International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2021; 4(3): 27-29
Received: 15-02-2021
Accepted: 16-03-2021

Dr. Ashish N Madiya
Assistant Professor,
Department of Pathology,
Government medical College,
Baroda, Gujarat, India

Dr. Bharat Muljibhai Chaudhari
Assistant Professor,
Department of Pathology,
Banas Medical College,
Palanpur, Gujarat, India

Corresponding Author:
Dr. Bharat Muljibhai Chaudhari
Assistant Professor,
Department of Pathology,
Banas Medical College,
Palanpur, Gujarat, India

Absolute lymphocyte count: A cost-effective substitute to CD4 count in HIV positive patients

Dr. Ashish N Madiya and Dr. Bharat Muljibhai Chaudhari

DOI: https://doi.org/10.33545/pathol.2021.v4.i2a.354

Abstract
Background and Aim: The most characteristic feature of HIV/AIDS is a selective depletion of CD4 T helper/inducer subsets of T- cells. 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. This study was done to evaluate the role of absolute lymphocyte count (ALC) as an alternative to CD4 count in HIV positive patients.
Material and Methods: A prospective observational study was carried out after taking the institutional ethics committee approval for the period of one year in the department of Pathology of a tertiary care hospital in Gujarat. 100 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: Out of 100 cases 54 of them had leucopenia. Out of 100 cases 65% had lymphopenia.35% had total WBC count ROC curve shows tradeoff between sensitivity (True positivity) and 1- specificity (1-False positivity rate).
Conclusion: ALC is a good surrogate test for HIV management and has a significant strong positive correlation with CD4 cell count. This methodology is also accessible to a larger population; flow cytometry being expensive and mostly unavailable.

Keywords: CD4 count, Complete blood counts, HIV, Lymphopenia

Introduction
Acquired immunod efificiency syndrome (AIDS) is a disease caused by the retrovirus human immunod efificiency virus (HIV) and characterized by immunosuppressant leading to opportunistic infections, secondary neoplasms and neurological manifestations [1]. Profound immunodefificiency, primarily affecting cell mediated immunity, is the hallmark of AIDS. This results chieflfly 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 most characteristic feature of HIV/AIDS is a selective depletion of CD4 T helper/inducer subsets of T- cells. The degree of T-cell depletion is currently the single most important laboratory finding considered when making recommendations regarding initiation of therapy 1. Highly active antiretroviral therapy has changed the landscape of HIV care in the developed world. Many patients with access to antiretroviral therapy have benefited from the dramatic reductions in mortality and morbidity and HIV disease has become of relative chronicity for most HIV-infected patients [4, 5].
Measurement of viral load and CD4 cell count are important tests in the management of HIV-infected individuals. With the progression of the disease, there is a gradual depletion of CD4 cell count. Prophylaxis for opportunistic infection (OI) is also dependent on CD4 cell count, especially when CD4 falls below 200/µL or CD4% < 20 [6]. The present recommendation for testing CD4 cell count is every 4–6 months, however, the test is costly and the equipment required to do so is sophisticated. To overcome this problem, WHO has recommended that in resource-constrained countries, absolute lymphocyte count (ALC) could be the replacement for CD4 cell count [7]. There have been various studies touching on this topic with contradictory results.

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 CD 4% < 14%) [8].

Hematologic abnormalities secondary to HIV infection also include venous thromboembolism, hemophagocytic syndrome, AIDS - related lymphoma including primary effusion lymphoma, Castleman disease and rarely Hodgkin's disease and myeloma [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 defificiency 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 fifind out any surrogate marker for CD4 count. Many studies like S. Srirangaraj *et al*. and Agrawal *et al*. found a signifificant 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. 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].

Materials and Methods

A prospective observational study was carried out after taking the institutional ethics committee approval for the period of one year in the department of Pathology of a tertiary care hospital in Gujarat. 100 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 fifive part differential cell counter. CD4 and CD8 counts were obtained by flow cytometry which was done on two lasers six colours flow cytometer. 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.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively. Chi-square test and Fischer test were used for qualitative data. A two tailed test with P – value< 0.05 was considered as significant.

Results

Table 1: Correlation of total leucocytes count with CD4 count

Total Leucocytes Count (/µl)	CD4 (<200)	CD4 (200-499)	CD4 (>500)	P value
<4000	37	8	12	0.01*
4000-11000	10	10	7	
>11000	5	6	5	

* indicates statistically significance at $p \leq 0.05$

Majority of cases belonged to age group of 30-39 years followed by 40-49 years. 64% were males and 36% were females. Out of 100 cases 54 of them had leucopenia. Out of 100 cases 65% had lymphopenia.35% had total WBC count ROC curve shows tradeoff 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.

