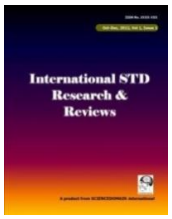

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Effect of HIV Infection and Antiretroviral Therapy Duration on CD4 Count, Total and Differential White Cell Count

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COO and GIA designed the study. Author COO wrote the protocol, and wrote the first draft of the manuscript. Author COO managed the literature searches and the experimental process and authors COO and MOI managed the statistical analysis. Authors EOO, ACO, NCI carried out manuscript revision. All authors approved the final manuscript.

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ABSTRACT

Aims: To assess CD4 counts, total and differential white cell counts in HIV positive patients on antiretroviral treatment (ART) and those not on antiretroviral treatments with varying durations of

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infection.
Study Design: Case-control study.
Place and Duration of Study: Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria from March to August 2013.
Methodology: We included 181 subjects; sixty HIV patients on ART with infection duration of <1 – 5, >5 – 8 and >8 – 17 years and ART duration of <1 – 5, >5 – 8 and >8 – 17 years; sixty HIV patients not on ART with an infection duration of <1 – 3, >3 – 6 and >6 – 11years; and sixty-one apparently healthy individuals control. CD4 counts, total and differential white cell counts as well as Human Immunodeficiency virus (HIV) status were determined.
Results: CD4 counts was significantly higher in the control compared to the ART and non-ART and lower in HIV duration of <1 – 3 years compared to >3 – 6 years and >6 – 11 years (P<0.05). TWBC was significantly higher in non-ART compared to ART and control (P<0.05). MXD was significantly lower in control compared to ART and non-ART (P<0.05). NEUT was significantly higher in non-ART than control while LYM was higher in non-ART than ART (P<.05). TWBC was lower in <1 -3 years than >6 – 11 years both in non-ART subjects (P<.05) TWBC was also higher in female controls than male (P<.05).
Conclusion: CD4 count is generally lower in HIV subjects. TWBC is elevated in non-ART, while MXD is higher in ART and non-ART subjects. There is no difference in white cell parameters for ART subjects irrespective of differences in duration of HIV infection and antiretroviral therapy.

Keywords: HIV/AIDS; total white cell counts; differential white cell counts; CD4 count; antiretroviral therapy.

ABBREVIATIONS

ART: Patients on Antiretroviral Therapy; Non-ART: Patients not on antiretroviral therapy; TWBC: Total white cell counts; NEUT: Neutrophil count; LYM: Lymphocyte counts; MXD: Mixed cell.

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) has emerged as a global disaster ever since its discovery [1]. Nigeria's HIV/AIDS prevalence rate is now 3.4 per cent, according to the report of a new national survey conducted by the federal ministry of Health (FMOH) for the 2012 National HIV/AIDS and Reproductive Health Survey-Plus (NARHS Plus). Meanwhile, HIV/AIDS continues to spread globally and remains a worldwide pandemic affecting over 40 million people [2,3]. It is now the leading cause of death in sub-Saharan Africa and the fourth leading cause of mortality worldwide. Highly active antiretroviral therapy (HAART) has generally been taken as the gold standard in the management of HIV patients [4]. With its introduction in 1996, HAART appears to have effectively controlled viral replication in HIV/AIDS patients and has successfully improved their quality of life and prolonged their life-expectancy [5], with a near normal turnover of both CD4 and CD8 T-cell populations [6]. HIV infection leads to a progressive reduction in the number of T cells expressing CD4, thus Medical professionals refer to the CD4 count to decide when to begin treatment during HIV infection as well as to determine efficacy of treatment.

Besides immunological complications of HIV disease, haematological abnormalities have been documented as strong independent predictors of morbidity and mortality in HIV infected individuals [7]. Although numerous complications occur in HIV infected patients, the most common haematological abnormalities are anaemia and leucopenia. These are generally caused by inadequate blood cell production because of bone marrow suppression by HIV infection mediated by abnormal cytokine expression and alteration of the bone marrow microenvironment [8,9]. White blood cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils) play a major role in the body defence against bacterial, fungal and viral infections like HIV. The changes in the total and differential white cell values are usually a reflection of infectious states. Patients with advanced HIV infection are vulnerable to infections and malignancies that are known as "opportunistic infections" due to their weakened immune system; these includes bacterial diseases like tuberculosis, bacterial pneumonia, septicaemia etc.; protozoal diseases like

toxoplasmosis, cryptosporidiosis, leishmaniasis and etc. fungal diseases like candidiasis, cryptococcosis and etc. viral diseases such as those caused by cytomegalovirus, herpes simplex and etc. and HIV-associated malignancies such as Kaposi's sarcoma, lymphoma etc. However, the introduction of highly active antiretroviral therapy (HAART) has dramatically reduced the incidence of opportunistic infections among HIV-positive patients.

