Original Article

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Absolute Lymphocyte Count: A Probable Substitute Marker for CD4 Count in HIV-Infected Patients in Economically Restrained Countries

Ruchee Khanna[1], Vaishnavi Pahwa[2], Shashidhar V.[3], Vinay Khanna[4]

Abstract

Objective: CD4 count is the mainstay criteria for initiation of HAART (Highly Active Anti-retroviral Therapy) and assessment of disease progression in HIV patients. CD4 percentage adds additional prognostic information. Our study was aimed to find out if Absolute Lymphocyte Count (ALC) could serve as a substitute marker for CD4 count and percentage. *Methods:* A total of 455 EDTA blood samples from HIV-infected patients were analyzed for their ALC, CD4 counts and CD4 percentages, over a period of 6 months, from January 2015 to June 2015 in Kasturba Hospital, Manipal. Correlation analysis of ALC with CD4 count and percentage, and receiver operating characteristic (ROC) analysis at CD4 ≤ 200/μL andCD4 ≤ 350/μL were conducted as proposed by WHO guidelines. *Results:* The male to female ratio was 2:1 and age ranged from 11 to 78 years. The median ALC was 1600/μL, median CD4 count was 258.58/μL and the median CD4 percentage was 16.4%. A strong positive correlation (Pearson coefficient, r= 0.741) was obtained between CD4 count and ALC. However, a weak positive correlation (r= 0.276) was seen between ALC and CD4 percentage. Areas under the ROC curve for ALC with CD4 count ≤ 200/μL andCD4 count ≤ 350/μL were 0.901 and 0.911, respectively, both of which showed an excellent correlation. But area under the ROC curve for ALC and CD4 ≤ 20% was 0.659, which is poor in accuracy. Also, from the ROC analysis, the ALC cut offs at CD4 count ≤ 200/μL (Sensitivity-83.87%, specificity-81.41%) and CD4 count ≤ 350/μL (Sensitivity-80.22%, specificity-86.44%) were ≤ 1450/μL and ≤ 1650/μL respectively. *Conclusion:* ALC has a strong correlation with CD4 count and the ALC cut offs corresponding to CD4 counts ≤ 200/μL and CD4 count ≤ 350/μL were ≤ 1450/μL and ≤ 1650/μL, respectively. But ALC did not have a good correlation with CD4 percentage. Hence, ALC is a credible alternate marker for CD4 count, but not for CD4 percentage.

Keywords: Absolute Lymphocyte Count; CD4 Count.

Introduction

CD4 cell count is one of the most important clinical parameters used to determine the timing of initiation of HAART (Highly active anti-retroviral therapy), measuring the efficacy of anti-retroviral therapy and prophylaxis against opportunistic infections. The latest WHO (World Health Organization) guidelines state that as a priority, HAART should be initiated in adults infected with HIV with CD4 count ≤ 350/ μL [1]. CD4 percentage is occasionally useful while evaluating significant reductions in an individual's CD4 count, which may be associated with transient lymphopenia due to inter-current infection or pregnancy [2]. Prophylaxis of opportunistic infections is also dependent on CD4 percentage, when it falls below 20% [3]. But these clinical estimations are not available to all and not affordable by all in financially constrained regions like Asia and Africa which also happen to inhabit about two-thirds of the HIV- positive population [4]. In lieu of this problem, the World Health Organization (WHO) in 2002 suggested that ALC can be used as a surrogate marker for CD4 count in settings where the latter is not available. WHO recommended using a TLC of 1200 cells/μLas a surrogate marker

Authors Affiliation: [1]Associate Professor, Department of Pathology [3,4]Associate Professor, Department of Microbiology, [2]Final Year MBBS Student, Kasturba Medical College & Kasturba Hospital, Manipal Academy of Higher Education (MAHE), Manipal, Karnataka 576104, India.

Corresponding Author: Vaishnavi Pahwa, Final Year MBBS Student, Kasturba Medical College, Manipal, MAHE Karnataka 576104, India.

