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RELATIONSHIP BETWEEN CD4 COUNT, NEUTROPHIL-TO-LYMPHOCYTE RATIO, PLATELET-TO-LYMPHOCYTE RATIO AND LEFT VENTRICULAR SYSTOLIC FUNCTION IN HIV/AIDS PATIENTS
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ABSTRACT

Background: **Inflammation is a cardinal feature of Human Immunodeficiency Virus (HIV) infection and accounts for most of its clinical manifestations. Cardiovascular involvement is common in HIV. Haematologic inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are elevated in some cardiovascular diseases in the non-HIV population. CD4 count correlates with left ventricular size. The relationship between haematologic inflammatory markers NLR and PLR and left ventricular (LV) systolic function and their prognostic value is not well studied in HIV patients.**

Objective: **To determine the relationship between CD4 count, NLR and PLR and LV ejection fraction (EF) and their diagnostic value as surrogates for the occurrence of LV systolic dysfunction (LVSD)**

Design: **Cross-sectional**

Setting: **The Cardiology unit of the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria**

Participants: **150 HIV positive patients**

Outcome measures: **The relationship between CD4 count, NLR and PLR and LV ejection fraction (EF) and their diagnostic value for the occurrence of LV systolic dysfunction (LVSD)**

***Results*: The mean age was 37.14+8.6 years, 86 (64%) female, median duration of illness 6(0.94-24) months. NLR and PLR had a negative correlation with LV EF, the CD4 count had positive correlation. On multivariate analysis only PLR was predictive of EF. A cut-off PLR of 169.5 was only weakly discriminatory of LVSD with sensitivity of 81.3% and specificity of 72.9%, AUC 0.794.**

***Conclusion*: CD4 count, and inflammatory markers correlate with LVEF in HIV patients. Only PLR was predictive, its value as a diagnostic surrogate for LVSD needs further investigation.**

INTRODUCTION

Cardiac involvement in Human Immunodeficiency Virus (HIV) has been recognized since the beginning of the pandemic. (1) Since the advent of Highly Active AntiRetroviral Therapy (HAART), HIV has been converted from a cause of inevitable death to a chronic manageable illness, systemic illnesses such as cardiac involvement in HIV infection are likely to increase as patients live longer. The nexus between HIV infection and cardiac involvement is related to HIV infection being an inflammatory condition. Research suggests that, paradoxically, chronic upregulation of inflammatory activity occurs in HIV patients and it is present even among those on anti-retroviral therapy with viral suppression and may play an important role in predicting mortality.(2) Inflammation has been shown to play an important role in the initiation and progression of cardiovascular diseases (CVDs). (3) White blood cells (WBCs) and their subtypes are associated with increased cardiovascular risk factors.(4) Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are inflammatory parameters used as prognostic factors in various diseases such as CVDs and cancers.(5) Durmus et al showed that they were higher in heart failure (HF) patients than in healthy controls.(6) Park et al demonstrated that a higher NLR is associated with a higher mortality rate in those with coronary artery disease (CAD). (7)

Several authors have shown that cardiac involvement in HIV/AIDS is not rare and have highlighted the importance of echocardiography in assessing cardiac function in patients.(8,9) On echocardiography, the assessment of left ventricular (LV) systolic function is pivotal in the overall assessment of cardiac function. Its core index the ejection fraction (EF) is the cornerstone of making a diagnosis of conditions such as HF, and it is the bedrock for disease classification and treatment. The greater occurrence of systolic dysfunction (SD) in HIV patients than in healthy controls has been reported variously.(10,11) Adebola et al and Mankwe et al in Nigeria have shown that the CD4 count correlated with LV systolic function. (9,12)

However, the influence of other common readily available haematological parameters of inflammation such as NLR and PLR on LV systolic function in HIV patients has not been well-studied. NLR has been shown to be an independent predictor of mortality in HF patients in the non-HIV population.(13) Because NLR and PLR are cheap, readily available and easily determinable from the full blood count study, it is important to determine their relationship to echocardiographically assessed LV function which may not always be readily available and is relatively more

expensive in the African setting. It is also important to see if any cut-off values of these parameters could provide an inexpensive surrogate for diagnosing LV systolic dysfunction (LVSD) in the HIV patient.