Leucopenia is a problem commonly encountered in patients with HIV infection. Leucopenia is frequently observed in advanced stages of HIV infection after development of AIDS and has been associated with certain types of antiretroviral medications used to treat HIV infection [10]. The pathogenesis of leucopenia in patients with HIV infection is multifactorial. Any infiltrative process involving the bone marrow (infection, malignancy) may produce leucopenia. In clinical practice, drug-induced leucopenia is common in patients with HIV infection. Drug toxicity is responsible for most of the leucopenia seen in patients with HIV infection and Zidovudine therapy is probably the most common cause of low leucocyte counts in patients with HIV infection. In a previous study, severe leucopenia (< 500 cells/mm3) developed in 16% of Zidovudine-treated patients in the original placebo-controlled study of Zidovudine therapy for advanced HIV disease and symptomatic middle-stage HIV disease [11]. Although low leucocyte counts may reflect the toxicity of therapies for HIV infection or associated conditions, studies of untreated patients have also shown a high incidence of leucopenia, particularly in patients with more profound immunodeficiency [11]. Leucopenia secondary to cancer chemotherapy also complicates treatment of HIV-infected patients, perhaps to an even greater extent as a result of impaired bone marrow function. When the leucocyte count falls below 500 cells/mm^3, the risk of infection and sepsis is significant, an observation in accord with similar findings in other disease states.

Because of the crucial role white blood cells play in defence of the body against opportunistic infections in HIV patients, this study was aimed at determining the effect of HIV infection and antiretroviral therapy duration on CD4 count, total and differential white cell count in HIV positive patients on antiretroviral treatment (ART) and those not on antiretroviral treatments.

2. MATERIALS AND METHODS

2.1 Study Site

This study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, a tertiary health institution serving patients of high, middle and lower socio-economic status. It houses a major HIV/AIDS Centre (IHVN Clinic) serving patients from all parts of the state and beyond, with a registered HIV Sero-positive patient population of over 6000.

2.2 Inclusion and Exclusion Criteria

The patients who were in the age range of 18 to 60 years were included in this study. This inclusion was made from HIV positive patients. The patients were all visiting the centre (NAUTH) with varying durations of HIV infection and with or without antiretroviral treatment. Patients who were on antibiotics, marrow toxic drugs, pregnant women and nursing mothers, paediatrics, geriatrics, those who have been transfused six months prior to the study, those with tuberculosis and those who refused to give their consent were excluded from the study.

2.3 Sample Size Calculation

The sample size was obtained using the formula stated by Naing et al. [12]; for the calculation of minimum sample size used in a study involving human subjects/patients, which is;

$n= Z^2xP(1-P)/d^2$. Where
n= Minimum sample size
d= Desired level of significance (0.05)
Z= 95% confidence interval (1.96)
P= Prevalence rate of HIV in Nigeria was 4.1% [13]; (4.1/100=0.041).
$n= 1.96^2x0.041\ (1-0.041)/0.05^2$.
n= 60 patients

Using the formula, the calculated minimum number of sample size was 60, but a total of 181 participants categorized into three groups were included for this study.

2.4 Consent

The authors declare that written informed consent was obtained from the patient before being recruited for this research.

2.5 Ethical Approval

The authors hereby declare that all experiments was examined and approved by the Nnamdi Azikiwe University Teaching Hospital ethics committee (NAUTHEC).

