



## **Factors Associated with a Low CD4 Count among HIV-1 Infected Patients at Enrolment into HAART in Jos, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author AOE Conception, design, data analysis/ results and manuscript writing and revision OOA, JAA, ASS, PAA, PO and SO. Manuscript revision. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** To determine the factors associated with a low CD4 count among HIV-1 positive patients.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** Adult HIV clinic at the Jos University Teaching Hospital, Jos, between October 2010 and April 2011.

**Methodology:** Data on demographic, clinical and laboratory variables for 218 HIV-1 infected patients aged 20 years and older were analysed. A low CD4 cell count was defined as CD4 cell count <200 cells/ml based on the WHO criteria for severe immune suppression. A multivariate logistic regression modeling was fitted to determine the

variables that were independently associated with a low CD4 count.

**Results:** Of the 218 HIV-1 infected patients, 119 (54.6%) had a low CD4 count at enrolment. The odds of having a low CD4 count was: 7 times higher in patients with WHO clinical stage 3 or 4 compared to those with stage 1 or 2 ( $P<.001$ ) and 4 times higher in those with HIV RNA viral load  $\geq 4.6 \log_{10}$  copies/ml compared to those with less ( $P<.001$ ); but the odds of having a low CD4 count was reduced by 63% in those patients that were resident in Plateau State compared to those resident outside the state ( $P=.01$ ).

**Conclusion:** Our study patients were more likely to have a CD4 count  $<200$  cells/ml which would suggest late presentation/ late HIV diagnosis and thus a delayed opportunity for timely access to HIV care and initiation of antiretroviral therapy. There is the need to intensify efforts in early routine HIV counseling and testing not only in health facilities in the cities but also in smaller towns and rural communities, so as to reduce the frequency of late HIV diagnosis with its potential implications.

*Keywords: Low CD4 count; HIV-1; HAART; severe immune suppression; late presentation; clinical stage; RNA viral load.*

## 1. INTRODUCTION

Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) constitute a major health problem in sub-Saharan Africa with an estimated 23.5 million people living with the infection; representing 69% of the global HIV burden [1]. HIV infection is one of the major causes of depletion in CD4+ cells and CD4 count is one of the parameters used to measure disease progression in HIV-positive persons [2]. Levels of CD4 count have been used for immunological classification of HIV infection and these levels have been shown to correlate with clinical staging of HIV-related diseases [3].

Several factors are capable of influencing the CD4 count levels of HIV positive persons at the time of diagnosis; the most important being HIV viral load [2,4,5]. The distance of a health care facility from a community could affect the accessibility of health care [6,7] by persons within that locality or region and this could influence the time to presentation and hence the duration of symptoms at the time of presentation [7,8]. It has been shown that HIV positive persons presenting late (late presenters) are often diagnosed with late HIV disease which corresponds to severe immune suppression, defined as CD4 count  $<200$  cells/ml [7,9-11]. Those with a low CD4 count at baseline besides having a higher risk of clinical events [12,13], are less likely to have a sustained virological response when commenced on highly active antiretroviral therapy (HAART) compared to those commencing treatment at higher counts [14-16].

In our setting no study has been carried out on the association of a low CD4 count with socio-demographic and clinical factors.

Identifying factors that may be associated with a low CD4 count in HIV positive patients could have implications for HIV care and management. In this study, we sought to determine factors associated with a low CD4 count among HIV-1 infected patients at enrolment into HAART at the adult HIV clinic of the Jos University Teaching Hospital (JUTH), Jos.

## **2. METHODOLOGY**

### **2.1 Study Setting**

The adult HIV clinic provides comprehensive HIV care services for the city of Jos, which is located in Jos North Local Government Area (LGA) of Plateau State and also serves as a referral centre for health facilities in other LGAs of the state and some neighbouring states in the country. The distance of these states to the city of Jos ranges from 200-800 Km or more. A 2006 census, estimated the population of Plateau State at 3,206,531, with the capital Jos city having a population of approximately 900,000 [17]. The current prevalence of HIV in the state was reported to be 7.7% [18].

