Original Article

Evaluation of Absolute Lymphocyte Count as a Surrogate marker for CD4+ cell count for the Initiation of Antiretroviral Therapy (ART) in Resource-limited Settings

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Background: In resource-limited settings, laboratory assessment of HIV-infected individuals requires the consideration of simple markers for the initiation and monitoring of HAART. Absolute lymphocyte count (ALC) could serve as a simple cost-effective tool for determining when to initiate ART in resource-constrained settings.
Objective: To determine the possibility of using absolute lymphocyte count as a surrogate marker for CD4+ cell count for the initiation of antiretroviral therapy (ART) in resource-limited settings.
Methods: Blood samples from 273 symptomatic HIV-positive individuals aged 18 to 57 years (33.3% males, 66.7% females) who presented for assessment for ART-eligibility were analyzed for CD4 count by flow-cytometry and ALC by automated haematology cell counter. Spearman rank correlation between CD4 count and ALC was assessed and positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of varying ALC cutoffs were computed for CD4 counts <200 cells/µL and <350 cells/µL.
Results: High degree of correlation was noted between CD4 and ALC counts (r = 0.71, p<0.0001). PPV, NPV, sensitivity, and specificity maximally aggregated at ALC <1400 cells/µL for CD4 <200 cells/µL and ALC <2300 cells/µL for CD4 <350 cells/µL. ALC <1400 cells/µL had a 90% PPV, 87% NPV, 86% sensitivity and 90% specificity for CD4 counts <200 cells/µL. ALC <2300 cells/µL had 89% PPV, 51% NPV, 83% sensitivity and 62% specificity for CD4 counts <350 cells/µL.
Conclusion: Our results have demonstrated the capability of absolute lymphocyte count to serve as a surrogate marker for CD4+ cell count for the initiation of antiretroviral therapy (ART) in resource-limited settings.

Key words*: *Absolute lymphocyte count, CD4+ cell count, antiretroviral therapy, resource-limited settings

Introduction

Since the first cases of acquired immunodeficiency syndromes (AIDS) were reported in 1981, infection with human immunodeficiency virus (HIV) has grown to pandemic proportions globally, and the resulting morbidity and mortality are major public health challenges. In sub-Saharan Africa an estimated 23.6 million people were living with HIV/AIDS as at the end of 2007 (1); with an annual estimate of 2.1 million persons newly infected and about 1.7 million deaths. Thus, sub-Saharan Africa remains the hardest hit continent: with less than eleven percent (<11%) of the total global population, accounting for 67% of all people living with HIV/AIDS

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and 75% of all AIDS deaths in 2007 worldwide (1).

In 1996, a revolution in the care of patients with HIV/AIDS both in the developed and developing world commenced with the advent of highly active antiretroviral therapy (HAART). However, it is estimated that in developing and transitional countries, 9.7 million people are in immediate need of antiretroviral therapy (ART) and regrettably only 2.99 million (31%) are receiving the drugs (1). This implies that despite the existence of affordable medications, only very few people living with HIV/AIDS are having access to treatment. One of the major obstacles to the administration of HAART is the fulfillment of the requirements for the initiation of antiretroviral therapy (ART). Currently, the existing number of CD4+ T lymphocytes is considered the best marker of the immediate state of HIV-induced immune impairment of a patient with HIV infection that would warrant initiation of ART.

However, accurate determination of CD4+ cell counts needs to be done by flow cytometry, an expensive technique not widely available in most resource-limited settings. Guidelines developed by the World Health Organization (WHO) for the use of antiretroviral therapy (ART) in low-income countries state that If CD4 cell counting is not available, using clinical staging alone, or in combination with absolute or total lymphocyte counts (TLCs) of <1200/μL could be used to determine ART eligibility (2). Many studies, however, have found both clinical staging and this TLC threshold to have poor sensitivity for low CD4 cell counts, leading researchers attempt to define other TLC thresholds which better correspond to CD4+ cell counts ≤ 200 or 350 cells/μL (3-5).

Only very few studies have attempted to study the correlation between absolute lymphocyte count and CD4+ count in Nigeria with varying results. To the best of our knowledge, a correlation study between absolute lymphocyte count and CD4+ count among HIV-infected individuals has not been carried out in this locality. This study, therefore, aim to evaluate the relationship between absolute lymphocyte count and CD4+ cell counts and determine different cutoff values of ALC to serve as surrogate for CD4+ counts in deciding when to initiate ART.

