

# Is total lymphocyte count a predictor for CD4 cell count in initiation antiretroviral therapy in HIV-infected patients?

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## ABSTRACT

**Background:** Since laboratory assessments of HIV-infected patients by flow cytometric methods are expensive and unavailable in resource-limited countries, total lymphocyte count by haematology cell counter is supposed to be a suitable surrogate marker to initiate and monitor course of the disease in these patients. The aim of this study was to evaluate the utility of total lymphocyte count as a surrogate marker for CD4 count in HIV-infected patients. **Patients and Methods:** In a prospective study 560 HIV-positive individuals evaluated for total and CD4 lymphocyte count. For correlation between CD4 count and total lymphocyte count, haemoglobin and haematocrit we defined cut-off values as 200 cell/ $\mu$ l, 1200 cell/ $\mu$ l, 12 gr/dl and 30%, respectively, and compared CD4 count with each parameter separately. Positive predictive value, negative predictive value, sensitivity and specificity of varying total lymphocyte count cutoffs were computed for CD4 count  $\leq$  200 cell/ $\mu$ l and  $\leq$  350 cell/ $\mu$ l. **Results:** Strong degree of correlation was noted between CD4 and total lymphocyte count ( $r$ : 0.610,  $P < 0.001$ ). Mean and standard deviation of total lymphocyte count, haemoglobin and haematocrit in relation to CD4 count were calculated which indicated significant correlation between these variables. Kappa coefficient for agreement was also calculated which showed fair correlation between CD4 200 cell/ $\mu$ l and total lymphocyte count 1200 cell/ $\mu$ l (0.35). **Conclusion:** This study reveals that despite low sensitivity and specificity of total lymphocyte count as a surrogate marker for CD4, total lymphocyte count is of great importance and benefit in resource-limited settings.

**Key words:** Antiretroviral therapy, CD4 cell, HIV, total lymphocyte count

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## INTRODUCTION

According to annual reports of UNAIDS 35.3 million (32.2-38.8) people were living with human immunodeficiency virus (HIV) infection at the end of 2012.<sup>1</sup> Although the prevalence of HIV infection is decreasing in a number of countries, the general trend is increasing all over the world. According to World Health Organization (WHO) report, the prevalence rate of HIV infection in Iran has risen from low to concentrate. Although the prevalence in overall population is below 1%, this rate is estimated to be 5% in some high-risk groups such as injection drug users.<sup>2</sup>

CD4 count is a standard method for measuring immunodeficiency in adults infected with HIV to initiate and monitor highly active antiretroviral therapy. However, the high cost and unavailability of CD4 count in resource-limited countries is a major challenge in monitoring of antiretroviral therapy.<sup>3</sup>

WHO guidelines for total lymphocyte count (TLC) acknowledge that total lymphocyte count is a useful marker of disease progression and in specific situations can be used as a surrogate marker for CD4 count to make treatment decision in resource poor settings.<sup>4</sup>

Although some of the previous studies have shown that there is a good correlation between CD4 cell count by flowcytometric methods and TLC by haematology cell counter,<sup>5-7</sup> opposite reports on the usefulness and validity of these tests are on record.<sup>8,9</sup>

It has been also suggested that TLC  $<1200$  cell/ $\mu$ l and haemoglobin  $<12$  gr/dl have a positive correlation with CD4 count  $\leq 200$  cell/ $\mu$ l;<sup>10,11</sup> however, there are also some

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discrepancies in this context.<sup>6,8</sup> The aim of this study is to investigate the correlation between total lymphocyte count, haemoglobin and haematocrit with CD4 count in HIV/AIDS patients and also to evaluate positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of varying TLC cutoffs for CD4 counts  $\leq 200$  cell/ $\mu$ l and  $\leq 350$  cell/ $\mu$ l.

## PATIENTS AND METHODS

This prospective study was carried out in Imam Hospital complex (central laboratory), Tehran, Iran, affiliated to Tehran University of medical science.

Five hundred sixty HIV positive individuals (diagnosis based on serology, PCR or western-blot test) were recruited for this study [We followed the national AIDS control organisation recommendation (NACO 2007) for diagnosis of HIV infection]. All patients who have referred to hospital while the study was under way have been included. Exclusion criteria were children, pregnant woman and other medical conditions such as tuberculosis, endocarditis and other viral and bacterial infections which could affect WBC. In order to prevent bias, the patients who had received anti-retroviral drugs were excluded from the study.