Aim and Objectives: To determine the relationship between CD4 count, NLR and PLR with LV ejection fraction (EF) and to determine the cut-off value of these parameters that predicts the occurrence of LVSD

MATERIALS AND METHODS

Study design: The research work was a cross-sectional study.

Study setting: The Cardiology unit of the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria

Study participants: Consecutively recruited adult HIV patients aged 18 years and above seen at the above location who satisfied the inclusion criteria.

Sample size: The required sample size was obtained using Cochran's formula (14) with the p value set at <0.05 using estimated prevalence rate of HIV infection in Nigeria of 5.7% giving a sample size of 150 patients.

Inclusion criteria: HIV positive patients ≥18 years old confirmed results based on serological confirmatory tests done in UITH. These comprised 75 patients who were already on HAART and 75 newly diagnosed patients who were HAART-naïve.

Exclusion criteria: Patients with pre-existing hypertension, other cardiovascular diseases, diabetes mellitus or sickle cell disease, pregnant women and patients who drank more than 2 units of alcohol per week or smoked tobacco were excluded.

Data collection:

Echocardiography

An ALOKA SSD (2004) 2-dimensional echocardiography machine was used using standard techniques and recommendations of the American Society of Echocardiography (ASE).(15) 2-D guided M-mode images of the left ventricle were displayed and the LV internal dimension in diastole (LVIDd) and in systole (LVIDs), interventricular septum in diastole (IVSd) and systole (IVSs), posterior wall dimension in diastole (PWd) and systole (PWs) were measured. The LV end diastolic (EDV) and end systolic volumes (ESV) were derived from these using the Teicholz formula. LV ejection fraction (EF) and fractional shortening (FS) were then derived from these earlier measurements. The echocardiography machine is already programmed to generate these parameters automatically using the formulae below.

$$EF = \frac{EDV - ESV}{LVEDV} \times 100$$

$$FS = \frac{LVIDd - LVIDs}{LVIDd} \times 100$$

LV systolic dysfunction (SD) was present when EF was <50% or FS was less than 28%.

Laboratory Procedures:
Haematological parameters:
Five mls of participants' venous sample was collected into an EDTA bottle using aseptic venipuncture technique. Samples were analysed using an Automated Haematology Analyser (Systems KX-21 Haematology Auto Analyser). The analyser uses electrical impedance method to determine the count and size distribution of red blood cell, white blood cell, neutrophil, lymphocyte and platelet counts and uses colorimetric method to determine haemoglobin.
The NLR was calculated from the absolute neutrophil count divided by the absolute lymphocyte count both obtained from the same blood sample, while PLR was calculated from the absolute platelet count divided by the absolute lymphocyte count, both obtained from the same blood sample. The baseline CD4 cell count was assayed using flow cytometry method with 5mls of blood drawn in ethylenediaminetetraacetic acid tube following standard procedure as well as observing universal precautions.
Data Analysis
Data was analyzed using SPSS version 23 (SPSS Inc., Chicago, Illinois). Normally distributed continuous variables were expressed as mean ± standard deviation while skewed variables were expressed as medians and interquartile range (IQR). Categorical variables were expressed as percentages. Correlation between CD4 count, NLR, PLR and other haematological parameters with EF was performed using Spearman's correlation for skewed variables and Pearson's correlation for normally distributed variables. Significant correlates were inputted into multiple linear regression to determine independent predictors of EF. A Receiver operating characteristic (ROC) analysis was used to assess the ability PLR to predict the occurrence of LVSD. Statistical significance was set at a p value < 0.05.
Ethical Consideration: Approval to undertake the study was obtained from the Ethics and Research Committee of the UITH, Ilorin.

RESULTS

The mean age of participants was 37.14±8.6 years. There were more women 96 (64%), the mean body mass index (BMI) was 21.44 ±4.98kg/m^2. Majority were employed 136 (90.7%), had formal education 122 (82.35) and were married. The mean systolic blood pressure (SBP) was 112.2 ± 12.29mmHg while the diastolic blood pressure (DBP) was 73.2 ± 9.14mmHg.

