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# Monitoring antiretroviral therapy in HIV/AIDS patients in resource-limited settings: CD4 counts or total lymphocyte counts?☆

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**Summary**
*Objective:* In order to improve the monitoring of disease progression and therapeutic effectiveness in the management of HIV/AIDS in a resource-limited setting, this study was carried out to establish a correlation between total lymphocyte counts (TLC) and CD4 lymphocyte counts in HIV-1 infected/AIDS adults in Yaoundé, Cameroon.
*Methods:* Full blood counts, differential white, and CD4 counts were measured in 149 patients using standard methods. The correlation coefficient established correlation between values. Sensitivity, specificity, and positive predictive values were calculated as required.
*Results:* The mean TLC, CD4 count, and CD4% as well as CD4/CD8 ratios were $1.932 \pm 0.895 \times 10^9$/L, $268 \pm 183$ cells/mm$^3$, $14.51 \pm 15.9$%, and $0.34 \pm 0.25$, respectively. Only a weak correlation was observed between TLC and CD4 counts ($r = 0.41$, $p = 0.05$). As a predictor of CD4 count, TLC cut-offs $<2.0$ and $<1.0 \times 10^9$/L were unable to predict these values reliably, but showed that at TLC cut-offs of $<1.0 \times 10^9$/L there was a high chance of CD4 counts being under 200 cells/mm$^3$.
*Conclusions:* These data suggest that TLC are of limited value in predicting CD4 counts and should not be substituted for CD4 counts whenever possible. However, TLC may be reliably used in designing algorithms and programs for initiating patient management and follow-up in this setting.


## Introduction

The prevalence of HIV/AIDS in Sub-Saharan Africa has reached alarming levels, with about 66% of the 39.4 million people infected living in countries of this region.[1] These are the countries with the least available resources to cope with the magnitude of the pandemic. Thus, the socio-economic impact has been overwhelming.

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Although prevention remains a major key to fighting the pandemic, appropriate management of those already infected is essential to reduce morbidity and mortality. Currently, management requires that disease stage, treatment, and progression be monitored with the use of sophisticated laboratory tests such as the measurements of plasma viral load (VL) and CD4 counts.[2–4] These tests require sophisticated and expensive equipment usually not readily available or accessible in most countries of Sub-Saharan Africa and other resource-limited settings.[5]

In Cameroon, there are currently six laboratories in which CD4 counts can be reliably performed at a cost of US$25–40, and two for viral load measurements at a cost of US$75–78. Cameroon is a country with a GNP per capita in the range of US$600–650. Thus, these tests are very expensive for an average Cameroonian. On the other hand, other routine tests also useful in monitoring therapy include the full blood count with a differential white cell count. This is more easily accessible through varying techniques, even in rural health institutions of the country. Thus, if a clear correlation was established between total lymphocyte counts (TLC) and CD4 counts, this could be an invaluable tool for monitoring patients in Cameroon and other resource-limited countries.

Hence, this study was undertaken to describe the correlation between TLC and CD4 counts in HIV/AIDS patients in Yaoundé at various stages of their disease, and to determine whether TLC could be used to predict CD4 counts in this population.

## Methodology

This was a cross-sectional study in which clinical and demographic data were obtained from an interview and physical examination of each patient after informed consent. Blood samples were collected during that visit from each participant, corresponding respectively to different stages of their disease evolution (HIV diagnosis and/or initiation/monitoring of anti-retroviral treatment (very few)). For each patient 5 mL of whole blood was drawn from a peripheral vein into tri-potassium EDTA tubes between 8 and 10 a.m. in the morning. All samples were analyzed within 4 h of collection for full blood counts, differential white cell counts, and lymphocyte subset measurements.

Full blood counts were measured using an automatic electronic particle counter (SYSMEX F820 micro cell counter, Kobe, Japan) and the differential white cell counts were estimated by microscopy on a May–Grunwald–Giemsa-stained thin blood film. T-cell subset analyses for CD4 counts were done by conventional flow cytometry using a Becton–Dickinson FACScount (California, USA). The revised 1993 Centers for Disease Control (CDC) classification of AIDS patients[6] was used to categorize the patients into categories A, B, and C.

All data were analyzed using Epi-Info™ software, version 6.0. The correlation coefficient was used to establish correlation between TLC and CD4 counts. Values of $r$ of $<0.59$ were considered a weak correlation, 0.60–0.69, a moderate correlation, and equal or $>0.70$ a strong correlation. The sensitivity, specificity, and positive and negative predictive values were calculated at various TLC cut-offs. The analysis of variance was used to determine the level of statistical significance between continuous variables and categorical variables. Values of $p$ less than or equal to 0.05 were considered statistically significant.

## Results

Of 149 patients studied, 87 (58.4%) were females and 62 (41.6%) males. The females were on average younger than the males, with mean ages of $33.9 \pm 6.7$ and $37.3 \pm 8.2$ years, respectively. About 84.5% of cases were symptomatic with 54.3% in category C of the CDC classification. Only 5.4% of the patients seen were on antiretroviral drugs.

