Research Paper

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Total Lymphocyte count - Can it be the replacement to CD4 count for deciding Anti-retroviral therapy in HIV positive patients in resource constraint settings?

Medical Science

KEYWORDS : Total Lymphocyte count, Absolute Lymphocyte count, CD4 count, Anti-retroviral therapy, HIV

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ABSTRACT

CD4 count is a standard measure of immunodeficiency in adults infected with HIV to initiate and monitor highly active Anti-retroviral therapy (HAART). WHO has recommended that, absolute lymphocyte count (ALC) of <1200/µl can substitute CD4 cell count of ≤ 200/µl in resource constrained countries throughout the world. This study was undertaken to evaluate the correlation of TLC to CD4 count and to determine the range of TLC cutoff for initiating HAART. In this prospective observational study a single blood sample was withdrawn from 156 HIV positive patients for both TLC&CD4 count. Spearman correlation between TLC and CD4 cell count was assessed. Sensitivity, Specificity, positive and negative predictive values of various TLC cut-off were computed for CD4 count (350cells/µl). These data were analysed by plotting receiver operating characteristic curve. Good correlation was noted between TLC and CD4 (r=0.583). TLC of 2000cells/µl had a sensitivity of 77% and specificity of 71% for predicting CD4 cell counts <350 /µl. TLC is simple and inexpensive surrogate marker for deciding on ART in a underserved resource-poor setting.

Introduction:

The number of people infected with HIV is increasing globally and the burden is more in the developing countries [1]. The AIDS epidemic has resulted in tremendous costs in terms of diagnosis and treatment in the healthcare setup. In well-resourced settings, the decision to initiate ART and monitor patients with HIV infection/AIDS is based on CD4 testing which requires the use of expensive tools .Whereas, in resource –limited settings, CD4 testing is very expensive and is not widely available because of lack of sophisticated equipment and trained personnel. In April 2002, the WHO has recommended the usage of Total lymphocyte count of <1000-1200 lymphocytes/cu.mm as a threshold value to initiate ART [2]. There are various studies which compared the utility of TLC as a predictor of CD4 count with contradictory results. There have been studies showing good correlation between Absolute lymphocyte count and CD4 counts[3,4,5,6] where as few others showing ALC as only a weak surrogate marker for monitoring highly active Antiretroviral therapy[7,8]. As per the latest guideline ART should be initiated in all patients with a history of an AIDS defining illness or the CD4 count <350 cell/µl. Hence this study is aimed to evaluate the correlation of Total lymphocyte count, to CD4 count in HIV patients in our setup and to determine the range of TLC cutoffs predicting the CD4 value of <350cells/µl.

Materials and Methods

Study population

Study design and setting: In this prospective observational study 156 HIV-positive patients, attending our ART Centre, on June & July of 2012 were selected.

CD4 & Total lymphocyte count analyses

Using standard precautions, single sample of 4 ml of venous blood was collected for complete blood count and CD4 cell count in a K2 EDTA BD vacutainer. The CD4 enumeration was done using the single platform PARTEC Cyflow counter, by strictly following the manufacturer's instructions. Internal quality control was performed as per the manufacturer's recommendations. External quality control was performed through an external quality assurance program with NARI (National AIDS Research Institute), Pune, India. CBCs were performed by using a Sysmcx Kx21 Hematology analyzer. TLC was derived from the CBC by multiplying lymphocyte percentage by the white blood cell count.

Statistical methods

Quantitative variable were expressed as mean with standard deviation and qualitative variable were expressed as numbers with percentages. Correlation between TLC,CD4 cell count and TLC&CD4% (all as continuous variable) were determined using Spearman's correlation coefficient.

The sensitivity, specificity, positive predictive value(PPV), negative predictive value(NPV) of the TLC cut-off in predicting severe immune-suppression as defined by CD4 thresholds were determined. Data was analysed to determine the optimal TLC cutoff that provide highest sensitivity and specificity for predicting CD4 count <350cells/µl.Statistical analysis was done using SPSS for windows version 16.0.Numerical data were calculated using Microsoft Excel and analysed.

Raising cutoff values of TLC to improve diagnostic utility

Since the recommended WHO TLC cutoff for identifying severe immune suppression had low specificity we explored whether raising the TLC cutoff would improve the specificity without unduly compromising sensitivity. We plotted receiver operating characteristic curve for simultaneously evaluating how sensitivity and specificity vary as the cutoff point is changed.

