Full Length Research Paper

Total and CD4+ T- lymphocyte count correlation in newly diagnosed HIV patients in resource-limited setting

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Few clinical settings in resource-limited countries perform CD4+ T-lymphocyte counts required as a baseline test for antiretroviral therapy. We investigated CD4 count in newly diagnosed HIV-infected patients attending our treatment centre and evaluated suitability of total lymphocyte count (TLC) as a surrogate marker for CD4+T-lymphocyte count required as a yardstick for initiating antiretroviral therapy. Usefulness of TLC as a surrogate marker for CD4+T-lymphocyte counts <200, ≤350 and <500cells/μL for HIV-positive patients in our facility was evaluated by 180 pairs of TLC and CD4 counts from 180 newly diagnosed HIV-infected patients and results were compared by linear regression and Spearman's correlation analytical tools. Approximately 72.8% of our patients were diagnosed late as revealed by CD4 count ≤350cells/μL. An overall good correlation was noted between TLC and CD4+T-cell counts (r=0.65, slope=0.69), mean total lymphocyte count of 1.04 ± 0.81, 1.39 ± 1.06 and 1.57 ± 1.13 x 10^9/L correspond to CD4 lymphocyte counts of <200, ≤350 and < 500cells/μL respectively. When considering initiating HAART for HIV-infected Nigerian clients, TLC can be considered as an inexpensive and easily accessible surrogate marker for predicting CD4+T-lymphocyte at two clinically important CD4 thresholds of CD4 count of ≤350 cells/μL and <500cells/μL.

Key words: CD4, total lymphocyte count, highly active antiretroviral therapy, enzyme immunoassay, HIV, flowcytometry.

INTRODUCTION

The CD4+T lymphocyte count is the determination of the concentration of CD4+ T lymphocyte in the blood. The associated immune deficiency in human immunodeficiency virus (HIV) patients leading to infection

by opportunistic pathogen is ascribed to depletion of CD4 T-cells (Shearer, 1983). CD4 count can therefore be regarded as the accurate measurement of the robustness and functionality of the immune capability to protect the body against general infection. CD4+T lymphocyte cell depletion is one of the hallmarks of progression of HIV infection and a major indicator of the stage of the disease in HIV infected individuals (Hogg et al., 2001; Mellors et al., 1997; WHO, 2005). World Health Organization recommended that most treatment initiation decisions be guided by CD4 measurement and clinical staging (Balter, 2003; Hanson et al., 1995). Previous study has shown good correlation between CD4 count and development of various complications in HIV/AIDS (Stein et al., 1992). Patients with low CD4+ T lymphocyte cell count have been reported as long-time infected patients than those with higher CD4 count (Hammer, 1997). It is clear that late starters of highly active antiretroviral therapy with CD4 count <200 cells/µl have significantly poor response to therapy and a worse prognosis when compared with early starters with higher CD4+ T cell count (Cheisson et al., 2000; Ledergerber,1999; Kilaru et al., 2004). The relative ease of CD4 cell monitoring also led to its advocacy in treatment guidelines for determining when to start, stop or change antiretroviral therapy and for deciding when to initiate prophylaxis for opportunistic infections (OIS) (Arthur and Sahan, 2006).

Retroviral disease has become a matter of relative chronicity in patients that have access to antiretroviral therapy and have benefitted greatly in marked reductions in morbidity and mortality. General treatment guidelines for the treatment of HIV-infected patients in many countries have adopted three approaches for the initiation of antiretroviral therapy. Early intervention in asymptomatic patients involves the commencement of antiretroviral therapy once the CD4 count is less than < 500 cells/µl. A less intensive approach is to recommend antiretroviral therapy when the CD4 count falls to 350 cells/µl.

In other countries where patients have limited financial resources, treatment decisions are typically delayed until the CD4 count becomes less than 200 cells/µl (Tarwater et al., 2001; BHIVA, 2000; Mellors, 1997). Recent study by the French researchers at the 7th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention in Kuala Lumpur, Malaysia (Hocqueloux et al., 2013) showed that people with HIV who start treatment with CD4 counts above 500, after the first phase of primary infection is over, are much more likely to experience substantial reductions in the reservoir of HIV-infected cells in their bodies, making them strong candidates for future research studies that seek to control HIV without medication. The French group found that people with HIV who started treatment with a CD4 cell count above 500 were 56 times more likely to experience a normalization of immune function and a reduction in HIV DNA to low levels when compared to people who started treatment at lower CD4 counts. Although it is well established that people who start HIV treatment with a CD4 count above 500 stand a better chance of achieving a CD4 cell count in the normal range (defined as 900 to 1000 cells/mm^3 according to the study), studies of people treated in chronic infection have not found evidence of a substantial reduction in HIV DNA (the reservoir of HIV within cells) over time. This outcome gives strong support to earlier interventions and showed the likely future direction in HIV treatment modalities. It then becomes very vital at every health institution involved in HIV/AIDS testing and treatment to establish the usefulness of total lymphocyte count as an alternative and inexpensive immunological marker for CD4 count where flowcytometric technique is not available. This is imperative not only at CD4 count threshold of < 200 and ≤ 350 cells/µl only but also at < 500 and ≥ 500 cells/µl thresholds.