E-mail: vaishnavi.pahwa2014@gmail.com

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for a CD4 count of 200 cells/ μLfor initiation of HAART [5]. Although the present guidelines do not mention the ALC cut off corresponding to CD4 count ≤ 350/ μL, but the debate regarding the use of ALC as a proxy marker for CD4 count in economically restrained settings hasn't died down yet. Many studies have been published on this subject all over the world, but with contradicting results [6-15].

Our study was aimed to determine the correlation of ALC with CD4 count and CD4 percentage respectively, and if any correlation existed, then to study the corresponding cut off values of ALC with maximum correlation at CD4 count ≤ 200/ μL, CD4 count ≤350/μL and CD4 percentage ≤20%, respectively.

Materials and Methods

This cross-sectional study was conducted in the Clinical Laboratory of Kasturba Hospital, a tertiary care hospital in Coastal Karnataka. All the HIV positive patients reporting to our hospital from 1st January 2015 to 30th June 2015 (6 months) were included in our study. Patients from the paediatric age group (<10 years of age) were excluded. Demographic details like age and gender of the patients were noted down. Finally a total of 455 blood samples were analyzed. A single sample of EDTA blood was collected for evaluating absolute lymphocyte count (ALC), CD4 count, CD4 percentage and CD45 count. ALC was calculated using a fully automated Coulter LH 780 Haematology Analyzer, and CD4 count, CD4 percentage and CD45 count were calculated using BD FACSCanto II Flow Cytometer.

Correlation of ALC with CD4 count and CD4 percentage was evaluated using Pearson correlation coefficient. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of ALC for predicting a CD4 count <200/ mm^3, CD4 count <350/mm^3 and CD4 percentage ≤20% respectively. Scatter plots depicting the relationship of ALC with CD4 count and CD4 percentage respectively, were also plotted. Statistical analysis was performed using SPSS software (version 21.0, SPSS, Chicago, USA).

Results

A total of 455 subjects were included in our study, of which 304 were males and 151 were females. The age of the subjects ranged from 11 to 78 years, mean age being 44 years. The median CD4 count was 258.58/μL, and it ranged from 2.72/μL to 2373/μL. The median CD4 percentage was 16.4% and the median CD45 count was 1580.68/ μL. Also the median ALC was 1600/μL, and it ranged from 100/μL to 6200/μL.

The mean ratio between CD45 and ALC was 1.03 with a standard deviation of 0.15. Hence, there was minimal error in lymphocyte count estimation while measuring ALC.

The Pearson correlation coefficient was calculated to determine any linear correlation of ALC with CD4 count and CD4 percentage, and it was found to be 0.741 and 0.276 respectively. Hence, a strong linear correlation was found between ALC and CD4 count, and a weak correlation was found between ALC and CD4 percentage. A scatter plot between CD4 count and ALC is shown in Figure 1, and that between ALC and CD4 percentage in Figure 2.

