ORIGINAL ARTICLE

ABSOLUTE LYMPHOCYTE COUNT AS A SURROGATE OF CD4+ T LYMPHOCYTE CELL COUNT IN INITIATING ANTIRETROVIRAL THERAPY IN HIV-INFECTED NIGERIANS.

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ABSTRACT

Background: Most laboratories in resource-constrained countries, cannot routinely use standard methods to measure markers of disease progression useful in staging and initiation of antiretroviral therapy due to high cost more so with the ongoing reduction of support by the implementing partners. The objective of this study is to determine whether Absolute Lymphocyte Count (ALC) can serve as a surrogate for CD4+ T-Lymphocyte Cell Count (CCC) for initiating highly active antiretroviral therapy (HAART) in HIV-infected treatment naïve patients in our setting. **Methods:** A total of 400 adult Nigerians infected with HIV-1 and who were previously antiretroviral naïve, were recruited into the study at Ahmadu Bello University Teaching Hospital, HIV subspecialty clinic. They were assessed clinically and immunologically and categorized into three clinical stages; A, B, and C according to CDC criteria. Absolute Lymphocyte Count and CCC values were tested for correlation and their validity determined using SPSS version 20 and Chi square statistics. **Results:** The patients comprised of 187 males (46.8%) and 213 (53.2%) females. One hundred and eight (27%) of the study subjects were in stage A, 153 (38.5%) in stage B, and 139 (34.8%) in stage C. The mean ALC of stage C subjects is significantly lower than that of stage A subject's $p < 0.05$. The mean CCC values declines significantly from stage A through B to C p 0. There was a positive and significantly correlation coefficient between ALC and CCC in stage C; r 0.28, $P < 0.05$. In all the subjects and in the stages, sensitivity and specificity of a low ALC value to predict a low CCC value were low. **Conclusion:** Absolute Lymphocyte Count is not a reliable and sensitive surrogate of CCC in all HIV infected patients however it is only useful in immunocompromised patients to determine the optimal time to initiate HAART.

KEYWORDS: Lymphocyte; count; surrogate; CD4+ cell count; initiation of antiretroviral therapy.

INTRODUCTION

Fundamental in understanding HIV/AIDS related opportunistic infections (OIs), is the appreciation of the relationship between the level of the underlying immune dysfunction as measured by the CCC, and the incidence of AIDS defining Ois.[1] CD4+ T-Lymphocyte Cell Count is presently considered as one of the best markers of HIV induced immune impairment.[2,3,4] The degree of immune deterioration correlates with the likelihood of development of OIs, which typically occurs when the CCC drops to critical levels as occurs in HIV infection.[1,3,5]

The CD4 antigen has a high affinity receptor for the HIV and this explains the selective tropism of the virus for CD4+ T cells and other CD4+ cells, [1,2,6] With successful infection, the viral particle enters the CD4+ cell replicates and destroys it predominantly by direct cytolysis. [6,7,8] Approximately 100 billion new viral particles are produced everyday, and 1-2 billion CD4+ T cells die everyday.[9] Other mechanisms of CD4+ T cell loss include;

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Apoptosis of uninfected CD4+ T cells, Fusion of infected and uninfected cells, (syncytia [giant cells] formation), with subsequent ballooning and death, direct infection of thymic progenitor cells and infection of accessory cells that secret cytokines essential for CD4+ T cells differentiation. [1, 8] CD4+ T cell plays a vital role in regulating the immune response, hence loss of this master cell has ripple effects on virtually every other cell of the immune system.[1, 8] Loss of CD4+ T cells is the hallmark of AIDS. [6,7, 8, 9] Therefore enumeration of CD4+ lymphocytes serve as an important tool in the classification, initiation of antiretroviral therapy, monitoring of treatment and prognosis of HIV/AIDS.[1,3,5]

