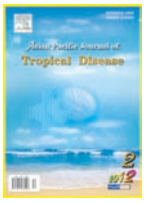
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd



Document heading doi:

Absolute lymphocyte count: A useful surrogate marker to initiate anti retroviral therapy in resource poor settings.

Tsewang Chorol1, Shukla Das[1*], Shilpee Choudhry[1], V G Ramachandran[1], Gajendra Singh Ranga[1], S N Bhattacharya[2], D P Bhadoria[3]

[1] *Department of Medicine University College of Medical Sciences & GTB hospital (University of Delhi), Dilshad Garden, Delhi-110095, India.*
[2] *Department of Dermatology & STD University College of Medical Sciences & GTB hospital (University of Delhi), Dilshad Garden, Delhi-110095, India*
[3] *Department of Medicine, Maulana Azad Medical College & Associated Hospital New Delhi, India.*

ARTICLE INFO

Article history:
Received 25 August 2012
Received in revised from 5 Semptember 2012
Accepted 7 December 2012
Available online 28 December 2012

Keywords:
Absolute lymphocyte count
AIDS
ART
CD4
Oral candidiasis

ABSTRACT

Objective: Initiation of anti retroviral therapy (ART) is routinely based on the algorithms that combine CD4, HIV load and clinical illness. But their high cost and unavailability at resource poor settings are one of its major limitations. An attempt has been made to find a correlation between CD4 count and Absolute lymphocyte count (ALC) so that timely initiation of ART could be done in peripheral areas of developing countries where automation and established technologies have not been reached. **Methods:** This cross sectional study included 200 patients between 18-60 year of ages who are HIV seropositive with and without clinical evidence of oral candidiasis (100 case; 100 control). ALC was calculated as per the percentage of lymphocyte in total leukocyte count as seen in peripheral smear. CD4 cell count estimation was done by flow cytometry method. **Results:** A good correlation was found between CD4 count and ALC in both cases (R=0.656) and controls (R=0.642). There was an increase in sensitivity (St) and decrease in specificity (Sp) of predicting CD4 count <200 cells/mm3 and < 350 cells/mm3 as the cut off value for ALC increased. ALC cut off value of 1700 cells/mm3 is likely to be the best predictor of CD4 count of < 200 cells/mm3 and ALC cut off value of 1800 cells/mm3 for CD4 count <350 cells/mm3. **Conclusions:** We recommend the use of ALC as a surrogate marker for or in combination with CD4 count to determine when to start therapy and to enable routine monitoring in resource poor settings .

1. Introduction

Acquired immunodeficiency syndrome (AIDS) was recognised in United States of America1 in 1981 and its causative agent HIV was first isolated in 1984.1 Since then the disease has spread virtually all over the world. HIV targets and destroys CD4 cells which predisposes the infected individual to various opportunistic infections.2,3 In areas where resources are available, decision to initiate anti retroviral therapy (ART) is routinely based on the algorithm that combine CD4, HIV load and clinical illness. But their high cost and unavailability at resource poor settings are one of its major limitations. Thus efforts are being made to find a parameter that is simple, easily available, and less costly and which can well correlate with CD4 cell counts so that the turn around time to provide treatment is faster. Prior studies have shown a correlation between absolute lymphocyte count (ALC), which is easily available, inexpensive laboratory test, and CD4 count in HIV infected patients4,5 but some studies have shown inconsistent results.6 There are very few published data examining the correlation of CD4 count to ALC in a cohort of HIV-positive patients in India. Keeping view these issues in mind an attempt has been made to find a correlation between CD4 count and ALC so that timely initiation of ART could be done in peripheral areas of developing countries where automation and established technologies have not been reached.

2. Material and methods

This cross sectional study included 200 patients between 18-60 year of ages who are HIV seropositive with and without clinical evidence of oral candidiasis (100 cases; 100

*Corresponding author: Shukla Das Professor,Department of Micobiology,University College of Medical Sciences& GTB hospital (University of Delhi),Dilshad Garden, Delhi-110095, India
Mobile No:- 9868234669; 91-011-25095252 ;Fax No:- 91-011-25095252
E-mail: drshilpee17@yahoo.in

controls). Patients on ART, or having clinical evidence of any other opportunistic infection, diabetes mellitus, any malignancy, on corticosteroid therapy, on anti neoplastic drugs, on antibiotic therapy or on antifungal drugs for more than 2 weeks, blood dyscrasias, xerostomia and pregnant women were excluded from the study. A detailed history and thorough physical examination and relevant routine blood testing was carried out in all cases.

For isolation of candida, sample was taken with moist sterile cotton swab, in duplicate, from buccal mucosa, floor of the mouth, dorsum of the tongue or from angle of the mouth. All samples were subjected to standard mycological techniques for identification and susceptibility testing.7

5 ml of peripheral venous blood was collected with anticoagulant and sera were stored in screw capped vial at −20oC. ALC was calculated as per the percentage of lymphocyte in total leukocyte count as seen in peripheral smear. CD4 cell count estimation was done by flow cytometry method.

All clinical laboratory data were entered into a relational database designed in SPSS software. Mean value of all laboratory parameters were calculated and linear regressions were applied.