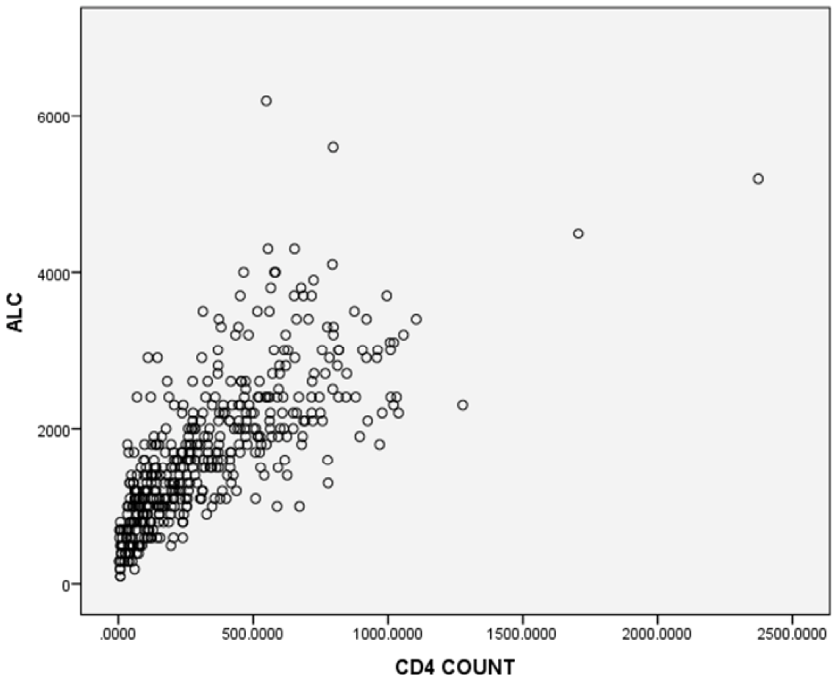

Fig. 1: Scatter diagram between CD4 count and ALC

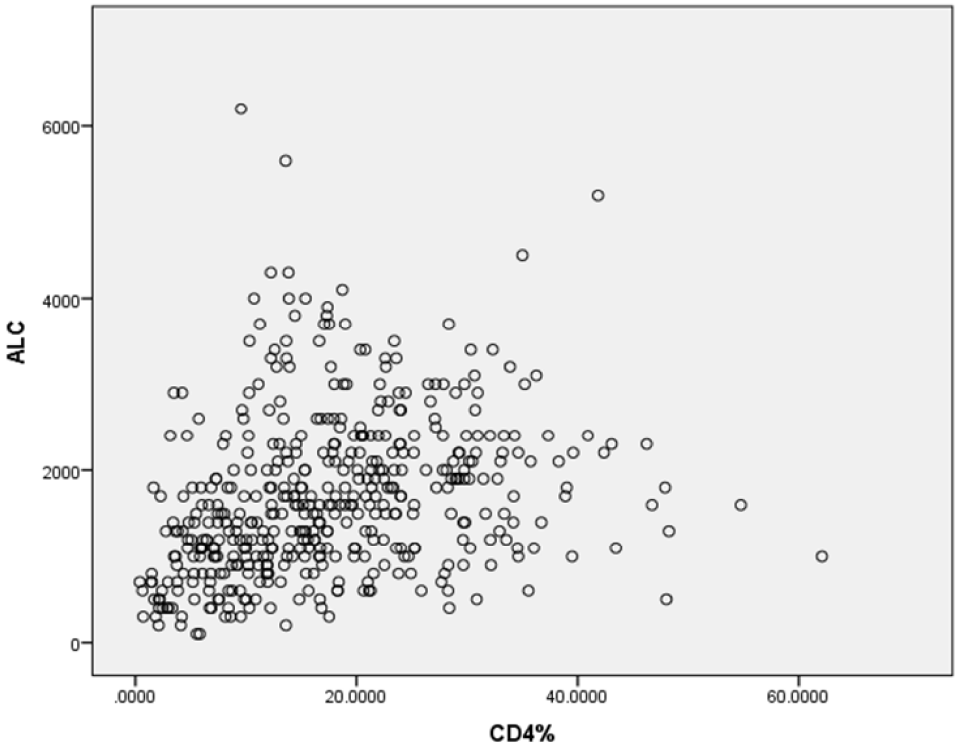

Fig. 2: Scatter diagram between CD4 percentage and ALC

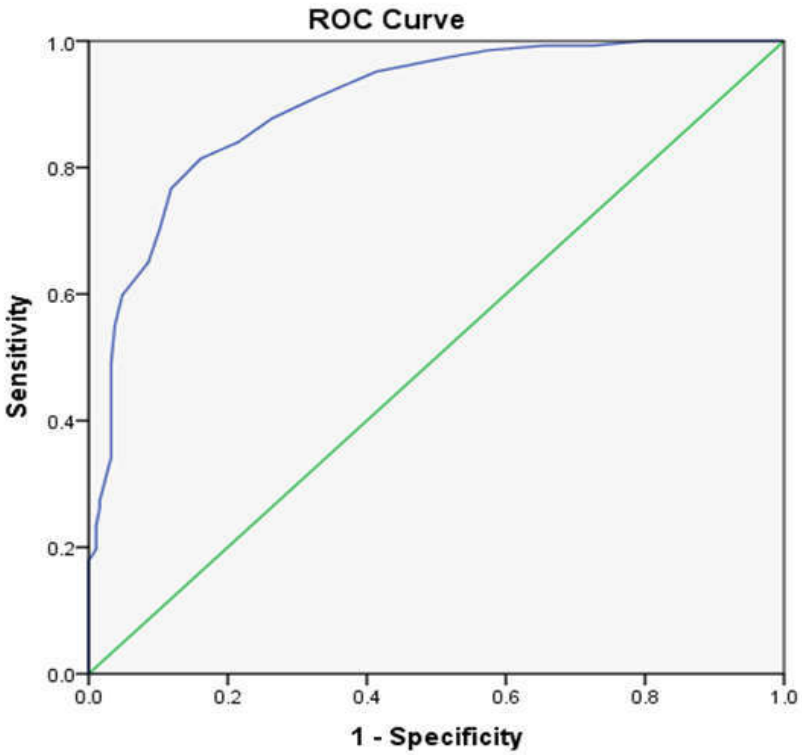

Fig. 3: ROC curve between ALC and CD4 countd"200/µL

The ROC curve was drawn between ALC and CD4 count ≤ 200/µL (Figure 3), and the area under the curve was calculated to be 0.901 (95% CI, 0.872-0.929). Therefore, ALC can be considered as an excellent predictor of the outcome at CD4 count ≤ 200/µL. Also, the optimum cut off value for ALC corresponding with CD4 count ≤ 200/µL would be 1450/µL (Table 1).

Table 1: Ability of ALC to predict CD4 count ≤ 200/μL

Statistic	Value	95% CI
Sensitivity	83.87%	0.777-0.888
Specificity	81.41%	0.762-0.858
Positive Predictive Value	75.73%	0.692-0.814
Negative Predictive Value	87.95%	0.832-0.917

Table 2: Ability of ALC to predict CD4 count ≤ 350/μL

Statistic	Value	95% CI
Sensitivity	80.22%	0.750-0.847
Specificity	86.44%	0.805-0.911
Positive Predictive Value	90.28%	0.858-0.936
Negative Predictive Value	73.56%	0.6701-0.794

The ROC curve was also drawn between ALC and CD4 count ≤ 350/μL (Figure 4), and the area under the curve was calculated to be 0.911 (95% CI, 0.885-0.937). Therefore, ALC is an excellent predictor of the outcome at CD4 count ≤ 350/μLalso. Moreover, the optimum cut off value for ALC corresponding with CD4 count ≤ 350/μL is 1650/μL (Table 2).

The ROC curve was drawn between ALC and CD4 percentage ≤ 20% (shown in Figure 5), and the area under the curve was calculated to be 0.659 (95% CI, 0.608-0.710). Hence, ALC is a poor predictor of the outcome at CD4 percentage ≤ 20%. Also, the optimum cut off value for ALC corresponding with CD4 percentage ≤ 20% would be 1650/μL (see Table 3).

Table 3: Ability of ALC to predict CD4 percentage ≤ 20%

Statistic	Value	95% CI
Sensitivity	64.3%	0.608-0.710
Specificity	65.2%	0.608-0.710
Positive Predictive Value	51.9%	0.449-0.590
Negative Predictive Value	75.7%	0.698-0.810