Materials and Methods

Two hundred and seventy three (273) symptomatic ELISA confirmed HIV-seropositive individuals who presented themselves for assessment for ART-eligibility in Bori General Hospital, Bori, Rivers State, Nigeria, between May, 2008 and June, 2009 were recruited for this study. Informed consent was obtained from each participant by FIB and all blood samples were collected between 8.00-11.00am only into K_3EDTA container to avoid circadian variation and analyzed within 6 hours on the same day of sample collection. The study received institutional ethical approval from the Management of the hospital.

CD4+ cell counts were determined by commercially available flow-cytometric technique (PARTEC CYFLOW COUNTER, Otto-Hahn-Str. 32 D-48161 Munster, Germany) and absolute lymphocyte count (ALC) was derived by multiplying the percentage lymphocyte count obtained from leukocyte differential count by the total white blood cell count (TWBC). The percentage lymphocyte counts and the total white blood cell counts (TWBC) were determined using an automated haematology cell counter (ABACUS JUNIOR HAEMATOLOGY ANALYZER-an 18 part haematological parameters auto-analyzer) (Diatron Messtechnik GmbH, Austria). Spearman-rank correlation between CD4+ count and ALC was assessed and positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of varying ALC cutoffs were computed for CD4 count <200 cells/μL and <350 cells/μL.

Statistical Methods: Data were entered and analyzed using SPSS statistical package for windows (version 16; SPSS Inc, Chicago). Statistical analysis included mean, standard deviation, chi square analysis and Spearman rank correlations. Differences were considered significant when P values were less than or equal to 0.05($P<0.05$)

Results

The patients had a mean age of 32.01±9.19 years with a range of 18-57 years. Of the 273 HIV-positive individuals, 91(33.3%) were men and 182 (66.7%) were women. The female patients were significantly more than the male patients with a male to female ratio of 1:2 ($p<0.05$). The mean absolute lymphocyte count of the patients was 1.6±0.95 x10^9/L with a range of 0.16 to 6.19 x 10^9/L, and the mean CD4+ cell count was 278.20±201.35 cells/μL with a range of 118 to 1216 cells/μL. Table1 shows that male patients were significantly older than the female patients (35.42±8.30 vs. 30.49±9.71 years; $p<0.05$). The male patients had mean absolute lymphocyte count and CD4+ cell count of 1.6.00±0.92 x10^9/L and 247.09±194.13 cells/μL, respec-

tively; whereas, the female patients had a mean absolute lymphocyte count of 1.62±0.96 x10^9/L and CD4+ cell count of 293.70±203.61 cells/µL. There were no statistically significant difference in the absolute lymphocyte counts of the male patients and the female patients (P>0.05). Females had comparatively higher mean CD4+ cell counts than their male counterparts. The difference was statistically significant (P< 0.05).Table2 shows that those between 18-37 years age group constituted the bulk (75.9%) of the study population. This implies that younger adults are more prone to HIV-infection. Table 3 shows that 129 (47.3%) of the study population had CD4+ cell count ≤200 cells/µL, 201 (73.6%) had CD4+ cell count ≤ 350 cells/µL, 237 (86.8%) had CD4+ cell count ≤ 500 cells/µL and 36 (13.2%) had CD4+ cell count >500 cells/µL. Table 4 shows the calculated positive predictive values (PPV), negative predictive values (NPV), sensitivities, and specificities of varying ALC cutoffs to predict CD4+ count <200 cells/µl. The Spearman-rank correlation determined between CD4+ and ALC show a significant correlation at the 0.0001 level (2-tailed) (r = 0.71). Absolute lymphocyte count <1400 cells/µl (<1.4 x 10^9/L) had a 89.9% PPV, 86.8% NPV, and had 85.9% sensitivity and 89.6% specificity for CD4+ count <200 cells/µl. Using an ALC cutoff of 1400 cells/µl or less would identify 85.9% of patients with a CD4+ <200 cells/µl. Table 5 shows the calculated positive predictive values (PPV), negative predictive values (NPV), sensitivities, and specificities of varying ALC cutoffs to predict CD4+ count <350 cells/µl. The Spearman-rank correlation determined between CD4+ and ALC show a significant correlation at the 0.0001 level (2-tailed) (r = 0.71). Absolute lymphocyte count <2300 cells/µl (<2.3 x 10^9/L) had a 88.6% PPV, 51.4% NPV, and had 83.2% sensitivity and 61.7% specificity for CD4+ count <350 cells/µl. Using an ALC cutoff of 2300 cells/µl or less would identify 83.2% of patients with a CD4+ <350 cells/µl.