Discussion

The availability of highly active antiretroviral therapy lymphocyte count may be more useful predicting CD4 (HAART) and the presence of effective facilities for lymphocyte count for symptomatic patients than for monitoring HIV infection has dramatically changed the asymptomatic patients with a high CD4 count and landscape of human immunodeficiency virus (HIV) care confirms earlier suggestion of possibility of using in the developed world. CD4 cell count of ≤200 cell/µL is an important land mark in the management of AIDS patients; it is at this stage that antiretroviral therapy (ART) is started and co-trimoxazole prophylaxis is required [6]. Although CD4 cell count is considered the best laboratory marker of HIV infection, it is an expensive test and not widely available because of lack of sophisticated equipment. This problem is more in resource-constrained developing countries where the majority of people infected with HIV are living. Here the facilities for CD4 cell count testing may not be available/ affordable. 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 resourceconstrained areas [7].

In our study, about 46.5% 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 years. Most common age group of 21-40 years with mean age of 36.59 ±9.12 was found in a study by Madhu Balla *et al*. There

were 64% males and 36% 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% 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% 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 50.5% cases were found having an absolute CD4 <200/µl followed by 25.8% with an absolute CD4 between 200-499/µl and 24.1% cases with an absolute CD4 >500/µl; this was in concordance with the study by Attili *et al*. [17]

There are many factors, such as diurnal variation, intercurrent illness besides age dependence, on which CD4 cell count depends. Some authors have suggested that adding hemoglobin, platelets, hematocrit, and clinical profile to ALC may improve the prediction of immunosuppression and studies done on this have shown a good correlation suggesting their use as surrogate markers. However, in India more studies need to be taken up before such criteria can be followed because of the difference in status of parity, co-infections, and nutrition. Adding clinical data and other hematologic parameters would also increase the efficacy of ALC test.

On using the ROC curve the area under curve is >90% when absolute lymphocyte count 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 were more. Hence a positive correlation between ALC and absolute CD4 count was seen.

Conclusion

Our study suggests that ALC is a good surrogate test for HIV management and has a significant strong positive correlation with CD4 cell count. 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.

References

1. Balla M, Kumar V, Koul KK. A study of haematological manifestations in HIV positive patients-correlation with cd4 count. J Evol Med Dent Sci 2019;8(19):1545–50.
2. Abbas AK, Aster JC. Robbins and Cotran pathologic basis of disease. Elsevier/Saunders, 2015.
3. Parinitha SS, Kulkarni MH. Haematological changes in HIV infection with correlation to CD4 cell count. Australas Med J 2012;5(3):157-62.
4. Cameron DW, Heath-Chiozzi M, Danner S *et al*. Prolongation of life and prevention of AIDS complications in a randomized controlled clinical trial of ritonavir in patients with advanced HIV disease. Lancet 1998;351:543-549.
5. Hogg RS, Yip B, Chan KJ *et al*. Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. JAMA 2000;286:2568 2577.
6. Sax PE. HIV Infection: In: Cunha BA, editor. Antibiotic Essentials. New York: Physicians press 2005, 227-33.
7. Scaling up antiretroviral therapy in resource-limited setting. Treating in resource limited setting. Treatment guidelines for public health approach. 2003 revision. Geneva: World Health Organization, 2004.
8. Ward MD, Buehler MJ, Jaffe MH, Berkelman RL. revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep 1992;41:1-9.
9. Mittal S, Mangal P. Study of anaemia in HIVaids patients and its relation with cd4 counts. Global J Res Anal 2009, 8(5).
10. Ray K, Gupta SM, Bala M, Muralidhar S, Kumar J. CD4/CD8 lymphocyte counts in healthy, HIV-positive individuals & AIDS patients. Indian J Med Res 2006;124(3):319-30.
11. Srirangaraj S, Venkatesha D. Absolute lymphocyte count as a surrogate marker for CD4 counts after six months of HAART initiation in a resource-limited setting in India. Indian J Med Res 2012;135(6):895.
12. Agrawal PB, Rane SR, Jadhav MV. Absolute lymphocyte count as a surrogate Marker of CD4 count in monitoring HIV infected individuals: A prospective study. J Clin Diagn Res: JCDR 2016;10(5):EC17.
13. Akinola NO, Olasode O, Adediran IA, Onayemi O, Murainah A, Irinoye 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 ResourceLimited Setting. Clin Infect Dis 2004;39(4):579-81.
14. Erhabor O, Uko EK, Ejele OA, Wachukwu C. Effect of long-term highly active antiretroviral therapy (HAART) on some haematological parameters of HIV, 2019.
15. Kumar V, Jayaswal M. A study of hematologic manifestations in patients with HIV infection. Paripex-Indian J Res, 2019, 8(2).
16. Treacy M, Lai L, Costello C, Clark A. Peripheral blood and bone marrow abnormalities in patients with HIV related disease. Br J Haematol 1987;65(3):289-94.
17. Attili SV, Singh VP, Rai M, Varma DV, Gulati AK, Sundar S, *et al*. Hematological profile of HIV patients in relation to immune status-a hospital-based cohort from Varanasi, North India. Turk J Hematol 2008;25(1):13-9.