At present there is no definitive cure for HIV/AIDS. The benefit of Highly Active Antiretroviral Therapy (HAART) lies in the timely and sustained suppression of viral replication, alongside the reversal of the progressive immune deficiency that is characteristic of HIV infection.[10] The reported benefits of antiretroviral therapy (ART) have encouraged its use in the clinical management of people living with HIV/AIDS (PLWHA) in several countries.[6, 11] Studies has shown that HAART has remarkably reduced HIV related morbidity and mortality, thereby improving the quality of life of PLWHA.[12,13]

In most laboratories particularly in developing countries, CCC are not measured routinely due to high cost of the equipments, reagents and lack of technical skill both in terms of usage and maintenance .[1,3, 5] Although test methods based on the use of light and ELISA techniques are now available instead of the gold standard which involves flow cytometry, all forms of measurement requires the use of monoclonal antibodies (this makes the cost of the test unaffordable).[12] Thus the need for a possible surrogate for CCC in our environment as is being documented by WHO and in other studies. [7,14,15,16,17]

The peripheral blood of man contains about 3000 lymphocytes per mm^3, of this number, 70 to 80 % are T lymphocytes and 15 to 20% are B-lymphocytes.[15] Of the T lymphocytes, about 65% express CD4 antigens.[16] Thus it is believed that if the CD4+ LC falls significantly as occurs in HIV infected persons, there should be a concomitant and proportionate decline in the ALC in the peripheral blood. Therefore ALC may be a useful surrogate marker of disease progression and have been recommended as a substitute in the management of HIV-infected individuals living in resource limited areas.[7, 17] In this study we have explored absolute lymphocyte count, as a possible surrogate test for CD4 cell count.

PATIENTS AND METHODS
Four hundred adult patients who were HIV-1 repeatedly reactive and who were previously antiretroviral naive were recruited into the study. They were assessed clinically and immunologically and categorized into three clinical stages; A, B, and C in accordance with CDC Clinical/Immunological categorization of HIV/AIDS.

The study was carried out at Ahmadu Bello University Teaching Hospital, Zaria-Haematology HIV Subspecialty clinic, one of the designated centre for the Federal Government of Nigeria's assisted antiretroviral treatment programme. Blood specimens were taken at enrolment, between 10am and 12 noon for full blood count (FBC) and CCC (10 mls was collected into plain sample bottles and 5 mls into EDTA anticoagulated bottle. Blood samples were analyzed within six hours of collection. Total white cell count and differential counts were determined according to standard methods.[18] Absolute lymphocyte count (ALC) was derived from the product of total white count and percentage differential count. CD4+ cell count was determined using monoclonal antibody labeled microspheres manual methods developed by Dynal Biotech SA,

Norway France. The following were computed.

1. All ALC and CCC values in each of the groups were tested for correlation (r)

2. Validity testing: Sensitivity and Specificity of low ALC value, as a predictor of low CCC Value was also determined. This was tested for significance using the Chi Square test where applicable.

3. Sensitivity was defined as the proportion obtained when number of measurements out of total number were ALC < 2000 /mm3 that have CD4 cell count below 200 /l (nominator) was divided by the total number of measurements with CCC < 200 /l (denominator).

4. Specificity was defined as the proportion obtained when number of measurements out of total number were ALC > 2000 /mm3 that have CCC > 200 /l (nominator) was divided by the total number of measurements with ALC > 2000 /mm3 (Denominator).

Approval was obtained from the Research and ethics committee of Ahmadu Bello University Teaching Hospital, Zaria. Data processing was performed by the Statistical Program (SPSS version 20). Data were tested by the student t-test for two means and the hypothesis test for two proportions. Coefficient of correlation (r) was determined between the two methods used. All tests were applied at a level of significance (α=0.05). P-values of 0.05 were considered as statistically significant.

RESULTS

A total of 400 patients comprising 187 (46.8%) males and 213 (53.2%) females. Their ages ranged from 15 to 64 years, with a mean age of 34.75 years 8.93. They were predominantly in the working age group of 25 to 44 years with only 6 in the age group of 15 to 24 years. Considering CDC clinical/immunological stage distribution, one hundred and eight (27%) of the 400 patients had stage A disease, 153 (38.5%) stage B, and 139 (34.8%) had stage C disease. The mean weight of the patients was 58.13 kg 12.25 and a range of 27 to 100 kg.