Table 1 Comparison of the mean age, CD4 cells/µL and ALC x10^9/L of the participants according to gender

Sex	Age (yrs)	CD4 cells/µL	ALC x10^9/L
Male	35.42±8.30	247.09±194.13	1.60±0.92 x10^9/L
Female	30.49±9.71	293.70±203.61	1.62±0.96 x10^9/L
P-value	(p<0.05)	(p<0.05)	(p>0.05)

Table 2 Frequency distribution of the study population according to age groups

Age group (years)	Frequency (%)
18-27	96 (35.2)
28-37	111 (40.7)
38-47	44 (16.1)
48-57	22 (8.1)
Total	273 (100.0)

Table 3 Frequency distribution of the various CD4+ cutoff values for the initiation of antiretroviral therapy

CD4+ cutoff values	Frequency (%)
≤200	129 (47.3)
≤350	201 (73.6)
≤500	237 (86.8)
>500	36 (13.2)

Table 4 Calculated positive predictive values (PPV), negative predictive values (NPV), sensitivities and specificities of ALC for CD4 Count <200 cells/µL in all the paired counts (n=273)

ALC	PPV	NPV	Sensitivity	Specificity
< 1000	65.1	95.8	93.3	75.4
< 1100	76.0	92.4	89.9	81.1
< 1200	79.1	89.6	87.2	82.7
< 1300	89.6	86.8	86.4	87.4
< 1400	89.9	86.8	85.9	89.6
< 1500	90.7	75.7	77.0	90.1
< 1600	93.0	71.5	74.5	91.0
< 1700	93.0	68.1	72.3	91.6
< 1800	93.8	66.7	71.6	92.3
< 1900	96.1	61.8	69.3	94.7
< 2000	96.9	54.2	65.4	95.1

Table 5 Calculated positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of ALC for CD4 Count <350 cells/µL in all the paired counts (n=273)

ALC	PPV	NPV	Sensitivity	Specificity
< 1500	69.2	81.9	91.4	48.8
< 1600	72.1	77.8	90.1	50.0
< 1700	73.1	73.6	88.6	49.5
< 1800	74.6	73.6	88.8	51.0
< 1900	78.6	70.8	88.3	54.3
< 2000	82.1	63.9	86.4	56.1
< 2100	84.6	58.3	85.0	57.5
< 2200	85.6	52.8	83.5	58.7
< 2300	88.6	51.4	83.2	61.7
< 2400	89.6	47.2	82.6	61.8
< 2500	90.5	43.1	81.6	62.0

Discussion

In trying to develop a model suitable for use in resource-limited settings, this study found absolute lymphocyte count (ALC) <1,400 cells/μL to be a strong predictor of CD4+ count < 200 cells/μL in this study environment. This finding show a strong correlation between ALC and CD4 (r = 0.71, p<0.0001). Earlier studies have shown similar correlations between ALC and CD4+ cell counts in HIV-infected patients in South Africa (6), the United Kingdom (7), the United States (8, 9) and India (3). Longitudinal follow-up of a South African cohort showed that TLC and CD4 cell counts were equal predictors of disease progression (10).

Using an ALC cutoff of 1,400 cells/μL or less would identify 85.9% of HIV-infected patients with a CD4+ cell counts <200 cells/μL. Hence, ALC has the capacity to serve as a surrogate marker for CD4+ cell count for the initiation of antiretroviral therapy (ART) in resource-limited settings. Moreover, TLC has been found to be an inexpensive and useful marker for staging HIV disease, predicting progression to AIDS and death and monitoring response to ART (11). However, the most recent ART guidelines from WHO state that "TLC is not suitable for monitoring therapy as a change in the TLC value does not reliably predict treatment success" (12). This WHO guideline is in agreement with Akinola *et al.* (13) who reported a poor correlation (r=0.43).

The findings in this study are similar to the findings of Kumarasamy *et al.* (3) who looked at the correlation between TLC and a CD4+ cell count in South Indian patients with HIV disease and found a high degree of correlation between 650 paired CD4 and TLC counts (r=0.744). They found that a TLC cutoff point of ≤1400 cells/μL had a 76% positive predictive value (PPV), 86% negative predictive value (NPV), and was 73% sensitive, 88% specific for CD4+ cell counts <200 cells/μL. They concluded that TLC could serve as a low-cost tool for determining both a patient's risk of opportunistic infection (OI) and when to initiate prophylaxis in resource-constrained settings. Similarly, Blatt *et al.* (9) found that a TLC cutoff point of ≤1400 cells/μL was 80% sensitive and 90% specific for CD4+ cell counts <200 cells/μL. Studies show variable levels of sensitivity of TLC to predict CD4 count.

