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Original Research Article

Correlation of Absolute Lymphocyte Count and CD4 Counts in HIV Infected Patients at a Tertiary Care Institute

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Abstract

Introduction: In HIV infected individuals, CD4 T cell counts along with viral load remains the mainstay for assessing the immune status and for clinical management. As these tests are expensive and not easily available, absolute lymphocyte count (ALC) has been suggested to reflect the CD4 counts. This can then be used to predict the immune status for initiation of chemoprophylaxis for opportunistic infections in resource limited settings. *Objectives:* This study was undertaken to evaluate ALC as an alternative marker for CD4 counts in HIV infected patients and to assess its clinical utility. *Materials and Methods:* Haematological parameters and CD4 counts for all HIV positive cases from January 2016 to August 2018 was assessed. The Beckman Counter FC 500 flow cytometer was used for CD4 counts and Sysmex XE 2100 for haematological profile. Spearman correlation and receiver operating curve (ROC) were used for calculating sensitivity, specificity and positive predictive values. *Results*: Sensitivity, specificity and positive predictive value of ALC < 1200 cells/µl to predict CD4 < 200 cells/µl, was found to be 92%, 53% and 64% respectively. There is a positive correlation between CD4 count and ALC of 0.655 which is statistically significant ($p < 0.05$). *Conclusion*: Our data shows a good correlation between ALC and CD4 cell counts, with ALC < 1210 cells/µl cut off to have the maximum sensitivity for predicting CD4 count < 200 cells/µl. Hence, ALC can be considered as an economical and easily available substitute in prediction of low CD4 counts.

Keywords: Absolute lymphocyte count; CD4 counts; HIV.

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Introduction

As of 2017, 36.9 million people globally are infected with Human Immunodeficiency virus (HIV), out of which, only 21.7 million are receiving treatment. 1.8 million new cases and 9, 40,000 acquired immunodeficiency syndromes (AIDS) related deaths were reported in 2017. Although HIV infection is on a decline, India still has the 3rd largest number of people living with HIV/AIDS. In 2017, 21, 00,000 cases were noted out of which only 11, 81,129 were receiving treatment; 88,000 new cases were reported with 69,000 AIDS related deaths [1].

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With the widespread availability and expansion of anti-retroviral therapy (ART), it is essential to monitor the response as well as to follow progression of the disease in an individual. AIDS, first revealed in 1981 in homosexual males in California and New York, caused by HIV, attacks the immune system in the body and leads to a significant reduction in CD4 counts [2].

Viral loads and CD4 counts are the mainstay for assessing the disease progression. CD4 count, a measure of CD4 T lymphocytes is one of the strongest predictor of immune status in HIV patients and hence is measured periodically for prognosis, modification of treatment and to assess the need for chemoprophylaxis for opportunistic infections [3,4].

The CD4 count monitoring requires special equipment, trained staff along with proper facility and finances, it's not easily available in a resource limited setup. In such conditions, where CD4 count facility is unavailable, World health organization (WHO) recommends the use of absolute lymphocyte count (ALC) to monitor the immune response in patients with HIV. Absolute lymphocyte count, an inexpensive and an easily available parameter, can be used as an alternative for CD4 count monitoring in various low income setups [5].

ALC obtained from multiplying total leukocytes with the percentage of lymphocytes present in blood; in the past has been studied for predicting CD4 counts < 200 cells/mm^3. The results of the past studies, showed a variable specificity and sensitivity along with different ALC cut off values in each study for prediction of declining CD4 counts [6].

In April 2002, WHO proposed the use of ALC (≤ 1200 cells/mm^3) in patients with stage II or stage III disease as an alternative for when CD4 counts were inaccessible [7].

This study was undertaken to evaluate ALC as an alternate marker for CD4 counts in HIV infected patients and to assess its clinical utility.

Material and Methods

The retrospective study was carried out from January 2016 to August 2018 in the Department of Pathology, M.S. Ramaiah Medical College and Hospitals, a tertiary level hospital in Bangalore. All HIV positive patients at all stages of illness, above 18 years of age were included in the study. Pregnant women and paediatric age group patients were excluded. Haematological parameters were assessed using Sysmex, Vestmatic 20 for complete blood counts (CBC) and ESR and Beckman Counter FC 500 flow cytometer for CD4 counts.

Statisical Analysis

Categorical variables were described using percentages, while continuous variables were described using mean, median and interquartile ranges. Statistical package for social sciences (SPSS version 20) and Microsoft Excel version 10 were used to analyse the data. Spearman correlation to establish a correlation between CD4 count and various haematological parameters was used, along with Receiver operator curve. Sensitivity, specificity, positive predictive value, and negative predictive value of ALC cut off value for CD4 counts were assessed.

Results

A total of 106 patients were included in the study, of which 59.4% were males and 40.6% were females. The patients ranged from 19 years to 72 years of age, with a mean age of 42.7 years (SD- 11.01).

