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Original Article

Pathology Section

Absolute Lymphocyte Count as a Surrogate Marker of CD4 Count in Monitoring HIV Infected Individuals: A Prospective Study

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ABSTRACT

Introduction: CD4 cell count has been proposed to be substituted by Absolute lymphocyte count in monitoring HIV infected individuals as methods of CD4 cell count and plasma viral estimation require expensive, specialized equipments and highly trained personnel.

Aim: To assess the clinical utility of the Absolute Lymphocyte Count (ALC) to serve as a surrogate marker for predicting a CD4 count < 200 cells/µl in patients with HIV infection in resource poor countries.

Materials and Methods: A prospective study of 61 patients with HIV/AIDS was conducted. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) of various ALC cut-offs were computed for CD4 cell count < 200 cells/µl for age < 30 or age ≥ 30 years. Pearson correlation, Linear regression and Receiver Operating Characteristics (ROC), were used.

Results: For patients aged ≥ 30 years, sensitivity, specificity, positive and negative predictive value of ALC <1200 cells/µl to predict CD4 cell count < 200 cells/µl were 34.48%, 67.5%, 43.48%, 58.69% respectively. For subjects aged < 30 years, these values were 27.27%, 67.5%, 18.75%, 77.14%, respectively. A ALC < 1643 was found to have maximal sensitivity for predicting a CD4 cell count <200/ µl.

Conclusion: Our data revealed good correlation between ALC and CD4 cell counts but ALC cut-off of 1200 was not a surrogate marker for CD4 cell count < 200 cells/µl. As we increase the cut-off to <1643/ µl it could be the cost-effective surrogate marker for CD4 cell counts < 200 cells/µl in resource limited settings.

Keywords: Acquired immune deficiency syndrome, Highly active antiretroviral therapy, Total lymphocyte count

INTRODUCTION

A global estimate showed that in 2012 there were 35.3 (32.2-38.8) million people with HIV. The new HIV treatment guidelines provided by the World Health Organization (WHO), issued in June 2013, recommend starting treatment when an individual's CD4 count falls below 500 cells/µl and immediately for pregnant women, HIV positive partner in serodiscordant couples, children younger than five and people with HIV associated tuberculosis and Hepatitis B [1].

The CD4 lymphocyte count is measured in HIV-1 infected people every three months. This is to judge the prognosis, modify prescriptions for Highly Active Antiretroviral Therapy (HAART) and to analyse the need for opportunistic infection prophylaxis [2]. But in regional hospitals with smaller set up, CD4 analysis and its routine evaluation might not be possible due to the expense involved or lack of the facility altogether [3].

To overcome this problem, in April 2002 WHO recommended that, when CD4 cell count is not available or is not affordable to be obtained for affected individuals, a total lymphocyte count of less than 1000-1200 lymphocytes/cmm^3 in individuals with stage 2 or stage 3 disease be used as an indication to initiate antiretroviral therapy [4].

Total Lymphocyte Count (TLC) is 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 patients would be 2.4×10^9/L [5].

To the best of our knowledge, few studies have compared the utility of ALC as a surrogate for CD4 count in monitoring HIV infected individuals in resource limited society [2-14].

In view of the high costs and limited availability of resources to estimate absolute CD4 counts, this study was initiated to assess the adequacy of using ALC as a suitable replacement for CD4 counts.

MATERIALS AND METHODS

This was a prospective study carried out over a period of one year (2004-2005) in the Department of Pathology, B J Govt. Medical College and Sassoon General Hospital (a tertiary level hospital), Pune. Prior permission from institutional ethical committee was sought before conducting the study. All HIV positive cases at all stages of illness, above 18 years were included. Patients on HAART therapy, pregnant women, and pediatric age group were excluded CD4 and CD8 T cell counts and haemograms were obtained using a Fluorescence Activated Cell Sorter (FACS) counter and FACS caliber automated machines at National AIDS Research Institute, Pune.