In HIV treatment, the implications are that a low sensitivity is a public health concern, and a low specificity is a cost and cost-effectiveness issue (14). The reference range for $CD4^+$ T lymphocyte count among apparently healthy HIV negative Nigerian adults is 365 – 1571 cells/μl (15).

In Africa and other parts of the developing world where there is a higher incidence of bacterial and parasitic infections that tend to occur at earlier stage of immunosuppression, the World Health Organization and others have recommended initiation of antiretroviral therapy at higher counts (≤350 cells/μL) and/or earlier HIV clinical stage than in developed countries (12, 16); primarily for those with factors which may limit the effectiveness of ART if treatment is much delayed (17). Consequently, this study adopted the approach of selecting ALC cutoffs for both CD4 count <200 cells/μL and CD4 count ≤350 cells/μL.

With a Spearman rank correlation of 0.71 (p<0.0001), an optimal ALC cutoff of 2,300 cells/μL was found to be the strongest predictor of CD4+ count <350 cells/μL with a sensitivity of 83.2%, specificity of 61.7%, PPV of 88.6% and NPV of 51.4%. These findings indicates that the appropriated ALC for predicting CD4+ counts < 350 cells/μL is ALC <2,300 cells/μL. These findings are similar to the findings of Moore *et al. (*16) who found a TLC threshold of 2250 cells/μL with a correlation of 0.73 that predict CD4 cell counts ≤350 cells/μL, yielding a sensitivity of 0.81 and a specificity of 0.54. In contrast, the findings are not in total agreement with Kumarasamy *et al.* (3) who found a total lymphocyte count (TLC) <1700 cells/μL to have an 86% positive predictive value (PPV), 69% negative predictive value (NPV), 70% sensitivity and 86% specificity for CD4+ cell counts <350 cells/μL. Akinola *et al.* (13) analyzing 151 TLCs and CD4+ cell counts obtained from 109 HIV-infected Nigerian patients who had not yet started ART concluded that TLC is not a reliable predictor of CD4+ cell count because 1 in 3 individuals were likely to be deprived of needed treatment.

Of the 273 HIV-positive individuals, 33.3% were men and 66.7% were women, giving a male to female ratio of 1:2 (p<0.05). This suggests higher carrier rate of HIV infection among females than their male counterparts or it is likely that differences in health seeking behaviour are more common in women than men. This study also shows that 18-37 years age group constituted the bulk (75.9%) of the study population. This implies that younger adults are more prone to HIV-infection.

While CD4+ cell counting has proven useful in allowing stratification of risk for disease progression on antiretroviral therapy (ART), individual variation of disease progression within CD4+ cell count strata is large (16). Thus, even if ALCs may not correlate perfectly with CD4+ cell counts, it has proven useful in predicting disease progression and in determining when ART

should be initiated.

The proportion of subjects in this study with CD4 cell counts ≤200 cells/μL and ≤350 cells/μL are very high (47.3% and 73.6%, respectively), resulting in a large number of subjects being ART-eligible. Though some of the participants came in response to voluntary counselling and testing (VCT) programme, it is not very surprising that the number of eligible individuals is large because many people who presented for assessment for ART-eligibility might have been tested for HIV because of ill health. As delaying ART until CD4 cell counts fall below 200 cells/μL results in increased mortality (18) and most studies from sub-Saharan African settings have shown high mortality rates in the first year on therapy (19-21), it seems reasonable to adopt the more liberal ART eligibility criteria of <350 cells/μL. This suggestion is in agreement with Moore *et al.* (16).

Because this study was conducted among asymptomatic HIV-positive individuals from rural and semi-urban environments, further studies need to be conducted to evaluate how well this inexpensive, surrogate marker predict CD4 lymphocyte counts in other geographical areas. CD4 cell counts differ from one locality to other and among different ethnic groups (22, 23). Thus one ALC cutoff may not necessarily apply to populations from different parts of the world. Different prevalent rates of viral, bacterial and parasitic co-infections or other biological and genetic factors may likely to affect the type of results. More so, as CD4 and absolute lymphocyte counts are expected to differ from one study to the other depending upon the region of study, number of subjects, method of enumeration, etc., more studies in different parts of the country are required in order to discern any regional difference in results.

Our results have demonstrated the capability of absolute lymphocyte count to serve as a surrogate marker for CD4+ cell count for the initiation of antiretroviral therapy (ART) in resource-limited settings. Given the greater availability and lower cost of ALC, there is a clear argument to proceed with HIV disease treatment and opportunistic infection prophylaxis based on ALC results. This would no doubt overcome one of the practical obstacles to more widespread provision of antiretroviral therapy in resource-poor settings.

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