Complete blood count was done with Sysmex-K21, Japan instrument on blood samples anticoagulated with EDTA. CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes were counted by flowcytometry device (PARTEC, Japan).

For correlation between CD4 count and TLC, haemoglobin and haematocrit we defined cut off values as 200 cell/ $\mu$ l, 1200 cell/ $\mu$ l, 12 gr/dl and 30%, respectively, and compared CD4 count with each parameter separately. PPV, NPV, sensitivity and specificity of varying TLC cutoffs were computed for CD4 count  $\leq 200$  cell/ $\mu$ l and  $\leq 350$  cell/ $\mu$ l.

Patients gave an informed consent before entering to the study and the institutional review board of Tehran University of Medical Sciences approved the study protocol. Data were analysed with statistical package for social science (SPSS, version 16, Chicago, Inc.) and a *P*-value of  $<0.05$  was considered statistically significant. T-test was also used to analyze the relationship between haematologic parameters and CD4 cell count.

## RESULTS

Five hundred and sixty HIV-positive patients enrolled in this study. Frequency distribution of the study population according to different age groups is shown in Table 1. As we see more than half of the populations were between 28 and 37 years, which implies that prevalence of HIV infection is more common in young population. The mean and standard deviation of CD4, haemoglobin, haematocrit and TLC are seen in Table 2.

After statistical analysis, high degree of correlation was found between CD4 and TLC ( $r$ : 0.61, *P*-value  $<0.001$ ).

Frequency distribution of various CD4 cutoffs is present in Table 3. CD4  $\leq 200$  cell/ $\mu$ l (36.4%) and CD4  $\geq 500$  cell/ $\mu$ l (16.4%) have the maximum and minimum number of the patients, respectively.

Tables 4 and 5 show the calculated PPV, NPV, sensitivity and specificity of varying TLC cutoffs to predict CD4 count  $\leq 200$  cell/ $\mu$ l and CD4  $\leq 350$  cell/ $\mu$ l.

Mean and standard deviation of TLC, haemoglobin and haematocrit in correlation to CD4 count was calculated. Mean and standard deviation for TLC  $<1200$  cell/ $\mu$ l in patients with CD4  $\leq 200$  cell/ $\mu$ l were 790 cell/ $\mu$  and 275, respectively, while mean and standard deviation of TLC  $<1200$  cell/ $\mu$ l in patients with CD4  $> 200$  cell/ $\mu$ l was 1020 cell/ $\mu$ l and 235. This indicates that there is statistically significant correlation between mean and standard

**Table 1: Frequency distribution of study population according to age groups and gender**

Age group (y)	Frequency %	Frequency in male %	Frequency in female %
18-27	71 (12.7)	45 (63.4)	26 (36.6)
28-37	313 (55.9)	234 (74.8)	79 (25.2)
38-47	130 (23.2)	100 (76.93)	30 (23.07)
48-57	32 (5.7)	23 (71.9)	9 (28.1)
Total	546 (100)	402 (73.62)	144 (26.38)
Missing system	14 (2.5)		

**Table 2: Mean and standard deviation of CD4, haemoglobin, haematocrit and TLC\***

CD4	Frequency	Percent
$\leq 200$ cell/ $\mu$ l	204	36.4
201-350 cell/ $\mu$ l	159	28.4
351-500 cell/ $\mu$ l	93	16.6
$> 500$ cell/ $\mu$ l	92	16.4
Total	548	97.9
Missing system	12	2.1

\*Total lymphocyte count; \*\*Standard deviation

**Table 3: Frequency distribution of various CD4 cutoffs**

Cutoffs	PPV	NPV	Sensitivity	Specificity
1000 cell/ $\mu$ l	89.2	69.8	28.4	97.9
1100 cell/ $\mu$ l	84.3	71.2	34.3	96.2
1200 cell/ $\mu$ l	73.5	72.2	40.7	91.3
1300 cell/ $\mu$ l	71.1	74.6	49.5	88.1
1400 cell/ $\mu$ l	70.1	77.2	57.4	85.5
1500 cell/ $\mu$ l	63.6	78.7	64.2	78.2
1600 cell/ $\mu$ l	61.4	81.2	71.1	73.5
1700 cell/ $\mu$ l	58.6	82.2	75	68.6
1800 cell/ $\mu$ l	56.8	84.3	79.9	63.9

deviation of TLC <1200 cell/ $\mu$ l with CD4  $\leq$  and > 200 cell/ $\mu$ l ( $P$ -value <0.001). This was also evident for haemoglobin <12 g/dl and haematocrit <30% with CD4  $\leq$  and > 200 cell/ $\mu$ l with  $P$ -values of 0.016 and 0.021, respectively.