3. Results

In our study a total of 100 patients who were HIV seropositive with oral candidiasis and 100 HIV seropositive people who were asymptomatic were included as case and control respectively. The mean CD4 counts amongst cases were significantly lower than the controls (Table 1). Majority of the patients amongst cases (77.14%) had CD4 count <200 cells/mm3. The mean ALC amongst cases were significantly lower than the controls (Table 1).

Table 1.
Mean count of CD4 and ALC

	Case	Control	p value
Mean CD4 cells/mm^3	151.69	390.89	<0.001
Mean ALC cells/mm^3	1456.23	1905.17	0.010

C.albicans was the predominant species, isolated in 54 subjects while non *C.albican* species were isolated in 46

Table 2.
Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of varying cut off for ALC; in identifying CD4 count < 200 cells/mm3 in HIV positive patients.

ALC (Cells/mm^3)	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	Case	Control	Case	Control	Case	Control	Case	Control
<1000	48.14	28.57	100	89.28	100	40.00	36.36	83.33
<1200	51.85	28.57	100	89.28	100	40.00	38.09	83.33
<1400	59.25	42.85	87.50	75.00	94.11	30.00	38.80	84.00
<1500	66.60	42.85	87.50	75.00	94.73	30.00	43.75	84.00
<1600	64.51	57.14	75.00	71.42	95.23	33.33	21.42	86.95
<1700	74.07	71.42	62.50	67.85	85.95	35.71	41.66	90.47
<1800	77.77	71.42	50.00	67.85	84.00	35.71	40.00	90.47
<1900	77.77	71.42	50.00	60.71	84.00	31.25	40.00	89.47
<2000	81.48	71.42	50.00	53.57	84.61	27.77	44.44	88.23
<2100	85.18	100	50.00	50.00	85.18	33.33	50.00	100
<2200	92.59	100	50.00	39.28	86.20	29.16	66.66	100
<2300	100	100	37.50	35.71	84.37	28.00	100	100
<2400	100	100	25.00	25.00	81.82	25.00	100	100

Table 3.
Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of varying cut off for ALC; in identifying CD4 count < 350 cells/mm3 in HIV positive patients.

ALC (Cells/mm^3)	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	Case	Control	Case	Control	Case	Control	Case	Control
<1000	41.93	18.75	100	89.47	100	88	18.18	56
<1200	45.16	18.75	100	89.47	100	88	19.04	56
<1400	45.16	31.25	100	73.68	98	78	19.04	56.66
<1500	61.29	31.25	100	73.68	98	75	25	56.66
<1600	64.51	37.5	75	68.42	96.23	69	31.42	56.52
<1700	67.74	43.75	69	66.15	95.3	60	36.66	57.14
<1800	74.19	73.75	65	63.15	90	40	50	87.14
<1900	74.19	75	55	59.89	90	39	50	87.89
<2000	77.41	76.25	50	50.63	90.59	35	52.22	88.82
<2100	80.64	85	48	50.63	90.3	34.14	55	91.42
<2200	87.09	87.5	48	47.36	91.9	30.33	66.33	91.81
<2300	96.77	93.75	39	47.36	91.75	24.69	66.66	95
<2400	100	93.75	30	42.1	93.13	24	100	98.88

out of which *C.tropicalis* (19) was the most common species followed by *C.guilliermondii* (11), *C.parapsilosis* (8), *C.krusei* (4) and *C.glabrata* (4). There was a trend towards non Candida albicans species being more resistant to antifungal agents than Candida albican, but the difference was not statistically significant (P=0.914).

A good correlation was found between CD4 count and ALC in both cases (R=0.656) and controls (R=0.642). There was an increase in sensitivity (St) and decrease in specificity (Sp) of predicting CD4 count <200 cells/mm3 and < 350 cells/mm3 as the cut off value for ALC increased (Table 2 & 3). ALC cut off value of 1700 cells/mm3 is likely to be the best predictor of CD4 count of < 200 cells/mm3 and ALC cut off value of 1800 cells/mm3 for CD4 count <350 cells/mm^3

4. Discussion

A free anti retroviral therapy (ART) initiative in India was launched on April 2004 by National AIDS Control Organisation (NACO) with key goals of strengthening the linkages & makes the treatment more effective by early intervention. Even though the availability of ART is gradually improving in resource poor settings, at present, the timely initiation of ART as well as opportunistic infection prophylaxis remains the most important and feasible intervention to reduce morbidity and lengthen survival time. According to WHO, the initiation of ART is based on the clinical stage and the CD4 count.8 The absolute CD4 count is a measurement of functional CD4 T-cells circulating in the blood while CD4 percentage represents the percentage of absolute lymphocytes that are CD4 cells. The CD4 counts may vary within individuals of different ethnicity; hence it is important to establish the reference ranges of CD4 count for the target population. The CD4 percentages in Indian adults were found to be lower as compared to Western countries. The mean CD4 per cent were found to be 37 per cent (range 14 to 65%). Various studies carried out have showed the range of absolute CD4 counts as 600 to 1200 cells/μl. Typically, HIV-negative people have CD4 percentage of about 40%, while HIV-infected people's CD4 percentage can be as low as 25% or less.9,10 However, sophisticated equipment is needed for lymphocyte subpopulation analyses, such as flow cytometers, that are not available in the majority of laboratories in resource-constrained countries. In large urban areas where such technology is sometimes accessible, serial CD4 count testing to monitor progression of HIV infection over time is limited by high cost. As ART should not be prevented just because of lack of monitoring laboratory test thus every effort should be made to introduce cost effective and appropriate monitoring assays.

