

# Correlation between Total Lymphocyte Count, Hemoglobin, Hematocrit and CD4 Count in HIV/AIDS Patients

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**Abstract:** Lymphocyte CD4<sup>+</sup> count, a standard laboratory test for staging of HIV infection, is expensive and unavailable in resource-restricted countries. Total lymphocyte count (TLC) and hemoglobin (Hb) are recommended as simple & inexpensive surrogates. The aim of this study was to assess the correlation, sensitivity and predictive power of these parameters as substitutes for CD4 count. One hundred HIV patients enrolled in this analytic descriptive study in Ahvaz, a city in the South of Iran, from 2005 to 2006. They were tested for CD4 count, TLC, Hb, and hematocrit (Hct). The cutoffs were determined as: 200 cells/ $\mu$ L, 1200 cells/ $\mu$ L, 12 g/dl and 30%, respectively. We used Sys Max SE 9500 for CBC and Flow cytometry for CD4 count. The correlation coefficient established correlation between values. Sensitivity, specificity and positive predictive values were calculated. 2 females (%2) and 98 males (%98) of the mean age of  $32\pm 5$  years were studied. 87 cases (%87) were IV drug users, the majority having a history of imprisonment. The mean CD4 count, TLC, Hb and Hct were  $279\pm 225$ ,  $2102\pm 1250$ ,  $10.7\pm 2.4$  and  $30.4\pm 9.0$ , respectively. A strong correlation was observed between CD4 count and TLC ( $R = 0.645$ ,  $P = 0.001$ ), but no correlation was seen between CD4 count and Hb or Hct ( $R = 0.451$ ,  $P = 0.056$  and  $R = 0.375$ ,  $P = 0.816$  respectively). This study shows that TLC is a suitable surrogate marker for CD4 count. Hb and Hct are of limited value in predicting CD4 counts and should not be substituted for CD4 counts.

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**Keywords:** TL-CD4 count, total lymphocyte count, hemoglobin, hematocrite, HIV/AIDS

## Introduction

40 million people live with human immunodeficiency virus (HIV) in low income countries and an estimated 6 million people in these countries currently require life-sustaining highly active anti retroviral therapy (HAART) (1). In resource-limited countries, widespread and routine use of T-lymphocyte CD4 positive (CD4) count and plasma viral load testing in the management of HIV infection has not been yet possible. Traditional methods of CD4 count measurement, such as immunophenotyping by flow cytometry or labeling with monoclonal antibodies, require expensive laboratory equipments and expertise. Plasma viral load testing has also been extremely challenging to scale up in resource-limited setting (2). Because of the lack of laboratory technologies due to the high prices of these tests, World Health Organization (WHO) guidelines suggest the use of simple laboratory tests such as hemoglobin (Hb) of  $<12$ g/dl and total lymphocyte count (TLC) of  $<1,200$

cells/ $\mu$ L as an indicator of initiation of antiretroviral treatment (ART) and also as a surrogate marker to monitor immune response to therapy in symptomatic HIV patients in resource-limited settings (1). TLC is easily obtained from the routine complete blood count (CBC) with differential through multiplication of lymphocyte percentage by white blood cell count. In developing and low income areas of Iran, for example Khuzestan, the cost of a single TLC obtained from a CBC is less than 10000Rls, while a single CD4 count by flow cytometry is approximately 250000Rls. In developing areas, even if laboratory technologies are available, the cumulative cost becomes a significant financial challenge (3). There are different and, occasionally, opposite reports on the usefulness and validity of these tests as surrogates of CD4 count. Akinolo and et al showed that 38% of patients with CD4 of  $<200$  cell / $\mu$ L had TLC of  $> 1200$  cells/ $\mu$ L, and they believed that TLC was not a valid predictor of CD4 (4). Spacek and his colleagues, Badri and et al, and Lee and et al reported that TLC  $<1200$

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**Table 1.** Mean and range of CD4count, TLC, hemoglobin and hematocrite of HIV infected patients

Marker	Mean	Median	SE Mean	SD	Range
CD4 count cells/ $\mu$ L	279	261	18.4	225	39-930
TLC cells/ $\mu$ L	2102	2223	81.2	1250	192-5454
Hemoglobin g/dl	10.7	11.1	0.162	2.4	4.2-17.1
Hematocrite %	30.4	30.2	0.331	9.0	13.0-46.6

Abbreviation:CD4,T-lymphocyte CD4positive; TLC,Total lymphocyte count; SD,standard deviation; HIV,Human immunodeficiency virus; SE Mean, Standard error Mean.

Data are given as numbers.

cells/ $\mu$ L and Hb <12 g/dl had a positive correlation with CD4 count < 200 cells/ $\mu$ L (5-7). It is estimated that more than 80000 people with HIV are living in Iran, and that a considerable proportion of them require HAART, the majority living in remote and deprived areas (8).

