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A prospective study comparing Total Lymphocyte Count (TLC) and CD4 counts in HIV patients in a resource limited setting in India

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INTRODUCTION

Even after 25 years of the first detection of Acquired Immunodeficiency Syndrome (AIDS), it remains a major health care problem without any cure.[1] Extensive research around the world and the subsequent introduction of highly active antiretroviral therapy (HAART) has produced dramatic reduction on morbidity, mortality and health care utilization. HAART regimens have revolutionized the treatment of human immunodeficiency virus (HIV), which consistently results in sustained suppression of HIV-1 RNA replication, resulting in gradual increases in CD4 T-lymphocyte count, sometimes to normal levels. Durable suppression of viral replication and the accompanying increases in CD4 count, reverse HIV disease progression, even in persons with advanced HIV infection.

Despite these great advancements, HAART poses a number of challenges. Many of the effective regimens are complex and have major adverse effects leading to problems with patient compliance and drug resistance. These problems continue to limit the effectiveness of HAART and present major challenges in managing HIV infection. Further, cost and intellectual property protections effectively limit access to antiretroviral drugs in countries most heavily affected by HIV.[2] Atripla™, (efavirenz 600mg, emtricitabine 200mg, tenofovir disoproxil 245 mg) a fixed dose, once a day tablet for treating HIV-1 infection in adults is a promising step to improve patient compliance.[3]

A HAART regimen should be able to delay disease progression, prolong survival and maintain quality of life through maximal viral suppression. Considering the conditions and challenges of a resource poor country, the goals of HAART are given in table 1.

Various guidelines have been published on HAART to make sure that the therapy is appropriate. These guidelines give clear information on indications to start HAART [4,5]

Test for CD4 count is too costly for resource poor countries. As highly active antiretroviral therapy (HAART) is now becoming available to large populations of HIV-infected patients in resource-poor countries, resource-appropriate markers need to be identified for clinicians to use in deciding when to initiate HAART. Also, monitoring individuals with HIV infection/AIDS involves the use of expensive tools, including CD4, which are not readily available in resource- limited settings. Previous studies suggested the absolute lymphocyte count (ALC) or total lymphocyte count (TLC, i.e. ALC plus all large lymphocytes such as lymphoblast or reactive lymphocytes) might be useful in identifying patients who would benefit from initiating prophylaxis for AIDS-related opportunistic infections. [6,7,8] Due to the lack of enough financial as well as qualified personnel support, initiation and monitoring of HAART based on CD4count becomes a significant challenge in India. Patients may have to wait for more than two months to get the CD4 count results even in National AIDS Control Organization (NACO) supported centers. This is a major obstacle in the proper management of HAART in a country like India where the HIV estimate for the year 2005 is 5.21 million infections and it is growing at a rapid scale. [9]

Initiation and monitoring of HAART based on TLC instead of CD4 count is particularly significant in a developing country like India. In India the cost of CD4 count by flow cytometry is approximately1500 Indian Rupees (INR) ($30.00 US) while the cost for TLC is less than 40 INR (<$1.00US). In a country where the

Indian Journal of Pharmacy Practice
Received on 09/09/2008 Modified on 19/09/2008
Accepted on 23/09/2008

per capita income is 34,825 Indian Rupees ($820.00 US)[10,11] and the per capita expenditure for health by government is about $27.00 US [12], this cost difference has a significant impact in treating AIDS patients.

WHO recommends CD4 count to monitor the patient's clinical status in AIDS cases; but in resource limited setting where there is no data on CD4 is available, TLC can be used as a substitute for symptomatic patients. According to the guideline by WHO for scaling up antiretroviral therapy in 2003, CD4 testing is the tool for making decision on HAART therapy and monitoring; however if this is not available, one can use TLC count less than 1200 /mm^3 as surrogate marker for CD4 count less than 200 cells/mm^3. [13]

This recommendation was based on rigorous evaluation of data obtained almost exclusively from developed countries.[6,7,8,14]