Results

The mean age of the study population was 38 years. Of the 156 patients, 87 were males and 69 were females. The mean CD4 count and mean TLC were 399cells/µl and 2822 cells/µl, respectively. Table 1 summarizes the various hematological parameters. Table 2&3 gives the distribution of CD4 count with respective TLC of <2000cells/µl and <1200cell/µl respectively.

The correlation between TLC and a CD4 cell count was highly significant (Spearman correlation coefficients of r=0.583, P<0.001). The positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, and likelihood ratio of TLC for CD4 count <350 cells/µl and <200cells/µl in all counts are given in [Table 4]. The ROC [Figure 1] curve was drawn between ALC and CD4 <350cells/µl and it was found significant area under the curve was .78. Our study did not have good correlation between CD4 % and ALC [Figure2] although there was a good correlation between ALC and CD4 cell count as also depicted in scatter diagram[Figure3]

Discussion

In ideal situations, HIV-infected persons are followed up longitudinally with serial CD4 cell counts to determine disease progression, risk for opportunistic infection and the need for prophylactic or therapeutic intervention.

Thus the use of TLC in predicting the CD4 count in HIV patients can be discussed in 2 clinical circumstances: first, disease staging and initiation of ART or opportunistic-infection prophylaxis

and second monitoring the response to ART [9].

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[10]

The latest WHO guidelines on anti-retroviral therapy recommend that all adolescents and adults including pregnant women with HIV infection and CD4 counts of ≤350 cells/cu.mm should start ART, regardless of the presence or absence of clinical symptoms [9].We therefore analysed the data from those patients with CD4 counts of <350 cells/µl to evaluate the performance of TLC.

A.Kakar et al found that ALC of 1520/µl was more sensitive than 1200/µl with higher sensitivity of 78 % for predicting the CD4 cell count of <200/µl[11].Whereas a study by S.Sreenivasan showed ALC of 1400cells/µl had a sensitivity of 71.08% and specificity of 78.26% for predicting CD4 cell counts less than 200 cells/µl[12].

A retrospective cohort study by Mahajan.A.P. et al showed that in the first 24 months of HAART, the sensitivity of a TLC increase, as a marker for CD4 count increase over the same time period ranged from 86-94% and the specificity ranged from 80-85%[3,13].

Several studies showed a good correlation between TLC and CD4 cell count, however the WHO recommended TLC cutoff of 1200 did not identify the majority of WHO stage 2 and 3 patients with CD4 counts less than 200 cells/µl.[4]

In our study we found that the TLC cutoff of <1200cells/µl as suggested by WHO for predicting CD4 cell count of <200/µl had a sensitivity, specificity ,positive predictive value (PPV), negative predictive value (NPV), of 93%, 48%,86% and 68% respectively.

Raising cutoff value of TLC to 2000cells/µl for predicting CD4 cell count <350/µl had improved the diagnostic utility with a sensitivity, specificity, positive predictive value and negative predictive value of 77%,71%,76%and 73% respectively with a good Spearman correlation of 0.583.

Several other authors have also suggested a higher cutoff value of TLC.[14,15,16,17] A similar correlation has also been suggested in studies done in India other parts of the world.[11,12] . A study by F.I Buseri suggested that ALC cutoff of <2300cells/µl for predicting CD4 cell count of <350/µl [18] with 83% sensitivity and 62% specificity. The advantage of using a higher TLC is that it would help reduce failure rate of identifying patients who might benefit from ART or OI prophylaxis. However this approach has the disadvantage of early initiation of ART in patients for whom it is not required.

In contrast to the CD4 count, there was a very poor correlation between TLC and CD4 %[r= - .082].Similar weak correlation was obtained in some other studies.[19]

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 TLC test.[20]

In terms of comparative costs, a single CD4 cell count may be 30-50 times more expensive than a complete blood cell count[9]. In India the cost of CD4 cell count is tremendous despite being subsidized by various organizations and facilities to measure this are not available universally. Such huge amount of money if saved can be invested for supplying medicine to HIV/AIDS patients and improving their quality of life. The main drawback of our study was a small sample size, therefore, to make these results reproducible, larger studies may be done and the results confirmed further.

Our findings suggest that TLC could have clinical utility in determining when HIV-infected patients in resource-poor settings should initiate HAART. However, more studies are required in resource-limited settings with larger study groups to ascertain the usefulness of TLC as a surrogate for CD4 counts both before and after HAART initiation.

