ORIGINAL ARTICLE

Absolute Lymphocyte Count As A Marker For Cd4 T-lymphocyte Count: Criterion For Initiating Antiretroviral Therapy In HIV Infected Nigerians

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ABSTRACT

Background: *Few laboratories in resource-constrained countries can afford to perform laboratory-monitoring tests required for the implementation of HIV therapy. In this case control study, we have investigated the relevance of absolute lymphocyte count as a surrogate marker for CD4 lymphocyte count as a criterion for initiating HAART in HIV-infected Nigerians.*

Methods: *A total of 100 consecutive recruited HIV-infected, previously antiretroviral naive persons and 30 HIV-negative individuals blood samples were run for absolute lymphocyte and CD4 lymphocyte counts and results were compared by a model of linear regression analysis.*

Results: *An overall modest correlation was observed between absolute lymphocyte count and CD4 lymphocyte (r = 0.51) and at CD4 lymphocyte threshold relevant for clinical management of HIV-infected; <200, 200-350 and > 350 cells/μL (r = 0.41, 0.30 and 0.21) respectively. Mean absolute lymphocyte count of 1.60 ± 0.77 x 10^9/L, 1.88 ± 1.11 x 10^9/L and 2.04 ± 0.54 x 10^9/L was equivalent respectively to CD4 of < 200, 200-350 and > 350 cells/μL.*

Conclusions: *This study indicates a modest correlation between absolute and CD4 lymphocyte counts of HIV-infected Nigerians and at CD4 lymphocyte count threshold significant for clinical management of HIV-infected. Absolute lymphocyte count can become a minimal inexpensive alternative to CD4 lymphocyte count in conjunction with WHO staging and clinical status of patient in determining the optimal time to initiate therapy particularly in resource limited settings where other expensive methods of CD4 enumeration are unavailable.*

KEY WORDS: *Absolute Lymphocyte; Marker; CD4 Lymphocyte; HIV-infected Nigerians.*

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INTRODUCTION

The most characteristic feature of HIV/AIDS is a selective depletion of CD4 T helper/inducer subsets of T-cells. The degree of T-cell depletion is currently the single most important laboratory finding considered when making recommendations regarding initiation of therapy [1]. Highly active antiretroviral therapy has changed the landscape of HIV care in the developed world. Many patients with access to antiretroviral therapy have benefited from the dramatic reductions in mortality and morbidity and HIV disease has become of relative chronicity for most HIV-infected patients [2,3].

Interestingly, concern related to drug toxicities, pill burden and the ability of patients to adhere strictly to these complicated regimens have complicated the decision making process for physicians and patients alike [4].

Despite promised price reductions and increased availability of generic drugs in some countries, cost remains a major factor in deciding when to start therapy in many parts of the world. The guidelines for initiation of therapy vary from country to country. Early intervention in asymptomatic patient involves the commencement of antiretroviral therapy once CD4 lymphocyte count is less than 500 cells/μL[5]. A less aggressive approach is to recommend therapy when the count falls to 350-cells/μL[6]. In some countries depending on the financial resources of the patient, treatment typically is delayed until CD4 count is 200 cells/l. Flow cytometry is the reference method for enumerating CD4+ lymphocyte[7]. However the infrastructure of clinical laboratories located in many developing countries is often inappropriate for a routine flow cytometry because of high cost of equipment and reagents, lack of trained manpower and maintenance-associated problems. Simple methods for enumeration of CD4 T-lymphocytes not requiring complex laboratory equipments have become available [8]. However, cost constraints and absence of trained manpower is a major hindrance in many resource-limited settings. In this study we have evaluated the relevance of absolute lymphocyte count in determining the optimal time to initiate HAART and monitor disease progression particularly in resource limited settings.

PATIENTS AND METHODS

One hundred Patients (100) HIV-infected and previously antiretroviral naive patients (91 symptomatic and 11 asymptomatic) enrolled into the antiretroviral therapy pilot project at the Department of Haematology of the University of Port Harcourt Teaching Hospital constituted the subjects for this case control study. The hospital is a 500- bed tertiary health

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facility and one of the designated centers for the Federal Government of Nigeria's assisted antiretroviral therapy programme rendering specialist HIV-related care to thousands of people in the Niger Delta of Nigeria. Thirty healthy HIV-negative individuals, age and sex matched were monitored as controls. Written informed consent was obtained from all participants.