Total lymphocyte count (TLC) is a derived immunological marker calculated from white blood cell count and relative lymphocyte count. For instance, if a patient has a total white blood cell count of 6.0 × 10^9/L and relative lymphocyte count of 40% obtained from differential leukocyte count, total lymphocyte count of such patient would be 2.4 × 10^9/L. There are series of controversial research outcomes over the use of TLC as a surrogate marker for CD4 count estimation (Anastos, 2004; Beck et al., 1996; Gitura et al., 2007; Akinola, 2005; Deresse and Eskindir, 2008; Chigu et al., 2006; Nyawira, 2011). Decisions became difficult to take as our health care providers insist on scientific evidence on the use of total lymphocyte count as an inexpensive point-of-care alternative for absolute CD4 count. Moreover, there are limited data on the use of TLC as surrogate marker for absolute CD4+T-lymphocyte count at a CD4 threshold of < 500 cells/µl especially in a facility located in a rural and resource-limited setting in Nigeria.

Recently published PEPFAR II goals stated that an estimate of 100,000 children needed to be newly initiated on highly active antiretroviral therapy before 2013 (PEPFAR, 2011). In line with this, new sites have been established throughout Nigerian states including Ekiti.

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Five new sites are receiving services from our treatment centre across Ekiti state as a comprehensive HIV treatment centre owing to lack of CD4 cyflow counter. Most of those sites across the country do not have cyflow counter to perform routine CD4 count analysis, and flowcytometry remains the reference method for the performance of CD4 count (Mellors et al., 1997). Even in centers where CD4 equipment are available, treatment decisions are sometimes delayed owing to varying factors ranging from proximity of the patients to testing and treatment sites, equipment breakdown, non-availability of reagents on consistent basis to late access to service engineers.

These therefore necessitate the need for the evaluation and validation of the correlation of TLC with CD4 count and its usefulness as a surrogate marker for CD4 count estimation. This is the first time it will be determined in this health institution in Ekiti state, South-West Nigeria. To the best of our knowledge, no research study in Ekiti state has determined the correlation between CD4 and total lymphocyte count to include CD4 count threshold of < 500 cells/µl but past studies have concentrated on correlations between absolute CD4 count and TLC at < 200 and < 350 cells/µl thresholds.

This study was carried out to determine at what stage of the disease HIV infected persons presented to our treatment facility, and to have comparison with our previous findings here and in other centres. Moreover, the total lymphocyte count as at the time of presentation will also determined and correlated with the CD4 count at different CD4 thresholds including < 500 cells/µl to establish its diagnostic utility as a surrogate marker for CD4 count.

The goal is to facilitate earlier treatment in newly diagnosed HIV patients based on total lymphocyte count results obtained from HIV positive samples at attached centres rather than travelling, in some cases, over 50 km distance to have CD4 count result done and asking the physicians to wait for results from testing sites. Delay associated with such old arrangement has been responsible for delay in initiation of highly active antiretroviral therapy, loss of follow-up, non-compliance to antiretroviral therapy, poor prognosis, immunologic failure and increased morbidity and mortality among HIV-infected patients.

METHODS

Study location

This study was carried out at the Haematology department of the Federal Medical Centre (FMC); Ido Ekiti, Nigeria over a period of three years. FMC is located in Ido Ekiti, the principal town in Ido Osi Local Government Area of Ekiti State with an estimated population of 107,000. It is geographically located in the northern part of Ekiti State which covers an estimated total area of 6353 km², 2,453 square mile and an estimated population of 2,737,186, where the routes

from Kwara and Osun states converge. FMC, Ido Ekiti was upgraded in 2006 to serve as a centre for HIV/AIDS referral, diagnosis and treatment in Ekiti State and serving five contiguous states. The Centre has since that time been offering free diagnosis and antiretroviral therapy.