2.6 Subject Recruitment

One hundred and eighty one (181) subjects aged between 18-60 years were recruited by simple random sampling and categorized into three groups comprising;

- Sixty adult HIV Sero-positive subjects on antiretroviral therapy (ART) - Lamivudine, Zidovudine, Nevirapine (30 males and 30 females), with HIV infection duration of <1 – 5 years (17 subjects), >5 – 8 years (26 subjects) and >8 – 17 years (17 subjects) and ART duration of <1 – 5 years (23 subjects), >5 – 8 years (25 subjects) and >8 – 17 years (12 subjects).
- Sixty adult HIV Sero-positive subjects not on antiretroviral therapy (25 males and 35 females) with an HIV infection duration of <1 – 3 years (31 subjects), >3 – 6 years (19 subjects) and >6 – 11years (10 subjects).
- Sixty-one apparently healthy adult Sero-negative control subjects (31 males and 30 females) respectively.

2.7 Sample Collection

Three millilitres (3 mls) were drawn into bottles containing di-potassium salt of Ethylenediamine tetra-acetic acid (K_2-EDTA) at a concentration of 1.5 mg/ml of blood and used for CD4 and white cell parameters, while two millilitres (2mls) were drawn into plain sample bottle and serum was obtained and used for HIV Screening. All the tests were carried out according to manufacturer's instructions.

2.8 Statistical Analyses

The data obtained were analysed using Statistical Package for Social Sciences (SPSS version 20). Data were expressed as mean ± SD. The significance of differences in mean values between groups were analysed using t-test, while significance of the differences in mean values among different groups was evaluated using one-way ANOVA. *P*<.05 was considered statistically significant.

2.9 Determination of HIV Status

2.9.1 Determine HIV 1/2 assay (Alere Medical co Ltd, Japan, 2012)

The abbot Determine™ HIV ½ is an in vitro test kit, visually qualitative immune assay for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood.

2.9.2 Chembio HIV ½ Stat-Pak™ assay (Chembio diagnostic system inc. 2012, USA)

The Chembio HIV ½ STAT PAK™ assay employs a unique combination of a specific antibody-binding protein, which is conjugated to colloidal gold dye particles and HIV ½ antigens which are bound to the membrane solid phase.

2.10 Full Blood Count (FBC) Estimation

This was carried out using Sysmex automated haematology analyser KX 21N model manufactured by Sysmex Corporation Kobe, Japan. This is a three part autoanalyser that differentiates white cells into Neutrophils (NEUT), Lymphocytes (LYM) and Mixed cells (MXD).

2.11 CD4 Cell Count (Partec GmbH Germany, 2011)

This was carried out using the Partec new model Cyflow Counter 2 which is an automated flow cytometer for the enumeration of CD4 and CD8-T lymphocyte cells in whole blood.

3. RESULTS

One hundred eighty one subjects, comprising 95 female and 86 male subjects. Out of the 95 females 30 (31.6%) were on ART, 35 (36.8%) were non-ART and 30 (31.6%) were control subjects. Similarly, of the 86 males 30 (34.9%) were on ART, 25 (29.1%) were non-ART, and 31 (36%) were control subjects. The mean age for ART, non-ART and control subjects were 43.4 years, 41 years and 40 years respectively (Table 1).

The mean values of CD4 count were significantly lower in both ART and non-ART when compared with the corresponding values in the control (*P*<.05). Similarly, the mean of TWBC was significantly higher in non-ART compared with mean values of ART and control (*P*<.05). In

addition, the Mean ± SD of NEUT was significantly higher in non-ART than Control (*P*<.05). Furthermore, the mean value of LYM was significantly lower in ART than non-ART (*P*<.05), while the Mean ± SD of MXD was significantly higher in ART and non-ART than Control (*P*<.05) (Table 2).
For non-ART patients the mean value of CD4 for HIV duration of <1 – 3 years was significantly lower than that for >3 – 6 years and >6 – 11 years (*P*<.05). The mean values of TWBC for <1 – 3 years was significantly lower than that of >6 – 11 years (P <.05) (Table 3).

In ART subjects there was no significant difference in the mean values of CD4 count, TWBC, NEUT, LYM, MXD for HIV durations of <1 – 5 years, >5 – 8 years and >8 – 17 (*P*>.05) (Table 4).

There was no significant difference in the mean values of CD4 count, TWBC, NEUT, LYM, MXD for ART durations of <1 – 5 years, >5 – 8 years and >8 – 17 (*P*>.05) (Table 5).

