

Comparing Absolute Lymphocyte Count to Total Lymphocyte Count, as a CD4 T Cell Surrogate, to Initiate Antiretroviral Therapy

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ABSTRACT

Background: The high cost of CD4 count estimation in resource-limited settings is a major obstacle in initiating patients on highly active antiretroviral therapy (HAART). Thus, there is a need to evaluate other less expensive surrogate markers like total lymphocyte count (TLC) and absolute lymphocyte count (ALC). **Objectives:** To evaluate the correlation of TLC and ALC to CD4 count. To determine a range of TLC and ALC cut-offs for initiating HAART in HIV-infected patients in resource-limited settings. **Materials and Methods:** In a prospective observational cohort study of 108 ART-naive HIV-positive patients, Spearman correlation between ALC and CD4 cell count, and TLC and CD4 cell count were assessed. Sensitivity, specificity, positive and negative predictive values of various ALC and TLC cut-offs were computed for CD4 count <200 cells/cu.mm. **Results:** Good correlation was noted between ALC and CD4 ($r=0.5604$) and TLC and CD4 ($r=0.3497$). ALC of 1400 cells/cu.mm had a sensitivity of 71.08% and specificity of 78.26% for predicting CD4 cell counts less than 200 cells/cu.mm. Similarly, TLC of 1200 cells/cu.mm had a sensitivity of 63.41% and specificity of 69.57%. **Conclusion:** Either ALC or TLC may be helpful in deciding when to initiate antiretroviral therapy in resource-poor settings, though ALC is better than TLC as a surrogate for CD4 counts.

Key Words: Absolute lymphocyte count, Total lymphocyte count, CD4 counts, HAART, Surrogate marker

INTRODUCTION

In India, the National AIDS Control Organization (NACO) has initiated the introduction of inexpensive and generic highly active anti-retroviral therapy (HAART).^[1] Current recommendations in western countries for initiation and monitoring of HAART are based on CD4⁺ T-cell counts and plasma human immunodeficiency virus (HIV) RNA levels.^[2] However, these methods are expensive. Due to this, the World Health Organization (WHO) stipulates that CD4 count testing is “desirable” but not essential for HAART use in resource-limited settings.^[3]

Several studies have demonstrated the usefulness of absolute lymphocyte count (ALC) or total lymphocyte count (TLC), (i.e. ALC plus all large lymphocytes such as lymphoblasts or reactive lymphocytes) in identifying patients who would benefit from initiating prophylaxis for acquired immunodeficiency syndrome (AIDS)-related

opportunistic infections.^[4-7]

We conducted this study to evaluate the correlation of TLC and ALC to CD4 count and to determine a range of TLC and ALC cut-offs for initiating HAART in HIV-infected patients, as there are fewer published studies on this subject, from resource-limited settings.^[8,9]

MATERIALS AND METHODS

Study design and setting

A prospective observational cohort study involving 108 HIV-positive patients, attending our ART Centre, from August 20 2006 onwards were selected. The duration of study period was 1 year from August 20, 2006 to August 19, 2007.

Selection and description of participants

After taking an informed consent (for HIV testing), these individuals, voluntarily attending our ICTC at the Department of Microbiology (or any of the Government designated ICTCs), underwent pre-test counseling by male or female ICTC counselors, followed by HIV testing as per the strategy III of the NACO guidelines (for HIV testing)^[10]. After post-

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test counseling, those found HIV positive were referred to the ART Centre, K.R. Hospital, Mysore Medical College and Research Institute, Mysore, where they underwent pre-ART counseling. After clinical evaluation, informed consent was taken from these patients and they were enrolled into the study if they satisfied the inclusion criteria. Those found eligible for ART as per the WHO guidelines^[11] were started on anti-retroviral therapy. This study was approved by the ethics Committee of our institution.

Inclusion criteria

The individuals should be above 18 years of age, they should be proven to be HIV-positive and they should not be on prior anti-retroviral therapy (ART).