Table 1: Pattern of ALC, CCC and correlation coefficient (r) by stage and gender stratification

Variable	Stage			Gender		
	A	**B**	**C**	**Male**	**Female**	**General**
	n = 108	**n = 153**	**N = 139**	**n = 187**	**n = 213**	**n = 400**
	Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
	SD ±	**SD ±**	**SD ±**	**SD ±**	**SD ±**	**SD ±**
ALC $\times 10^9$/l	2.6 2.9	2.3 1.5	1.9 1.0	2.2 1.3	2.3 2.2	2.2 1.3 ``
CCC cells/l	506.1 260.2	256.6 128.0	134.5 80.4	277.9 211.4	284.8 226.3	282.4 219.0
r P value	- 0.11	+ 0.054	+ 0.280	+ 0.23	0.006	0.074
	0.255. P > 0.05	0.504 P > 0.05	0.001 P < 0.05	0.002 P < 0.05	0.92 P > 0.05	0.141 P > 0.05

Where n is number of sample in the study group.

Table 2: Sensitivity and specificity of low ALC value as a predictor of low CCC value

Variable	Stage			Gender		
	A n = 108	**B** N = 153	**C** n = 139	**Male** n = 187	**Female** n = 213	**General group** n = 400
Sensitivity %	3.7	37.3	84.4	52.1	68.5	48.1
Specificity %	98.2	78.2	21.3	65.6	57.9	68.2
P value (x^2)	*	< 0.05	> 0.05	< 0.05	< 0.05	< 0.05

n Where n is number of sample in the sub group
* cannot be subjected to a test of statistical significance due to single observation in one of the sub group.
P value statistical significance by Chi Square (x^2)

No correlationship
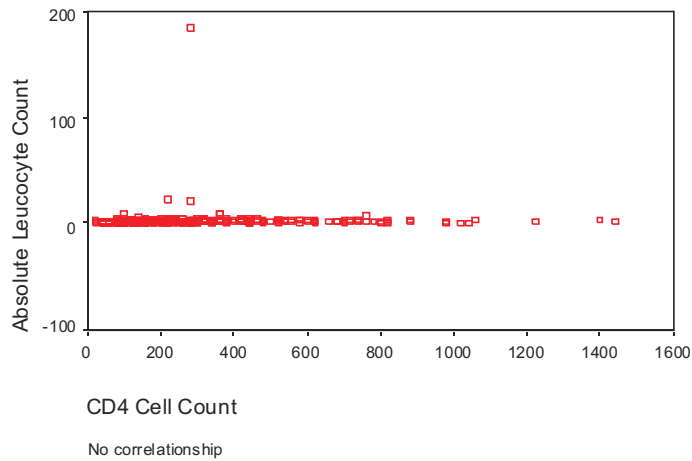
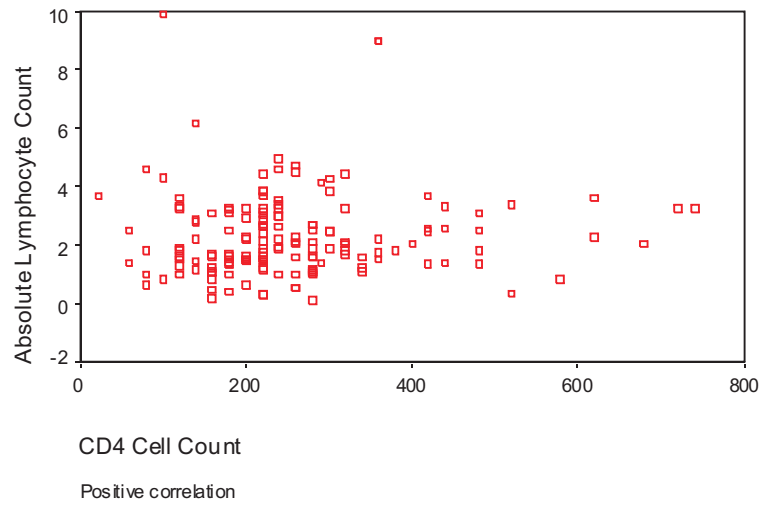**Figure 1:** Scattered diagram of ALC and CCC (General group)