The mean value of TWBC for the male controls was significantly lower when compared with the mean value of the female controls (*P*<.05) (Table 6).

For non-ART subjects, the mean value of CD4 count was significantly higher in >2-4years than for <1-2 years (*P*<.05) (Table 7).

4. DISCUSSION

Haematological abnormalities are among the most common complications of HIV infection and involve all lineages of blood cells; thus anaemia, thrombocytopenia and leucopenia are common findings [14]. From our findings, total white cell counts (TWBC) and neutrophil counts (NEUT) were significantly elevated in HIV seropositive patients not on ART than the control. This could be a haematological response to the increase in opportunistic infections prevalent in HIV infected patients. However this result negates the findings by some previous authors [15,16,17,18]. They related their observed decrease to reduced colony growth of the progenitor cells due to HIV suppression of bone marrow that leads to decreased production of both granulocytes and monocytes, as well as the presence of anti-granulocyte antibodies in HIV infected persons [19] and presence of soluble inhibitory substances produced by HIV infected cells noted to suppress neutrophil production in vitro [20].

Total white cell counts was higher in HIV seropositive subjects not on ART compared to those on ART (Table 2). This could be explained by previous findings that some antiretroviral drugs like Zidovudine causes leucopenia in HIV patients [21]. However, the effects of specific antiretroviral agents were not carried out as part of this study.

Lymphocytosis is usually a common finding in viral infections like HIV infection. In this study Lymphocyte count (LYM) was higher in HIV seropositive subjects not on ART compared to those on ART (Table 2). The decrease in ART subjects could also be explained by previous findings of the adverse effect of some antiretroviral drugs like Zidovudine in HIV patients [21].

CD4 cell counts was lower in HIV seropositive patients (ART and non-ART) than control (Table 2); this agrees with a previous finding [15] and could be attributed to the destruction of CD4+ T cells by the HIV virus. It is well documented that CD4 cells are the primary target of HIV and is most commonly used marker to determine HIV progression. It also helps to obtain information on immune responses, staging of HIV disease, risk of mother to child transmission, use and response of antiretroviral treatment [22].

The finding that the mean CD4 counts in ART compared to non-ART showed no significant difference (Table 2) may be explained by the fact that ART is administered to HIV patients with decreasing CD4 counts<350 cells/µl. Hence, those not placed on ART initially have a higher CD4 counts while those placed on ART tend to have an improved CD4 counts recovery with ART treatment over a period of time [23]. A higher CD4 count with longer HIV duration in non-ART was observed (Table 3). However with the exclusion of patients with HIV duration above 6 years (Table 7) a significant increase in CD4 count was observed at the initial stage (<1-2 years to >2-4 years) followed by a non-significant decline after 4 years (>4-6 years) this could suggest that the increased CD4 count observed with longer duration in Table 3 could be as a result of Long term non-progressors (LTNP) which has been well documented to maintain healthy CD4+ counts after being HIV infected for along period of time [24].

MXD cells was significantly elevated in HIV seropositive subjects (ART and non-ART) than control (Table 2); this could be explained by the

knowledge that cells of the macrophage lineage (including monocyte) play an important role in initial infection with HIV and contribute to the pathogenesis of the disease throughout the course of infection [25].

Table 1. Sex demographic parameters of the HIV seropositive on ART, not on ART and HIV negative control subjects

Group	Gender		Total number	Mean age (years)	Age range (years)
	Females (%)	Males (%)			
ART	30 (31.6)	30 (34.9)	60	43.4	28 - 60
Non-ART	35 (36.8)	25 (29.1)	60	41	18 - 59
Control	30 (31.6)	31 (36)	61	40	18 - 50
Total	95 (100)	86 (100)	181	-	-

Table 2. Mean ± SD of parameters compared for seropositive on ART, seropositive not on ART and control subjects