Methods

Ten milliliters of venous blood was collected from the antecubital vein of each participant into EDTA anticoagulated tubes (5 milliliters) and plain non-anticoagulated tubes (5 milliliters). Blood samples were analyzed within four hours of collection. Serum from the non-anticoagulated tubes were confirmed for HIV using a double ELISA confirmatory tests of Immunocomb HIV 1 & 2 kits (Orgenics, Israel), an enzyme immunoassay test for the qualitative and differential diagnosis of HIV. Initially reactive samples were confirmed using the Genscreen HIV 1 & 2 kits (Bio Rad, France). Total white cell count was analyzed using the Turks method. Blood films were stained by Leishman stain. Differential leucocytes count was carried out by Battlement method. Manual methods as described by Dacie and Lewis [9] were used for all haematological analysis.

The CD4 T-cell enumeration was carried out using the Dynal beads technique (Dynal Asa, Oslo, Norway). This technique uses paramagnetic polymer beads coated with anti-CD4 monoclonal antibodies to capture and isolate CD4 T-lymphocytes from whole blood. The average cost for single test is £10 (about ₦2500). Previous report by Diagbouga and co-workers [10] indicated that CD4 lymphocyte count values from Dynal beads correlated positively with values from flow cytometry (r = 0.90).

Study Design

This case control study consisted of enumeration of total and CD4 T-helper lymphocyte counts of 100 HIV-infected subjects at baseline before the initiation of highly active antiretroviral therapy (HAART). The absolute and CD4 lymphocyte counts of 30 healthy HIV-negative individuals were monitored as controls. A model of linear regression analysis was applied on the data.

Statistical Analysis

Data were computed using SPSS statistical software (Version 10 SPSS Inc. Chicago IL). Continuous variables were summarized as means and standard deviation. Correlation data obtained with the alternative test (absolute lymphocyte count) were compared to the standard Dynal beads CD4 count and the correlation coefficient was calculated. We also determined the proportion of results classified by the absolute lymphocyte counts at various CD4 cell count threshold relevant for clinical management of HIV-infected patients (<200, 200 350 and > 350 cells/μL). A p-value of = 0.05 was considered significant for all statistical comparison.

RESULTS

One hundred (100) HIV-infected patients made up of 53 males and 47 females aged 18-56 years, mean age 34.13 ± 8.45 constitute the subjects for this case control study. Thirty HIV-negative persons consisting of 16 males and 14 males aged 18-54 (mean ± SD = 33.33 ± 5.28) years were monitored as controls. Each participant was monitored for CD4 cell count, total white cell count, differential leucocyte count and absolute lymphocyte count. The overall mean Cd4 and absolute lymphocyte counts of HIV-infected persons were 235.00 ± 112.6 cells/μL and 1.77 ± 0.89 x 10^9/L, while the mean CD4 and absolute lymphocyte count of HIV-negative controls were 743.67 ± 139.79 cells/μL and 1.96 ± 0.53 x 10^9/L respectively. Table I shows the mean and correlation between CD4 and absolute lymphocyte counts of HIV-positive subjects and HIV-negative controls.

The overall correlation coefficient between CD4 and absolute lymphocyte count of HIV infected individuals was modest (r = 0.51**, p < 0.01). Comparatively there was no correlation between CD4 and absolute lymphocyte count of HIV-negative controls (p > 0.05). Correlation between CD4 and absolute lymphocyte count was compared based on the CD4 lymphocyte thresholds relevant for clinical management of HIV-infected (< 200, 200-350 and > 350 cells/μL). The correlation between absolute lymphocyte count and CD4 lymphocyte count was modest at a CD4 threshold of 200-350 cells/l (r = 0.41**, p < 0.01) compared to a low correlation at a CD4 threshold of < 200 cells/μL (r = 0.30 *, p < 0.05) and at a CD4 lymphocyte threshold of > 350 cells/μL (r = 0.21*, p < 0.05). Absolute lymphocyte count of 1.60 ± 0.77, 1.88 ± 1.11 and 2.04 ± 0.54 corresponded with CD4 lymphocyte count of <200, 200-350 and > 350 cells/μl respectively as shown in Table II.

Table I. Correlation between CD4 and absolute lymphocyte count of HIV-infected subjects and HIV-negative controls

Subject Group	CD4 count ($\bar{x}$ ± SD)	Absolute Lymphocyte count ($\bar{x}$ ± SD)	r	P-value
HIV-infected	235.6 ± 112.6	1.77 ± 0.89	0.51**	< 0.01
HIV-negative	743.67± 139.79	1.96 ± 0.53	0.43	> 0.05

* Correlation is significant p = 0.005 (2 tailed)

** Correlation is significant at p = 0.01 (2 tailed)

Table II. Correlation between CD4 and absolute lymphocyte count of HIV-infected persons based on clinically significant CD4 lymphocyte threshold.