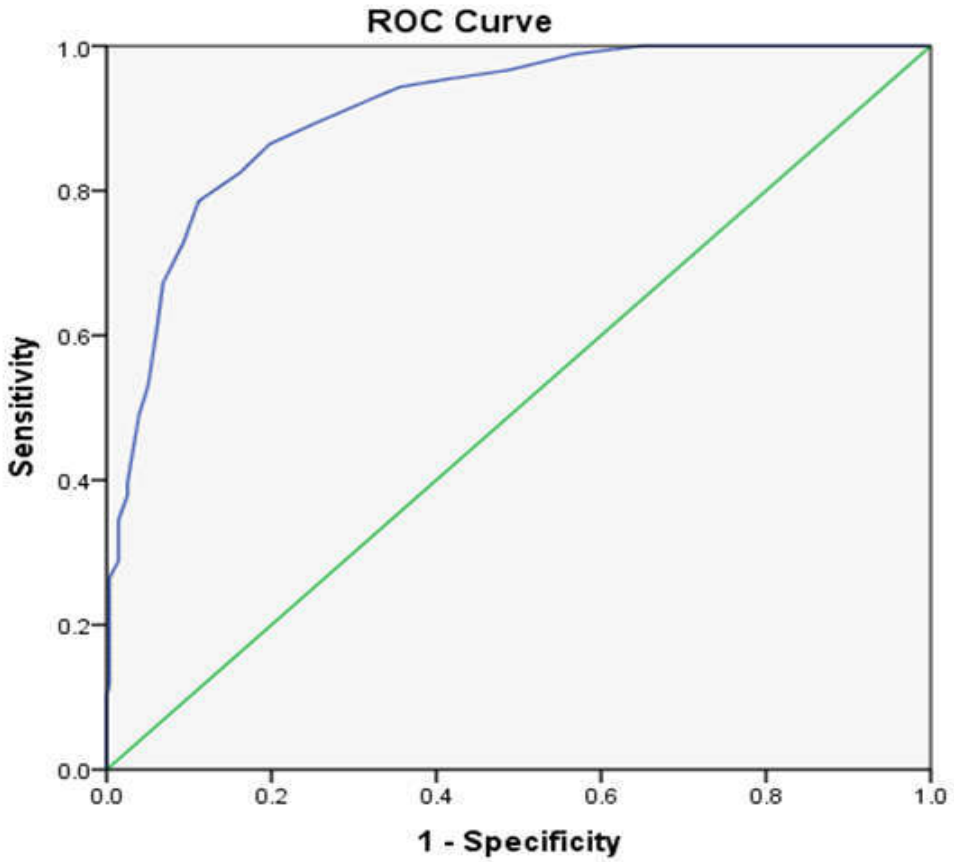

Fig. 4: ROC curve between ALC and CD4 count ≤ 350/μL

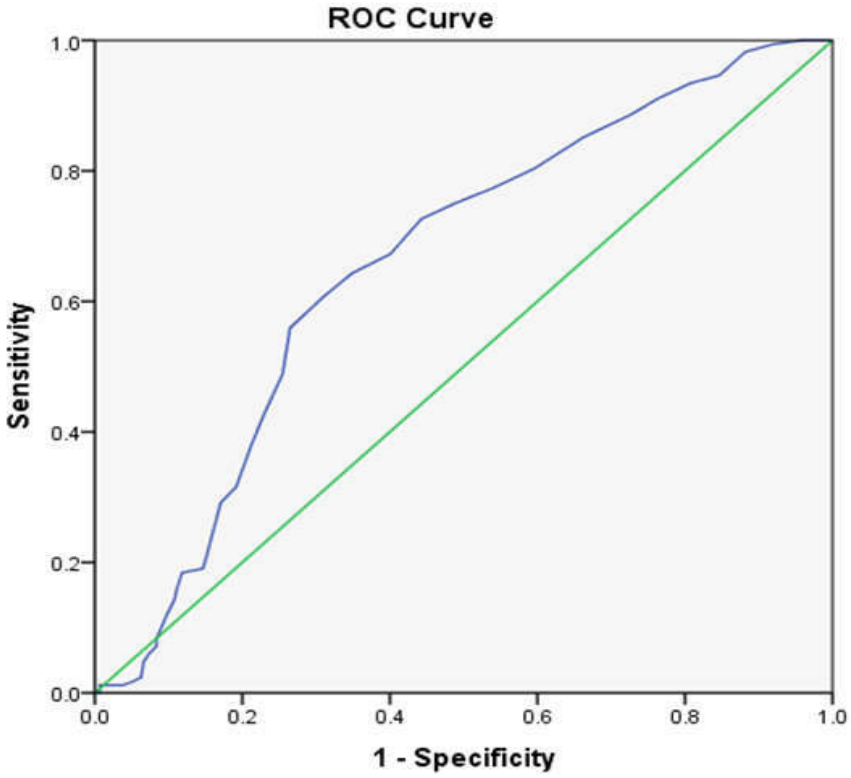

Fig. 5: ROC curve between ALC and CD4 percentd"20%

There were 54.7% of patients with an ALC ≤ 1450/μL and 45.3% patients with an ALC > 1450/μL. There were 59.1% patients with CD4 count ≤ 200/μL and 40.1% patients with CD4 count > 200/μL. Table 4 depicts the distribution of ALC with respect to CD4 count at CD4 count ≤ 200/μL.

