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Original Research Article

Absolute lymphocyte count as a cost effective alternative to CD4 count in HIV positive patients

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ABSTRACT

Introduction: Acquired immunodeficiency Syndrome (AIDS) is caused by Human Immunodeficiency virus leading to profound immunosuppression and its consequences. Depletion of CD4+ T cells is a hallmark of AIDS apart from anaemia, leucopenia and thrombocytopenia. Monitoring of CD4 count is crucial for an effective treatment. Absolute lymphocyte count (ALC) less than 1000/μl i.e. lymphopenia correlates with lower CD4 counts as per various studies and recommendation by the WHO. Detection of lymphopenia and measurement of ALC are routinely obtained parameters using a basic haematology analyser. This study was done to evaluate the role of absolute lymphocyte count (ALC) as an alternative to CD4 count in HIV positive patients.

Materials and Methods: This prospective observational study was conducted for the period of two years in the department of Pathology of a tertiary care hospital in western India. 110 HIV positive patients consenting to be the part of the study were included. Complete blood counts (CBC), CD4 and CD8 counts were done for all patients following stringent quality control protocols. Statistical analysis was done to evaluate the correlation between various parameters.

Results : 65.5% cases had lymphopenia with an absolute CD4 count <200cells/μl. Using ROC curve, we found that ALC of less than 575/μl has a significant statistical association with CD4 count of less than 200/μl.Correlation of lymphopenia with CD4:CD8 ratio was not found to be statistically significant in our study.

Conclusion : ALC can be considered as a cost effective alternative to absolute CD4 counts in the monitoring of HIV positive patients.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a disease caused by the retrovirus human immunodeficiency virus (HIV) and characterised by immunosuppression leading to opportunistic infections, secondary neoplasms and neurological manifestations.[1]

Profound immunodeficiency, primarily affecting cell-mediated immunity, is the hallmark of AIDS. This results chiefly from infection and a severe loss of CD4+T cells as well as impairment in the function of surviving helper T cells.[2]

The common haematological abnormalities seen with HIV include anaemia followed by leucopenia and thrombocytopenia.[3]

The CDC classification system from the revision in the year 1993 combines three categories of the CD4 count with three symptom categories and is closer to a staging system. According to the CD4+ T cells the categories are - Category 1: > 500 cells/μl (or CD4% > 28%), Category 2: 200-499 cells/μl (or CD4% 14% - 28%), Category 3: < 200 cells/μl (or CD4% < 14%).[4]

Hematologic abnormalities secondary to HIV infection also include venous thromboembolism, hemophagocytic

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syndrome, AIDS - related lymphoma including primary effusion lymphoma, Castleman disease and rarely Hodgkin's disease and myeloma.[5]

In 2018 it was estimated that there are around 37.9 million people living with HIV.[6] As a priority, antiretroviral therapy (ART) should be initiated in all adults with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and adults with CD4 count $\leq$350 cells/μl[3,7]

Lymphopenia is defined as absolute lymphocyte count (ALC) <1000/μl.[8,9] In a resource limited country like India, viral load assays as a marker of the disease status are not affordable to majority of the patients infected with HIV. Absolute CD4 counts and CD4: CD8 ratios are used as the surrogate markers to assess the degree of immune deficiency in these circumstances. Flow cytometry is an accepted standard method for determination of absolute counts of CD4 and CD8.[10]

The immune system of patients with HIV infection is characterized by a profound increase in lymphocyte turnover that is immediately reduced with effective anti-retroviral therapy. Due to expanding access to HAART (highly active anti-retroviral therapy) in resource limited settings, there has been a need to find out any surrogate marker for CD4 count. Many studies like S. Srirangaraj et al and Agrawal et al found a significant correlation between absolute lymphocyte count (ALC) and CD4 count (p value <0.05)[11,12]

CD4 count is measured every 3 months in HIV-1 infected patients. This is done to assess the effectiveness of HAART and to analyse the need for prophylactic treatment for the opportunistic infections. However, in resource limited settings absolute CD4 count is not always available.[12]

In April 2012 WHO recommended that when CD4 cell count is not available, an absolute lymphocyte count of <1000-1200 lymphocytes/μl with stage 2 and 3 disease is an indication to initiate antiretroviral therapy.[13]

2. Materials and Methods

A prospective observational study was carried out after taking the institutional ethics committee approval for the period of two years in the department of Pathology of a tertiary care hospital in western India. 110 HIV positive patients consenting to be the part of the study were included. Patients less than 18 years of age and those not willing to undergo testing were excluded from the study.