However, there are also studies indicating that substitution of TLC for CD4 count monitoring might not be a good clinical decision. A study conducted in Nigeria evaluating the reliability of total lymphocyte count as a substitute for CD4 cell count found that total lymphocyte count is not suitable for CD4 cell count in a resource limited setting. The sensitivity of total lymphocyte count as a predictor of CD4 cell count was 45.5% and the specificity was 62.2%. The study's author concluded that if WHO recommendation of 1200 cells/ mm^3 were used to determine treatment, 1 in 3 individuals would have been deprived of needed treatment. So in that particular setting **TLC is not a reliable predictor of CD4 cell count in HIV-infected individuals.**[14]

Based on these differing evidence accounts, this study's primary aim was to analyze the reliability and clinical utility of total lymphocyte count as a surrogate marker for CD4 count and to check the reliability of WHO recommendation in resource limited setting in India. The main objective was to calculate the sensitivity and specificity values of using total lymphocyte count as a surrogate marker for CD4 count and to determine if there is a linear correlation between the two parameters.

Methodology

Study setting and data collection

The study was conducted in the Antiretroviral Treatment (ART) unit in the Department of Medicine of Government Medical College Hospital, Thiruvanathapuram, Kerala, India. This is a 1500 bed tertiary care teaching hospital with specialty and super specialty clinical setting. ART unit works all days from 9.00 am to 12.00 noon except Sunday. The ART center is funded by the National AIDS Control Organization (NACO) of India and subsequently, NACO is funded by various programs under WHO. All patients attending ART clinic gets treatment and related tests including CD4 count test free of cost.

Study data was collected from patients who satisfied the inclusion and exclusion criteria and gave consent for the study. The inclusion criteria consisted of patients who are 18-65 years of age and patients who are receiving triple regimen of HAART from the clinic during the study period. Exclusion criteria were pediatric patients less than 18years of age and pregnant patients. Permission to conduct the study in the ART unit was given by the Head of the Department of Medicine. The study was approved by the Human Ethical Committee of Government Medical College, Thiruvananthapuram. An informed consent form, approved by the Human Ethical Committee, was signed by all patients and this process was in accordance with Good Clinical Practice (GCP). The following demographics of the study patients were collected: HAART regimen, age, sex, urban/rural, place and source of infection, disability status, employment status, marital status, and income. CD4 count was recorded from the report from the department of dermatology (CD4 count was ordered from this department). WBC and Total lymphocyte count were obtained from medical laboratory report. (Both CD4 count and TLC count were done on same day).

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to establish the relationship between TLC and CD4 counts. A receiver operator characteristic (ROC) curve was also drawn to determine the best cut-off points. Statistical significance was calculated utilizing linear regression. Paired t-test was utilized to determine statistical significance of pre and post treatment CD4 counts. For all tests, $p<0.05$ was set as the level of significance and all were two-tailed.

Results

Demographics

During the study (6 months, from April to October, 2005) using inclusion and exclusion criteria, 146 patients were found eligible to enroll in the study and out of these 142 patients consented and were enrolled in the study. The demographics of the study population are described in Table 2. Sixty-nine pairs of TLC and CD4 counts were obtained for the study period. Twenty-six patients had pre and post treatment CD4 counts. Total number of HIV

patients taking ART enrolled for the study was 142. The mean age of the population was 37.88 with a standard deviation of 7.24. Total number of patients from rural area was 111 (78%) and from urban area were 31(22%). All patients were detected to have HIV 1 strain. Based on self reporting in the interview conducted by the ART physician and the investigator, the possibility of place and source of infection was recorded. An analysis on the place and source of infection in this study indicated that 55% of the infection occurred from outside Kerala. The source of infections from Kerala was indicated in 56 (39%) of the patients. Of these patients whose place of infection was Kerala, 40 (71%) patients reported that the method of infection was by sexual contact with the male partner. Five persons blamed blood transfusion as the cause but no substantial evidence was present to prove that. Most of the patients (88%) were ambulatory; during the course of the study period; 71% of the non-ambulatory patients improved their status. Among the 142 patients, 85 were non-employed and 46 were employed initially; during the course there was an increase of 7 patients to the employed group. Out of the 142 patients, 71% were in a married relationship, 21% reported as their partner dead and 8% were not married. Sixty-four percent of the patients earned below the Rs. 30,000/year and the government hospital was their only source for the HIV medications.