Table 1

Socio-Demographic and clinical variables of Study Participants

Variable	All Participants N =150 n(%)/Mean±SD/Median (IQR)
Age (years)	37.14±8.6
Gender	
Male	54 (36)
Female	96 (64)
Weight (kg)	58.2± 13.3
Height (m)	1.64 ± 0.09
BMI (kg/m^2)	21.44 ± 4.98
Waist circumference (cm)	81.72 ± 9.33
Hip circumference (cm)	86.8 ± 10.83
Waist: Hip Ratio	0.94 ± 0.06
Duration of diagnosis (months)	6 (0.94-24)
Systolic blood pressure (mmHg)	112.2 ± 12.29
Diastolic blood pressure (mmHg)	73.2 ± 9.14
Employment status	
Unemployed	14 (9.3)
Employed	136 (90.7)
Education status	
No formal education	28 (18.7)
Has formal education	122 (82.3)
Marital status	
Single	22 (15)
Married	109 (72.3)
Divorced/Separated	19 (12.7)
Religion	
Islam	89 (59.3)
Christianity	61 (40.7)
On HAART	
Yes	75 (50)
No	75 (50)

BMI- Body mass index; HAART- Highly active anti-retroviral therapy

Table 2 shows that the median CD4 count at diagnosis was 138 (IQR 47-237). The mean Packed cell volume was 31.64 ± 6.2%. The median White Blood Cell count (WBC) was 4800 (IQR 3500-7200) /mm^3. The median neutrophil count was 2268 (IQR 1545.5 - 4450) /mm^3, the median lymphocyte count1656 (IQR 1011.5- 2339.5) /mm^3. The median platelet count was 246000 (IQR 205000-331000) /mm^3, Neutrophil-to-Lymphocyte ratio was 1.59 (IQR 0.83-2.89) and the Platelet-to-Lymphocyte ratio was 146.8 (95.53-272.2)

Table 2

CD4 Count and Haematological parameters of HIV patients

Haematological parameter	**n(%)/Mean±SD/Median (IQR)**
CD4 Count (cells/mm^3)	138 (47-237)
Packed cell volume (%)	31.64 ± 6.2
White Blood Cell count (/mm^3)	4800 (3500-7200)
Neutrophil count (/mm^3)	2268 (1545.5 - 4450)
Lymphocyte count (/mm3)	1656 (1011.5- 2339.5)
Eosinophil count (/mm3)	598 (420.5-863)
Platelet count (/mm3)	246000 (205000-331000)
Neutrophil-to-Lymphocyte ratio (NLR)	1.59 (0.83-2.89)
Platelet-to-Lymphocyte ratio (PLR)	146.8 (95.53-272.2)

The echocardiography parameters of the systolic function of patients are shown in Table 3. LVSD was present in 27 patients (18%). The mean EF was 60.49 ±12.37% and the mean FS was 33.17 ± 8.7%.

Table 3

The Echocardiographic Characteristics of Patients

Variable	**Patients N=150 (mean ±SD)**
Echo left ventricular systolic dysfunction	27 (18%)
LVIDd (cm)	4.63+ 0.76
LVIDs (cm)	3.1+ 0.64
IVSd (cm)	1.16+ 0.32
IVSs (cm)	1.5+ 0.32
PWd (cm)	0.83+0.33
PWs (cm)	1.27+0.33
Ejection fraction	60.49 +12.37
Fractional Shortening	33.17 + 8.7
Ejection Time (msec)	269.62 + 47.9
LVM (gm)	188.1 +69.06
LVMI	115.4+36.74
RVIDd (cm)	1.55+ 0.49

Key- IVSd- Interventricular Septal Thickness in diastole, IVSs-Interventricular Septal Thickness in systole, LVIDd-Left Ventricular Dimension in diastole, LVIDs-Left Ventricular Dimension in systole, PWd-Posterior Wall Thickness in diastole, PWs-Posterior Wall Thickness in systole RVIDd- Right ventricular dimension in diastole, LAD-Left Atrial Dimension, LVM- Left ventricular mass, LVMI-Left ventricular mass indexed to body surface area.

