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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

Introduction
Human Immunodeficiency Virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), has been a challenge to medical fraternity since it was first discovered in 1981 [1]. Globally 36.7 million people were living with HIV and approximately 2.1 million people became newly infected with HIV and 1.1 million people died from AIDS-related illnesses at the end of 2015 [2]. The first case of HIV in India was reported in 1986; As per National AIDS Control Organization (NACO) the prevalence of HIV infection in India is estimated at 0.26%. The total number of People Living with HIV (PLHIV) in India is estimated at 21.17 lakhs and in Rajasthan approximately 1.03 lakhs at the end of 2015 [3]. HIV has become a major threat in relatively poor and developing countries including the Indian sub-continent and causes large number of morbidity and mortality [4]. The World Health Organization (WHO) currently recommends initiation of antiretroviral therapy (ART) in people living with HIV/AIDS with CD4$^+$ T lymphocyte counts < 350 cells/µl irrespective of the WHO clinical staging [5]. In India as per National AIDS Control Organization (NACO) recommendations, ART is initiated when CD4$^+$ T lymphocyte counts are less than 350 cells /µl and in symptomatic HIV patients ART is given to all patients irrespective to their CD4$^+$ T lymphocyte counts [6]. CD4$^+$ T lymphocyte count is a cumbersome test that requires special equipment and well trained staff. The test facility is not available at each and every place. In our country for HIV positive patients CD4$^+$ T lymphocyte counts test facility is provided by NACO at various ART centers. To get their CD4$^+$ T lymphocyte counts patients have to travel to nearby ART centers. It is difficult for HIV positive patients to determine their

$CD4^+$ 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].

$$\text{Total lymphocyte count (TLC)} = \frac{\text{Total leukocyte count X Percentage of lymphocytes}}{100}$$

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 83.3% specificity for predicting a $CD4^+$ T lymphocyte count of < 200 cells/µl in this study.

Table 1: Correlation of TLC and CD4

	Mean	Std. Deviation	N	R	R Square	Sig. F Change
TLC	1439.56	399.486	140	.698[a]	.488	.000
CD4	254.85	150.908	140			

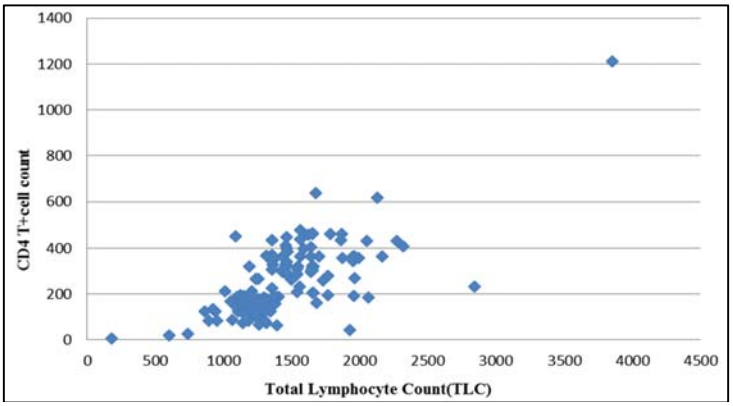

Fig 1: Distribution of TLC and CD4 counts in HIV patients

Table 2: Association of CD4 range with TLC

Total Lymphocyte Count					
CD4 Range	**Less than 1200**		**More than 1200**		**Total**
	No	**%**	**No**	**%**	
< 200	29	90.63	39	36.11	68
200- 350	2	06.25	34	31.48	36
350-500	01	03.12	32	29.62	33
>500	00	00.00	03	02.78	03
Total	32	100.00	108	100.00	140

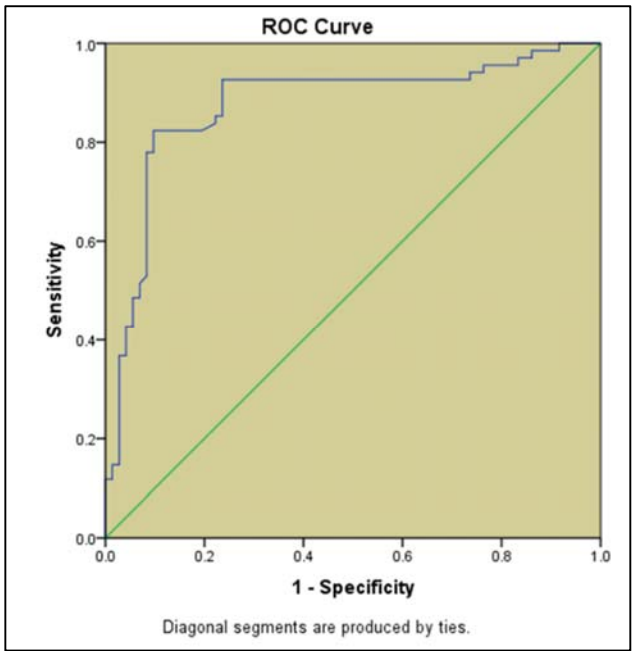

Fig 2: ROC plot of TLC in reference to CD4$^+$ T lymphocyte count < 200

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 = 0.449] 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 Atere *et al*, [21] Kakar A *et al* [22] and Ushakrishnan K *et al* [23] Studies from abroad have also concluded TLC as a equal predictor of disease progression and can be used as a cutoff for starting prophylaxis. Post FA *et al* [24] 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.

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