Whole blood EDTA samples were collected for the tests of CBC and flow cytometry. CD4 count, CD8 count and CD4:CD8 ratios were carried out on all samples. CBC samples were processed on automated five part differential cell counter (LH-750 from Beckman coulter). CD4 and CD8 counts were obtained by flow cytometry which was done on two lasers six colours flow cytometer (Navios from Beckman Coulter). Chi-square test and Fischer test were used for qualitative data. A two tailed test with P - value <0.05 was considered as significant. ROC curve was used to find the association between two variables. Anaemia, leucopenia and thrombocytopenia ware graded as per WHO criteria.

Stringent quality control protocols were deployed for complete blood counts and for CD4 and CD8 counts. The quality control data was monitored and immediate corrective actions were taken for any deviations. Satisfactory participation in a national EQA programme was also ensured. The quality control processes ensured accuracy of results. Other relevant patient parameters were obtained from the patients' hospital records and laboratory information system.

3. Results

Majority of cases belonged to age group of 31-40 yrs. (47.3%) followed by 41-50 yrs. (26.4%). 65.5% were males and 34.5% were females.

Out of 110 cases 60 (54.5%) of them had leucopenia. Out of 110 cases 72 (65.5%) had lymphopenia. 40(36.4%) had total WBC count <4000/μl with an absolute CD4 count <200cells/μl. These values were statistically significant (p value = 0.014) (Table 1)

The mean CD4 count was 407.85 cells/μl. 56 (51%) of the patients in our study had CD4 count <200 cells/μl. 48 (43.6%) patients with lymphopenia had CD4 values <200 cells/microliter and these values were statistically significant (p = <0.001) (Figure 1). 63 (57.3%) cases had an ALC <1000cells/μl with CD4:CD8 ratio <1. These values were not statistically significant with a p value of 0.238.

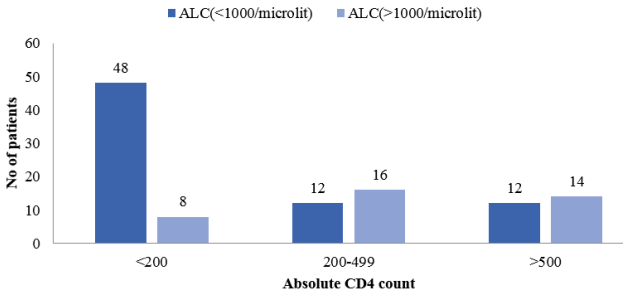

Fig. 1: Correlation of ALC with absolute CD4 count

ROC curve shows trade off between sensitivity (True positivity) and 1- specificity (1- False positivity rate). Classifiers that give curve closer to the left upper corner indicate a better performance. We have used ROC curve to analyse sensitivity of absolute lymphocyte count versus absolute CD4 count.

Using the ROC curve the area under curve is >90% when absolute lymphocyte count is 575/μl with a specificity of 90%. This implies that if the absolute lymphocyte count is <575/μl, the probability of CD4 count being <200/μl are high. (Figure 2).