### **2.2 Study Subjects**

These were adult patients aged 20 years and above presenting to the HIV clinic, who were diagnosed with HIV at presentation and yet to be commenced on HAART.

### **2.3 Study Design**

This was a cross-sectional study in which data, collected over a period of 7 months (October 2010 – April 2011), on 218 consecutive patients were analyzed. We used data that were already being captured in an electronic data management system at the HIV clinic. The information on the mode of transmission of HIV was obtained by patient self-report through direct questioning. The data obtained included the following variables: demographic (age, sex, marital status, residence, occupation, education level, mode of HIV transmission, spouse HIV status and spouse ARV treatment status), clinical (World Health Organization (WHO) HIV clinical stage, chronic diarrhoea, Kaposi's sarcoma and oropharyngeal candidiasis) and laboratory (viral load, CD4 cell count and hepatitis B virus status) .

### **2.4 Case Definitions**

A diagnosis of a case of PTB was made using the WHO criteria [3]. Patients with clinical features of PTB and whose sputum smears were positive for AFBs were considered as having PTB; while those with negative smears and chest radiographs consistent with PTB were taken as smear negative PTB. Kaposi's sarcoma was diagnosed based on its clinical features [3].

### **2.5 Laboratory Methods**

Laboratory tests carried out were part of the existing HIV treatment programme. Two different rapid HIV tests: Uni-Gold (Trinity Biotech Plc Bray Co Wicklow, Ireland) and Determine HIV-1/2 test (Determine Alere Medical Co., Ltd 357 Matsuhidai, Japan) were used for HIV serodiagnosis. Flow cytometry (Partec GmbH, Munster Germany) was used to determine the CD4+ lymphocyte count and Roche Cobas Amplicor HIV-1 Monitor, version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany) was used to determine HIV-1 RNA viral load. Enzyme immunoassay (EIA) (Monolisa HBsAg Ultra3; Bio-Rad) was used to determine the HBsAg. Patients were not screened for hepatitis C virus (HCV).

## 2.6 Statistical Methods

The outcome variable defined as: low CD4 cell count (CD4 cell count <200 cells/ml), was obtained using the WHO cut-off level of 200 cells/ml which is regarded as severe immune suppression [3]. All other variables were considered as independent variables. WHO clinical stage (stage 3 or 4 versus stages 1 or 2) was based on clinical severity [3] while HIV RNA viral load (<4.6 log<sub>10</sub> versus ≥4.6 log<sub>10</sub> copies/ml) was obtained using the median cut-off value. For the univariate analysis, associations of each independent variable with low CD4 cell count was examined using the Chi squared test or Fisher's exact test for categorical variables and Wilcoxon-Mann-Whitney test for comparison of two medians.

The variable age was used as a dichotomous variable (≤34 years versus >34 years using the median cut-off age of 34 years) in the logistic regression analyses to give a more parsimonious model with fewer degrees of freedom. Factors associated with low CD4 cell count in the univariate logistic regression at  $P<.05$  were included in the multivariate modelling. Age and sex were included *a priori* in the multivariate model since these could influence HIV infection [19]. A forward stepwise modelling strategy was used in building the final multivariate model. Results of regression analyses were expressed as odds ratio. The area under the receiver operating characteristic (ROC) curve was determined in order to assess the performance of the model. Analyses were done using Stata software version 10.0 (Stata Corporation, College Station, Texas, USA) and all tests were two-sided with a  $p$ -value of <.05 considered significant.

## 3. RESULTS

Out of the 218 HIV-1 infected adult patients, 119 (54.6%) had a low CD4 count (CD4 count <200 cells/ml) at enrolment to HAART. Majority were: in the younger age group (<30 years), females (60.1%), resident in Plateau state (64.7%), had secondary or tertiary education (67.9%), employed (77.1%), married (65.6%) and were in WHO clinical stage 3 or 4 (56.3%). The main mode of HIV transmission was heterosexual sex (97.7%). One hundred and three (47.3%) of the patients had spouses who were HIV positive with only 10.1% of these spouses on antiretroviral drugs (ARVs). Only 6.4% had hepatitis B virus infection, 9.6% pulmonary TB, 20.6% oropharyngeal candidiasis, 16.1% chronic diarrhoea and 3.7% Kaposi sarcoma. The median viral load of the 218 patients was 43019 copies/ml (IQR, 17190-109049 copies/ml) while the median CD4 count was 185 cells/ml (IQR, 92-280 cells/ml) with the median count for the low and high CD4 count groups being 101 (IQR, 50-161 cells/ml) and 291 (IQR, 251-389 cells/ml) respectively and the difference was significant,  $P<.001$  Table 1.