The correlation co-efficients of the haematologic parameters to EF are shown in Table 4. Positive significant correlations were found to CD4 count, Packed cell volume, Hemoglobin concentration and lymphocyte count while significant negative correlations were found to NLR and PLR.

Table 4

Correlation between Haematological parameters and Left ventricular ejection fraction

PARAMETERS	CORRELATION CO-EFFICIENT	P VALUE
CD4 count	0.307	0.003*
Packed cell volume	0.279	0.006*
Neutrophil count	-0.015	0.884
Lymphocyte count	0.361	<0.001*
Eosinophil count	0.137	0.203
Platelet count	-0.044	0.688
Neutrophil-to-lymphocyte ratio (NLR)	-0.270	0.008*
Platelet-to-lymphocyte ratio(PLR)	-0.316	0.003*

Significant correlates of EF were compared with EF using single linear regression (Table 5). Significantly associated parameters were then inputted into a multiple linear regression model with EF as the outcome variable. Multicollinearity of variables was tested for and excluded using Variance Inflation Factor (VIF) values. Only PLR was significantly predictive of EF.

In order to determine a PLR cut-off value that could diagnose the presence of Figure 2 shows the ROC curve of the predictive capacity of PLR on LV dysfunction. The Area under the curve (AUC) was significant- 0.794, P <0.001, (0.653-0.934). The PLR cut-off value that showed greatest sensitivity and specificity was 169.5, with sensitivity of 81.3% and specificity of 72.9%

Table 5

Predictors of Left ventricular systolic function

Single Linear Regression			Multiple linear regression		
PARAMETERS	**CO-EFFICIENT**	**P VALUE**	**PARAMETERS**	**CO-EFFICIENT**	**P VALUE**
CD4 count	0.278	0.008*	CD4 count	0.133	0.378
Packed cell volume	0.279	0.006*	Packed cell volume	-0.024	0.853
Lymphocyte count	0.251	0.014*	Lymphocyte count	-0.019	0.912
Neutrophil lymphocyte ratio	-0.242	0.018*	Neutrophil lymphocyte ratio	-0.054	0.692
Platelet lymphocyte ratio	-0.419	<0.001*	Platelet lymphocyte ratio	-0.355	0.025*