These 142 patients were administered three drug combinations. Fifty-two patients (37%) were administered zidovudine, lamivudine, and nevirapine; fifty-one patients (36%) were administered lamivudine, stavudine, and nevirapine; and thirty-nine (27%) were administered lamivudine, stavudine, and efavirenz.

Linear regression analysis

Out of these 142 patients only 68 patients had both CD4 count and TLC count. From these 68 patients we collected 69 paired observations of CD4 counts and TLC counts. To find out if linearity exists between the CD4 count and TLC, the linear regression method was utilized. As shown in Figure 1 it is seen that an increase in 1 unit of CD4 correlates to 4.6 units change in TLC, and this was considered to be clinically significant at $p<0.05$

Analysis of pre and post CD4 counts

Out of the 142 patients monitored for the study, only 26 patients had a pre and post treatment CD4 count during the course of the study. Out of the 26 patients 4 patients had a decrease in the post treatment CD4 count. The increase in CD4 in this subset of patients was 107.46 cells/mm^3 (SD: 106.25 cells/mm^3). Paired t-test indicated that this was a significant difference at $p<0.05$.

Sensitivity, Specificity, PPV, NPV, and ROC Analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were conducted in order to find out the suitable range of TLC count which can predict CD4 count. The data from 69 TLC and CD4 values were analyzed to determine sensitivity, specificity, PPV, and NPV, as shown in Table 3. To find out the clinical utility of TLC count as a surrogate for CD4 count, sensitivity, specificity, PPV, and NPV of different groups of CD4 count namely, Cd4 $\leq$400 cells/mm^3, CD4 $\leq$350cells/mm^3, CD4 $\leq$300cells/mm^3, CD4 $\leq$ 250cells/mm^3, and Cd4 $\leq$200cells/mm^3 were compared to TLC groups of TLC $\leq$ 3000cells/mm^3, TLC $\leq$ 2500 cells/mm^3,TLC $\leq$ 2000 cells/mm^3,and TLC $\leq$ 1500 cells/mm^3. The best estimate appears to be that if the TLC count is $\leq$ 3000cells/mm^3, the CD4 count will be $\leq$ 400cells/mm^3 with sensitivity of 75.8, specificity of 100, PPV of 100, and NPV of 31.8, which is also seen in the ROC curve (Figure 2). The ROC curve was also evaluated to determine the best cut-point and the results indicated that TLC counts are best associated with predicting CD4 of <400cells/mm^3.

Discussion and Conclusion

Initiation and monitoring of HAART based on CD4 count becomes a barrier for the widespread use of HAART medication in resource poor setting. Even in the financially supported and well equipped centers, proper and timely CD4 testing has been a challenge because of large number of patients. In a country where 5.21 million (based on 2005 NACO data) infections are reported and this number is growing rapidly, CD4 testing becomes practically impossible to many of these patients. An alternative testing system which is easily available and less costly is highly indicated. Effort of various governments and organizations brought down the cost HAART regimens dramatically especially in the developing countries. To fully utilize this advancement, cheap and equally reliable monitoring methods have to be implemented.

Much research has been conducted on this area around the world in the quest for an alternative method. As a result , a number of alternative methods for monitoring antiretroviral therapy in resource limited setting have been proposed, such as HAART administration by directly observed therapy programs, enumeration of CD 4count by simpler and less expensive methodologies and the development of a low cost technique to track p24 antigen as a surrogate marker for viral load.[15,16]

Though these methods are under intensive research, WHO recognizes the immediate need for a low cost method for scaling up of antiretroviral therapy in countries where spreading rate of HIV infection is startling. Monitoring of HAART is a must to minimize the more dangerous possibility of developing drug resistant which may cause a catastrophe to the already **less than optimal HAART program in developing and poor countries.**[17,18]

This study was conducted in the ART unit (in the Department of Medicine) of Government Medical College Hospital, Thiruvanathapuram, Kerala, India. This hospital is funded by NACO in support of WHO. Even in such a center CD4 testing is irregular for the patients. Because of the high patient load they may have to wait up to 3 months to get their CD4 count done. This shows the importance of a reliable surrogate marker for CD4 count for the better administration of HAART. This study was designed to check the reliability and clinical utility of TLC as a surrogate marker for CD4 count. Along with this we also compared out results with WHO recommendation.

