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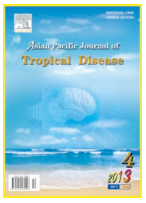

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Absolute lymphocyte count as a surrogate marker for CD4+ cell count in monitoring of antiretroviral therapy, Northwest Ethiopia: retrospective evaluation

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PEER REVIEW

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Comments

This is a well-designed study addressing an important issue regarding options to support treatment of HIV patients in resource-limited settings. The results are also interesting to support treatment and provoke researchers to think of prospective studies that can avoid the limitation of retrospective studies.
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ABSTRACT

Objective: To determine the use of total lymphocyte count as a surrogate marker for CD4+ cell count among HIV infected patients at the University of Gondar Hospital.
Methods: A retrospective cross sectional study was conducted at the University of Gondar Hospital antiretroviral therapy laboratory from December 2011 to May 2012. Data on CD4+ cell count, total lymphocyte count, sex, and age were collected from 2964 HIV infected patients and analyzed using SPSS version 16 computer software.
Results: Total lymphocyte count was significantly correlated with CD4+ cell count ($P<0.001$; $r^2=0.434$). The sensitivity, specificity, positive predictive value, negative predictive value of total lymphocyte count<1 200 cells/mm^3 to predict CD4+ cell count <200 cells/mm^3 was 57.8%, 86.4%%, 34.1%, 86.39%, respectively. A total lymphocyte count<1 000cells/mm^3 was found to have suboptimal sensitivity (69.0%), and specificity (85.0%) for predicting a CD4+ cell count <200 cells/mm^3.
Conclusions: Total lymphocyte count and CD4+ cell count was positively correlated. Hence, lymphocyte count less than or equal to 1 000/mm^3 can be used as a cutoff value in place where there is no CD4+ cell counting machine.

KEYWORDS
Absolute lymphocyte count, CD4+ cell count, HIV patients

1. Introduction

Sub-Saharan Africa remains the region most heavily affected by Human Immunodeficiency Virus (HIV). An estimated 22.4 million people were living with HIV/AIDS at the end of 2009; with an annual estimate of 1.9 million persons newly infected and about 1.4 million died. The sub-Saharan reagin was accounting for 67% of all people living with HIV/AIDS and 68% of all AIDS deaths in 2009 worldwide[1]. According to an established single point estimate based on "AIDS in Ethiopia-sixth report", there were 977 394 people living with the virus and those 258 264 require antiretroviral therapy (ART)[2].

The determination of CD4+ cell count has become a standard measure of immunodeficiency in adults infected with HIV in resource rich areas where the burden of

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the pandemic is low[3]. Cognizant of this problem, the current guidelines from World Health Organization (WHO) acknowledge that total lymphocyte count (TLC) may be used to make treatment decision in resource poor settings when CD4+ cell count is not available and patients are mildly symptomatic[4,5]. Total lymphocyte count of <1 200 cell/mm^3 confined with symptoms is recommended as a surrogate for CD4+ cell count of 200 cell/mm^3[5]. Moreover, a number of previous studies indicated that TLC may be useful as a surrogate marker of immune status in certain settings[6]. However, the use of TLC count as a surrogate marker to evaluate CD+ cell count remains unpractised. Nevertheless, most studies concluded that a decline in TLC was strongly correlated with a decline in CD4+ cell count, though there were some discrepancies[7]. On the other hand, there is a recent report warned that TLC<1 200 cells/mm^3 was not optimal for identifying patients requiring HAART since it showed low sensitivity and specificity to predict CD4+ cell counts below 200 cells/mm^3[7,8].

The disagreements documented in various studies commence to undertake the current study. The aim of this study was therefore to ascertain the relationships between CD4+ cell count and TLC and determine if TLC could be used as a surrogate marker for CD4+ cell counts in the initiation and monitoring of ART in resource-limited localities, particularly in Ethiopia.

2. Materials and methods

2.1. Study design and setting

This retrospective study was carried out at the University of Gondar Hospital ART treatment and follow up center. The ART center provides services for more than 1 000 patients per month. Routine laboratory services in the center includes hematological and immunological tests. A total of 2 964 HIV positive patients attended the ART centre between October 2011 to January 2012 and included in the study.