Cd4 lymphocyte Threshold (cells/mL)	n	CD4 count ($\bar{x}$ ± SD)	Absolute Lymphocyte count ($\bar{x}$ ± SD)	r	P-value
<200	48	142.5 ± 28.17	1.60 ± 0.77	0.30*	<0.05
200-350	36	273.06 ± 68.46	1.88 ± 1.11	0.41**	<0.01
>350	16	430.63 ± 68.50	2.04 ± 0.54	0.21*	<0.05

** Correlation is significant at p = 0.01 (2 tailed)

* Correlation is significant p = 0.005 (2 tailed)

DISCUSSION

The availability of highly active antiretroviral therapy (HAART) and the presence of effective facilities for monitoring HIV infection has dramatically changed the landscape of human immunodeficiency virus (HIV) care in the developed world. This possibility however is beyond the reach of a vast majority of people living with HIV in sub-Saharan Africa where paradoxically more than 23 million people are living with the virus but with less than 8% having access, an ethical and public health issue that should be of great concern to the developed world [11]. In this case control study, we have evaluated the relevance of absolute lymphocyte count as a surrogate for CD4 lymphocyte count in determining the optimal time to initiate HAART in HIV-infected Nigerians.

In this study we observed a modest overall correlation coefficient of ($r = 0.51$, $p < 0.01$) between paired CD4 and absolute lymphocyte counts of 100 HIV-infected Nigerians. This values although higher than a value of ($r = 0.25$) obtained on a similar study involving 32 HIV-infected Nigerians [12], is however lower than that obtained in a study involving 2774 HIV-positive persons in South Africa [13] ($r = 0.70$) and also among 1535 asymptomatic HIV-infected in the United Kingdom ($r = 0.64$) [14]. The lower correlation coefficient observed in our study may have been accounted for by the fact that unlike in the others, ours included 9 asymptomatic HIV-infected patients. We hypothesize that the correlation may be higher with a larger sample population.

In this study, we observed a modest correlation between the absolute lymphocyte count and CD4 lymphocyte count at CD4 thresholds significant for clinical management of HIV-infected persons (<200, 200-300, >300 cells/μL, r=0.41**, 0.30* and r =0.21* respectively p<0.05). This finding is consistent with previous study[15], which indicated that absolute lymphocyte count was a better predictor of CD4 for symptomatic patients than for patients that are asymptomatic and have higher CD4 cell counts.

We observed that a mean absolute lymphocyte count of 1.60 ± 0.77 $x10^9$/L was equivalent to CD4 count of <200 cells/l while 1.88 ± 1.11 $x10^9$/L and 2.04 ± 0.55 were equivalent to CD4 count of 200-350 cells/l and >350 cells/μL respectively.

We obtained a modest correction between the absolute lymphocyte count and CD4 lymphocyte count of HIV-infected individuals at CD4 lymphocyte count threshold relevant for the clinical management of HIV-infected Nigerians (r = 0.40, 0.30 and 0.21) respectively for CD4 lymphocyte count threshold of <200, 200-350 and >350 cells/l. This observation although discordant with the suggestion in a previous study that total lymphocyte count is not a substitute for CD4 lymphocyte count [16], it is however consistent with previous studies [17,18] which indicated that absolute lymphocyte count may be more useful predicting CD4 lymphocyte count for symptomatic patients than for asymptomatic patients with a high CD4 count and confirms earlier suggestion of possibility of using absolute lymphocyte count in determining the optimal time to initiate antiretroviral therapy particularly in resource limited settings where expensive methods of CD4+ enumeration is unavailable[15].

Our study with a correlation of r = 0.51 between absolute lymphocyte count and CD4 lymphocyte count has indicated that absolute lymphocyte count is positively correlated with CD4 lymphocyte count and at CD4 lymphocyte threshold relevant for the clinical management of HIV-infected Nigerians and that absolute lymphocyte count could be used as a readily available minimal alternative for CD4 lymphocyte count in conjunction with the World Health Organization staging system and clinical status in determining the prognosis and the optimal time to initiate HAART, particularly in resource-limited settings where CD4 monitoring facilities are unavailable.

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