The most common clinical finding in the patients was of fever, followed by respiratory tract infections. Tuberculosis was found to be one of the most common respiratory infections. Patients in addition also had presented with weight loss, diarrhoea, meningitis, pancytopenia and urinary tract infections.

Out of the 106 patients studied, 73 patients (68.87%) had CD4 counts < 200 cells/mm^3.

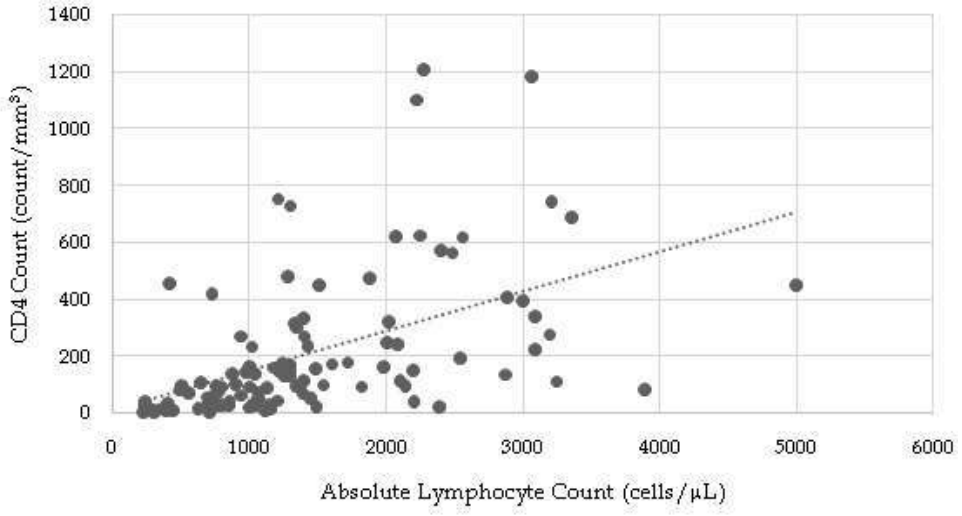
There was a positive correlation of r= 0.655 between CD4 count and ALC, which was statistically significant (p value < 0.05) (Graph 1).

The mean Hemoglobin (Hb), erythrocyte sedimentation rate (ESR), total leucocyte count and CD4 to CD8 ratio in this study was found to be 10.g/dl (SD 2.49), 52.57 mm/hr (SD 40.6), 6819.43 (SD 3617.44) and 0.41 (SD 0.37) respectively.

Sensitivity, specificity and positive predictive value (PPV) of ALC < 1200 cells/µl to predict CD4 < 200 cells/µl, was found to be 92%, 53% and 64% respectively in our study. The ALC cut-off value of 1210 cells/mm^3 was found to have the highest sensitivity and specificity for predicting CD4 counts <200 cells/mm^3. The ALC cut off of 1210 cells/mm^3 had 92% sensitivity and 53% specificity for predicting CD4 counts < 200 cells/mm^3.

Table 1: Various parameters with minimum, maximum and mean values

Parameters	Minimum	Maximum	Mean	Std. Deviation
Age (years)	19	72	42.70	11.01
HB (g/dl)	4.3	16.9	10.6	2.49
ESR (mm/hr)	3	140	52.57	40.6
Total leucocyte count (cells/mm^3)	1280	20040	6819.43	3617.44
ALC (cells/mm^3)	221	5000	1436.01	916.93
CD4 Counts (cells/mm^3)	4	1212	210.99	251.03
CD4/CD8 Ratio	0.02	1.49	0.41	0.37



Graph 1: Relationship between CD4 count and ALC in cells/µl

Discussion

Ever since the detection of first case of HIV in India, in Chennai, the epidemic has gradually spread to urban and the rural areas. The virus acts by utilising the CD4 T lymphocytes, decreasing their number and leading to a decline in immune response of the body. Hence, CD4 T lymphocytes are used for monitoring the disease activity [2]. The current WHO guidelines have stated the need of starting ART irrespective of viral loads or CD4 cell counts [9]. WHO recommends CD4 count testing to be done at the time of HIV diagnosis, before and at the time of ART, and for treatment failure cases. For the purpose of monitoring, CD4 counts should be done every 6 months [9].

With the declining CD4 cell counts, the host becomes susceptible to various infections. Patients with CD4 counts of 400 cells/mm^3 are suceptible for Herpes Zoster infections, at counts of 350 cells/mm^3 tuberculosis is seen and at 300 cells/mm^3 oral candidiasis is noted. Patients with very low immune response having CD4 counts of <200 cells/mm^3 are highly susceptible for opportunistic infections such as Pneumocystis carinii, esophageal candidiasis, mucocutaneous herpes, cryptococcosis, coccidioidomycosis, cryptosporidiosis, mycobacterium avium complex, etc. In such patients CD4 counts are essential for starting chemoprophylaxis. However, in resource limited setup, CD4 counts are not easily available [2]. Hence, WHO proposed the used of ALC with the cut off of < 1200 cells/mm^3 for predicting such declining immune functions [7].

