ORIGINAL ARTICLE

Value of the use of absolute lymphocyte as surrogate for CD4 count in resource poor situations

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ABSTRACT

Background: The initiation of antiretroviral (ARV) drugs and monitoring of human immunodeficiency virus (HIV) treatment in developing nations such as sub-Sahara Africa is based on the clinical stage and level of CD4 count. Clinical stages can easily be determined using the World Health Organisation (WHO) criteria, this is not so with CD4 count where the right equipment and expertise are not easily available. This lead to various studies being carried out in search of surrogates for CD4 count with use of total lymphocyte count (TLC) being suggested by some studies. **Objective:** In situation where determination of CD4 cell count is not available or feasible, lymphocyte count is believed to be one alternative method for immunological classification of Acquired Immunodeficiency Syndrome (AIDS). Such assumption may not be true of every population. The objective is, therefore, to examine the correlation between the absolute lymphocyte count and the CD4+ lymphocyte count in HIV positive patients. **Materials and Methods:** One hundred and sixty-five consecutive HIV positive patients were recruited for the study before the commencement of ARV drugs over a period of 13 months. The haemotological parameters such as the CD4 count was done by flow cytometry using Partec cyflow counter machine made in Germany, with strict adherence to the manufacturer's standard operating procedure. TLC were also determined using Sysmex haematology blood analyser, following the manufacturer's standard operating procedure. Patients were then grouped into CD4 and Total lymphocyte (TLC) categories. These were then compared to determine if there is any correlation as shown in previous studies. Statistical analysis of data was done using Statistical Package for Social Sciences (SPSS) and statistical significance of data was based on *P* value of less than 0.05. There was significant positive correlation (*P* value 0.000) between TLC and CD4 count. **Results:** Majority of the patients with TLC less than 1000/mm^3 had CD4 count <200 cells/µl. Using TLC <1000/mm^3 threshold, there was high sensitivity of 81.8% but low specificity and positive predictive value of 47.5% and 19.4%, respectively, for CD4 count <200 cells/µl. Further assessment using TLC of <1,200/mm^3 for the currently accepted CD4 count cut-off of <350 cells/µl for initiation of antiretroviral drugs, the sensitivity, specificity, positive predictive value were found to be 76.5%, 26.7%, 21.3%, respectively. **Conclusions:** Considering the low specificity and positive predictive value, it was concluded that the use of TLC of as a surrogate for CD4 count is unreliable. However, where there is no alternative, it could be used with caution bearing in mind its limitations.

Key words: Antiretroviral (ARV) therapy, CD4 count, predictive values, total lymphocyte count

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INTRODUCTION

The human immunodeficiency virus (HIV) (causative agent of Acquired Immunodeficiency Syndrome (AIDS)) has been reported to produce a slow but progressive deterioration in the host immune system, leading to infections, neurologic disorders and neoplasms.[1] HIV infection has been found to be commoner among commercial sex workers, and people with other sexually transmitted diseases.[2]

The situation in HIV-infected people involves continuous viral replication and destruction and replacement of CD4+ cells. There is eventual deterioration of the host immune system when the rate of CD4+ T cells destruction by HIV supersedes the rate of replacement. While the CD4+ cell count is used as a measure of HIV disease progression, quantifying the viral load is currently the most direct measurement of the HIV disease process.[3] It has also been used to assess the risk of disease progression and the response to antiretroviral therapy (ART).[4]

Government of Nigeria, as part of its care and support strategies initiated the National Antiretroviral (ARV) Drug Access Programme in 2002.[4] Initiation of ARV drugs is based on CD4 count and World Health Organisation (WHO) clinical stage of the disease.[5] WHO has recommended CD4 count of <350 cells/μl and at least Clinical stage 3 disease for commencement of ARV drugs.[5]

CD4 count, though very important, is still expensive, and needs high expertise, which is obtainable in only few centres in resource poor countries like Nigeria. Thus, there is need for a surrogate test that could correlate closely to the CD4 count. Among the suggested surrogates for CD4 is the use of Serum albumin as surrogate for CD4 count in the study done by Olawumi and Olatunji.[6]

Another suggested surrogate for CD4 count, which is the focus of this study, is the use of total lymphocyte count (TLC) (which is readily available in most centres). Positive correlation has been found between TLC in some studies.[7] This led to suggestions of possible use of TLC as surrogate for CD4 count. Thus, the need arises for studies to confirm the usefulness or not of the use of TLC as surrogate for CD4 count.