2.2. Enumeration of total lymphocyte and CD4+ T cells

The total lymphocyte count and CD4+ T cell were enumerated as part of the routine test to follow the status of HIV positive patients on HAART. In brief, the blood samples were taken from the patients and the white blood cell count plus differential WBC count were determined using automated haematology analyzer (CELL-DYN 1800, Abbott Laboratories Diagnostics Division, USA). The CD4 T lymphocytes count was enumerated using the Becton Dickinson FACS count system (Becton, Dickinson). The Becton Dickinson FACS Count system used flow Cytometry for the quantification of the CD4+ T Lymphocytes. The socio-demographic characteristics, Absolute lymphocyte count and CD4+ T cell count results were taken from the ART record book.

2.3. Data analysis

Data were entered and analyzed using SPSS version 16 statistical software. The results were interpreted with mean ±standard error of mean (SEM). The frequent distributions of the variables were worked out and the association between variable was explored. Correlations were evaluated using the Pearson's correlation test. Sensitivity, specificity, positive and negative predictive values of various cut-off points of the TLC to predict CD4+ T cell count >200 cells/mm^3 and <200 cells/mm^3 were calculated. For all statistical comparisons, the level of significance was set at P<0.05.

2.4. Ethical consideration

Approval for this study was granted by the Ethics Committee of the school of Biomedical and Laboratory Sciences of the University of Gondar and an official letter was submitted to the University of Gondar Hospital. The purposes and the importance of the study were explained and permission was obtained from the Director of the hospital.

3. Results

3.1. Socio-demography of study subjects

A total of 2 964 HIV infected patients were included in this study. Of these HIV patients, 1 078 (36.4%) were males and 1 886 (63.6%) were females. Mean age of the participants were 30.63 year and 68% of patients had the age under 30.63±10.52. The majority, 1 545 (52.1%), were in the age range 30 to 50. Socio-demographic characteristics of the study participants are shown in Table 1.

Table 1
Socio-demographic profile of HIV infected patients seeking diagnosis and treatment at the University of Gondar Hospital between October 2011 to January 2012.

Character		Frequency	Percentage
Sex	Male	1078	36.4
	Female	1886	63.6
Age range	<18	283	9.6
	18-29	1011	34.1
	30-50	1545	52.1
	>50	125	4.2

Table 2
Comparison of Total lymphocyte count and CD4+ T cell count among HIV infected patients attained for diagnosis and treatment at the University of Gondar Hospital between October 2011 to January 2012.

Characteristics		Male Number (%)	Female Number (%)	Total Number (%)	*Chi*-square	*P*-value
Lymphocyte (cells/mm^3)	< 1200	126(11.7)	178(9.4)	304(10.25)	1.256	0.075
	>1200	952(88.3)	1708(90.6)	2660(89.74)		
CD4 count (cells/mm^3)	< 200	253(23.5)	289(15.3)	542(18.30)	12.354	0.0045
	>200	825(76.5)	1597(84.7)	2422(81.70)		

3.2. Total lymphocyte and CD4+ T cell count

The mean absolute lymphocyte count of the patients was 2.16±1.21×10^3/μL with a range of 0.02 to 4.42×10^3/μL, and the mean CD4+ cell count was 407±264 cells/μL with a range of 40 to 3235 cells/μL. Male patients had mean absolute lymphocyte count and CD4+ cell count of 2.05.00±1.92×10^3/μL and 394±294 cells/μL, respectively; whereas, the female patients had a mean absolute lymphocyte count of 2.25±1.86×10^3/L and CD4+ cell count of 418±272 cells/μL. There was no statistically significant difference in the absolute lymphocyte counts of the male versus female patients (P>0.05). However, females had comparatively higher mean CD4+ cell counts than their male counterparts and the difference was statistically significant (P<0.05). Five hundred forty two (18.3%) of the patients had CD4+ cell count less than 200 cells/mm^3, while the majority 2422 (81.7%) had CD4+ cell count greater than 200 cells/mm^3. Similarly, 304 (10.25%) of the patients had TLC<1200 cells/mm^3, while the majority 2660 (89.74%) had TLC greater than 1200 cells/mm^3 (Table 2).