In April 2002, WHO recommended that if CD4 testing is unavailable, ART is indicated for patients in WHO clinical stage 4 and for patients in WHO clinical stage 2 or 3 with ALC below 1200 cells/ mm3. Absolute lymphocyte count is easily obtained from routine complete blood cell count by multiplying percentage of lymphocytes by white blood cell count. Also it is an inexpensive test. In India the cost of CD4 count is 5-10 times more than the ALC count in spite of subsidised rate provided by various organisations.

Our findings showed a good correlation between ALC and CD4 count in both cases (R=0.656) and control (R=0.642). Similar correlation between ALC and CD4 count has also been reported from North America (r=0.77) 11, England (0.76) 4 and India (0.744). 12 As per WHO 2006 guidelines ART and OI prophylaxis should be started at CD4 count <200 cells/mm3 in asymptomatic patients while in symptomatic patients it should be started at CD4 count <350 cells/ mm3.13 The present study showed that in our population, ALC cut off value of 1700 cells/ mm3 would identify 71-74% of patients with CD4 <200 cells/ mm3 while 1800 cells/ mm3 cut off value of ALC would identify 73-74% of patients with CD4 count <350 cells/mm3. However various studies showed variable level of sensitivity of ALC cut off value to predict CD4 count.6 Some studies have not found ALC to be a good predictor of CD4 count and some observed low predictive values among patients with relatively early stage of HIV infection.5,11 Thus one ALC cut off value may not necessarily apply to the populations from different parts of the world but selection of appropriate ALC cut offs for prophylaxis administration should be made on a regional basis. Further studies with larger sample size and variant population would be of additional value.

To summarise, though WHO guidelines released in 2006 do not use ALC in the national ART programme for deciding on the initiation and monitoring the response of ART but in our study population we recommend the ALC cut off value of 1700 and 1800 cells/mm3 to screen CD4 count <200 cells/mm3 and <350 cells/mm3 respectively in HIV seropositive people and to be used as a surrogate marker for or in combination with CD4 count to determine when to start therapy and to enable routine monitoring.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Barre SF, Chermann JC, Rey F. Isolation of T- lymphotropic retrovirus from a patient at risk for aquired immunodeficiency syndrome (AIDS). *Science* 1983;**220**:868-71.

[2] Kaplan JE, Benson C, Holmes KK, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MM*WR Recomm Rep*. 2009;**58**:1-207.

[3] Sinai AP, Kaneshiro ES, Ward H, Weiss LM, Cushion MT. The State of Research for AIDS-Associated Opportunistic Infections and the Importance of Sustaining Smaller Research Communities. *Eukaryotic Cell* 2012, **11**:90-7.

[4] Beck EJ, Kupek EJ, Gompies MM. Correlation between total and CD4 lymphocyte counts in HIV infection: not making the good an enemy of the not so perfect. I*nt J STD AIDS* 1996;**7**(6):422-28.

[5] Blatt SP, Butzin CA, Hendrix CW, Lucey DR. Total lymphocyte

count as predictor of absolute CD4 count and CD4 percentage in HIV infected persons. *JAMA* 1993;**269**(5):622-6.

[6] Post FA, Wood R, Maartens G. CD4 and total lymphocyte counts as predictors of HIV disease progression. *QJM* 1996;**89**(7):505-8.

[7] Rippon JW. Candida and pathogenic yeasts. In:Wonseiwicz M, editor. Medical Mycology:The pathogenic fungi and the pathogenic actinomycetes,3rd ed.1988;WB Saunders Company:Philadelphia.532-81.

[8] NACO. National Guidelines on Second-line ART for adults and adolescents. Ministry of Health & Family Welfare,Government of India April 2011.

[9] Pattanapanyasat K, Thakar MR. CD4+ T cell count as a tool to monitor HIV progression & anti-retroviral therapy. *Indian J Med Res* 2005;**121**: 539-49.

[10] Gebo K. Absolute CD4 versus CD4 percentage for predicting the risk of opportunistic illness in HIV infection. Journal of Acquired Immune Deficiency Syndromes 2004.

[11] Fournier AM, Sosenko JM. The relationship of total lymphocyte count to CD4 lymphocyte count in patient infected with human immunodeficiency virus. *Am J Med Sci* 1992;**304**(2):79-82.

[12] Kakar A,Beri R,Gogia A,Byotra SP,Prakash V,Kumar S,Bhargava M. Absolute lymphocyte count: A cost- effective method of monitoring HIV-infected individuals. *Ind J Pathol Microbiol* 2011.**54**;107-11.

[13] World Health Organization. WHO HIV/AIDS Programme: Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach 2010.