The mean $\pm$ standard deviation (SD) of TLC and CD4 counts/CD4% were respectively $1.932 \pm 0.895 \times 10^9$/L and $268 \pm 183$ cells/mm$^3$/$14.51 \pm 15.9$%, while the mean $\pm$ SD of CD8 counts and CD4/CD8 ratio were $874 \pm 459$ cells/mm$^3$ and $0.34 \pm 0.25$, respectively (Table 1).

An overall weak correlation was observed between TLC and CD4 counts for the whole group (Table 1). This correlation was generally further weakened when samples were stratified into groups according to age, sex, and clinical category. A moderate correlation was, however, observed between TLC $<2.0 \times 10^9$/L and CD4 $<100$ cells/mm$^3$ ($r = 0.60$; Table 1). The correlations between TLC, and CD4% and the CD4/CD8 ratio were generally found to be weak.

**Table 1** Mean total lymphocyte counts (TLC) and CD4 counts and correlation between mean TLC and CD4 counts at various TLC cut-off values

| Parameter | Mean $\pm$ 1SD | Correlation with TLC ($r$ value) | $p$ value |
|---|---|---|---|
| TLC ($\times 10^9$/L) | $1.932 \pm 0.895$ | | |
| CD4 (cells/mm$^3$) | $268 \pm 183$ | 0.41 | 0.05 |
| CD4% | $14.51 \pm 15.9$ | 0.16 | 0.10 |
| CD4/CD8 ratio | $0.34 \pm 0.25$ | 0.03 | 0.10 |
| Correlation with TLC cut off points: | | | |
| CD4 $<100$ (cells/mm$^3$) | | | |
| TLC $<1.0 \times 10^9$/L | | 0.41 | 0.10 |
| TLC $<2.0 \times 10^9$/L | | 0.60 | 0.10 |
| CD4 $<200$ (cells/mm$^3$) | | | |
| TLC $<1.0 \times 10^9$/L | | 0.31 | 0.10 |
| TLC $<2.0 \times 10^9$/L | | 0.31 | 0.10 |

**Table 2** Prediction of CD4 counts by TLC cut-off values

| | Sensitivity (%) | Specificity (%) | PPV (%) |
|---|---|---|---|
| CD4 <100 (cells/mm$^3$) | | | |
| TLC cut-off $<1.0 \times 10^9$/L | 34.6 | 95.1 | 60.0 |
| TLC cut-off $<2.0 \times 10^9$/L | 84.6 | 42.3 | 23.7 |
| CD4 <200 (cells/mm$^3$) | | | |
| TLC cut-off $<1.0 \times 10^9$/L | 20.3 | 96.7 | 80.0 |
| TLC cut-off $<2.0 \times 10^9$/L | 76.3 | 46.7 | 48.4 |

PPV: Positive predictive value.

As a predictor of CD4 count, TLC $<2.0 \times 10^9$/L had a high sensitivity (84.6%) to detect CD4 counts less than 100 cells/mm$^3$ but a low specificity (42.3%) and positive predictive value (23.7%). When the TLC cut-off was lowered to $<1.0 \times 10^9$/L, the specificity and positive predictive values were respectively 95.1% and 60% while the sensitivity reduced considerably to 34.6% (Table 2).

On the other hand, to predict CD4 counts <200 cells/mm$^3$, TLC $<2.0 \times 10^9$/L had a high sensitivity (76.3%), a specificity of 46.7%, and a positive predictive value of 48.4%. When the TLC cut-off was lowered to $<1.0 \times 10^9$/L, the specificity and positive predictive values increased to 96.7% and 80% respectively, and the sensitivity was 20.3% (Table 2).

## Discussion

Although several studies have been carried out in different contexts in an attempt to recommend TLC use over CD4 counts, the results have varied widely. It is essential for each race and community to establish its own correlation because of possible racial and environmental influences.[7,8] Such environmental factors may include endemic disorders that are known to impact leukocyte counts. For example, malaria is an endemic infection in some countries including Cameroon, and has been shown to cause neutropenia.[9]

Of 149 cases analyzed, 84.5% were symptomatic with 54.3% in category C of the revised 1993 CDC classification. The cost of healthcare as well as the low socio-economic level of the country could explain why people seek medical care only in advanced stages. Based on the CDC definition for AIDS,[6] antiretrovirals (ARV) are proposed for patients in categories A3, B3, and C1, C2 and C3. Thus, all patients with CD4 counts <200 cells/mm$^3$ are recommended to start ARV. Yet only 5.4% of our patients were already on ARV drugs. Many patients may not have qualified for treatment based on this classification because this classification requires prior knowledge of CD4 counts. Furthermore, the lack of financial access to the drugs may be a contributing factor even when they are appropriately prescribed.