In this study, from 68 patients we collected 69 paired observations of CD4 counts and TLC. From this data we find out the direction of change of TLC count with increase in CD4 count is positive. In addition to correlating direction of change between TLC and CD4 count, this study also examined the average change in TLC per unit change in CD4 count. In this study population, the average individual specific mean change in TLC per 1 CD4 cell/mm^3 was 4.6 cells/mm^3. To find out the best sensitivity and specificity correlation we analyzed various combinations of paired observations of CD4 count and TLC. Based on the clinical utility and best descriptive analysis value we came to the most suitable CD4 count TLC combination. The best estimate appears to be that if the TLC count is $\leq$ 3000cells/mm^3, the CD4 count will be $\leq$ 400cells/mm^3 which is also seen in the ROC curve. Though the combination of CD4 count $\leq$ 200cells/mm^3 & TLC count $\leq$2500cells/mm^3 is most clinically relevant in the view of WHO recommendation, it lacks reasonable sensitivity and specificity (56.7 & 76.7 respectively).

In this study we had 39 patients with CD4 count less than 200cells/mm^3. When the TLC was compared (those having 1500 cells/mm^3 or less and more than 1500 cells/mm^3,close to the WHO recommendation.) only 6 patients had less than 1500 cells/mm^3 TLC count. Though WHO recommends TLC less than 1200 cells/mm^3 can be used as a predictive for CD4 count less than 200 cells/mm^3, in this study population, it does not seem to be predictive. Had the WHO recommendation followed, only 15.4% of patients would have got treated and the rest 84.6% of patients were left untreated. This result could be due to the small sample size of the study. But the positive correlation of CD4 count with TLC promises further investigation for appropriate levels of TLC which would predict more precisely the CD4 level less than 200 cells/mm^3. Further more this study was conducted for 6 months which is not enough to recruit more number of patients for the study. Total number of paired observations of CD4 count and TLC in this study is 69, and only 26 patients had a pre and post treatment CD4 count.

Table – 1: Goals of HAART[4]*

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Maximal and durable suppression of viral replication (measured by viral load assays)
Restoration and/or preservation of immune function
Reduced human immunodeficiency virus (HIV)-related morbidity and mortality
Improved quality of life
Limit the likelihood of viral resistance to preserve future treatment options
Provide maximum access to HAART regimens

Table –2: Study Population Demographics

Demographic Characteristics	N (%)
Males	91 (64%)
Females	51 (36%)
Age Mean (SD)	37.88 (7.24)
Rural	111 (78%)
Urban	31(22%)
Place of Infection	
Kerala	56 (39%)
Outside Kerala	77(55%)
No Data	9(6%)
Source of Infection	
Spouse	40 (28%)
Other	102 (72%)
Disability Status	
Ambulatory	125 (88%)
Non-ambulatory	17 (12%)
Improved During Study	12 (71%)
Employment Status	
Employed	48 (34%)
Unemployed	94 (66%)
Marriage Status	
Married	130 (92%)
Not-Married	12 (8%)
Income	
More than 30000 INR per year	51 (36%)
Less than 30000 INR per year	91 (64%)

Figure 1: Linear Regression of Lymphocytes versus CD4 counts (p<0.05)