The usefulness of ALC as a surrogate marker has been studied by many investigators from around the world for predicting CD4 count < 200 cells/ mm^3 in HIV infected individuals [3].

In our study, ALC of ≤ 1200 cells/mm^3 as proposed by WHO for predicting CD4 counts < 200 cells/ mm^3 has sensitivity, specificity and positive predictive value of 92%, 53% and 64% respectively. Thus indicating that the cut off proposed by WHO having a high sensitivity can be used as a screening tool for identifying patients with deteriorating immune status. Comparable results were seen in several other studies [10,11,12].

According to our study, ALC of ≤ 1210 cells/ mm^3 had a slightly higher sensitivity to predict CD4 count of < 200 cells/mm^3. Various studies which

have looked into the relationship between CD4 counts and ALC have proposed their own cut off values with maximal sensitivity and specificity. (Table 2).

Table 2: Various studies and their ALC cut off values along with sensitivity and specificity for predicting CD4 count <200 cells/mm^3

Studies	ALC cut-off	Sensitivity	Specificity
Daka *et al.* [13]	≤ 1780 cells/µl	61%	62%
Angelo ALD *et al.* [14]	≤ 1700 cells/µl	59.4%	75.8%
Karnath S *et al.* [11]	≤ 1500 cells/µl	82%	88.2%
Kakar A *et al.* [15]	≤ 1400 cells/µl	78%	
Sreenivasan S *et al.* [16]	≤ 1520 cells/µl	71.08%	78.26%
Obirikorang C *et al.* [10]	≤ 1200 cells/µl	72.22%	100%
Agrawal PB *et al.* [3]	≤ 1643 cells/µl	93.33%	20%
Kumaraswamy N *et al.* [17]	≤1400 cells/µl	73%	88%
Darmana IMS *et al.* [18]	≤1450 cells/µl	80%	67%
Kotwal J *et al.* [19]	≤ 1250 cells/µl	87.3%	70%
Hassan MK *et al.* [20]	≤ 1360 cells/µl	82.4%	90.3%
Present study	≤ 1210 cells/µl	92%	53%

There was a statistically significant correlation noted between ALC and CD4 counts in our study with r value of 0.65 which was similar to the studies conducted by Dalela G *et al.* (r value 0.73), Kumaraswamy N *et al.* (r value of 0.74), Fasakin KA *et al.* (r value of 0.65), Chen J *et al.* (r value of 0.60), Riyanti R *et al.* (r value of 0.65) Hassan MK *et al.* (r value of 0.69) and slightly higher than the studies conducted by Birajdar SV *et al.* (r value of 0.388), Kwamboka AD *et al.* (r value 0.399) and Moolla Y *et al.* (r value of 0.25) [5,11,12,17,20-24]. The variation in the result of these studies may be attributed to various factors such as sample size, treatment, opportunistic infections and the fact that ALC includes both T and B lymphocytes [6].

Other studies in the past have shown a positive and a statistically significant correlation between ALC and CD4 counts. Studies conducted by Daud MY *et al.*, Dalela G *et al.*, Balak DA *et al.* and Kim HJ *et al.* all have shown such correlation [12,17,25-27].

A study conducted by Balak DA *et al.*, in Fiji did not recommend the use of ALC as a predictor of CD4 counts as a result of a weak correlation and low sensitivity; instead they had proposed a higher value of 2300 cells/mm^3 as the cut off having a much greater sensitivity [26]. Similarly Daka *et al.* had shown a low sensitivity and specificity of ALC as a surrogate marker and had proposed a higher cut off value of 1780 cell/mm^3 [13].

The study by Sen *et al.* showed a poor association between ALC and CD4 counts [28].

Young B *et al.* in 2015 found a positive correlation (r= 0.589, p value < 0.001) between CD4 counts and ALC; and stated that while ALC may accurately measure lymphocyte count, its ability to predict CD4 count is limited [29]. ALC was similarly judged unsuitable to predict CD4 values in few other studies [30-32]. Furthermore, Nwabuko OC concluded that although ALC can be used as a predictor of HIV disease progression, however, it's utility as a CD4 surrogate is limited [33].

Clinical features presented by the patients in our study were found to be comparable to various other studies. Fever was one of most common finding in this as well as other studies [5,34].

Few limitations of our study were its modest sample size, single values of ALC and CD4 and exclusion of pregnant and paediatric patient groups. Furthermore, this was a single institute study which included only a certain subset of Indian population.

Conclusion

Our study has shown a positive correlation between the CD4 counts and ALC which can be used in monitoring and surveillance of immune status of HIV infected individuals living in less equipped areas. Despite the positive and significant correlation between the parameters we advise further and larger scale studies to look upon ALC as a diagnostic and prognostic tool to judge the declining immune status of HIV patients and to correlate its trends during the phases of the disease.

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