3.3. Correlation between TLC and CD4 count

The overall correlation between absolute CD4+ T cell counts and the TLC among HIV positive population was significant (P<0.001, r^2=0.434). Correlation between CD4+ T cell counts and TLC was then explored among different age groups, and stratified by sex of the study subjects. Accordingly, correlation of CD4+ T cell count with the TLC among patients <18 years of age was relatively strong (r^2=0.59) (P<0.001). On the other hand, for patients between 18-29, 30-50 and >50 years of age, the correlation of CD4+ T cell count with the TLC was 0.44, 0.41, 0.30 with P<0.001. The trend shows that the strongest correlation was seen among patients within the youngest age group (<18 years). Correlation between CD4+ T cell count and TLC among female patients was 0.53 (P<0.001), which was stronger than their male counterpart 0.40 (P<0.001). However, the overall correlation between CD4+ T cell count and that of differential lymphocyte percentage was weak (P<0.001, r^2=0.29).

3.4. Sensitivity and specificity at different cut off TLC count

The sensitivity, specificity, PPV and NPV of various TLC ranges for CD4+ cell count <200 cells/mm^3 are listed in Table 3. The maximum sensitivity was found at the lowest TLC range (<1000 cell/mm^3) and, maximum positive predictive value was found at the highest TLC range (<1600 cells/ mm^3). TLC less than 1000 cells/mm^3 would identify the patients who had CD4+ cell count<200 cells/mm^3 with sensitivity 69.0%, specificity 85%, and positive predictive value 23.0% and negative predictive value of 97.6%.

Table 3
The sensitivity, specificity, positive predictive value, and negative predictive value of TLCs for CD4+ T cell counts less than 200 cell/μL among HIV patients seeking diagnosis and treatment at the University of Gondar Hospital between October 2011 to January 2012.

TLC (cells/μL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<1000	69.0	85.0	23.0	97.6
<1200	57.8	86.7	34.1	94.4
<1400	47.0	88.2	47.4	88.1
<1600	39.5	90.3	62.1	78.7
<1800	34.3	88.2	47.4	88.1

4. Discussion

The CD4+ lymphocytes are the principle target cells for HIV virus infection. The number of CD4+ T cell count remains a useful marker of disease progression and widely used as indicators for starting ART, monitoring of treatment or primary prophylaxis for opportunistic infections. However, accurate determination of CD4+ cell count needs to be established by the use of flow Cytometry, an expensive technique that is not available in the majority of general hospital laboratories particularly in developing and resource poor countries[9,10]. Several studies in rural and poor resource settings and WHO guidelines recommend the use of TLC to predict the CD4+ cell count in HIV/ AIDS patients[2,9,10].

In our study, statistically significant positive correlation was observed between TLC and CD4+ cell count of HIV patients (P<0.001, r^2=0.434). This finding is also supported by other reports from different African countries. For example, correlation between CD4+ cell counts with TLC was reported

significant in Kenya, Uganda and in India[11,12,13]. Although the association between CD4+ cell counts with TLC was significant in the current study, the correlation coefficients were relatively lower than previous reports. In the current study, CD4+ cell count and TLC correlation was statistically significant both among female and male patients. However, correlation was relatively higher in females (P<0.001, r^2=0.53) than male patients (P<0.001, r^2=0.40). The correlation of CD4+ T cell count and TLC under the age of 18 years was (P<0.001, r^2=0.591) but it was less in the age greater than 50 years (P<0.001, r=0.30)). This finding indicated that correlation of CD4+ T cell count and TLC was affected by age and sex of the participants.

Current WHO guidelines recommend the use of TLC<1 200 cells/mm^3 as a surrogate marker for CD4+ cell count <200 cells/mm^3 in HIV infected patients. In line with that, we assessed the sensitivity and specificity of the use of TLC below 1 200 cells/mm^3 to predict CD4+ T cell count <200 cells/mm^3, and found the sensitivity to be 57.8%, specificity 86.4%, PPV 34.1% and NPV 94.4%. Similar study in Hawassa, southern parts of Ethiopia, reported that TLC<1 200 cells/mm^3 had sensitivity of 41% specificity of 83.5%, PPV of 87.9% and NPV of 32.5% for predicting CD4 count of <200 cells/mm^3[14]. Another study conducted in Kenya found a sensitivity, specificity, and NPV of TLC<1 200 cells/mm^3 to predict CD4 <200 cells/mm^3 as 37%, 99%, and 56% respectively[13]. Again, another study conducted in Khon kaen Hospital found a sensitivity, specificity, PPV, NPV with a value of 40%, 98.36%,97.87%,46.51% respectively[15]. From these reports we can deduce that the correlation of CD4+ cell count with TLC of specific locality need to be standardized to utilize TLC as a biomarker for the initiation of anti-retroviral treatment. Moreover, previous reports and the result of the current study found low sensitivity and the test with low sensitivity might result in missing patients that had CD4+ cell cont <200 cells/mm^3 which will be difficult to initiate anti-retroviral treatment.