Table 1: Correlation of total leucocytes count with CD4 count

Total Leucocytes Count (/μl)	CD4 (<200)	CD4 (200-499)	CD4 (>500)	P value
<4000	40	9	13	
4000-11000	11	12	8	0.014
>11000	5	7	5	

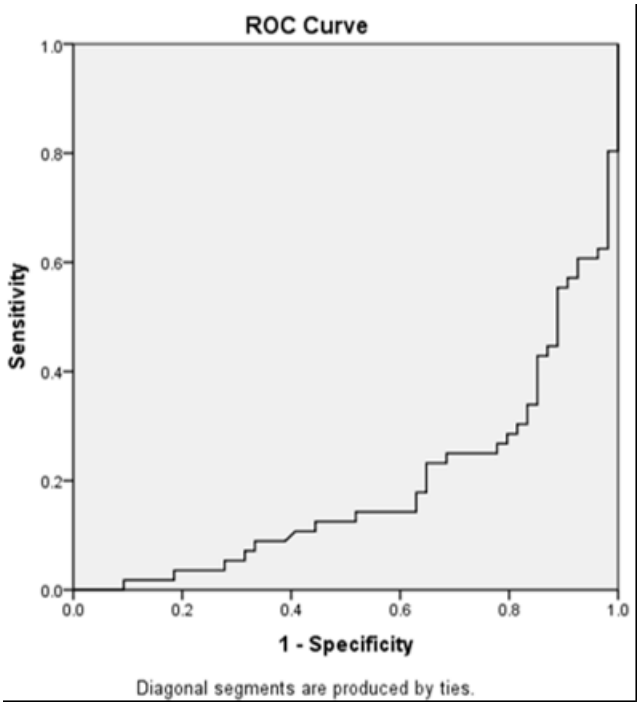

Fig. 2: ROC curve for absolute lymphocyte count and CD4 count

4. Discussion

HIV infection is associated with significant immunodeficiency and leads to depletion of CD4+ T cells. Enumeration of CD4 count is routinely used for monitoring of these patients worldwide. However, it requires sophisticated instrument and expertise; making it largely inaccessible. Haematology analysers used in routine laboratories provide absolute lymphocyte count along with the other parameters.

In our study, about 47.3% cases were in the age group of 31-40 year group. In a study by Shilpa Mittal et al 48% of patients belong to the age group of 31-40 yrs.Most common age group of 21-40 years with mean age of 36.59 $\pm$9.12 was found in a study by Madhu Balla et al. [1,5]

There were 65.5% males and 34.5% females which was in concordance with the study by Madhu Balla et al where there were 58% males and 42% females. [1]

Leucopenia was seen in 54.5% patients, whereas in a study done by Erhabor et al and Vijay Kumar et al showed leucopenia in 62% and 40% cases respectively. [14,15] This variation could be due to unavailability of antiretroviral therapy status in our study.

Lymphopenia was found in 65.5% cases. These findings were in concordance with other studies done by Parinitha et al (65.2%) and Treacy et al (70%). [3,16]

In the present study 51% cases were found having an absolute CD4 count <200/μl followed by 25.4% with an absolute CD4 between 200-499/μl and 24% cases with an absolute CD4 >500/μl; this was in concordance with the study by Attili et al. [17]

In studies by S. Srirangaraj et al and Agrawal et al they found a significant correlation between absolute lymphocyte count and CD4 count with a specificity of 88.9% and 69.6% respectively. [11,12]

On using the ROC curve the area under curve is >90% when absolute lymphocyte count is <575/μl with a specificity of 90%. This means that if the absolute lymphocyte count is <575/μl the chances of having a CD4 count <200/μl were more. Hence a positive correlation between ALC and absolute CD4 count was seen.

5. Conclusion

Absolute lymphocyte count obtained on a routine haematology cell counter can be a suitable cost effective alternative for the monitoring of HIV positive patients. This methodology is also accessible to a larger population; flow cytometry being expensive and mostly unavailable. Robust quality control protocols need to be in place for dependable use of this alternative.

6. Acknowledgment

RN was responsible for designing the study. AC and AD performed the tests, basic data collection and preliminary analysis. AN supervised the data analysis and prepared final manuscript. AK did the critical review of the manuscript.

7. Source of Funding

None.

8. Conflicts of Interest

None.

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