Because of the major obstacle to the initiation of HAART in such areas is the expensive and technology-limited setting of CD4 count testing, we decided to investigate the capability of TLC , Hb and hematocrit (Hct) as surrogates for CD4 count in HIV/AIDS patients. The aim of this study was to assess the correlation, sensitivity and predictive power of these parameters as substitutes for CD<sub>4</sub> count to initiate HAART in HIV/AIDS patients visiting our infectious disease clinic.

## Patients and Methods

Study subjects included all HIV seropositive individuals (diagnosed based on HIV antibody-Elisa and confirmed by western blot test) visiting the infectious disease clinic in Razi hospital, an educational Medical Center affiliated with Joundishapour University of Medical Sciences(JUMS) in Ahvaz, a city in the South west of Iran, from 2005 to 2006. Inclusion criteria were at least 15 years of age and HIV-1 seropositivity. Exclusion criteria were antiretroviral therapy and co-morbidity with other medical conditions (e.g. tuberculosis, endocarditis and acute viral infections) which could affect CBC. Following approval by Ethic Committee of JUMS and informed consent of patients, two blood samples (2ml) were obtained from each patient, one for CBC (WBC,

Hb and Hct) and another for CD4 cell counting. Samples were rapidly transported to laboratory (Shafa reference hematology laboratory). We used Sys Max SE 9500 for TLC, Hb and Hct and Flow cytometry for CD4 count. For correlation between CD4 count and TLC, Hb or Hct, we defined cutoffs as 200 cells/ $\mu$ L, 1200 cells/ $\mu$ L, 12g/dl and 30% respectively (1), and compared CD4 count with each parameter separately. Data including sex, age, injection drug usage (IDU) and previous imprisonment were collected by using questionnaires for each patient. Data was analyzed in SPSS 11.5 by using differential statistics. The correlation coefficient established correlation and Kappa coefficient showed agreement between CD4 count and these parameters. Sensitivity, specificity and positive and negative predictive values for using direction on TLC, Hb and Hct changes as a marker for direction of CD4 changes were calculated.  $p < 0.05$  was considered as statistically significant for all tests.

## Results

Of one hundred and six patients who enrolled in this study, 6 patients were excluded due to tuberculosis and endocarditis. Of total 100 patients, 98 (98%) were males of mean age of  $32 \pm 5$  ranging between 21 and 43. 87 patients (87%) were IV drug users with the imprisonment rate of 80%. The mean and SD of CD4 count, TLC, Hb, and Hct are shown in Table1. There was a strong correlation between CD4 and TLC ( $R = 0.645$ ,  $P = 0.001$ ). Among 76 patients, 48 cases had TLC > 1200

**Table 2.** Validity and predictive value for surrogate markers of CD4count in HIV infected patients

Marker	N	P	SE	SP	PPV	NPV	Kappa*
CD4 count <200 cell/ $\mu$ L	46	46	100	100	100	100	1.00
TLC <1200 cell/ $\mu$ L	34	34	74.0	82.0	82.4	72.7	0.488
Hemoglobin < 12 g/dl	47	47	69.5	79.0	68.1	75.0	0.248
Hematocrite < 30%	35	35	45.6	68.0	60.0	63.0	0.160

Abbreviation:N,number; P,Prevalence (%); SE,Sensitivity (%); SP,Specificity (%);

PPV,Positive predictive value; NPV,Negative predictive value; CD4,T-lymphocyte CD4positive;TLC,Total lymphocyte count; HIV,Human immunodeficiency virus.

Data are given as numbers.

\*Kappa coefficient for agreement between CD4 count and TLC,Hb,and Hct.

**Table 3.** Agreement between CD4 count and TLC,Hb and Hct

Parameters	CD4 count( cell/ $\mu$ L)		Kappa	Approx.Sig
	<200	>200		
TLC: >1200 cell/ $\mu$ L	18	47	0.488	0.001
<1200 cell/ $\mu$ L	28	7		
Hb: >12 g/dl	19	38	0.202	0.070 (NS)
<12 g/dl	27	16		
Hct: >30%	26	39	0.160	0.101 (NS)
<30%	20	15		

Abbreviation:CD4,T-lymphocyte CD4positive; TLC,Total lymphocyte count; Hb,hemoglobin;

Hct,haematocrit; NS,non significant;

Data are given as numbers.

Kappa coefficient for agreement

P value < 0.05 ,significant

cells/ $\mu$ L and CD4 > 200 cells/ $\mu$ L, whereas 28 patients had TLC < 1200 cells/ $\mu$ L and CD4 < 200 cells/ $\mu$ L.