STATISTICAL ANALYSIS

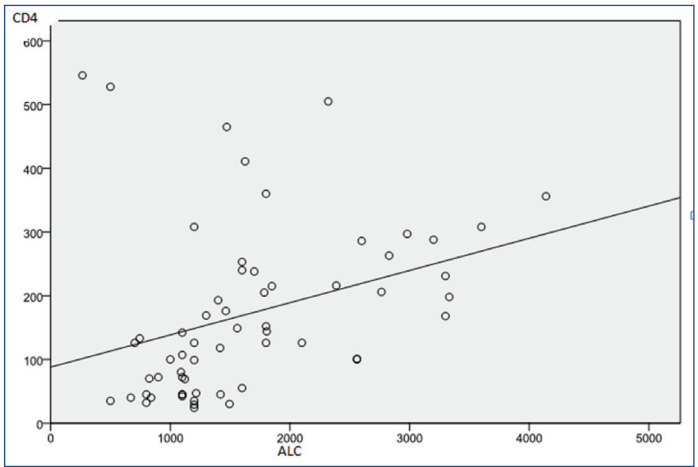
Pearson correlation between ALC and CD4 cell count, receiver operating characteristics were assessed. Sensitivity, specificity, positive predictive value, and negative predictive values of various ALC cut-offs were computed for CD4 cell count < 200, for age group < 30 and ≥ 30 years. All these statistical analysis were performed using SPSS software (version 17.0, SPSS, Chicago, USA).

RESULTS

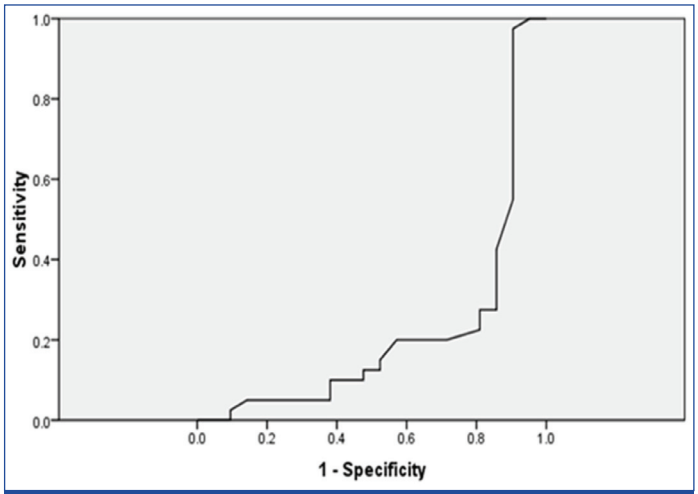
A total of 61 patients were included in this study, among which 38 were male (62.29%). The mean (standard deviation) of age was 35.34 (±7.76) years ranging from 19-60years. The mean (SD) of CD4 cell count and ALC were 120.25(± 62.89) cells/µl and 1324.33 (±441.26) cells/µl for subjects less ≤than 30 years age respectively. The mean (SD) of CD4 cell count and ALC were 183.41(±142.62) cells/µl and 1721.43(±918.99) cells/µl for subjects ≥ 30-year-old respectively.

ALC cut-off (cells/µl)	Mean CD4 count (cells/µl)		Sensitivity %		Specificity %		Positive predictive value %		Negative predictive value %	
	<30 years	≥30 years	<30 years	≥30 years	<30 years	≥30 years	<30 years	≥30 years	<30 years	≥30 years
≤1000	80.33	64.57	27.27	24.13	75	75	23.07	41.17	78.95	57.69
≤1200	66.66	71.5	27.27	34.48	67.5	67.5	18.75	43.48	77.14	58.69
≤1400	169	120	9.09	6.89	92.5	92.5	25	40	78.72	57.81
≤1600	176	79.4	9.09	17.24	85	85	14.28	45.45	77.27	58.62
≤1800	139	0	18.18	0	95	95	50	0	80.85	56.71

[Table/Fig-1]: Different cut-off values of ALC predicting CD4< 200 cells/µl for subjects aged less than 30 years and aged 30 years and above.



[Table/Fig-2]: Relationship between CD4 and ALC in cells/µl.



[Table/Fig-3]: The Receiver Operating Charateristic Curve.

The mean CD4 count, sensitivity, specificity, positive predictive value, negative predictive value for different levels of ALC cut-offs among those who were less than 30 years of age and ≥ 30 years of age are depicted in [Table/Fig-1].

Considering the best cut-off value of ALC that are with highest sensitivity, a ALC < 1643 cells/µl was found to have sensitivity of 93.9% and specificity of 20% for predicting a CD4 cell count of < 200 cells /µl.

The correlation coefficient (r) for CD4 cell count and ALC was significant with r-value= 0.327 (p< 0.05) [Table/Fig-2].