Exclusion criteria

HIV-seronegative individuals and those on prior ART were excluded.

CD4, total lymphocyte count and absolute lymphocyte count analyses

Using standard precautions, 4 ml of venous blood was collected between 9 am to 12 noon using two 2 ml K3-EDTA Vacutainers (BD), one for CD4 testing and the other for complete blood counts (CBCs).

The CD4/CD3 enumeration was done using the single platform BD FACS Calibur™ machine (Becton, Dickinson and Company, San Jose, United States of America), by strictly following the manufacturer's instructions. Internal quality control was performed with process controls using the manufacturer's recommendations. External quality control was performed through an external quality assurance program with NARI (National AIDS Research Institute), Pune, India. This machine also gave the ALC along with the CD3 and CD4 counts.

CBCs with differential were performed by using a Sysmex K21 Hematology analyzer (Sysmex Corporation, Kobe, Japan). TLC was derived from the CBC by multiplying lymphocyte percentage by the white blood cell count.

Statistical analysis

Spearman correlations between ALC and CD4 cell count and TLC and CD4 cell count were assessed. Sensitivity, specificity, positive predictive value, and negative predictive values of various ALC and TLC cut-offs were computed for CD4 count <200 cells/cu.mm.

All statistical analyses were performed using SPSS software (version 16.0, SPSS, Chicago, USA).

RESULTS

The mean age of this cohort was 34.69 years. Of the 108 patients, 71 (65.74%) were males and 37 (34.26%) were females. 70 (64.8%) of the patients were from rural areas. 90 (83.4%) of the patients stated that they acquired HIV through heterosexual intercourse.

During the study period, 108 ALC, TLC, and CD4 counts were generated from as many patients. The mean CD4 count and mean TLC at baseline were 129.65 ± 76.84 cells/cu.mm and 1262.96 ± 738.98 cells/cu.mm, respectively. The mean ALC at baseline was 1347.9 ± 760.57 cells/cu.mm.

The correlations between ALC and a CD4 cell count, and between TLC and a CD4 cell count were highly significant (spearman correlation coefficients of $r=0.5604$, $P<0.0001$ and 0.3497 , $P<0.001$ respectively). The positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, and likelihood ratio of ALC and TLC for CD4 count <200 cells/mm³ in all paired counts are given in Tables 1a and 1b, respectively.

An ALC of 1400 cells/cu.mm or less had maximal combined sensitivity, 71.08% (95% CI: 60.11-80.51%), and specificity, 78.26% (95% CI: 56.26-92.53%), for a CD4 cell count of less than 200 cells/cu.mm. However, a TLC cut-off value of 1200 cells/cu.mm μ l had a sensitivity of 63.41% [(95% CI: 52.09 to 73.74%) and a specificity of 69.57% (95% CI: 47.10 to 86.80%), with $P=0.0081$, considered very significant].

Notably, ALC and TLC of less than 800 cells/cu.mm had a positive predictive value of 100.00% and 96.15%, respectively, for a CD4 cell count <200 cells/cu.mm.

Similarly, the (PPV, NPV, sensitivity, specificity, and likelihood ratio of ALC and TLC for CD4 count 200-350 cells/cu.mm in all paired counts are given in Tables 2a and 2b, respectively.

DISCUSSION

In this study, we have demonstrated that both TLC and ALC can be used in the place of CD4 count as a routine marker of immune status.