Degree of agreement between CD4 count and TLC, haemoglobin and haematocrit is shown in Table 6. Calculating of K-coefficient for agreement indicates that there is a fair correlation (K: 0.35) between TLC and CD4. Degree of agreement for haemoglobin and haematocrit was 0.12 and 0.08, respectively, which reveals slight correlation to CD4.

In this study we calculated PPV, NPV, specificity and sensitivity for both cutoffs of CD4  $\leq$ 200 cell/ $\mu$ l and CD4  $\leq$ 350 cell/ $\mu$ l.

As we see in Table 4, increasing in sensitivity is associated with reduction in specificity, but considering the best cut-

**Table 4: Calculated PPV\*, NPV\*\*, sensitivity and specificity of varying TLC \*\*\*cutoffs to predict CD4 count 200 cell/ $\mu$ l**

	Number	Mean	SD**
CD4 cell/ $\mu$ l	548	310.56	234.04
Hemoglobin g/dl	560	13.87	2.42
Hematocrit (%)	560	39.6	6.19
TLCcell/ $\mu$ l	560	1782	778.64

\*Positive predictive value; \*\*Negative predictive value; \*\*\*Total lymphocyte count

**Table 5: Calculated PPV\*, NPV\*\*, sensitivity and specificity of varying TLC\*\*\*cutoffs to predict CD4 count 350 cell/ $\mu$ l**

Cutoffs	PPV	NPV	Sensitivity	Specificity
1500 cell/ $\mu$ l	90.3	48.2	51.2	81.2
1600 cell/ $\mu$ l	89.8	51.6	58.4	87.3
1700 cell/ $\mu$ l	87.7	53.3	63.1	82.7
1800 cell/ $\mu$ l	85.7	55.2	67.8	77.8
1900 cell/ $\mu$ l	83.3	58.2	74.1	70.8
2000 cell/ $\mu$ l	80.9	59.9	78.2	63.8
2100 cell/ $\mu$ l	79.3	62.2	82.1	57.8
2200 cell/ $\mu$ l	77.7	65.5	86.2	51.4
2300 cell/ $\mu$ l	75.2	64	87.6	43.2

\*Positive predictive value; \*\*Negative predictive value; \*\*\*Total lymphocyte count

**Table 6: Degree of agreement between CD4 count and TLC\*, haemoglobin and haematocrit**

Parameters	CD4 $\leq$ 200	CD4 > 200	Kappa**
TLC > 1200 cell/ $\mu$ l	121	314	0.352
TLC < 1200 cell/ $\mu$ l	83	30	
Hemoglobin >12gr/dl	154	299	0.128
Hemoglobin <12gr/dl	50	45	
Hematocrit >30%	184	355	0.087
Hematocrit <30%	20	9	

\*Total lymphocyte count; \*\*The Kappa values <0 indicates no agreement; 0-0.20 indicates as slight; 0.21-0.40 as moderate; 0.61-0.80 as substantial and 0.81-1 as almost perfect agreement.

off values of the TLC with the most acceptable PPV, NPV, sensitivity and specificity, TLC <1600 cell/ $\mu$ l was found to be the strongest predictor of CD4 count  $\leq$ 200 cell/ $\mu$ l with sensitivity of 71.1%, specificity 73.5%, PPV 61.4% and NPV of 81.2%

Sensitivity and specificity of TLC <1200 cell/ $\mu$ l to predict CD4  $\leq$ 200 cell/ $\mu$ l were 40.7% and 91.3%, respectively.

For CD4  $\leq$ 350 cell/ $\mu$ l, TLC <2100 cell/ $\mu$ l was found to have the most acceptable prediction power with sensitivity 82.1%, specificity 57.8%, PPV 79.3% and NPV 62.2%.

## DISCUSSION

According to the WHO guidelines for decision making in HIV-infected patients, the scarcity of flow cytometry should not be a cause to delay antiretroviral therapy while there is access to TLC and clinical staging of the patient.<sup>4</sup>

In trying to substitute a suitable model in resource-limited settings, this study examined the correlation between TLC and CD4.