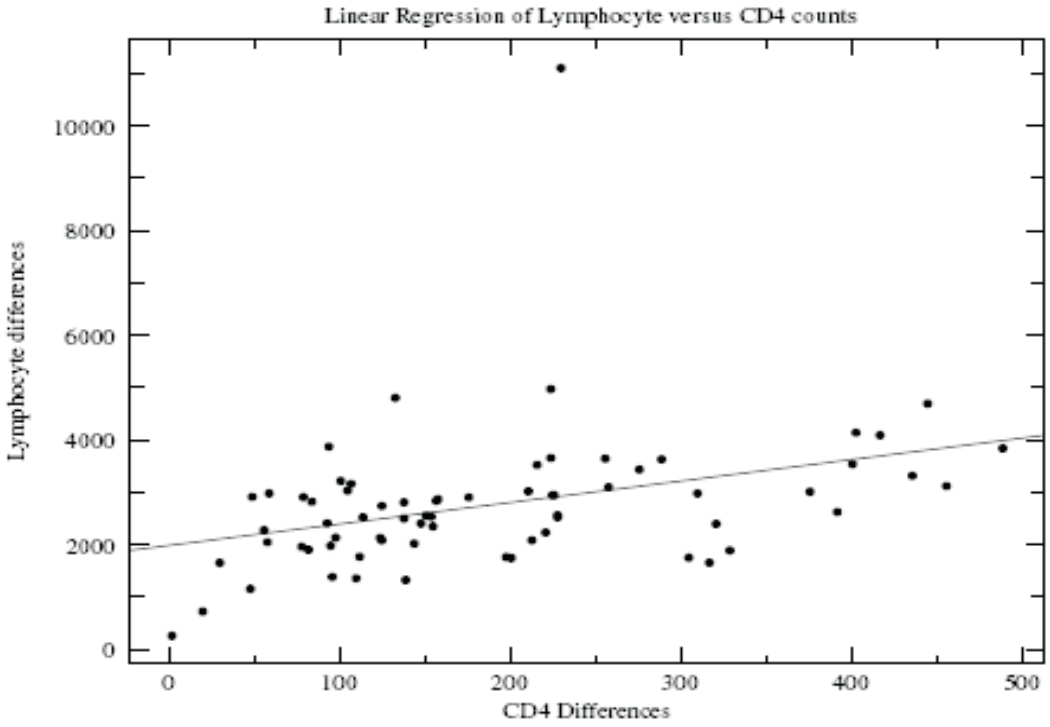
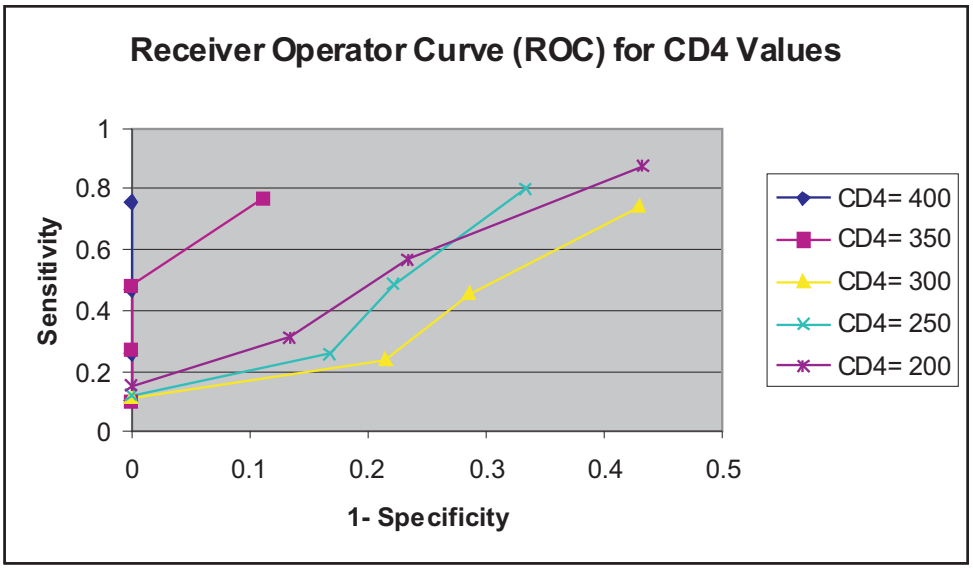