In remaining 24 patients, there were no positive correlations between TLC and CD4 count, of whom 18 patients had TLC > 1200 cells/ $\mu$ L, but CD4 < 200 cells/ $\mu$ L and 6 patients had TLC < 1200 cells/ $\mu$ L, but CD4 > 200 cells/ $\mu$ L. Positive predictive value (PPV) for concurrent decreasing of CD4count and TLC was 82% and negative predictive value (NPV) for concurrent increasing of CD4 count and TLC was 72%. A weak correlation was observed between CD4 cell count and Hb ( $R = 0.451$ ,  $P = 0.056$ ). Of 72 patients, 32 cases had Hb < 12g/dl and CD4 < 200 cells/ $\mu$ L, whereas 40 had Hb > 12g/dl and CD4 > 200 cells/ $\mu$ L. In 28 patients, 15 had Hb of < 12g/dl and CD4 > 200 cells/ $\mu$ L, whereas 13 patients had Hb > 12g/dl and CD4 < 200 cells/ $\mu$ L. PPV for concurrent decreasing and NPV for concurrent increasing of CD4 count and Hb were 68% and 75% respectively. Prevalence, sensitivity and specificity of each test are shown in Table 2. We did not observe a correlation between CD4 cell count and Hct ( $R = 0.375$ ,  $P=0.816$ ). Of sixty two patients, 21 had Hct < 30% and CD4 < 200 cells/ $\mu$ L whereas, 41 had Hct > 30% and CD4 > 200 cells/ $\mu$ L. Of 38 patients, 24 had Hct > 30%, but CD4 < 200 cells/ $\mu$ L, whereas 14 patients had Hct of < 30%, but CD4 > 200 cells/ $\mu$ L. PPV for concurrent decrease and NPV for concurrent increase of CD4 count and Hct were 60% and 63% respectively. Kappa coefficient for agreement between CD4 count and TLC, Hb, and Hct was 0.488, 0.202 and 0.160 respectively. Significant agreement was observed between CD4 count and TLC (Table 3).

## Discussion

Results of this study demonstrate that in the majority of HIV/AIDS patients, there is a positive correlation between CD4 count and TLC. PPV of 82% shows a direct

relationship between CD4 count and TLC, so in the remote and deprived areas of Iran with the scarcity of laboratory technologies (*i.e.* CD4 counting is not available). TLC is a useful and acceptable surrogate for CD4 count.

The present study shows that in more than three fourths of patients, TLC is a suitable predictor of CD4 count. This finding is consistent with other reports (5, 6, 9-11). Support for this claim is provided by the 75% sensitivity of TLC as a predictor of CD4 counts. In this study, we found that 18% of patients had TLC > 1200 cells/ $\mu$ L in spite of CD4 < 200 cells/ $\mu$ L that is lower than 38% reported by Akinol and et al (4). Obviously, the findings of such studies are conflicting in different countries. This difference can be due to: 1- different racial, ethnic, socioeconomic and epidemiological factors in HIV/AIDS patients, 2- different male to female ratio of patients (1:1.4 vs. 49:1). This difference may also be a result of high percentage of IV drug users in our study (87% vs.18%) that could affect parameters such as CBC and TLC. Interpretation of hematological tests in IV drug users is complicated by a number of factors. First, toxins or impurities in the injected substance rather than infection may cause depletion in hematological index such as CD4 Tcells. Second, variety of associated conditions e.g. concurrent viral infection may affect on lymphocytes or red blood cells.

This study shows a poor correlation between CD4 and Hb, the finding being far from Spacek and et al (5) with 70% and Lau et al (12) with 75% of concurrent decreasing values for CD4 and Hb. This disagreement may be due to: 1- malnourishment and various socioeconomic factors in our patients with a history of imprisonment and IDU resulting in chronic anemia and low Hb values, 2- differences in various countries as mentioned before. Our study also demonstrates that Hct is not a valid test for predicting CD4 count, with only 21% concurrent decreasing values for CD4 and Hct (Hct

## TLC a surrogate of CD4 count

< 30% and CD4 <200 cells/ $\mu$ L) which was consistent with Akinolo and colleagues report (4). There were some limitations in our study; First, more than three fourths of our subjects were IV drug users that might not reflect the real HIV/AIDS situation; second, nearly all of our patients were male; and third, the sample size of our study was small. We believe that the results of this study should be confirmed by further investigations. In conclusion, this study reveals that TLC is a suitable and useful surrogate marker for CD4 count, but in the case of TLC > 1200 cells/ $\mu$ L, it is necessary to test CD4 counting. Hemoglobin and hematocrit are of limited value in predicting CD4 counts and should not be substituted for CD4 counts.

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## Ethical Consideration

This work has been approved by the ethical committees of Jondishapour University of Medical Science and the subjects were appropriately informed about the work.

## Conflicts of interests

This study was approved and funded by infectious disease and tropical medicine research center of JUMS. The authors declare no competing interests.

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