MATERIALS AND METHODS

One hundred sixty-five consecutive adult subjects were selected for the study from patients attending University of Ilorin Teaching Hospital, who are being screened for HIV infection.

Patient's consent and approval of Hospital Ethical Review committee were obtained before the study was conducted.

HIV screening was done using the WHO parallel testing algorithm using rapid kits (DETERMINE and UNIGOLD). These were further validated using ELISA kit (GENSCREEN^{R} PLUS HIV Ag-Ab made by BIO-RAD).[8] Western blot or other confirmation testing was not done.

The CD4+ lymphocyte count was done on fresh samples taken by aseptic procedure into Ethylenediaminetetraacetic acid (EDTA) bottles using the Partec Flow cytometry based technique. TLC were also determined using Sysmex haematology blood analyser, with strict adherence to the manufacturer's standard operating procedure.

Statistical analysis of data was done using statistical package for social sciences (SPSS) and statistical significance was based on *P*-value of less than 0.05. Results were presented in tables and figures where applicable.

Comparisons were made using standard statistical methods in which categorical data was compared by Chi-square and discrete variables by *t*-test; 95% confidence level was observed. Conclusion and recommendations were based on scientific evidence from the results.

RESULTS

One hundred and sixty-five samples were analysed, 22 (13.3%) had TLC less than 1,000/mm^3, 74 (44.8%) had TLC between 1,000-2,000/mm^3, while 69 (41.8%) had TLC >2,000/mm^3 [Table 1].

Using non-parametric analysis, the mean total lymphocyte count was 2024 ± 988/mm^3 while the mean CD4 count was 270 ± 282 cells/μl. The minimum and maximum counts were 5 and 1599 cells/μl for CD4 count and were 300 and 7,500/mm^3 for TLC.

At TLC less than 1000/mm^3, 81.8% of these patients had CD4 count less than 200 cells/μl, 13.6% had CD4 count within 200-499 cells/μl and only 4.5% had CD4 count greater than 500 cells/μl.

At TLC between 1,000-2,000/mm^3 range (1-2 × 10^9/l), 62.1% had CD4 count less than 200 cells/μl, 28.4% had CD4 count within 200-499 cells/μl, while 9.5% had CD4 count >500.

And at TLC >2000/mm^3, 42.0% had CD4 <200 cells/μl, 30.4% had CD4 count within 200-499 cells/μl, while 27.5% had CD4 count greater than 500 cells/μl [Table 2].

Using CD4 count threshold of <350 cells/μl, showed that at TLC less than 1,200/mm^3, 26 out of 34 (76.5%) of these patients had CD4 count less than 350 cells/μl, seven (20.5%) had CD4 count between 350-499 cells/μl, while one (3%) had CD4 count greater than 500 cells/μl [Table 3].

Looking at all the cases that had TLC <2,000/mm^3, 79 out of 96 (82.2%) had CD4 count <350 cells/μl, nine (9.3%) had CD4 count between 350-499 cells/μl, while eight (8.3%) had CD4 count greater than 500 cells/μl [Table 3].

Linear regression Analysis showed positive correlation between CD4 count and TLC with R-value = 0.08 and *P*-value of 0.00 [Figure 1].

Table 1: Frequency of total lymphocyte group

Total lymphocyte count (/mm^3)	Frequency	Percent	Mean CD$_4$ count (cells/μl)	Standard Deviation
<1,000	22	13.3	162.545	294.4916
1,000-2,000	74	44.8	362.667	317.3259
>2,000	69	41.8	215.730	215.3043
Total	165	100.0	270.085	282.8105

Table 2: Frequency of total lymphocyte count and CD4 categories

Lymphocyte count	CD4 <200		CD4 200-499		CD4 >500		Total
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	
<1,000/mm³ (1 × 10⁹/l)	18	81.8	3	13.6	1	4.5	22
1,000-2,000/mm³ (1-2 × 10⁹/l)	46	62.1	21	28.4	7	9.5	74
<2,000/mm³ (<2 × 10⁹/l)	64	66.7	24	25	8	8.3	96
>2,000/mm³ (>2 × 10⁹/l)	29	42	21	30.4	19	27.5	69
Total	93		45		27		165