Negative correlation
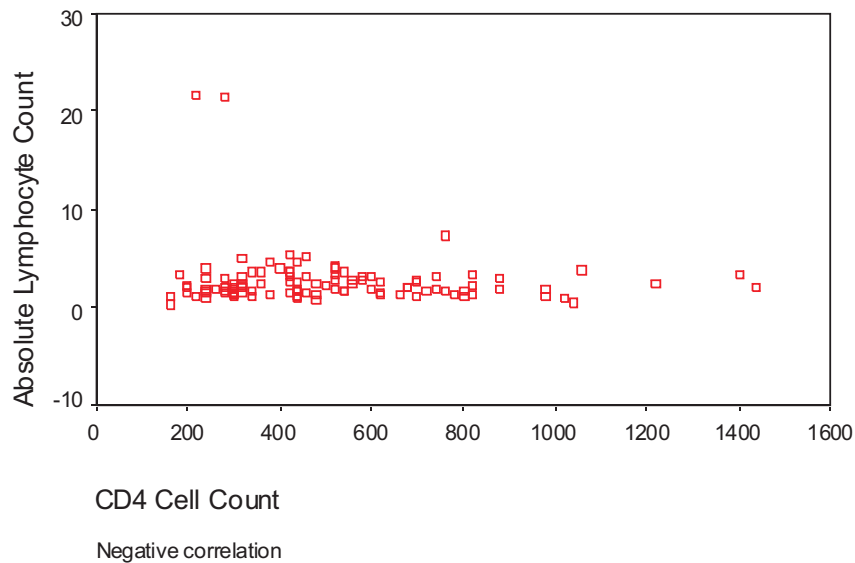
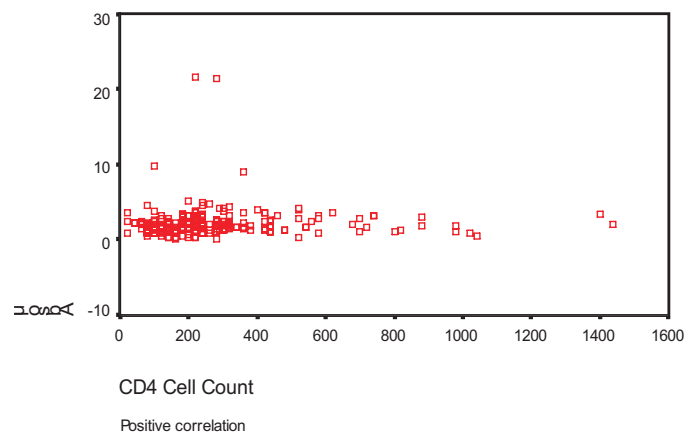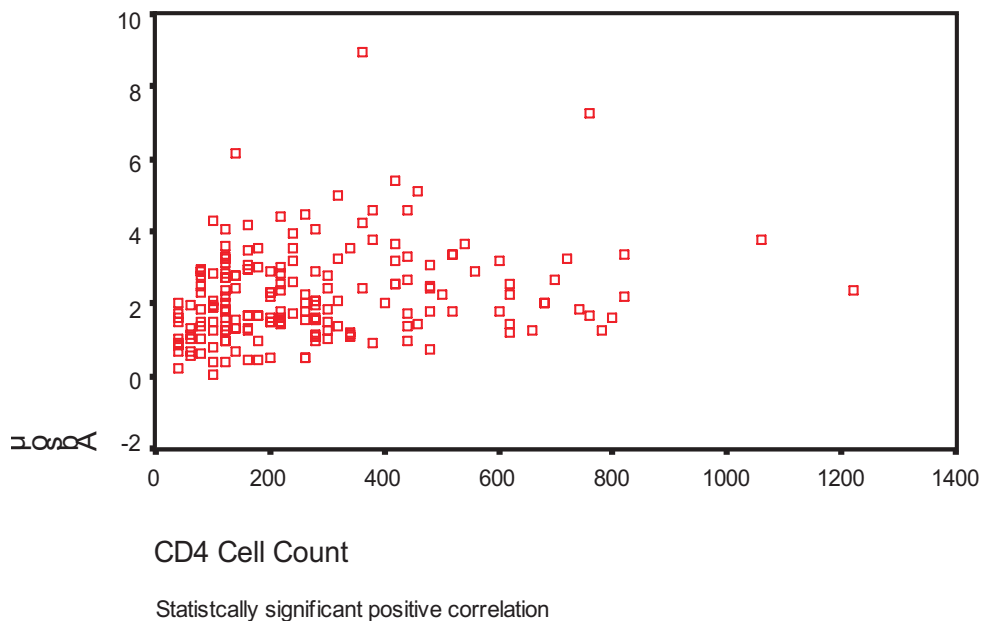**Figure 2:** Scattered diagram of ALC and CCC (Stage A)