Study design

This study consisted of enumeration of total and CD4 T-helper lymphocyte counts of 180 HIV- infected subjects at baseline before initiation of highly active antiretroviral therapy (HAART). A model linear regression analysis was applied to the data and the sensitivity and specificity of the World Health Organization recommended TLC thresholds corresponding to CD4 count <200, ≤350 and <500 cells/µL were determined.

HIV counseling and testing

Early and accurate diagnosis of human immunodeficiency virus (HIV) in retroviral patients is sine quanon to successful management, good prognosis, effective follow-up and increased reduction of morbidity and mortality due to opportunistic infections among infected patients. All enrolled subjects were newly diagnosed for HIV at our HIV counseling and testing (HCT) site and PEPFAR-Supported laboratory at the Haematology Department according to the Centre for Disease Control and Prevention serial Algorithm II guidelines. We performed HIV testing using two rapid enzyme immunoassay (EIA) techniques. Whole blood samples obtained by capillary puncture or plasma samples separated from 4 millilitres of whole blood collected into K2EDTA spray-dried collection tubes were used for the procedures and the tests were performed according to CDC-UMD HIV rapid testing serial algorithm II guideline. We first used Determine kit, an immunochromatographic technique (Abbot Laboratories, Abbot Park, USA). Negative result by Determine ended testing but research patients were requested to repeat test 1 month to confirm negative results. Positive results observed were repeated with Unigold Kit (Trinity Biotech, Wicklow, Ireland).

Discordant results were first repeated by senior research counsellor and tester to ascertain true inconclusive results and finally tested with the tie-breaker kit-Stat-Pak (Chembio Medford, NY, USA), an immunoconcentration technique. Final test results were considered positive/negative on the basis of the tie-breaker result and corresponding similar result from one of previous test procedures (WHO, 2007). Similar results were obtained from Genscreen HIV 1&2 kits (Biorad, France). The rapid HIV screening technique was an enzyme immunoassay based on the detection of antibodies to HIV in the patients' sera or plasma. The Genscreen ULTRA HIV Ag-Ab is an enzyme immunoassay based on the principle of sandwich technique for the detection of HIV antigen and of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma. Diagnostic techniques/algorithm was quality controlled using oneworldAccuracy HIV samples with already known positive and negative HIV results. Patients with two concurrent positive HIV results and who were repeatedly reactive were enrolled in the study.

Blood sample requirements, CD4 count and complete blood count analyses

Patients already confirmed HIV positive had their blood samples taken (following informed consent as part of the ethical guideline)

Table 1. The Overall data showing the Mean age, CD4 and TLC, the correlation coefficient, slope and p-value of HIV infected patients

Age	CD4 count (Cells/µL)	Total lymphocyte count (x 10⁹/L)	r	Slope	p-value
37.89±10.47	250.81 ±226.59	1.79±1.43	0.65**	0.69**	0.01

Correlation is significant at p=0.004.

Table 2. Correlation of CD4 T-lymphocytes and total lymphocytes count of HIV-infected patients

CD4 Count Thresholds (Cells/µl)	CD4 Count (Cells/µL)	Total lymphocyte count (× 10⁹/L)	r	Slope	P -value
<200	82.96±57.03	1.04±0.81	0.30	0.46	<0.05
≤350	140.23±104.05	1.39±1.06	0.55**	0.64	<0.01
<500	188.22±144.80	1.57±1.13	0.58**	0.68	<0.05
≥500	687.86±224.34	3.29±2.27	0.58*	-0.013	>0.05

**Correlation is significant at p=0.004

for baseline CD4 count and total lymphocyte count estimation at initial visit as part of routine work up to access their baseline status and eligibility for antiretroviral therapy. CD4 cell count and complete blood count (CBC) were determined using a freshly collected blood sample drawn into two separate 4 ml spray-dried dipotassium ethylenediaminetetraacetic acid (K$_2$EDTA) anticoagulant bottles and processed within 4 hours of venepuncture. Samples for CD4 count were prepared and run on the Partec cyflow counter (Partec flow cytometer, GMBH, Germany) according to the manufacturer's instructions.

Principle of flow cytometry for cd4 count

The cyflow counter operation is based on the simultaneous measurement of multiple physical characteristics of CD4 T-cells in a single file as it flows through the cyflow counter. The counter separated the CD4+ T cell from the monocytes-CD4 bearing cells and noise using a gating system. We prepared the samples and analyzed CD4 count according to the manufacturer's instructions.

