Total lymphocyte count as a surrogate marker for CD4+ T lymphocyte count in newly diagnosed HIV positive patients in tertiary care Hospital in Eastern Rajasthan

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Abstract
Background: CD4+ T lymphocyte count is a standard measure of immunodeficiency in adults infected with HIV to initiate and monitor highly active antiretroviral therapy (HAART); however, it may not be feasible in resource poor countries. There is need to have another marker of immunodeficiency that is less resource demanding.

Objective: The aim of this study was to assess the relationship between total lymphocyte count and CD4+ T lymphocyte count in newly diagnosed HIV positive patients.

Materials and Methods: One hundred forty treatment naive HIV seropositive patients were enrolled over a period of one year. Blood sample was collected in K2 EDTA vial to carry out Complete Blood Count (CBC), Total lymphocyte count (TLC) and CD4+ T lymphocyte count. Pearson correlation and receiver operating characteristic (ROC) curves were used to calculate the relationship between TLC and CD4+ T lymphocyte count.

Results: A significant correlation between TLC and CD4+ T lymphocyte count was observed (r = 0.698, p < 0.001). TLC cut off of 1360 cells/µl as a predictor of CD4 count < 200 cells/µl had 82.4% sensitivity and 90.3% specificity. The ROC curve demonstrated highest area under curve (AUC= 0.8) for TLC of 1360 cells/µl.

Conclusion: TLC can be used as a surrogate marker for CD4+ T lymphocyte count for monitoring treatment in resource poor settings.

Keywords: CD4, TLC, Human Immunodeficiency Virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), National AIDS Control Organization (NACO), World Health Organization (WHO), antiretroviral therapy (ART).
CD4+ T lymphocyte counts to monitor their immunity status. So there is a need to evaluate alternate markers that are easily available, cheap and can match CD4+ T lymphocyte counts. According to the WHO guidelines, in the absence of CD4+ T lymphocyte counts, total lymphocyte count (TLC) < 1200 cells/µl can be used for starting ART in individuals with symptomatic HIV disease [7]. There are studies in HIV infected adults that have demonstrated association of TLC < 1200 cells/µl and subsequent disease progression or mortality [8-11].

For resource limited settings, which usually have ill equipped laboratories to perform CD4+ T lymphocyte counts and HIV RNA viral load; it becomes imperative for the clinicians to search for other available markers for disease progression and management. Hence, we evaluated the usefulness of TLC as a surrogate marker for CD4+ T lymphocyte counts initiating antiretroviral therapy and prophylaxis against opportunistic infections.

2. Materials and Methods

2.1 Study Design: This study was carried out in Department of Microbiology, Sawai Man Singh Medical College, Jaipur from August 2014 to July 2015. The study protocol was approved by the Ethics Committee of SMS Hospital. We enrolled 140 ART naive HIV sero-positive patients who visited Integrated Counseling and Testing Centre (ICTC) in SMS Medical College. The HIV status of patient was confirmed at ICTC by three tests with different antigen or principle as per NACO guidelines [12]. After obtaining informed consent from the patients, the socio-demographic details, clinical sign and symptoms, occupation, education and history of risk behavior were filled on a Standard Proforma. HIV positive patients above 18 years of age and ART naive were included in the present study.

2.2 Sample Collection and Methods: Three ml of blood was collected in K2 EDTA vacutainer for CD4+ T lymphocyte count and CBC. The complete blood count (CBC) was performed through 5 part automated hematology analyzer (Siemens, ADVIA® 2120i) and CD4+T lymphocyte count was determined by single platform BD FACS Calibur™ (Becton, Dickinson and Company, San Jose, United States of America), as per manufacturer instructions. All the samples were processed within six hours of collection. TLC was derived by multiplying the total leukocyte count by the percentage of lymphocytes [13].

### 2.3 Statistical analysis

Statistical analyses were done using computer software (SPSS Trial version 20 and primer). The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. Significance levels for tests were determined as 95% (P<0.05). Correlation between CD4+ T lymphocyte count and TLC was evaluated using Pearson’s correlation coefficient (r). ROC curve analysis was performed to determine the optimal cut-off values of significant variables (MPV) detected between the two groups.

3. Results

A total of 140 HIV positive patients were included in this study, among which 94(67.14%) were males and 46(32.86%) were females. The mean age was 37.66 ± 8.99 years ranging from 19-65 years. The mean CD4+ T lymphocyte count and mean TLC were 254.85±150.91 cells/µl and 1439.56±399.48 cells/µl respectively. (Table 1)

Out of 140 HIV positive patients, 68(48.57%), had CD4+ T lymphocyte count less than 200 cells/µl, 36 (25.71%) had CD4+ T lymphocyte count between 200-350 cells/µl, 33(23.57%) had CD4+ T lymphocyte count between 350-500 cells/µl and 03(2.14%) patients had CD4+ T lymphocyte count greater than 500 cells/µl. A significant association was observed between CD4+ T lymphocyte count and TLC. Lower CD4+ T lymphocyte count is significantly associated with low TLC count at <1200 (P<0.001). Distribution of CD4+ T lymphocyte count with TLC <1200 cells/µl and TLC > 1200 cells/µl both are summarized in Table 2. A TLC 1360 cells/µl was found to have 61.5 % sensitivity and 85.3% specificity for predicting a CD4+ T lymphocyte count of < 200 cells/µl in this study.