Groups	CD4 (Cells/µl)	TWBC ($X10^9$/L)	NEUT ($X10^9$/L)	LYM ($X10^9$/L)	MXD ($X10^9$/L)
(A) Seropositive on ART(n=60)	451.88±264.29	5.00±1.70	2.67±1.65	1.91±0.77	0.56±0.38
(B) Seropositivenot on ART (n=60)	572.40±334.85	6.03±1.83	3.01±1.50	2.38±1.01	0.59±0.31
(C) control (n=61)	902.38±263.36	4.55±1.46	2.14±0.91	2.10±0.64	0.31±0.16
F (P) values	39.39(.00*)	12.45(.00*)	6.05(.00*)	4.97(.01*)	16.05(.00*)
A vs B*P*-value	.08	.01*	.45	.01*	.92
A vs C*P*-value	.00*	.27	.08	.33	.00*
B vs C*P*-value	.00*	.00*	.00*	.16	.00*

- *Significant at P <.05,*

Key: F (P) value = Mean ± SD of parameters compared among Seropositive on ART, Seropositive not on ART and Control subjects using ANOVA. A vs B: (P) value = Mean ± SD of parameters compared between Seropositive on ART and Seropositive not on ART using t-test. A vs C: (P) value = Mean ± SD of parameters compared between Seropositive on ART and Control subjects using t-test. B vs C: (P) value = Mean ± SD of parameters compared between Seropositive not on ART and Control subjects

Table 3. Mean ± SD of parameters compared for seropositive not on ART based on duration of HIV infection

Duration (years)	CD4 (Cells/µl)	TWBC ($X10^9$/L)	NEUT ($X10^9$/L)	LYM ($X10^9$/L)	MXD ($X10^9$/L)
(A) < 1 – 3(n = 31)	409.94±215.67	5.70±1.96	2.80±1.48	2.24±1.01	0.50±0.20
(B) > 3 – 6(n = 19)	641.58±291.09	6.03±1.79	2.82±1.18	2.49±1.07	0.69±0.38
(C) > 6 – 11(n = 10)	944.60±394.43	7.04±1.03	4.02±1.80	2.61±0.96	0.67±0.38
F(P) values	15.14 (.00*)	2.13 (.13)	2.90 (.06)	0.65 (.53)	3.00 (.06)
A vs B: *P*-value	.01*	.82	1.00	.70	.11
A vs C: *P*-value	.01*	.02*	.17	.56	.38
B vs C: *P*-value	.12	.15	.18	.95	.99

- *Significant at P<.05*

F (P) value = Mean ± SD of parameters compared within the Seropositive not on ART based on duration of HIV infection using ANOVA. A vs B: (P) value = Mean ± SD of parameters compared between Seropositive not on ART whose duration of infection is < 1 – 3 years and > 3 – 6 years using t-test. A vs C: (P) value = Mean ± SD of parameters compared between Seropositive not on ART whose duration of infection is < 1 – 3 years and > 6 - 11 years using t-test. B vs C: (P) value = Mean ± SD of parameters compared between Seropositive not on ART whose duration of infection is > 3 – 6 years and > 6 – 11 years using t-test

Table 4. Mean ± SD of parameters compared for the seropositive on ART based on duration of HIV infection

Duration (Years)	CD4(Cells/µl)	TWBC($X10^9$/L)	NEUT($X10^9$/L)	LYM ($X10^9$/L)	MXD ($X10^9$/L)
<1 – 5(n =17)	454.06±289.98	4.52±1.14	2.71±2.44	1.75±0.59	0.65±0.48
> 5 – 8(n = 26)	462.65±263.04	5.48±2.02	2.72±1.40	2.18±0.89	0.53±0.32
> 8 – 17(n = 17)	433.24±254.58	4.74±1.53	2.54±1.03	1.66±0.63	0.53±0.36
F-values	0.60	1.95	0.07	2.96	0.57
P-values	.94	.15	.93	.06	.57

- *Significant at P<.05*

Table 5. Mean ± SD of parameters compared for the seropositive on ART based on duration of antiretroviral therapy

Duration (Years)	CD4 (Cells/µl)	TWBC ($X10^9$/L)	NEUT ($X10^9$/L)	LYM ($X10^9$/L)	MXD ($X10^9$/L)
< 1 – 5 years(n=23)	423.96±261.16	4.62±1.42	2.58±2.19	1.80±0.59	0.67±0.46
> 5 – 8 years (n=25)	482.44±286.99	5.58±1.96	2.88±1.37	2.14±0.93	0.49±0.29
> 8–17 years(n=12)	441.75±233.83	4.52±1.36	2.37±0.88	1.63±0.62	0.52±0.36
F-values	0.30	2.64	0.44	2.23	1.39
P-values	.74	.08	.65	.12	.26