Figure 3: Scattered diagram of ALC and CCC (Stage B)
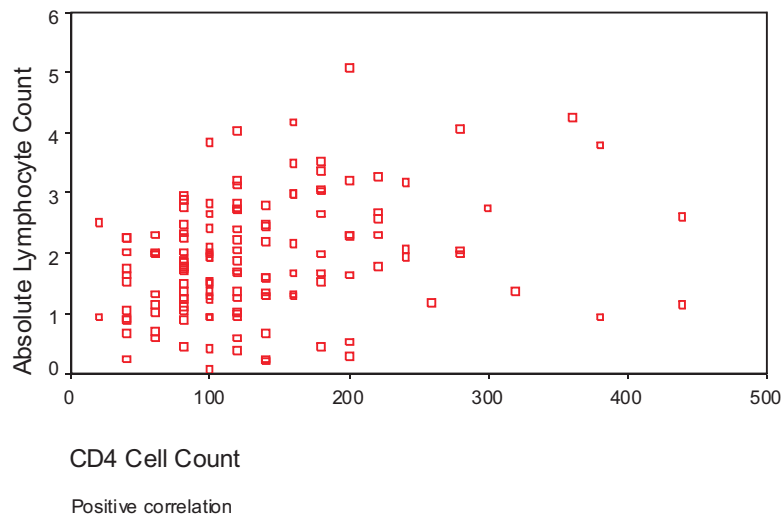
Figure 4: Scattered diagram of ALC and CCC (Stage C)

Figure 5: Scattered diagram of ALC and CCC (Female group)

Figure 6: Scattered diagram of ALC and CCC (Female group)

DISCUSSION

The mean CD4 counts of the general group declines significantly between the stages see table 1, this study thus confirms several 'old' findings that have been previously reported in several studies and in textbooks where CD4 are said to decline with increasing clinical severity of the infection.[1, 8, 19, 20, 21] Although a decline was noted in the ALC values between the stages see table 1, however this was statistically significantly only between stage A and C, P < 0.05. Neither the mean ALC between the sexes nor in the general group were significantly different P > 0.05.

In the general group the correlation coefficient between ALC and CCC was very weak r 0.074 but this was statistically not significant P > 0.05 see figure 1 for scattered diagram. Following CDC/Immunological stratification r - values became weaker in stage A where it was negatively correlated r - 0.11 and statistically not significant P > 0.05; figure 2 shows the scattered diagram. Although it still remained weak in stage B, it became positively correlated r 0.054 but remained statistically not significant p > 0.05; figure 3 shows the scattered diagram.

However a stronger association was seen in stage C r 0.28, which was statistically significant P < 0.05; figure 4 shows the scattered diagram.

When gender influences were tested a very weak association was obtained in the female gender r = 0.006 but was statistically not significant P > 0.05, figure 5 shows the scattered diagram. However a statistically significant and positive correlation was noted in the male gender r = 0.23 p < 0.05; figure 6 shows the scattered diagram.