Our finding also points that the use of the WHO cutoff value for TLC count of 1 200 cells/mm^3 had less sensitivity(57.8%) to predict a CD4+ cell count <200 cells/mm^3. To improve the sensitivity, one must increase the cutoff value for the TLC count by the expense of specificity. The cutoff level should be a balance between sensitivity and specificity in the relationship between TLC and CD4+ cell count. Accordingly, we report here that a TLC<1 000 cells/mm^3 for the prediction of CD4+ cell count <200 cells/mm^3 was a maximum value of sensitivity 69%, specificity of 85%, PPV of 23%, NPV of 97.6%. Other study conducted in Hawassa showed that TLC <1 780cells/mm^3 was optimal to indicate CD4+ cell count <200 cells/mm^3[14]. Different from this, in Uganda a TLC of 1250 cells/mm^3 was reported optimal to detect CD4+ cell <200 cells/mm^3[12]. We suggest here that the difference in sensitivity and specificity was varied from locality to locality or from place to place as a result of difference in physiology and environment such as temperature, nutrition, ethnicity and other factors that may affects CD4+ cell and TLC count.

In conclusion, there was a positive correlation between total lymphocyte count and CD4+ T cell count. Total lymphocyte count of 1000 cells/mm^3 had suboptimal sensitivity and specificity to predict CD4+ cell count<200 cells/mm^3. The sensitivity and specificity of TLC<1 000 cells/mm^3 to predict CD4+ cell count <200 cells/mm^3 was 69.0% and 85.0% respectively. However, the WHO cut off value (1 200 cell/mm^3) has low sensitivity to predict CD4+ cell count <200 cells/mm^3. We recommended here that, there needs to standardize the WHO total lymphocyte cutoff value (1 200 cell/mm^3) depending on the CD4+ cell count and TLC of specific community for i nitiation of HAART. Hence, lymphocyte count less than or equal to 1 000/mm^3 can be used as a cut off value in place where there is no CD4+ cell counting facilities.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

The continuing rise in the population of people living with HIV reflects the combined effects of continued high rates of new HIV infections and the beneficial impact of antiretroviral therapy. CD4 testing is the recognized gold standard used to stage HIV/AIDS, guide treatment decisions for HIV-infected persons and evaluate effectiveness of therapy. However, in resource poor settings testing HIV patients for CD4 counts seems expensive. WHO acknowledge the use of TLC to make treatment decision in resource poor settings when CD4+ cell count is not

available. Evaluating the WHO total lymphocyte cut off value for treatment of HIV patients is recommended by different reports, as there are discrepancies.

Research frontiers

This study utilized large number of routinely collected date and draw clinically important findings about the correlation of total lymphocyte count with CD4 counts of patients with HIV/AIDS. This research is very important as it indicates the possible option in the treatment of HIV patients in an area where there is shortage of CD4 counting facilities.

Related reports

Authors reported that, statistically significant correlation was observed between TLC and CD4+ cell count of HIV patients, which is supported, by other reports from different African countries. For example, correlation between CD4+ cell counts with TLC was reported significant in Kenya and Uganda.

Innovations & breakthroughs

Conducting clinically relevant research in developing countries is very expensive. Extracting clinically important data for monitoring of treatment in HIV/AIDS patients from hospital based records is relatively less expensive. This study utilizes such an option to generate important information.

Applications

Clinical research of such a kind is very important in guiding treatment, follow up and management of patients with HIV/AIDS. Thus, the findings of this study are highly applicable in the health institutions of resource-limited countries by replacing the very expensive CD4 counting techniques.

Peer review

This is a well-designed study addressing an important issue regarding options to support treatment of HIV patients in resource-limited settings. The results are also interesting to support treatment and provoke researchers to think of prospective studies that can avoid the limitation of retrospective studies.

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