There are several studies which indicate that TLC is a reliable surrogate marker for CD4 count to initiate and monitor course of the disease in HIV-infected individuals;<sup>11-14</sup> however, studies with discordant results are also on record.<sup>8</sup>

As previously mentioned the analysis of results of this study has found strong correlation between CD4 cell count and TLC ( $r$ : 0.61,  $P$ <0.001).

In parts of the developing world with higher incidence of bacterial and parasitic infection that tend to occur in earlier stage of immunodeficiency, the WHO have recommended initiation of antiretroviral therapy at higher counts of CD4 (350 cell/ $\mu$ l) and/or earlier clinical stage.<sup>7</sup> In this study we calculated PPV, NPV, specificity and sensitivity for both cutoffs of CD4  $\leq$ 200 cell/ $\mu$ l and CD4  $\leq$ 350 cell/ $\mu$ l.

As we see in Table 4, increasing in sensitivity is associated with reduction in specificity, but considering the best cut-off values of the TLC with the most acceptable PPV, NPV, sensitivity and specificity, TLC <1600 cell/ $\mu$ l was found to be the strongest predictor of CD4 count <200 cell/ $\mu$ l with sensitivity of 71.1%, specificity: 73.5%, PPV: 61.4% and NPV of 81.2%. This optimal cut-off was between obtained cutoffs of two other studies; one, performed by F. I. Buseri in Nijeria<sup>7</sup> that found TLC 1400 cell/ $\mu$ l with the strongest prediction power and 90%PPV, 87%NPV, 86%sensitivity and 90% specificity and another study performed by D. Daka in Ethiopia<sup>9</sup> which indicated TLC 1780 cell/ $\mu$ l had maximum sensitivity of 61% and specificity of 62%.

Sensitivity and specificity of TLC <1200 cell/ $\mu$ l to predict CD4  $\leq$ 200 cell/ $\mu$ l were 40.7% and 91.3%, respectively,

which was rather concordant to results obtained by D. Daka<sup>9</sup> with sensitivity and specificity of 41% and 83.5% and lower than reported results of an Indian study<sup>15</sup> with 59.9% and 94% sensitivity and specificity.

For CD4  $\leq 350$  cell/ $\mu$ l, TLC  $< 2100$  cell/ $\mu$ l was found to have the most acceptable prediction power with sensitivity: 82.1%, specificity: 57.8%, PPV: 79.3% and NPV: 62.2%. This cutoff was lower than TLC  $< 2300$  cell/ $\mu$ l which was obtained by F. I. Buseri in Nigeria.<sup>7</sup>

These differences in results and various cutoffs could be due to different racial, ethnic, socioeconomic and epidemiologic factors and also differences in the most common causes of HIV infection in study populations.<sup>6</sup>

Despite low sensitivity and specificity of TLC as a surrogate marker for CD4, TLC is an important tool in the absence of flow cytometry to measure CD4.

Among 113 patients who had TLC  $< 1200$  cell/ $\mu$ l, 83 patients found to have CD4  $\leq 200$  cell/ $\mu$ l and 30 patients were found to have CD4  $> 200$  cell/ $\mu$ l and among 435 patients with TLC  $> 1200$  cell/ $\mu$ l, 121 were found to have CD4  $\leq 200$  cell/ $\mu$ l and 314 had CD4  $> 200$  cell/ $\mu$ l.

After measuring K-coefficient for agreement, fair degree of correlation was found between these markers (k: 0.352) that was lower than degree of agreement which was found in study performed by Alavi *et al* (k: 0.448).<sup>6</sup>

As previously mentioned, there are also studies on record that suggest the use of TLC combined with haemoglobin  $< 12$  g/dl as a simple laboratory surrogate marker for initiation of antiretroviral therapy.<sup>10,11</sup> We also calculated the degree of agreement for haemoglobin and haematocrit in correlation with CD4 which indicated slight degree of agreement with kappa of 0.128 and 0.087 respectively. This result was similar to degree of agreement calculated by Alavi *et al*<sup>6</sup> with kappa of 0.202 and 0.160. However, some of the previous studies like studies performed by Spacek and Lau<sup>10,15</sup> found strong degree of correlation between CD4 and haemoglobin. These discordant results may be due to socioeconomic factors and predominance of injection drug users in our country which could lead to chronic anaemia and affect the level of haemoglobin in this group.