CBC analysis and TLC calculation.

The CBC samples were run directly on Sysmex KX-21N three-part differential haematology analyzer (Sysmex Corporation, Kobe, Japan). TLC was calculated from white blood cell count and relative lymphocyte count. Sysmex KX-21N is a three-part open tube differential haematology analyzer according to manufacturer's instructions.

Statistical analysis

Data were computed using SPSS statistical software (Statistical Package for Social Sciences Inc, Chicago IL), version 17. The summary of the continuous variables were presented as means and standard deviation using one sample t-test. Correlation data obtained with the surrogate marker (total lymphocyte count) were compared to the reference flow cytometric CD4 count and the correlation coefficient was calculated. The degree of correlation

between the reference CD4 count and total lymphocyte count was further established using bivariate Spearman's analytical tool. We also determined the proportion of results classified by the total lymphocyte counts at various CD4 T-cell count thresholds relevant for clinical management of HIV-infected patients (< 200, ≤350 and < 500 cells/µL). A p-value =0.05 was considered significant for all statistical comparison.

RESULTS

A total of 180 HIV research patients comprising 54 males and 126 females aged 20-80 years, mean age 37.89±10.47 years were involved in the study. 89 of the patients who are approximately 49.4% were asymptoma-tic HIV-infected patients. The overall mean baseline CD4 count and total lymphocyte count were 250.81±226.59 and 1.79±1.43 × 10⁹/L, respectively as shown in Table 1. Of the 180 research patients, 51.67, 72.78 and 87.22% had CD4 count values of <200, ≤350 and <500, respec-tively. The remaining 12.78% had CD4 count of ≥500 cells/µL. The overall correlation coefficient between CD4 and total lymphocyte count of HIV-infected subjects was modest (r=0.65**, p< 0.01). Table 2 showed the com-parison of correlation between CD4 and total lymphocyte counts based on CD4 T-lymphocyte count thresholds (<200, ≤350, <500 and >500 cells/µL) relevant to clinical management of HIV-infected Nigerians.

The correlation between total lymphocyte count and CD4 count was modest at a CD4 count threshold of ≤350 cells/µL (r=0.55**, p < 0.01) compared to a low correlation at a CD4 threshold of < 200cells/µL (0.30, p<0.05) and slightly higher correlation at a CD4 threshold of <500 and ≥500 cells/µL (r=0.58, p<0.01). The slope was further determined to establish the degree of association between total lymphocyte count and CD4

count thresholds. The slopes for CD4 count thresholds <200, ≤350, <500 cells/µL were 0.46, 0.64, 0.68, respectively at p<0.05. CD4 count threshold of >500 cells/µL showed negative association at p> 0.05, p=0.95 specifically. Total lymphocyte count of 1.04 ± 0.81, 1.39 ± 1.06 and 1.57 ± 1.13 × 10^9/L correspond to CD4 lymphocyte counts of <200, ≤350 and <500 cells/µL, respectively.

DISCUSSION

With the current trend in the clinical management of retroviral patients, the use of highly active antiretroviral therapy (HAART) has brought a panoramic change of positive impact in the treatment of HIV-infected patients. In fact, HIV patients live relatively normal lives as non-infected subjects when diagnosed early and aggressive interventions are adopted. However, in many resource-poor countries, most facilities do not have cyflow counter for the performance of CD4 count analysis required for the initiation of antiretroviral therapy. Amazingly, in Sub-Saharan Africa where nearly 24 million people are living with HIV, less than 8% have access to HAART partly due to lack of access to CD4 count test. (WHO, 2002). In this study, we have evaluated the relevance of TLC as a readily accessible and alternative immunological marker for CD4 count in determining the optimal time to initiate highly active antiretroviral therapy in HIV-infected Nigerians especially those presenting late to testing and treatment centres.

More than 50% (51.7%) of patients enrolled in this study had CD4 count <200cells/µL and 57 (61.3%) of these had CD4 count less than 100cells/µL at presentation. 72.8% had CD4 count ≤350cells/µL. The World Health Organization (Nyawira et al., 2011) recommended the following parameters for initiating antiretroviral therapy:

1. Initiate ART for all patients with CD4 Count <200 cells/µl regardless of clinical stage.
2. Consider ART for all patients with stage I and II disease and CD4 count ≤350 cells/µl. Treatment should be initiated before CD4 count drops below 200 cells/µl.
3. Initiate ART for patients with stage III disease and CD4 count ≤350 cells/µl.
4. Initiate ART for patients with stage IV disease regardless of CD4 count.