P value = 0.02

Table 3: Relationship between CD4 group and total lymphocyte count using CD4 threshold of <350 cells/μl

CD4 count/μl	Absolute lymphocyte count/mm³				Total
	<1,200	1,201-2,000	Total <2,000	>2,000	
<350	26	53	79	43	122
350-499	7	2	9	7	16
>500	1	7	8	19	27
Total	34	62	96	69	165

P-value = 0.01

Table 4: Sensitivity, specificity, positive and negative predictive value of total lymphocyte count for CD4 count <200cells/μL

Total lymphocyte count threshold for cd4 count <200 cells/μl	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
<1000/mm³	81.8	47.5	19.4	94.2
<2000/mm³	66.7	58	68.8	55.6

In comparing the sensitivity, specificity and positive predictive value of using TLC of <1,000/mm³ and <2,000/mm³ for CD4 count threshold of <200 cells/μ, it was found that using TLC of <1,000/mm³ as surrogate for CD4 count threshold of <200 cells/μ, has sensitivity of 81.8%, but positive predictive value of 19.4%. However, the use of TLC of <2,000/mm³ for CD4 count threshold of <200 cells/μ, gave a sensitivity of 66.7% and positive predictive value of 68.8% [Table 4].

Using the latest recommended CD4 count of <350 cells/μl for initiation of antiretroviral drugs,[4] the sensitivity, specificity, positive and negative predictive value of TLC of <1,200/mm³ and 2,000/mm³ for CD4 count threshold of <350 cells/μl, was calculated. It was found that using TLC of <1,200/mm³ as surrogate for CD4 count threshold of <350 cells/μl, gave a sensitivity of 76.5%, but positive predictive value of 21.3%. However, the use of TLC of <2,000/mm³ for CD4 count threshold of <350 cells/μ, gave a sensitivity of 82.3% and positive predictive value of 64.8% [Table 5].

DISCUSSION

In this study, the evaluation of the relationship between TLC and CD4 count, showed a statistically significant positive correlation (*P* value = 0.02) [Table 2].

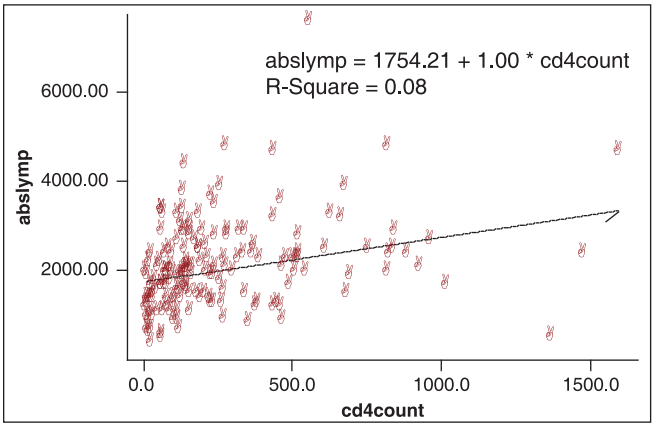

Figure 1: Linear regression analysis for CD4 count and total lymphocyte count correlation. *P* value = 0.00

It is also of note that a high percentage of patients in this study who had total lymphocyte count <2,000/mm³ (66.7%) also had CD4 count less than 200 cells/μl [Table 2]. This suggest that most patients with absolute lymphocyte count less than 2000/mm³ will most likely have CD4 count less than 200 cells/μl. In a similar study by Beck *et al.*, it was found that total lymphocyte count less than 1,250 × 10⁶/l approximates to CD4 count less than 200.[7]

Linear regression graph showed R square as 0.08, and significance of 0.000 [Figure 1]. This agrees with WHO finding that total lymphocyte count of <1,000/mm³ correlates with CD4 count of less than 200 cells/μl (WHO Improved clinical staging). This is the basis of WHO recommendation for centre where CD4 count could not be done that HIV patient with TLC of <1,200/mm³ with at least stage II disease can be started on ARV drugs.[9]

In this study, it was found that using absolute lymphocyte count threshold of <1,000/mm³ for CD4 count <200 cells/μl, gave a sensitivity of 81.8%, with positive predictive value of 19.4%. Increasing the absolute lymphocyte count threshold to <2,000/mm³ for CD4 count <200 cells/μl gave the sensitivity of 61.5% and positive predictive value to 68.8% [Table 4]. It may be inferred that using absolute lymphocyte count threshold of <2,000/mm³ for CD4 count <200 cells/μl l will be a more reliable