There were 45.7% of patients with an ALC ≤ 1650/μL and 54.3% patients with an ALC>1650/μL. There were 38.9% patients with CD4 count ≤ 350/μL and 61.1% patients with CD4 count>350/μL. Table 5 depicts the distribution of ALC with respect to CD4 count at CD4 count ≤ 350/μL.

There were 45.7% of patients with an ALC ≤1650/μL and 54.3% patients with an ALC>1650/μL. There were 36.9% patients with CD4 percent ≤ 20% and 63.1% patients with CD4 percent>20%.

Table 4: Distribution of ALC with respect to CD4 count at CD4 count ≤ 200/μL

	CD4 count≤200/μL (n=269)	**CD4 count>200/μL(n=186)**
ALC≤1450/μL (n=249)	88.0% (219)	12.0%(30)
ALC>1450/μL (n=206)	24.3%(50)	75.7%(156)

Table 5: Distribution of ALC with respect to CD4 count at CD4 count ≤ 350/μL

	CD4 count≤350/μL (n=177)	**CD4 count>350/μL(n=278)**
ALC≤1650/μL (n=208)	73.6% (153)	26.4% (55)
ALC>1650/μL (n=247)	9.7% (24)	90.3% (223)

Table 6: Distribution of ALC with respect to CD4 percentage at CD4 percent ≤ 20%

	CD4%≤20% (n=168)	**CD4%>20%(n=287)**
ALC≤1650/μL (n=208)	51.9%(108)	48.1% (100)
ALC>1650/μL (n=247)	24.3% (60)	75.7% (187)

Discussion

CD4 count and percentage are cardinal aids in the management of an AIDS patient. But, these are expensive investigations, which are not readily available or affordable in developing and under-developed countries, where majority of HIV-positive patients reside. Hence, it becomes necessary to find a substitute marker which is inexpensive, easily retrievable and clinically plausible, but at the same time provides accuracy similar to the gold-standard, in order to provide quality healthcare to the patients. To overcome this problem, WHO has recommended that irrespective of the CD4 cell count, ART can be started on patients who have WHO stage III or IV disease and on patients who have WHO stage II disease with an ALC of ≤ 1200 /μL (which can substitute CD4 cell count of ≤ 200/μL), especially in resource constrained areas [5].

In our study, we found a strong correlation between ALC and CD4 count (Pearson correlation coefficient=0.741), but a weak correlation between ALC and CD4 percentage (r=0.276). Several authors have found varying results in this respect. A good correlation between ALC and CD4 count has been observed by workers from India like Kumaraswamy et al. [6] (r=0.744), Jain et al.[7](r=0.77) and Gogia et al.[8] (r=0.714), and also by workers from other parts of the world like Beck et al. in England[9] (r=0.76), Ryste et al. in South Africa[10] (r=0.70), Chen et al. in China[11] (r=0.60) and Badri & Wood in South Africa[12] (r=0.61). In contrast, some authors did not find a very strong correlation between CD4 count and ALC, like Akinola et al.[13] (r=0.43), Sagar et al.[14] (r=0.38) and Angelo et al. [15] (r=0.581).

Similar to our results, studies conducted by Gogia et al.[8], Angelo et al.[15] (r= -0.019), Beck et al.[9], Blatt et al.[16] and Van Der Ryst et al.[18] also found either a poor correlation or no correlation between ALC and CD4 percentage.