The mean $\pm$ SD of TLC, CD4 and CD8 counts, CD4%, and CD4/CD8 ratio were respectively $1.932 \pm 0.895 \times 10^9$/L, $268 \pm 183$ cells/mm$^3$, $874 \pm 459$ cells/mm$^3$, $14.51 \pm 15.9$, and $0.34 \pm 0.25$. These mean values, although higher in males than females, were not statistically different ($p > 0.05$). These findings, however, contradict those reported by Reichert et al.,[10] Tugume et al.,[11] Zekeng et al.,[12] and Mbanya et al.,[13] who reported higher values for females than for males. This inconsistency may be due to our relatively smaller sample size compared to those of the other studies. In fact, Reichert et al.[10] had earlier reported the possibility that small sample sizes could be the reason for contradictory findings on gender variations in lymphocyte subset values.

An overall weak correlation was observed between TLC and CD4 counts for the whole group ($r = 0.41$, $p = 0.05$), with a moderate correlation observed between TLC $<2.0 \times 10^9$/L and CD4 <100 cells/mm$^3$ ($r = 0.60$, $p = 0.10$). Other authors have reported similar findings. Guarner et al.[14] found a poor correlation of TLC and CD4 counts ($r = 0.59$). Van der Ryst et al.[15] found a moderate correlation between these values, but which was considerably weakened when the CD4 counts were grouped into three classes. When CD4 counts <100 cells/mm$^3$ or <200 cells/mm$^3$ and TLC $<1.0 \times 10^9$/L or $<2.0 \times 10^9$/L were matched, a moderate correlation ($r = 0.60$, $p = 0.10$) was observed only between CD4 <100 cells/mm$^3$ and TLC $<2.0 \times 10^9$/L. With a TLC cut-off of $<1.0 \times 10^9$/L, there is a 96.7% chance that CD4 counts are less than 200 cells/mm$^3$, suggesting that it may be cost effective not to measure CD4 counts when TLC is $<1.0 \times 10^9$/L. Incidentally, the World Health Organization (WHO)[16] recommended that where CD4 counts cannot be available, that TLC of $<1.2 \times 10^9$/L be considered sufficiently low to initiate ARV therapy. Our findings suggest that the TLC is a poor predictor of CD4 counts, comparable to those of Van der Ryst et al.,[15] in which they demonstrated that a TLC less than or equal to $2.0 \times 10^9$/L had a sensitivity of 90.3% to detect patients with a CD4 count of <200 cells/mm$^3$, but a specificity of only 53.7%, confirming that TLC is not a good predictor of CD4 counts. Interestingly, other studies have observed a good correlation between TLC and CD4 counts. Beck et al.[3] reported this correlation in patients in London, UK to be more marked among the symptomatic cases. In another study in Uganda, Kamya et al.[17] reported a good correlation between TLC and CD4 counts. However, they also noted that the TLC cut-off of $1.2 \times 10^9$/L recommended by the WHO did not serve to identify WHO stage 2 and 3 patients with CD4 counts <200 cells/mm$^3$.

At late stages of HIV infection there is severe depletion of CD4 cells. Since most of our patients were already in category C, this explains the very low CD4 counts and possibly the poor correlation of CD4 counts to TLC. Furthermore, since we measured TLC by multiplying the percentage of lymphocytes (from the differential count) by the white blood cell count (WBC), any variations in the other components of the WBC would indirectly influence the percentage of lymphocytes and hence the TLC. The high prevalence of opportunistic infections at late stages of HIV infection would certainly lead

to alterations in the relative percentages of the different components of the differential white cell count. These virtual modifications of the TLC could also be another reason for the poor correlation found between TLC and CD4 counts.

In the advent of HIV/AIDS there has been an upsurge in tuberculosis and other infections that may impact TLC,[18] and hence misdirect the decision to start or modify ARV treatment in some patients. Other sexually transmitted infections such as syphilis are also closely associated with HIV/AIDS and may modify TLC.[19,20] Thus, it is essential that each patient be reliably monitored with TLC only based on studies established within their given population.

There are more and more initiatives being created and implemented worldwide in order to increase access to ARV therapy for HIV/AIDS patients in resource-limited settings. These include the WHO '3 by 5', the PEPFAR, Melinda and Bill Gates, Hope for the African Child Initiative, and others. This means that more patients will soon be able to start ARV. However, the pre-treatment laboratory requirements as well as adequate monitoring of efficacy, tolerance, and toxicity during treatment are indispensable in this process. Thus, although our findings suggest that TLCs are of limited value in predicting CD4 counts and should not be substituted for CD4 counts in patient management and follow-up in this population, nevertheless, TLC can be used in resource-restrained settings; a very low TLC could be used to develop appropriate algorithms for managing these cases and could serve as a useful guide for initiating chemoprophylaxis for opportunistic infections in severely immune-suppressed individuals (CD4 $<$200 cells/mm$^3$).

Hence we recommend that while TLC values may be useful, that less sophisticated and less costly methods of determining CD4 counts such as microvolume fluorimetry[21] and ELISA techniques be evaluated and made available for use in resource-limited settings.

*Conflict of interest:* No conflict of interest to declare.

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