Table 5: Sensitivity, specificity, positive and negative predictive value of using total lymphocyte count for CD4 count threshold of <350cells/μL

Total lymphocyte count threshold for cd4 count <350 cells/μl	Sensitivity (TP/TP+FN) (%)	Specificity (TN/TN+FP) (%)	Positive predictive value (TP/TP+FP) (%)	Negative predictive value (TN/TN+FN) (%)
<1200/mm^3	76.5	26.7	21.3	81.4
<2000/mm^3	82.3	37.7	64.8	60.5

predictor in view of the higher sensitivity and positive predictive value.

Similar study was done by Brites and colleagues to evaluate the absolute lymphocyte count as a substitute for CD4 count in the follow-up of patients under HAART in Brazilian patients.[10] Using the absolute lymphocyte count threshold of 1,000 cells/mm^3, they found a positive predictive value (PPV) of 70.2% for CD4 count <200 cells/μl. Increasing the absolute lymphocyte count to 2,000/mm^3, was found to increase the sensitivity to 96.7% but decreases the positive predictive value to 26.7% (unlike the better positive predictive value found when TLC was increased to 2,000/mm^3 in this study). They concluded that using higher limit of absolute lymphocyte count (such as 2,000 cells/mm^3) for estimation of CD4 <200 cells/μl, would save the use of CD4 tests in only one-third of patients.[10] This study was, however, conducted on patients already on HAART.

Study by S. P. Blatt and colleague found that the likelihood ratio of the TLC in predicting absolute CD4 count <200 cells/μl increased from 2.4 (95% confidence interval) for all TLC <2,000/mm^3 to 33.2 (95% confidence interval) for all TLC less than 1,000/mm^3.[11] The specificity for this prediction was found to increase from 57% to 97% over this range. They concluded that TLC between 1,000/mm^3 and 2,000/mm^3 appears to be useful predictor of significant immunosuppressant as measured by a CD4+ T cells less than 200 cells/μl in HIV infected persons.[11] This agrees with the finding in this study where a large percentage of patients with TLC <2,000/mm^3 has CD4 count of <200 cells/μl [Table 2].

However, using the latest accepted CD4 count of <350 cells/μl for initiation of ARV drugs,[4] the sensitivity, specificity, positive and negative predictive value of using TLC of <1,200/mm^3 and 2,000/mm^3 for CD4 count threshold of <350 cells/μl, were respectively 76.5%, 26.5%, 21.3%, 81% for TLC <1,200/mm^3 and 82.3%, 37.7%, 64.8%, 60.5 for TLC 2,000/mm^3 [Table 5].

Comparing the sensitivity, specificity, positive predictive value of using total lymphocyte count as surrogate for CD4 count of <200cells/μl and <350 cells/μl, it was observed that though both values showed a relatively high sensitivity value, the specificity and positive predictive values were low.

This agreed with the findings by Deresse and Eskindir[12] where they also recorded low specificity for the use of TLC as a surrogate for CD4 count. They however submitted that TLC as surrogate for CD4 count in resource poor situations can still be used with the understanding of its low sensitivity and specificity.

It was found that out of 122 patients that had CD4 count <350 cells/μl, only 26 (21.3%) had TLC <1,200/mm^3 [Table 3]. Thus, using TLC <1,200/mm^3 in the absence of CD4 count would have excluded 78.7% of patients that should have been on ARV drugs based on CD4 count <350 cells/μl. This corroborates similar findings by Akinola *et al*.,[13] where they also concluded that TLC is not a reliable predictor of CD4 cell count in HIV-infected individuals.

CONCLUSION

From this study, it was found that there was statistical positive correlation between TLC and CD4 count, which is consistent with findings in other studies.[13]

That, based on the low specificity and positive predictive value as recorded in this study and some other studies, the use of TLC as a surrogate for CD4 count is unreliable.

That in areas where there are no alternative, or the alternatives (as suggested by Didier *et al*.)[15] are not affordable; it could be used with caution as expressed by Deresse and Eskindir,[12] bearing in mind its low PPV.

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