Univariate analysis showed that sex, spouse ARV status, WHO clinical stage, pulmonary tuberculosis, oropharyngeal candidiasis, chronic diarrhoea and log HIV RNA viral load were significantly associated with a low CD4 count Table 1. The HIV RNA log viral load was negatively correlated with CD4 count,  $r = -0.4$ .

The unadjusted logistic regression analyses showed the following variables to be significantly associated with low CD4 count: sex (OR, 1.95), residence (OR, 0.56), spouse ARV status (OR, 3.13), WHO clinical stage (OR, 7.73), oropharyngeal candidiasis (OR, 2.43), chronic diarrhoea (OR, 4.04) and HIV RNA viral load (OR, 4.01) Table 2.

In the multivariate analyses, the odds of having a low CD4 count was: 7 times higher in patients with WHO clinical stage 3 or 4 compared to those with stage 1 or 2 ( $P<.001$ ), 9

times higher in those whose spouses were on ARVs compared to those whose spouses were not on ARVs ( $P=.008$ ), 4 times higher in those with HIV RNA viral load  $\geq 4.6$  log<sub>10</sub> copies/ml compared to those with less; but the odds of having a low CD4 count was reduced by 63% in those patients that were resident in Plateau State compared to those resident outside the state. Spouse ARV status, oropharyngeal candidiasis and chronic diarrhoea did not make it into the final model as they were not significantly associated with low CD4 count Table 2. The area under the ROC curve for our model was 0.84.

**Table 1. Characteristics of HIV-1 positive patients according to low CD4 count**

Characteristics	CD4 count level			P value*
	Total	Low CD4 count	High CD4 count	
	N (%)	N (%)	N (%)	
<b>Age (yrs)</b>				0.34
<30	64 (29.4)	32 (26.9)	32 (32.3)	
30-40	98 (44.9)	52 (43.7)	46 (46.5)	
>40	56 (25.7)	35 (29.4)	21 (21.2)	
Median (IQR)	34 (28-41)	34 (29-42)	34 (28-38)	0.24†
<b>Sex</b>				0.02
Male	87 (39.9)	56 (47.1)	31 (31.3)	
Female	131 (60.1)	63 (52.9)	68 (68.7)	
<b>Residence</b>				0.04
Plateau	141 (64.7)	70 (58.8)	71 (71.7)	
Others	77 (35.3)	49 (41.2)	28 (28.3)	
<b>Education level</b>				0.07
Illiterate/ Primary	70 (32.1)	32 (26.9)	38 (38.4)	
Secondary/Tertiary	148 (67.9)	87 (73.1)	61 (61.6)	
<b>Occupation</b>				0.46
Student	19 (8.7)	8 (6.7)	11 (11.1)	
Unemployed	31 (14.2)	16 (14.5)	15 (15.2)	
Employed	168 (77.1)	95 (79.8)	73 (73.70)	
<b>Marital status</b>				0.44
Married	143 (65.6)	82 (68.9)	61 (61.6)	
Widowed/Divorced/ Separated	31 (14.2)	14 (11.8)	17 (17.2)	
Single	44 (20.2)	23 (19.3)	21 (21.2)	
<b>Spouse HIV status</b>				0.07
Positive	103 (47.3)	63 (52.9)	40 (40.4)	
Negative	115 (52.7)	56 (47.1)	59 (59.6)	
<b>Spouse on ARV drugs</b>				0.03
On ARV	22 (10.1)	17 (14.3)	5 (5.1)	
Not on ARV	196 (89.9)	102 (85.7)	94 (94.9)	
<b>Mode of HIV transmission</b>				1.00
Heterosexual	213 (97.7)	116 (97.5)	97 (98.0)	
Blood transfusion	5 (2.3)	3 (2.5)	2 (2.0)	
<b>WHO clinical stage</b>				0.000
Stages 3 & 4	112 (56.3)	88 (75.9)	24 (28.9)	
Stages 1 & 2	87 (43.7)	28 (24.1)	59 (71.1)	
<b>Pulmonary tuberculosis</b>				0.000
Present	21 (9.6)	21 (17.6)	0 (0.00)	
Absent	197 (90.4)	98 (82.4)	99 (100)	

Table 1 Continued.....