- *Significant at P<.05*

Table 6. Mean ± SD of parameters compared within the seropositive on ART, seropositve not on ART and control subjects based on gender

Groups	Gender	CD4(Cells/µl)	TWBC ($X10^9$/L)	NEUT ($X10^9$/L)	LYM ($X10^9$/L)	MXD ($X10^9$/L)
ART	Male (n=30)	461.73±261.84	5.36±1.68	3.08±2.02	1.96±0.86	0.61±0.44
	Female (n=30)	442.03±270.83	4.63±1.68	2.25±1.06	1.88±0.68	0.51±0.30
***P*-value**		**1.00**	**.55**	**.35**	**1.00**	**.91**
Non-ART	Male (n=25)	593.68±368.12	6.28±1.94	2.90±1.37	2.75±0.97	0.60±0.34
	Female (n= 35)	557.20±313.59	5.84±1.75	3.09±1.60	2.12±0.98	0.58±0.29
***P*-value**		**1.00**	**.94**	**1.00**	**.15**	**1.00**
Control	Male (n=31)	819.71±228.69	3.98±0.95	1.85±0.66	1.87±0.54	0.26±0.12
	Female (n=30)	987.80±273.03	5.13±1.67	2.44±1.04	2.33±0.67	0.37±0.17
***P*-value**		**.11**	**.02***	**.12**	**.05**	**.06**

- *Significant at P<.05*

Table 7. Mean ± SD of parameters compared for seropositive not on ART for duration of HIV infection less than 6 years

Duration (years)	CD4 (Cells/µl)	TWBC ($X10^9$/L)	NEUT ($X10^9$/L)	LYM ($X10^9$/L)	MXD ($X10^9$/L)
(A) < 1 – 2(n = 23)	368.74±198.13	5.74±2.09	2.92±1.58	2.13±1.05	0.48±0.19
(B) > 2 – 4(n = 15)	636.07±323.40	6.24±2.02	2.85±1.21	2.71±0.99	0.68±0.37
(C) > 4 – 6(n = 12)	573.00±210.43	5.45±1.24	2.54±1.15	2.27±0.98	0.61±0.33
F(P) values	6.16 (0.00*)	0.60 (0.55)	0.31 (0.74)	1.49 (0.24)	2.23 (0.12)
A vs B: *P*-value	0.01*	0.71	0.99	0.21	0.11
A vs C: *P*-value	0.06	0.91	0.72	0.93	0.45
B vs C: *P*-value	0.79	0.54	0.83	0.51	0.80

- *Significant at P<.05*

F (P) value = Mean ± SD of parameters compared within the Seropositive not on ART for duration of HIV infection less than 6 years using ANOVA. A vs B: (P) value = Mean ± SD of parameters compared between Seropositive not on ART whose duration of infection is < 1 – 2 years and > 2 – 4 years using t-test. A vs C: (P) value = Mean ± SD of parameters compared between Seropositive not on ART whose duration of infection is < 1 – 2 years and > 4 - 6 years using t-test. B vs C: (P) value = Mean ± SD of parameters compared between Seropositive not on ART whose duration of infection is > 2 – 4 years and > 4 – 6 years using t-test

5. CONCLUSION

CD4 counts is generally lower in HIV positive patients. ART administration lowers TWBC. MXD is increased irrespective of ART status. However, white cell parameters in ART subjects are not affected irrespective of differences in duration of HIV infection and antiretroviral therapy. Similarly, there are no gender variations in white cell parameters in HIV subjects. The individual effects of the various antiretroviral drugs were not studied.

6. RECOMMENDATIONS

It is recommended that patients on antiretroviral therapy like Zidovudine be monitored regularly to avoid drug-induced leucopenia which could make

the patient susceptible to various opportunistic infections. Regular monitoring of CD4 counts should also be encouraged.

It is also recommended that in future studies, the effect of specific antiretroviral drugs on these white cell parameters should be monitored to determine the effect of specific drug toxicity on these parameters.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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