In this cohort of Indian patients, there was a good correlation between TLC and CD4 count by Spearman rank order correlation ($r=0.3497$), indicating a moderately positive association between TLC and CD4 counts. However, Spearman correlations between TLC and CD4 count reported in North America ($r=0.77$)^[12], England ($r=0.76$)^[13], and India ($r=0.744$)^[9] were higher, when compared with this

Table 1a: Positive predictive value, negative predictive value, sensitivity and specificity of absolute lymphocyte count for CD4 count <200 cells/cu.mm in all paired counts (n=108)

ALC (cells/mm ³)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR
<2000	79.38	27.27	90.59	13.04	1.042
<1900	79.79	28.57	88.24	17.39	1.068
<1800	80.22	29.41	85.88	21.74	1.097
<1700	81.11	33.33	85.88	26.09	1.162
<1600	80.00	26.09	80.00	26.09	1.082
<1500	80.25	25.93	76.47	30.43	1.099
<1400	92.19	42.86	71.08	78.26	3.270
<1300	95.00	43.48	68.67	86.96	5.265
<1200	94.34	37.74	60.24	86.96	4.618
<1100	97.78	36.07	53.01	95.65	12.193
<1000	97.50	33.33	46.99	95.65	10.807
<900	96.97	30.14	38.55	95.65	8.867
<800	100.00	29.11	32.53	100.00	—*

ALC=Absolute lymphocyte count; PPV=Positive predictive value; NPV=Negative predictive value; LR=Likelihood ratio; *Likelihood ratio could not be calculated as the specificity was 100%

Table 2a: Positive predictive value, negative predictive value, sensitivity and specificity of absolute lymphocyte count for CD4 count 200 to 350 cells/cu.mm in all paired counts

ALC (cells/mm ³)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR
<2000	20.62	72.73	86.96	9.412	0.9599
<1900	20.21	71.43	82.61	11.76	0.9362
<1800	19.78	70.59	78.26	14.12	0.9113
<1700	18.89	66.67	73.91	14.12	0.8606
<1600	20.00	73.91	73.91	20	0.9239
<1500	19.75	74.07	69.57	23.53	0.9097
<1400	7.813	57.14	21.74	28.92	0.3058
<1300	5.00	56.52	13.04	31.33	0.1899
<1200	5.66	62.26	13.04	39.76	0.2165
<1100	2.22	63.93	4.35	46.99	0.08202
<1000	2.5	66.67	4.35	53.01	0.09253
<900	3.03	69.86	4.35	61.45	0.1128
<800	0	70.89	0	67.47	0

ALC=Absolute lymphocyte count; PPV=Positive predictive value; NPV=Negative predictive value; LR=Likelihood ratio

Table 1b: Positive predictive value, negative predictive value, sensitivity, and specificity of total lymphocyte count for CD4 count <200 cells/cu.mm in all paired counts (n=108)

TLC (cells/mm ³)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR
<2000	68.18	27.27	90.36	7.89	0.9811
<1900	69.52	28.57	87.95	11.11	0.9895
<1800	79.78	29.41	85.54	21.74	1.093
<1700	80.68	33.33	85.54	26.09	1.157
<1600	79.52	26.09	79.52	26.09	1.076
<1500	79.75	25.93	75.90	30.43	1.091
<1400	79.73	25.00	71.08	34.78	1.090
<1300	82.35	27.03	67.47	45.45	1.237
<1200	88.14	34.78	63.41	69.57	2.084
<1100	88.89	33.33	58.54	73.91	2.244
<1000	92.86	30.16	46.99	86.36	3.446
<900	94.44	29.17	40.00	91.30	4.600
<800	96.15	27.50	30.12	95.65	6.928

TLC=Total lymphocyte count; PPV=Positive predictive value; NPV=Negative predictive value; LR: Likelihood ratio

Table 2b: Positive predictive value, negative predictive value, sensitivity and specificity of total lymphocyte count for CD4 count 200 to 350 cells/cu.mm in all paired counts

TLC (cells/mm ³)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	LR
<2000	21.05	72.73	86.96	9.64	0.9623
<1900	20.65	71.43	82.61	12.05	0.9392
<1800	20.22	70.59	78.26	14.46	0.9149
<1700	19.32	66.67	73.91	14.46	0.8641
<1600	20.48	73.91	73.91	20.48	0.9295
<1500	20.25	74.07	69.57	24.10	0.9165
<1400	20.27	75.00	65.22	28.92	0.9175
<1300	17.65	72.97	54.55	32.53	0.8084
<1200	11.86	65.22	30.43	36.59	0.4799
<1100	11.11	66.67	26.09	41.46	0.4457
<1000	7.14	69.84	13.64	53.01	0.2902
<900	5.56	70.83	8.70	60.00	0.2174
<800	3.85	72.50	4.35	69.88	0.1443