Table – 3: Sensitivity, Specificity, PPV, and NPV Results

CD4 count (cells/mm^3)	TLC range (cells/mm^3)	N	Sensitivity	Specificity	PPV	NPV
= 400	=3000	34	75.8	100	100	31.8
	=2500	29	46.8	100	100	17.5
	=2000	16	25.8	100	100	13.2
	=1500	6	9.7	100	100	11.1
= 350	=3000	46	76.7	88.9	97.9	36.4
	=2500	29	48.3	100	100	22.5
	=2000	16	26.7	100	100	17
	=1500	6	10	100	100	14.3
= 300	=3000	41	74.5	57.1	87.2	36.4
	=2500	25	45.5	71.4	86.2	25
	=2000	16	23.6	78.6	81.3	20.8
	=1500	6	10.9	100	100	22.2
= 250	= 3000	41	80.4	66.7	87.2	54.5
	= 2500	25	49	77.8	86.2	35
	= 2000	13	25.5	83.3	81.3	28.3
	= 1500	6	11.8	100	100	28.6
= 200	= 3000	34	87.2	56.7	72.3	77.3
	= 2500	22	56.4	76.7	75.9	57.5
	= 2000	12	30.8	86.7	75	49.1
	=1500	6	15.4	100	100	47.6

Figure 2: Receiver Operator Curve (ROC) for CD4 Values



References

1. Fletcher V, Kakuda NK, Collier AC. Human immunodeficiency virus infection. In: Dipiro TJ, Tallbert LR , Yee CG, Matzke RG, Wells GB, Posey LM, editors. Pharmacotherapy: A Pathophysiological approach. 5th ed. United States of America: McGraw-Hill Medical publishing division; 2002: 2151-2174.
2. Kojic EM, Carpenter CJ. Initiating antiretroviral therapy. Available from University of California, San Francisco, CA, (US); hivinsite; 2006.
3. U.S Food and drug administration (US).FDA approves the First Once-a-Day Three-Drug Combination Tablet for Treatment of HIV-1. Silver Spring: FDA news;2006.
4. New York State Department of health (US). Antiretroviral therapy. New York :2008.
5. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. National Institute of Health, Maryland, (US); aidsinfo; October 10, 2006.
6. Jacobson MA, Liu L, Bashi HK , Deeks S, Hecht FM, Kahn J. Absolute or total lymphocyte count as a marker for the CD4 T lymphocyte criterion for initiating antiretroviral therapy. AIDS 2003;17: 917-919.
7. Spacek LA, Griswold M, Quinn TC, Moore RD. Total lymphocyte count and hemoglobin combined in an algorithm to initiate the use of highly active antiretroviral therapy in resource-limited settings. AIDS 2003; 17:1311–1317.
8. Mahajan AP, Hogan JW, Snyder B, Kumarasamy N, Mehta K, Solomon S. et.al. Changes in Total Lymphocyte Count (TLC) as a surrogate for changes in CD4 count following initiation of HAART: Implications for monitoring in resource – limited settings. J Acquir Immune Defic Syndr 2004; 36: 567-575.
9. National AIDS control organization (India) HIV/AIDS epidemiological surveillance & estimation report. New Delhi:2005
10. World Bank (US).World development indicators, India- data and statistics. Washington: Data profile; 2008
11. Reserve bank of India (India).RBI reference rate on 31/07/08.Mumbai:Exchange rate; 2008
12. World heath organization (Switzerland).Selected national health accounts indicators. Geneva: The world health report; 2006.
13. World heath organization (Switzerland).Scaling up of Antiretroviral Therapy in resource limited setting. Geneva: Guidelines for a public health approach;2002
14. Akinola N O, Olasode O, Adediran I. A, 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 Resource-Limited Setting. Clin Infec Diseases. 2004; 39:579–581.

15. Farmer P, Léandre F, Mukherjee JS, Claude M, Nevil P, Smith-Fawzi MC. et.al. Community-based approaches to HIV treatment in resource–poor setting. Lancet 2001;358:404-409
16. Mitty JA, Stone VE, Sands M, Macalino G, Flanigan T. Directly observed therapy for the treatment of people with human immunodeficiency virus infection: a work in progress. Clin infec diseases 2002;34:984-990.
17. Harries AD, Nyangulu DS, Hargreaves NJ, Kaluwa O, Salaniponi FM. Preventing antiretroviral anarchy in Sub-Saharan Africa. Lancet 2001; 358:410-414.
18. Weidle PJ, Mastro TD, Grant AD, Nkengasong J, Macharia D. HIV/AIDS treatment and HIV vaccines for AFRICA. Lancet 2002; 359: 2261-67.