Our findings thus contradicts earlier Nigerian studies where an association was reported in stage A and in the female gender.[22,23] Akinola et al reported a correlation of r = 0.49 p < 0.00l, which was stronger in the females r = 0.55 than males r = 0.42.22 Beck et al also reported higher correlation than was obtained in this study.[16, 24] Although the sensitivity (48%) and specificity (68%) of low ALC to predict a low CCC was low in the general subjects, statistically significant numbers of subjects with low ALC have low CCC by chi square statistics, p < 0.05, table 2. Sensitivity and specificity remained

low even after gender influences and disease stratification effects were considered see table 2, except in stage C were the sensitivity was high (84%), but this was of no statistical significance P >0.05 table 2. This result is in line with the low sensitivity of 58% and specificity of 75.6% reported by Akanmu et al in Nigeria, [23] and differs markedly from the high sensitivity of 96% and specificity of 60% obtained by Blatt et al. [25] which was statistically significant p < 0.05. Contradicting results have also been reported from other studies in other parts of the world where high validity were obtained. [26]

The differences observed in this study as compared to others may be largely due to methods of cells enumeration. Akinola et al used automated methods for all cell counts. Akanmu et al used manual methods for blood cell counts, and Coulter manual methods, while in this study manual cell count and Dynabeads method was used for CD4 + T cell enumeration. Studies from other parts of the world (Blatt, Beck, Kumarasany) used automated cell count and flow cytometers. The inherent errors associated with manual method is large, coefficient of variation for leucocytes for manual method is 16% and for automated analytical method is 1.5%. Dynabeads correlates well with the flow cytometric methods with a coefficient of correlation between the two techniques of between 0.89 to 0.97 for low counts in overseas studies [27, 28] while in Nigerian studies r value of > 0.75 was obtained. [29] Flow cytometry is the standard technique used in determining CD4+ T lymphocyte subset. However, the possibility of using this technique for routine monitoring of the immunological profiles of patients in developing countries are limited because of the sophisticated technology and high cost involved.

Secondly in our study a large sample size was used as opposed to the other Nigerian studies, where smaller sample size was used. Akinola used sixty-six patients while Akanmu used thirty-two patients. [23, 23]

The main draw back in our study was while other studies monitor the changes with therapy this study only considers the values at first presentation or prior to commencement of any form of drug (antiretroviral naïve). The presence of co-infections (Hepatitis B, C, HTLV – 1 and Syphilis) will also influence the CD4+ LC. [7, 20] Hepatitis B and C could not be screened for in all the study subjects due to finance.

Other reasons for the variations in results may be due to seasonal variation, diurnal variation, exercise, and drug use such as tobacco or snuff.[7] Heterogeneity of the CD4 epitopes molecules and cell loss during lysis of blood in CCC estimation is another factor. Problems of lysing African blood due to unknown reasons, probably due to the presence of a high proportion of nucleated cells, as a response to heightened erythropoiesis in an environment with a high prevalence of multiple infectious agents has been reported.[27] The high prevalence of sickle cell gene in Nigeria that is estimated to be between 23-30% and sickle cell anaemia of between 1-3% may also account for this.[30,31] So also is the undocumented prevalence of thalassaemic gene in the country may be responsible for this high erythropoietic drive.

CONCLUSION
Absolute lymphocyte count is correlated with CD4 + lymphocyte count in severely immunocompromised patients however it is an insensitive surrogate of CD4+ lymphocyte count in all HIV infected patients. Very accurate prediction cannot be made of CD4+ lymphocyte count from Absolute lymphocyte count in our under resource laboratories, more modern less cumbersome and more reliable analytical methods need to be put in place for this to be accomplished. Hence it cannot be absolutely and reliably used for the initiation of treatment or prophylaxis at all times in our setting. Thus Government intervention for a uniform global use of CD4+ lymphocyte count is advocated to initiate antiretroviral therapy.

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