ROC (Receiver Operating Characteristic) curve were used to display the result of sensitivity and false positive error rate (1-specificity) of ALC cut-off values and CD4 cell count groups [Table/Fig-3].

DISCUSSION

Many investigators from different countries and regions of the world are focused on evaluating the usefulness of TLC as surrogate marker of a CD4 cell count less than 200 cells/µl for HIV infected patients of different ethnicities [3].

Studies	Sensitivity	Specificity
Daka et al., [9]	41%	83.5%
Angelo ALD et al., [10]	46.5%	92.8%
Karanth SS et al., [11]	73%	100%
Kakar A et al., [12]	64.4%	91.1%
Sreenivasan S et al., [13]	63.41%	69.57%
Obirikorang C et al., [14]	72.22%	100%
Present study	34.48%	67.50%

[Table/Fig-4]: Comparison of sensitivity and specificity of ALC cut-off < 1200 cells /µl for predicting CD4 cell count <200 in present study and similar studies in past.

Studies	ALC cut-off	Sensitivity	Specificity
Daka et al., [9]	≤1780 cells /µl	61%	62%
Angelo ALD et al., [10]	≤1700 cells /µl	59.4%	75.8%
Karanth S et al., [11]	≤1500 cells /µl	82%	88.2%
Kakar A et al., [12]	≤1400 cells /µl	78%	
Sreenivasan S et al., [13]	≤1520 cells /µl	71.08%	78.26%
Obirikorang C et al., [14]	≤ 1200 cells /µl	72.22%	100%
Present study	≤1643cells /µl	93.93%	20%

[Table/Fig-5]: Comparison of ALC cut-offs with highest sensitivity in present study and similar studies in past.

In our study, we found that ALC of ≤ 1200 cells/µl as suggested by WHO, had sensitivity of 34.48% and specificity 69.57%; positive predictive value 43.48%; negative predictive value 58.69%. To the best of our knowledge many studies showed low sensitivity of ALC less than 1200/µl for predicting CD4 cell count less than 200 cells/µl [Table/Fig-4] [6,9,10,12,13]. However, study by Karanth SS et al., and Obirikorang C et al., show higher sensitivity(73% and72.22%) and specificity (100% and 100%), respectively, for TLC cut-off of 1200 cells/µl to predict CD4 cell count less than 200/ µl [11,14]. This difference could be due to different ethnic, racial, epidemiological and socioeconomic factors.

According to our finding, ALC of ≤ 1643 cells/µl was more sensitive (93.93%) to predict CD4 cell count of <200 cells/µl. Kumarasamy N et al., found ALC cut-off < 1400 cells/µl had sensitivity of 73%, specificity 88%, PPV 76% and NPV 86% for predicting CD4 cell count of <200 cells/µl [6]. Other studies from India also suggested higher ALC cut-off for predicting CD4 cell count of <200 cells/µl [11-13]. Study from Ethiopia and Brazil also agree with higher cut-off of ALC for predicting CD4 cell count <200 cells/µl [9,10].

We found significant correlation between ALC and CD4 cell count with r value 0.327 (p-value < 0.05) [Table/Fig-2]. Kumarasamy N et al., and other studies from India [6], Karanth SS et al., Kakar A et al., Sreenivasan S et al., also found high degree of correlation between CD4 cell count and ALC count with r-value 0.744,0.682,0.714,0.560 respectively [11-13]. A similar has also been suggested in studies from other parts of world, Fasakin et al., studied r-value 0.65, Daka et al., also showed high correlation with r-value 0.398, same proved by Angelo ALD et al., with r-value 0.581 [5,9,10].

In our study, ALC obtained a relatively low diagnostic performance (Area Under Curve=0.217) for predicting a CD4 cell count less

than 200 cells/µl with sensitivity of 93.93% and specificity of 20% at threshold of ≤ 1643 cells/µl [Table/Fig-3]. However, study from Chen J et al., showed high diagnostic performance (Area Under Curve=0.80) for predicting CD4 cell count less than 350 cells/µl [3]. This difference may be due to larger study group in Chen J et al., study [3]. [Table/Fig-4,5] shows the compilation of similar studies.

CONCLUSION

Our study suggest that ALC could have clinical utility in monitoring HIV infected individuals as there is significant positive correlation between ALC and CD4 cell count. However, studies with larger sample size are still required to prove the usefulness of ALC as surrogate marker for CD4 cell count in monitoring HIV infected individuals in resource limited settings.

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