The results of this study could be limited due to some factors. We were not aware of the cause of HIV-infection in this population; as some individuals like injection drug users were more prone to bacterial infections and chronic anaemias which both intervene in the results, also racial and socio-economic differences may also contradict this conclusion.

Finally we believe that as long as CD4 cell count differ from one locality to other and among different ethnic groups, one TLC cutoff may not necessarily apply to other populations.

So, results of studies are better to be analyzed for specific groups in one country or even different parts of one country in order to discern any regional differences.

## CONCLUSIONS

Strong degree of correlation was found between CD4 count and TLC, but we did not find considerable sensitivity and specificity for TLC  $< 1200$  cell/ $\mu$ l to predict CD4  $\leq 200$  cell/ $\mu$ l.

It should be mentioned that even with low reliability of TLC as a surrogate marker for CD4, TLC is an important tool in the absence of flow cytometry in resource-limited settings.

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## REFERENCES

1. UNAIDS. UNAIDS report on the global AIDS epidemic, 2013. Available from: [http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS\\_Global\\_Report\\_2013\\_en.pdf](http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf).
2. Haghdoost A, Mostafavi E, Mirzazadeh A, Navadeh S, Feizzadeh A, Fahimfar N, *et al*. Modelling of HIV/AIDS in Iran up to 2014. J AIDS HIV Res 2011;3:231-9.
3. Yitayih Wondimeneh, Getachew Ferede, Gizachew Yismaw, Dagnachew Muluye. Total Lymphocyte Count as surrogate marker for CD4 Cell Count in HIV-Infected Individuals in Gondar University Hospital, Northwest Ethiopia. AIDS Research and Therapy 2012;9:21.
4. WHO. Antiretroviral therapy for HIV infection in Adults and Adolescents Recommendations for a public health approach 2010 revision, Available from: [http://whqlibdoc.who.int/publications/2010/9789241599764\\_eng.pdf](http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf).
5. Jafari S, Rasulinezhad M, Emadikuchak H, Mokarrami F. The relationship between total lymphocyte count and CD4 count in patients infected with HIV. Tehuni Med J 2009;67:477-82.
6. Alavi SM, Ahnadi F, Farhadi M. Correlation between TLC, hemoglobin and hematocrit and CD4 count in HIV/AIDS patients. Acta Medica Iranica 2009;47:1-4.
7. Buseri FI, Mark D, Jeremiah ZA. Evaluation of absolute lymphocyte count as a surrogate marker for CD4 cell count for the initiation of antiretroviral therapy in resource limited settings. Int J Biomed Lab Sci 2012;1:44-9.
8. Akinola NO, Olasode O, Adediran IA, 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 Infect Dis 2004;39:579-81.
9. Deresse Daka, Eskindir Loha. Relationship between Total Lymphocyte count (TLC) and CD4 count among peoples living with HIV, Southern Ethiopia: a retrospective evaluation. AIDS Res Ther 2008;5:26.
10. 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;17:1311-7.
11. Badri M, Wood R. Usefulness of total lymphocyte count in monitoring highly active antiretroviral therapy in resource-limited settings. AIDS 2003;17:541-5.



12. Lee SS, Wong KH. The use of total lymphocyte count as an independent criterion for initiating HAART in resource-poor countries. *J Infect* 2005;50:66-7.
13. Kumarasamy N, Mahajan AP, Flanigan TP, Hemalathe R, Mayer KH, Carpenter CC, *et al.* Total lymphocyte count is a useful tool for the timing of opportunistic infection prophylaxis in India and other resource-constrained countries. *J Acquir Immune Defic Syndr* 2002;31:378-83.
14. Gupta A, Gupte N, Bhosale R, Kakrani A, Kulkarni V, Nayak U, *et al.*, Byramji Jeejeebhoy Medical College-Johns Hopkins University Study Group. Low sensitivity of total lymphocyte count as a surrogate marker to identify antepartum and postpartum Indian women who require antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007;46:338-42.
15. Lau B, Gange SJ, Phair JP, Ridler SA, Detels R, Margolick JB. Rapid declines in total lymphocyte counts and hemoglobin concentration prior to AIDS among HIV-1-Infected men. *AIDS* 2003;17:2035-44.

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