<b>HBV status</b>				0.09
Positive	14 (6.4)	11 (9.2)	3 (3.0)	
Negative	204 (93.6)	108 (90.8)	96 (97.0)	
<b>Oropharyngeal candidiasis</b>				0.02
Present	45 (20.6)	32 (26.9)	13 (13.1)	
Absent	173 (79.4)	87 (73.1)	86 (86.9)	
<b>Chronic diarrhoea</b>				0.001
Present	35 (16.1)	28 (23.5)	7 (7.1)	
Absent	183 (83.9)	91 (76.5)	92 (92.9)	
<b>Kaposi's sarcoma</b>				1.00
Present	8 (3.7)	4 (3.4)	4 (4.0)	
Absent	210 (96.3)	115 (96.6)	95 (96.0)	
<b>HIV RNA viral load (copies/ mL)</b>				0.000
≥43,019	109 (50.0)	76 (63.9)	33 (33.3)	
<43,019	109 (50.0)	43 (36.1)	66 (66.7)	
Median (IQR)	43019 (17190-109049)	67,818 (31144 - 176021)	22193 (9399 - 56519)	0.000†
<b>HIV RNA Log viral load (copies/ mL)</b>				0.000
≥4.6	123 (56.4)	85 (71.4)	38 (38.4)	
<4.6	95 (43.6)	34 (28.6)	61 (61.6)	
Median (IQR)	4.6 (4.2-5.0)	4.8 (4.5-5.2)	4.3 (4.0-4.8)	0.000†

\*P value for chi squared test, †P value for comparison of two medians

Table 2. Factors associated with low CD4 count in HIV-1 positive patients

Characteristic	Crude OR (95% CI)	P value	Adjusted OR* (95% CI)	P value
<b>Age (yrs)</b>				
<34	1.00 (Ref)		1.00 (Ref)	
≥34	0.86 (0.50 – 1.48)	0.59	1.09 (0.51 – 2.35)	0.82
<b>Sex</b>				
Female	1.00 (Ref)		1.00 (Ref)	
Male	1.95 (1.12 – 3.40)	0.02	1.70 (0.78 – 3.73)	0.18
<b>Residence</b>				
Others	1.00 (Ref)		1.00 (Ref)	
Plateau	0.56 (0.32 – 1.00)	0.04	0.37 (0.16 – 0.82)	0.01
<b>Education level</b>				
Illiterate/ Primary	1.00 (Ref)			
Secondary/ Tertiary	1.69 (0.95 – 3.0)	0.07		
<b>Occupation</b>				
Student	1.00 (Ref)			
Unemployed	1.47 (0.46 – 4.64)	0.51		
Employed	1.79 (0.68 – 4.67)	0.23		
<b>Marital status</b>				
Married	1.00 (Ref)			
Widowed/Divorced/ Separated	0.61 (0.28 -1.34)	0.22		
Single	0.81 (0.41 – 1.60)	0.55		