Table: 1 The demographic and laboratory profile of patients included in the study n156

	Age	Total Lymphocyte count	Neutrophils	Lymphocytes	Eosinophils	CD4 (cells/µl)	CD4%
Minimum	13	405	24	9	2	22	1.85
Maximum	69	6156	96	60	23	1887	42.4
Range	56	5751	72	51	21	1865	40.5
Mean	38	2822.4	58.2	36.4	5.97	399	16.22
Std.deviation	9.2	1775.9	12	12.5	4.81	257	8.79
Median	37.5	2172	57	38	5	376.5	15.88
Std.error of mean	0.738	142.29	.9663	.1	0.385	20.59	.704

Table: 2 The Distribution of CD4 cell count with respective TLC2000/µl

	CD4>350	CD4<350
TLC>2000/µl(n=77)	59(76%)	18(23.3%)
TLC<2000/µl(n=79)	21(26.5%)	58(73.4%)

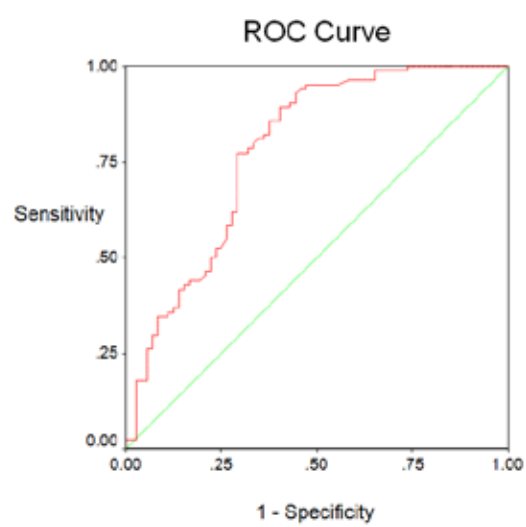
Table: 3 The Distribution of CD4 cell count with respective TLC1200/µl

	CD4>200	CD4<200
TLC>1200/µl(n=131)	113(86%)	18(13.7%)
TLC<1200/µl(n=25)	8(32%)	17(68%)

Table:4 Taking CD4 cell count as the gold standard, correlation of TLC with CD4 cell count.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
TakingTLC<2000				
CD4 <350	77	71	76	73.4
Taking TLC<1200 CD4<200	93	48	86	68

Figure 1: ROC curve between TLC & CD4 cell count <350cell/µl



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Figure 2: Scatter diagram between ALC & CD4%

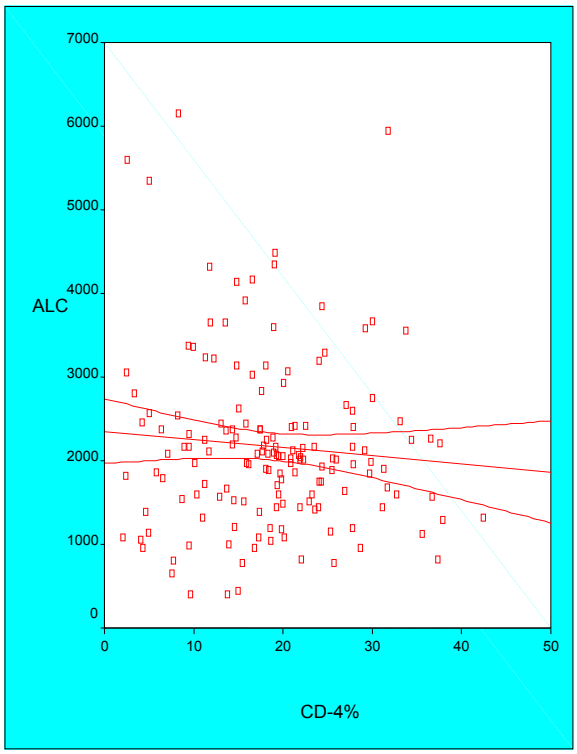
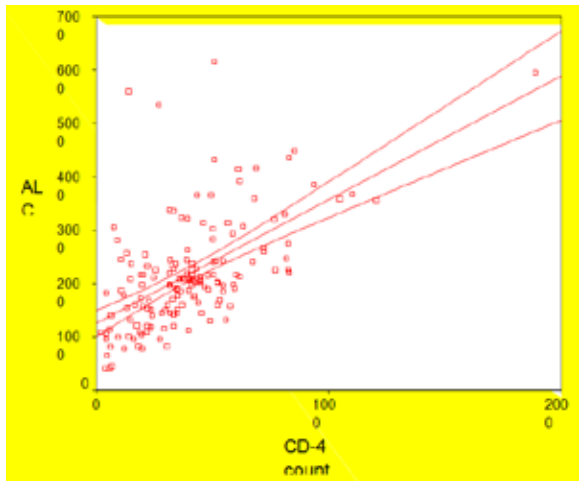


Figure 3: Scatter diagram between ALC & CD4 cell count



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