The variability of results can be accounted by ethnic differences, diurnal variation, inter-current illness and age differences. For example, CD4 counts in HIV-infected Asians have been reported to be lower than those of European and North American HIV-infected patients. Some studies have also shown that West African adults have physiological lymphocytosis which leads to higher ALC and CD4 counts as compared to those of Europeans [7].

In our study, we also found out the ALC cut off value corresponding to CD4 count ≤ 200/μL, CD4 count ≤350/μL and CD4 percent ≤20% respectively. CD4 count ≤ 200/μL corresponds to a cut-off value of ALC ≤ 1450/μL in our study. Kumaraswamy et al.[6] found a cut off value of ALC ≤ 1400/μL, Jain et al.[7] found a cut off of ALC ≤1700/μL, Gogia et al.[8] found a cut off of ALC ≤ 1520/μL in India. Blatt et al.[16] found a cut off of ALC ≤ 1400/μL in USA, Mwamburi et al.[18] found a cut off of ALCd"1500/μL in USA, Stebbing et al.[19] found a cut off of ALC ≤ 1500/μL in London and Spaeck et al.[20] found a cut off of ALC ≤ 1200/μL in USA, corresponding to CD4 count ≤ 200/μL.

CD4 count ≤350/μL corresponds to a cut off of ALC ≤1650/μL in our study. Kumaraswamy et al.[6] found that ALC ≤ 1700/μL was suitable for predicting CD4 count ≤ 350/μL in India (Sensitivity 70%, specificity 86%, PPV 86%, NPV 69%). Moore et al.[21] found an ALC threshold of 2250 cells/μL as the most accurate predictor of CD4 cell count ≤ 350/μL in Uganda (Sensitivity 81% and specificity 54%). Chen et al.[11] found that ALC ≤ 1570/μL corresponded to CD4 count ≤ 350/μL in a study conducted in China (Sensitivity 65% and specificity 80%).

Also, we found that CD4 percent ≤ 20% corresponds to ALC ≤ 1650/μL with a significant compromise in sensitivity (64.3%) and specificity (65.2%).

Pertaining to the variations in region-wise results and in CD4 cell counts seen among patients of different ethnic and geographic backgrounds, there is need to establish region-wise ALC cut offs. The major limitation of our study was a small sample size. Hence, larger studies should be done to reproduce and confirm these results.

The cost of CD4 analysis imposes a tremendous economic burden on the patients suffering from HIV infection. On the basis of our study, ALC can be used as an inexpensive alternative to reduce the financial burden of the patients.

Conclusion

Our study suggests a strong correlation between ALC and CD4 count (Pearson correlation coefficient=0.741) which is statistically and clinically significant. But there is a very weak correlation between ALC and CD4 percentage. Hence, ALC can be used as a substitute marker for CD4 count, but not for CD4 percentage. We would recommend a cut off of ALC ≤ 1450/μL corresponding to CD4 count ≤ 200/μL, and ALC ≤ 1650/μL corresponding to CD4 count ≤ 350/μL.