![Fig 1: Distribution of TLC and CD4 counts in HIV patients](image-url)
Table 2: Association of CD4 range with TLC

<table>
<thead>
<tr>
<th>CD4 Range</th>
<th>Less than 1200</th>
<th>More than 1200</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>29</td>
<td>90.63</td>
<td>39</td>
</tr>
<tr>
<td>200-350</td>
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<td>34</td>
</tr>
<tr>
<td>350-500</td>
<td>01</td>
<td>96.12</td>
<td>32</td>
</tr>
<tr>
<td>&gt;500</td>
<td>00</td>
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<td>03</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100.00</td>
<td>108</td>
</tr>
</tbody>
</table>

Fig 2: ROC plot of TLC in reference to CD4+ T lymphocyte count < 200

4. Discussion

HIV infection is associated with clinical latent period in which the infected patient remains symptom free. During this period the virus actively replicated resulting in clinical illness. It is important for physicians to understand factors affecting disease progression which can facilitate them to monitor and take treatment decisions. In developing and resource limited countries that usually do not have laboratories to perform CD4+ T lymphocyte count and HIV RNA viral load; it becomes imperative for the clinicians to search for other available markers for disease progression and management.

Our results showed a significant correlation between CD4+ T lymphocyte counts and TLC with spearman correlation (r=0.669); which consistent with other studies reported from India [4, 14, 15]. Studies conducted by Fournier et al from North America (r= 0.77) [16], Van Der Ryste et al from South Africa (0.704) [17] and S. M Alavi et al from Iran (r=0.645) [18] also found high degree of positive correlation between paired CD4+ T lymphocyte counts and TLC counts. In contrast to these studies, Akinola et al [19] and Angelo et al [20] reported poor correlation between paired CD4+ T lymphocyte counts and TLC counts. Our results are consistent with study conducted by Akinola et al [19] who reported this in 38% of patients however S.M Alavi et al [18] reported only 18% of patients with TLC >1200 having low CD4 counts. It is obvious the findings of studies are conflicting. This difference can be due to many factors such as differences in racial, ethnic, socioeconomic and epidemiological conditions of HIV/AIDS patients, different male to female ratio in their study and variety of associated conditions i.e. concurrent viral infections which may have effect on lymphocytes and red blood cells.

In this study, we found TLC ≤ 1200, as suggested by WHO had a sensitivity of 30.8% and specificity of 97.2%. We also found that TLC of 1360 cells/µl was more sensitive than 1200 cells/µl with an area under the curve (AUC = 0.875); Sensitivity 61.5 % and specificity 83.3% [SE± 0.032; Youdon index = 0.499] for a CD4 count < 200 cells/µl. However, at a TLC value of 1554 cells/µl the sensitivity increases to 81.7% but specificity decreases to 61.7%.

Our findings are consistent with several other studies suggesting a higher cut-off like N Kumasamy et al, [14] Adedeji David Atene et al, [21] Kakar A et al [22] and Ushakrishnan K et al [23] and from South Africa, Spaeck et al [25] from U.S.A, Mwamburi et al [26] from Massachusetts and Blatt et al [27] The advantage of using a higher TLC is to reduce failure rate of finding eligible patients for ART who may be immuno-compromised and to initiate early prophylaxis against opportunistic infections.

In this study optimal cut-off values of TLC to predict CD4+ T cell counts was found 1360 cells/µl. The optimal cut-off values described by others show great variation ranging from 1000 by Liu et al,28 to 2250 cells/µl by Moore et al [29] for a CD4+ T lymphocyte count <200 cells/µl. This variation can be explained by the influence of patient characteristics like age, parity, nutrition, co-infections etc.

5. Conclusion

Our findings in this study suggest that Total Lymphocyte count is a good surrogate marker for HIV management and has a strong positive correlation with CD4+ T lymphocyte count. We recommend a higher cut off i.e. 1360 cells/µl which would pick maximum number of patients having CD4+ T lymphocyte counts <200 cells/µl.

6. References

7. WHO: Scaling up antiretroviral therapy in resource-limited settings: treatment guidelines for a public health


27. Blatt SP, Lucey CR, Butzin CA, Hendrix CW, Lucey DR. Total lymphocyte count as a predictor of absolute CD4+ T cell count and CD4+ percentage in HIV-infected persons. JAMA. 1993; 269:622
