chi^2=5.20$	0.267
	20-29	1(25.0)	3(75.0)	4(100)		
	30-39	1(14.3)	6(85.7)	7(100)		
	40-49	1(25.0)	3(75.0)	4(100)		
	$\geq 50$	3(75.0)	1(25.0)	4(100)		
n=20		6(30.0)	14(70.0)	20(100)		
Mean age		48.00 $\pm$ 14.57	34.93 $\pm$ 9.68		t=2.01	0.059

**Table 2. Distribution of red cell indices [mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC)] among the HIV infected anaemic subjects**

MCV (fl)	Frequency (%)	MCH (pg)	Frequency (%)	MCHC (g/dL)	Frequency (%)
<74	4(10.0)	<25	21(52.5)	<30	15(37.5)
74-94	29(72.5)	25-31	18(45.0)	30-35	25(62.5)
>94	7(17.5)	>31	1(2.5)	>35	0(0.0)
Total	40(100.0)		40(100.0)		40(100.0)
<b>Using count statistics</b>					
MCV	MCH	MCHC			
74-94fl	<25	15(51.72)	<30	9(31.03)	
	25-31	14(48.28)	30-35	20(68.97)	
	>31	0(0.0)	>35	0(0.0)	
Total=29		29(100.0)		29(100.0)	

The HIV infected anaemic patients had a mean WBC of  $7.20 \times 10^9/L \pm 4.93$  (95% CI, 5.63-8.79) and the HIV infected non-anaemic patients had a mean WBC of  $5.66 \times 10^9/L \pm 1.74$  (95% CI, 4.85-6.48). There was a significant difference in the mean WBC values ( $p=0.008$ ).

**Table 3. Distribution of red cell morphology in HIV infected anaemic patients**

Red cell morphology	HIV/Anaemic	Percentage
Normocytic/normochromic	22	55.0
Normocytic/hypochromic	1	2.5
Normocytic/poikilocytes	1	2.5
Dimorphic picture	1	2.5
Microcytic/normochromic	3	7.5
Microcytic/hypochromic	6	15.0
Anisopoikilocytes	6	15.0
Total	40	100.0

The HIV infected anaemic patients had a mean platelet count of  $277.27 \times 10^9/L \pm 126.76$  (95% CI, 236.74-317.81). The HIV infected non-anaemic patients had a mean platelet count of  $207.40 \times 10^9/L \pm 61.86$  (95% CI, 178.45-236.35). These are also significantly different values ( $p=0.005$ ) as shown in Table 4.

**Table 4. Comparison of mean white blood cell count (WBC) and platelet count in the patients**

	HIV subjects		P value
	Anaemic	Non-anaemic	
Mean WBC ( $\times 10^9/L$ ) (SD)	7.20 (4.93)	5.66 (1.74)	0.008
Mean Platelets ( $\times 10^9/L$ ) (SD)	277.27 (126.76)	207.40 (61.86)	0.006

#### 4. DISCUSSION

Anaemia is a frequent complication of HIV infection, and its incidence is associated with

progression of HIV disease, prescription of certain chemotherapeutics, black race, and female sex. Immunosuppression predisposes to anaemia. Anaemia is associated with shorter survival of HIV-infected patients and also a risk factor for early death in patients with AIDS [5,13].

The mean corpuscular volume (MCV) is used as a pointer to distinguishing between the different types of anaemia. In this study, distribution of red cell indices among the HIV infected anaemic patients largely reflects normocytic anaemia. Majority (72.5%) had normocytic anaemia. Only 10.0% had microcytic anaemia and 17.5% had macrocytic anaemia. Hypochromia was found in 52.5%, normochromia in 45.0% and hyperchromia in 2.5%. The finding of normocytic, normochromic red blood cells in this study is not unexpected as HIV associated anaemia is a chronic anaemic state where such picture may be seen and this is in keeping with the general belief that anaemia in HIV infection is a normocytic, normochromic anaemia [4,14].

Although other authors have described other haematological abnormalities in HIV infected individuals [15-18], for example, Sharma and Panchonia [15] found abnormal red blood cell shapes while Kulkarni et al. [16] found dysplastic neutrophils more commonly with neutropenia being their least common finding. In this study, there were few abnormal shapes noted namely: 15% anisopoikilocytes and 2.5% of the study population showed a mixture of normocytic cells and poikilocytes. These observations further highlight the wide range of haematologic abnormalities that could be associated with HIV infection.

Similar to earlier reports by other authors [17,18], patients with more severe immunodeficiency in this study had higher degree of anaemia. This corroborates the findings by Wankah et al. [17] which showed that HIV patients with advanced disease were more prone to anaemia and other blood cell disorders. In the same vein, Akinbami et al. [18] found that haematologic abnormalities like anaemia, neutropenia and thrombocytopenia are common among HIV patients and the degree of abnormalities correlate with the severity of immunodeficiency. These observations may not be unconnected with the complications that may accompany severe immunosuppression such as opportunistic infections, development of malignancies and impaired response of the bone marrow. Hence, CD4+ cell count may be used as a predictor of the development of anaemia in HIV

infected patients and for identifying those that will require therapy.

The HIV infected anaemic patients in this study had a higher WBC count than the non-anaemic patients. The observed high WBC count in these patients may be a response of the body to infections which are common in HIV patients.

## 5. CONCLUSION

Anaemia in HIV infection was found to be normocytic, normochromic anaemia. The degree of anaemia may differ with the stage of HIV disease. The use of peripheral blood film is a simple, less invasive and easy way of characterising the types of anaemia in HIV infected individuals and this could be of great value in resource poor settings for prompt diagnosis and appropriate intervention.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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