**Table 2 Continued...**

<b>Spouse HIV status</b>				
Negative	1.00 (Ref)	0.07		
Positive	1.66 (0.97 – 2.84)			
<b>Spouse on ARV</b>				
Not on ARV	1.00 (Ref)		1.00 (Ref)	
On ARV	3.13 (1.11 – 8.83)	0.03	9.33 (1.79 – 48.68)	0.008
<b>Mode of HIV transmission</b>				
Blood transfusion	1.00 (Ref)			
Heterosexual	0.80 (0.13 – 4.87)	0.81		
<b>WHO clinical stage</b>				
Stages 1/ 2	1.00 (Ref)		1.00 (Ref)	
Stages 3/ 4	7.73 (4.08 – 14.61)	<0.001	7.19 (3.40 – 15.19)	0.000
<b>Pulmonary TB</b>				
Absent	1.00			
Present	<i>Predicts success perfectly**</i>			
<b>HBV status</b>				
Negative	1.00 (Ref)			
Positive	3.23 (0.88 – 12.03)	0.08		
<b>Oropharyngeal candidiasis</b>				
Absent	1.00 (Ref)			
Present	2.43 (1.20 – 4.95)	0.01		
<b>Chronic diarrhoea</b>				
Absent	1.00 (Ref)			
Present	4.04 (1.68 – 9.72)	0.002		
<b>Kaposi's sarcoma</b>				
Absent	1.00 (Ref)			
Present	0.83 (0.20 – 3.39)	0.79		
<b>HIV RNA Log viral load (copies/ mL)</b>				
<4.6	1.00 (Ref)		1.00 (Ref)	
≥4.6	4.01 (2.27 – 7.08)	<0.001	4.05(1.91 – 8.58)	0.000

\* Adjusted ORs for variables that remained in the final model.

\*\*Stata did not provide the OR (CI) because there were no subjects with PTB in those with high CD4 count

#### 4. DISCUSSION

WHO clinical stage 3 or 4, residing outside Plateau State and HIV RNA viral load  $\geq 4.6$  log<sub>10</sub> copies/ml were independently associated with a low CD4 count.

Our finding that WHO clinical stage 3 or 4 was associated with a low CD4 count was similar to findings in other studies [3,20] where WHO clinical stage 3 or 4 was associated with advanced immune suppression (CD4 count <200 cells/ml) in patients with HIV infection. WHO stage 3 or 4 has been defined as advanced or severe HIV symptoms [3]. In the unadjusted logistic regression analyses, oropharyngeal candidiasis and chronic diarrhea which are symptoms of clinical stage 3 or 4, were associated with low CD4 count (ORs: 2.43 and 4.04, respectively).

Our finding of a 63% reduction in the odds of having a low CD4 count among patients residing in Plateau State compared to those who were not may be due to an early

presentation to our health facility in the former group because of a closer proximity and easier access to the facility. Some studies have shown that lower CD4 count was commoner in late presenters than early presenters among HIV infected patients [7-9]. Again, based on the definition by Buchacz et al. of "late HIV diagnosis" as a CD4 count <200 cells/ml [21], we could argue that patients residing in Plateau State were less likely to have a late HIV diagnosis compared to those residing outside the state.

The increase in the odds of having a lower CD4 count in those with higher HIV RNA viral load and the negative correlation ( $r=-0.4$ ) between viral load and CD4 count that we observed was similar to the findings of a very large systematic review [4] and of other studies [2,5] that as viral load increases, CD4 count declines over time in the progression of untreated HIV infection.

In this study we were unable to look at the association between residence and duration of symptoms (which often correlates with early or late presentation) at the time of presentation to our HIV clinic, as we did not collect data on the latter. This would have enabled us to directly look at the statistical association between these two variables to help support our finding of an association between low CD4 count and residential location. We had to use residential location as a proxy variable for duration of symptoms instead. This is thus another limitation of our study. But the finding of an association between low CD4 count and residence could also have been due to some confounding variables that we were unable to measure in our study.

## **5. CONCLUSION**

Patients with WHO clinical stage 3 or 4, with HIV RNA viral load  $\geq 4.6$  log<sub>10</sub> copies/ml and who were residing outside Plateau State; were more likely to be have a CD4 count <200 cells/ml which would suggest late presentation and hence late HIV diagnosis. These patients thus have a delayed opportunity for timely access to HIV care and initiation of ARV therapy due to late presentation. There is therefore the need to intensify the present efforts on early routine HIV counselling and testing not only in well established donor-supported facilities in the cities; but also in smaller towns and rural communities, so as to reduce the frequency of late HIV diagnosis and its potential implications.

## **CONSENT**

A written informed consent was obtained from all patients for use of their data.

## **ETHICAL APPROVAL**

This study was as approved by the Ethics committee of the Jos University Teaching Hospital, Jos.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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