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References

1. WHO Consolidated Guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach [Internet]. Apps.who.int. 2016 [accessed 9 July 2016]. Available from: http://apps.who.int/iris/bitstream/10665/208825/1/9789241549684_eng.pdf?ua=1.
2. Walker B, Colledge N, Ralston S, Penman I. Davidson's principles and practice of medicine.
3. Sax PE. HIV Infection: In: Cunha BA, editor. Antibiotic Essentials. New York: Physicians press; 2005.p.227-33.
4. UNAIDS Report- Global AIDS Update 2016 [Internet]. Who.int. 2016 [accessed 9 July 2016]. Available from: http://www.who.int/hiv/pub/arv/global-AIDS-update-2016_en.pdf?ua=1.
5. World Health Organization (2002) Scaling up antiretroviral therapy in resource-limited settings: Treatment guidelines for a public health approach [Internet]. Who.int. 2002 [accessed 9 July 2016]. Available from: http://www.who.int/hiv/pub/prev_care/en/ScalingUp_E.pdf?ua=1.
6. Kumarasamy N.M. A., Flanigan T.P., Hemalatha R., et al. Total lymphocyte count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India and other resourceconstrained countries. JAIDS 2002;31:378-83.
7. Jain S, Singh AK, Bajaj J, Singh RP, Damle AS. Evaluation of total lymphocyte count (TLC) as a surrogate marker for CD4 count in HIV-positive patients for resource-limited settings. Muller J Med Sci Res 2015;6:23-6.
8. Gogia A, Byotra S, Prakash V, Kumar S, Bhargava M, Kakar A et al. Absolute lymphocyte count: A cost-effective method of monitoring HIV-infected individuals. Indian Journal of Pathology and Microbiology. 2011;54(1):107.
9. Beck EJ, Kupek EJ, Gompels MM, Pinching AJ. Correlation between total and CD4 lymphocyte counts in HIV infection: Not making the good an enemy of the not so perfect. Int J STD AIDS 1996;7:422-8.
10. Van der Ryst E, Kotze M, Joubert G, Steyn M, Pieters H, van der Westhuizen, et al. Correlation among total lymphocyte count, absolute CD4+ count, and CD4+ percentage in a group of HIV1-infected South African patients. J Acquir Immune Deû c Syndr Human Retrovirol 1998;19:238-44.
11. Chen J, Li W, Huang X, Guo C, Zou R, et al. Evaluating Total Lymphocyte Count as a Surrogate Marker for CD4 Cell Count in the Management of HIV-Infected Patients in Resource-Limited Settings: A Study from China. PLoS ONE 2013;8(7):e69704. doi:10.1371/journal.pone.0069704.
12. Badri M., Wood R. Usefulness of total lymphocyte count in monitoring higly active antiretroviral therapy in resourcelimited settings. AIDS 2003;17:541-45.
13. Akinola O.O., Adediran I.A., Onayemi O., et al. The search for a predictor of CD4 cell count continues: Total lymphocyte count is not a substitute for CD4 cell count in the management of HIV infected individuals in a resource-limited setting. Clin Infect Dis 2004;39:579-81.
14. Sagar A, Pathak A, Ambiya V, Naithani N, Vasudevan B, Agrawal S. Utility of absolute lymphocyte count as a surrogate marker of CD4 cell counts: Is it useful?. Medical Journal Armed Forces India. 2014;70(1):48-52.
15. Angelo A, Angelo C, Torres A, Ramos A, Lima M, Netto E et al. Evaluating total lymphocyte counts as a substitute for CD4 counts in the follow up of AIDS patients. Brazilian Journal of Infectious Diseases. 2007;11(5).
16. Blatt S.P., Butzin C.A., Hendrix C.W., Lucey D.R. Total lymphocyte count as a predictor of absolute CD4+ count and CD4+ percentage in HIV-infected persons. JAMA 1993;269(5):622-6.
17. Van Der Ryste K. M., Joubert G., Steyn M., et al. Correlation among total lymphocyte count. absolute CD4+ count. and CD4+ percentage in a group of HIV-1 infected South African patients. J Acquir Immune Defic Syndr and Human Retrovirology 1998;19(3):238-44.
18. Ammann A, Burrowes S. Use of Total Lymphocyte Count vs. CD4 Cell Count as a Marker of Immunity in HIV-infected Adults and Children. 2006. Available from: http://www.womenchildrenhiv.org/ whiv. [Last accessed on 2016 Jul 11].
19. Stebbing J, Sawleshwarkar S, Michailidis C, Jones R, Bower M, Mandalia S, et al. Assessment of the efû cacy of total lymphocyte counts as predictors of AIDS deû ning infections in HIV-1 infected people. Postgrad Med J 2005;81:586-8.
20. Spacek LA, Griswold M, Quinn TC, Moore RD. Total lymphocyte count and hemoglobin combined in an algorithm to initiate the use of highly active antiretroviral therapy in resource-limited settings. AIDS 2003;17:1311-7.
21. Moore DM, Awor A, Downing RS, Were W, Solberg P, et al. Determining eligibility for antiretroviral therapy in resource-limited settings using total lymphocyte counts, hemoglobin and body mass index. AIDS Res Ther 2007;4:1.

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