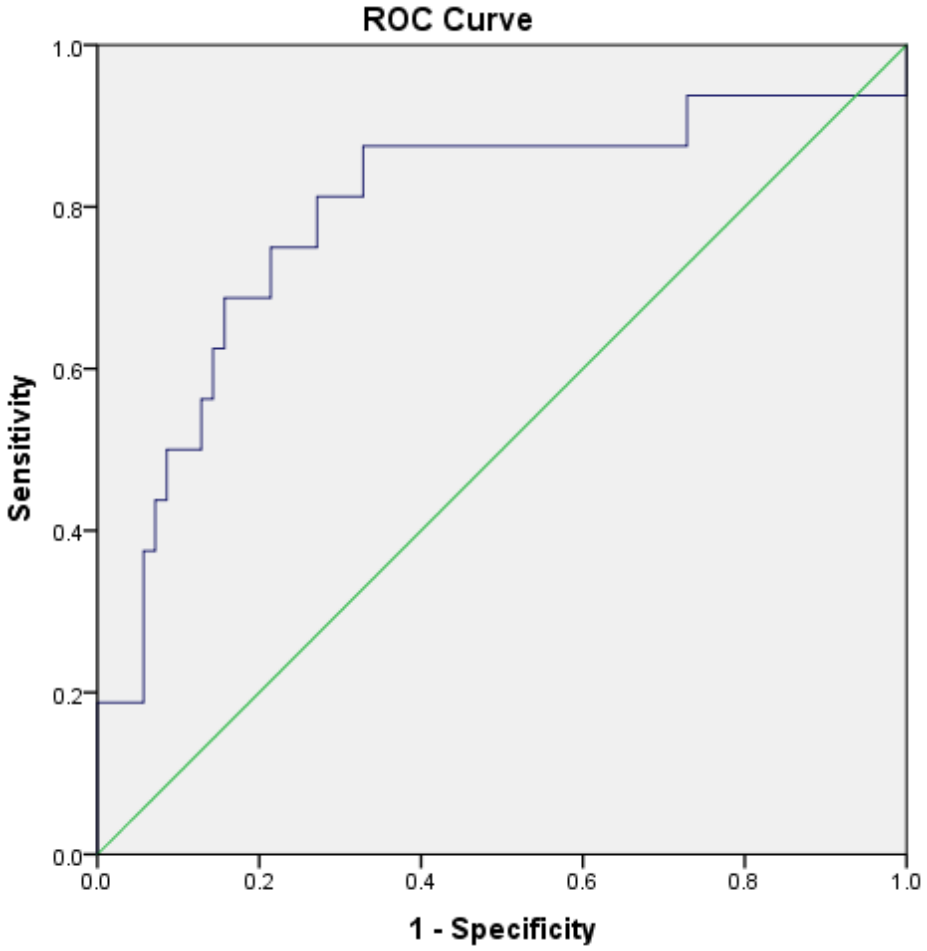

Figure 1: Receiver Operator Characteristic curve of the prediction of Platelet lymphocyte ratio and occurrence of Left ventricular systolic dysfunction. Area under the curve (AUC)=0.794

DISCUSSION

The positive correlation between the CD4 count and EF is similar to observations by Adebola et al (9) and Mishra et al (10). However, in our study CD4 count was not predictive of the EF on multivariate regression analysis.

In our study, the NLR and PLR were negatively correlated to the EF. Studies exploring the relationship between NLR and PLR and EF in HIV patients are rare, and to our knowledge, this is the first such study from Nigeria. Most studies of NLR and PLR in HIV patients studied association with mortality. In this light, Hanberg et al observed that NLR and PLR correlated with mortality but found that only NLR was independently predictive of mortality.(16) In patients with myocardial infarction, Arbel et al also observed NLR was independently predictive of mortality.(17) In HF patients, however, Pourafkari et al found that neither PLR nor NLR were independently predictive of mortality.(18)

A negative correlation of NLR and PLR to EF is consistent with the observation of a positive

correlation between EF and lymphocyte count. A high NLR or PLR implies a relatively low lymphocyte count compared to neutrophil or platelet count and vice versa. A possible explanation for this relationship is that in inflammatory conditions, neutrophils release several pro-inflammatory cytokines, including TNF-α, IL-6, C-reactive protein, and proteolytic enzymes. These cytokines have destructive effects on the myocardium, resulting in decreased LV function. Lymphocytes play an important role in healing by modulating mononuclear cell phenotypes and inducing the expression of tissue inhibitor of metalloproteinase-1.(19) In high NLR and PLR scenarios, there is a relative depletion of lymphocytes which is related to decreased myocardial healing and hence possibility of reduced function. Incidentally, in stressful conditions such as cardiac dysfunction, lymphopenia can also occur due to increased cortisol secretion caused by the activation of the hypothalamic-pituitary-adrenal axis.(20) Lymphopenia has been shown to be an independent prognostic factor and is also associated with decreased survival in patients with LV dysfunction of HF. (13)

Though both NLR and PLR are inflammatory markers found to have prognostic value in various diseases, this study found only PLR to be predictive of EF on multivariate analysis. Having an additional discriminatory capacity to diagnose LVSD would have made PLR a useful surrogate for echocardiography derived EF. Echocardiography is relatively expensive and may not be readily available in some settings unlike a complete blood count study from which PLR can be easily determined. In this study, the PLR of 169.5 gave the best sensitivity and specificity of 81.3% and 72.9% respectively. A larger sample size may be able to further determine the prognostic value of PLR in the development of LVSD.

CONCLUSION

The CD4 count and markers of inflammation such as NLR and PLR correlate with LVEF in HIV patients. Only PLR was however predictive of it. Its value as a diagnostic surrogate for LVSD needs further investigation probably with a larger sample size.

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