We inferred that there was late diagnosis of HIV in the patients attending our treatment centre which is similar to findings by Kilaru and Co-workers (1997) in Barbados and Guarner and his group (1996) in Mexico. It is interesting to note that this study showed 13.4% cut in the overall previous findings of 86.2% among enrolled patients having a relatively advanced disease as reflected by their CD4 + cell count of <350 cells/µL in 2009. Of relevant interest is also the fact that while 65.5% of the study population in 2009 had CD4+ T-lymphocyte cell count of <200 cells/µL, the present study showed that 61.3% had CD4+ cell count of < 200cells/µL, representing a concomitant cut of 4.2% (Ajayi et al., 2009). The observed difference between the present outcomes and Ajayi and his group's findings at this health care facility was due to improved clinical setting (multiple testing sites)approach, trained counselors and testers, more aggressive 'Know your HIV status' campaign and policy formulations on reduction of discrimination and stigmatization. The World Health Organization recommended that in treatment centres where there is no facility for performing absolute CD4 count test, clinicians need not wait until CD4 counters are available but total lymphocyte counts of <1200 and <1,500 µL corresponding to CD4 count of <200 and <350 cells/µL should be used as a surrogate marker. In this research study, we observed a modest overall correlation coefficient of r= 0.65 (p < 0.01) between paired CD4 and total lymphocyte counts of 180 HIV-infected Nigerians in Ekiti, South West, Nigeria. This value correlates well with that of Beck and his colleagues (1996) (r=0.64) obtained from a study involving 1535 asymptomatic HIV-infected patients in the United Kingdom. While it is slightly lower than that obtained from a South African study in a larger population (n=2774) of HIV-infected patients (r=0.70) (van Der Ryst et al., 1998) it is higher than that obtained from similar studies involving 100 and 32 HIV- infected Nigerians with correlation coefficients of r=0.51 and 0.25 respectively (Akanmu et al., 2001; Erhabor et al., 2006)). Our results and those of previous studies showed that the higher the population of asymptomatic patients involved in the study, and early antiretroviral therapy intervention decision adopted, the better the correlation.

Comparison of correlations between CD4 and total lymphocyte count at different CD4 count thresholds showed a correlation coefficient of r= 0.30, slope 0.46; 0.55,**slope 0.64 and 0.58,**slope 0.68 for CD4 count <200, ≤350 and <500 cells/µL respectively (p < 0.05).

Mean total lymphocyte counts of 1.04 ± 0.81, 1.39 ± 1.06 and 1.57 ± 1.13 × 10^9/L correspond to CD4 lymphocyte counts of <200, ≤350 and <500 cells/µL, respectively. TLC is more positively correlated with CD4 count ≤350 and ≤500 cells/µL than <200 threshold. We inferred that TLC does not accurately predict corresponding CD4 count values in clients being severely immuno-suppressed as at presentation to the HIV clinic.

There was a negative correlation between TLC and CD4 count ≥ 500 (r=0.68, slope= -0.013, p=0.95). The

cause of this negative slope was unclear but we assume that a lower population of patients (15%) with CD4 count of ≥ 500 cells involved in the study are responsible. If a study on evaluation of TLC as a suitable substitute for CD4 count at a CD4 count threshold of ≥500 cells/µL involves a higher population of CD4 count-TLC pair, a better picture may be obtained.

Conclusion

When considering initiating highly active antiretroviral therapy in HIV-infected patients, our findings of overall modest correlation of r=0.65 and slope=0.69 (p=0.004) between total and CD4+ T-lymphocyte count has shown that total lymphocyte count can be used as an alternative inexpensive and readily available surrogate marker at two clinically significant CD4 thresholds of ≤ 350 and < 500 cells/µL. It must be used in conjunction with the clinical status and WHO clinical staging system in determining the prognosis and the optimal time to initiate HAART in resource-poor settings without CD4 count facility. The findings of a cut of 13.4% in the overall findings of our patients presenting with relatively advanced disease as at diagnosis (CD4 count <350cells/µL) in 2009 and concomitant cut of 4.2% (CD4 < 200 cells/µL) showed a working system. A more aggressive campaign and public enlightenment on, 'Know your HIV status' in Ekiti communities and neighbouring states where patients come to our treatment facility to access care, evidently, will further lead to dramatic reduction in late diagnosis of HIV, hence, improve TLC diagnostic utility as a surrogate marker for CD4+ cell count.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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