TLC=Total lymphocyte count; PPV=Positive predictive value; NPV=Negative predictive value; LR=Likelihood ratio

study. This difference could be due to the small size of the study sample. However, the correlation between ALC and CD4 counts in this study was higher ($r=0.5604, P<0.0001$), indicating that ALC was a better marker when compared with TLC as a surrogate for CD4 counts.

We found that an ALC of 1400 cells/cu.mm was 71.08% sensitive and 78.26% specific for a CD4 cell count <200 cells/cu.mm. However, Jacobson *et al.*^[8] reported similar findings at a slightly higher ALC cut-off of 1500 cells/cu.mm.

But, we found that all the four statistical indices (PPV, NPV, sensitivity, and specificity) maximally aggregated at

TLC <1200 cells/cu.mm for CD4 <200 cells/cu.mm, thus proving that the WHO recommended cut-off (of 1200 cells/cu.mm)^[11] was adequate though the sensitivity was less.

A critical issue in taking an ALC cut-off of 1400 cells/cu.mm is that a proportion of patients with a CD4 cell count <200 cells/cu.mm who would be misclassified by ALC as having a CD4 cell count greater than 350 cells/cu.mm, and who would thus mistakenly have antiretroviral treatment deferred.

Using an ALC of 1400 cells/cu.mm or less as the threshold to initiate HAART, 24 of the 108 patients (25.92%) with a CD4 cell count <200 cells/cu.mm

would have been misclassified as not being in need of antiretroviral treatment, by having a simultaneous ALC greater than 1400 cells/cu.mm. Of note is the fact that 7 of these 108 (7.56%) patients had a CD4 cell count <100 cells/cu.mm and although at very high risk of opportunistic infection, would have been misclassified as not needing antiretroviral treatment, by an ALC greater than 1400 cells/cu.mm.

ALC and TLC cut-off values could be defined in such a way that PPV is very high, so that CD4 cell enumeration might not be needed to initiate HAART. However, lower ALC and TLC values of <800 cells/cu.mm cannot be taken as cut-offs [in spite of high PPV (100% for ALC and 96.15% for TLC respectively)] because of the low sensitivity.

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.^[14] We therefore analysed the data from those patients with CD4 counts of 200-350 cells/cu.mm to evaluate the performance of ALC and TLC. The positive predictive values (PPV) for ALC and TLC at this higher CD4 count of 200-350 cells/cu.mm were poor [Tables 2a and 2b]. We were also not able to draw any further conclusions as the results obtained were not statistically significant though the sensitivity and NPV maximally aggregated at ALC of <1600 cells/cu.mm and TLC of <1400 cells/cu.mm, respectively. We believe that this discrepancy was mainly due to the small size of the study sample. Hence, more studies with larger sample sizes need to be done to analyse this aspect.

The main limitations of this study were the small size of the study sample, which has an implication on determining the cut-offs for both ALC and TLC. The second limitation was that we could not rule out the latent cases of intercurrent infections like tuberculosis (TB) and malaria, which might affect the interpretation of the ALC/TLC.

TB-related immune activation and anti-TB therapy may lead to fluctuations in CD4 cell count.^[14] Malaria is another prevalent endemic disease known to have specific interactions with HIV infection. Though its effect on CD4 cell count is less well documented, patients with HIV infection are known to have an increased likelihood of developing malaria, as well as a decreased response to prophylaxis.^[15] Thus, further studies are needed to examine this aspect of impact of TB and malaria based on the correlation between TLC/ALC and CD4 cell counts.

CONCLUSIONS

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

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