Use of Total Lymphocyte Count for Monitoring Response to Antiretroviral Therapy

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The CD4 cell count has become a key laboratory measurement in the management of human immunodeficiency virus (HIV) disease. In ideal situations, HIV-infected persons are followed up longitudinally with serial CD4 cell counts to determine disease progression, risk for opportunistic infection, and the need for prophylactic or therapeutic intervention. However, the use of the CD4 cell count in resource-limited settings is often not possible because of lack of availability and high cost. Thus, other laboratory markers have been proposed as substitutes for the CD4 cell count. The data regarding the clinical utility of the total lymphocyte count (TLC) as a potential surrogate marker of immune function in patients with HIV disease are examined. The role of the TLC in the initiation of antiretroviral therapy and opportunistic infection prophylaxis, as well as the role of the TLC in monitoring the response to antiretroviral therapy, are also addressed.

CD4 cell count and CD4 cell percentage are key markers for determining disease progression and risk for opportunistic infection in HIV-infected patients. These markers are of greatest use in treating the asymptomatic patient, in whom disease stage is more difficult to assess clinically and for whom laboratory measurements serve as guidelines for the initiation of therapy and opportunistic-infection prophylaxis. However, providers in resource-constrained settings may not have access to this laboratory measurement or its cost may be prohibitive, resulting in the need for an alternative, surrogate marker. Given the decreasing costs and increased availability of antiretroviral therapy (ART) in the developing world, this is an issue of critical and increasing importance [1]. A number of previous studies indicate that the total lymphocyte count (TLC) may be useful as a surrogate marker of immune status in certain settings. However, controversy regarding the utility of the TLC remains.

The laboratory determination of the CD4 cell count requires the use of a fluorescence-activated flow cytometer, thus making the process both expensive and labor-intensive. However, the TLC (derived by multiplying the percentage of lymphocytes by the WBC count) is calculated from the complete blood cell count and is thus a relatively inexpensive measurement. In terms of comparative costs, a single CD4 cell count may be 30–50 times more expensive than a complete blood cell count. For example, in 2000, a single CD4 cell count in Chennai, India, cost $30, and the cost of a single complete blood cell count was $0.80 [2]. Costs for these tests in Durban, South Africa, (in 2003) are comparable. Furthermore, the increasing availability and decreasing cost of ART in the developing world indicate that the cost of monitoring patients receiving ART may soon equal or actually exceed the cost of the drugs themselves [3].

Many guidelines exist regarding the use of laboratory markers in monitoring ART in resource-limited settings. Although World Health Organization guidelines [4] advocate the use of TLC as a surrogate marker for CD4 cell count, the data regarding this issue have been mixed. We will review the available studies, their limitations, and their implications for the use of the TLC in 2 clinical circumstances: first, disease staging and initiation of ART or opportunistic-infection prophylaxis, and, second, monitoring the response to ART.

USE OF TLC FOR DISEASE STAGING AND INITIATION OF ART OR OPPORTUNISTIC-INFECTION PROPHYLAXIS

A variety of recent small studies have sought to determine the utility of the TLC in predicting the stage of HIV disease. The majority of these studies indicate a positive correlation between TLC and CD4 cell count, although the specific data on correlation coefficients, sensitivity, specificity, and positive predic-
TLC as a surrogate marker for CD4 cell count: supportive data. One of the earliest studies addressing the issue of TLC as a surrogate marker for CD4 cell count was performed by Blatt et al. [5]. These investigators studied 828 US Air Force personnel with HIV disease. They found that 98% of those who had a TLC of $<1000$ cells/mm$^3$ had a CD4 cell count of $<200$ cells/mm$^3$, indicating a specificity of 98%. In another study of a cohort of 831 South African patients with HIV disease, Post et al. [6] concluded that a TLC of $<1250$ cells/mm$^3$ and a CD4 cell count of $<200$ cells/mm$^3$ were equal predictors of disease progression. However, both of these studies used low cutoff points for the TLC (i.e., a TLC of $<1000–1250$ cells/mm$^3$), thus maximizing specificity but lowering sensitivity. For example, in Blatt et al. [5], the sensitivity of a TLC of 1000 cells/mm$^3$ was only 53%. Additional studies have used a higher TLC cutoff point, thereby maximizing sensitivity but lowering specificity.

Many of these recent data have been presented as abstracts and have not yet been published in peer-reviewed journals [7–12]. Jacobson et al. [7] followed up 2044 patients with HIV disease in San Francisco and compared CD4 cell count and TLC by using receiver-operator characteristic curves. A TLC of $<1900$ cells/mm$^3$ was found to have maximal sensitivity (74%) and specificity (73%) for predicting a CD4 cell count of $<350$ cells/mm$^3$. The authors indicate that lower TLC values had similar combined sensitivity and specificity in predicting a CD4 cell count of $<200$ cells/mm$^3$, although the data are not shown. A recent study by Kumarasamy et al. [2] looked at the correlation between the TLC and a CD4 cell count of $<350$ cells/mm$^3$. This CD4 cell count value was chosen on the basis of the recommendation to initiate trimethoprim-sulfamethoxazole prophylaxis, at higher CD4 cell counts, in the developing world, given the increased risk for other diseases, such as infection with nontyphi Salmonella, Streptococcus pneumoniae, and Isospora belli. This study of 405 South Indian patients with HIV disease (122 with active tuberculosis [TB]) examined 650 paired TLCs and CD4 cell counts and found a high degree of correlation ($r = 0.744$) between all paired counts. It was concluded that a TLC cutoff point of $<1400$ cells/mm$^3$ would identify 73% of patients with CD4 cell counts of $<200$ cells/mm$^3$ (sensitivity, 73%; specificity, 88%; PPV, 76%; negative predictive value [NPV], 86%) and a TLC $<1700$ cells/mm$^3$ would identify 70% of patients with a CD4 cell count of $<350$ cells/mm$^3$ (sensitivity, 70%; specificity, 86%; PPV, 86%; NPV, 69%).

TLC as a surrogate marker for CD4 cell count: less supportive data. Other authors have had less convincing results and found a weaker correlation between CD4 cell count and TLC. Several abstracts presented at the 14th International AIDS Conference concluded that there was a poor correlation between CD4 cell count and TLC. Okiror et al. [13] followed up 377 Vietnamese patients with HIV disease, and, after performing a regression analysis, they found only a slight correlation between TLC and CD4 cell count ($r = 0.52$). Similarly, Mbanya and Assah [14] studied 149 patients with HIV disease from Cameroon and found a weak correlation between CD4 cell count and TLC ($r = 0.41$).

A common concern among authors of studies less supportive of TLC as a surrogate marker is the variability in sensitivity and specificity according to the TLC cutoff point used. Van der Ryst et al. [15] performed a study among 2777 South African patients and found that, although a statistical correlation existed between TLC and CD4 count ($r = 0.704$), the PPV and specificity (54%) of the TLC in predicting the CD4 cell count were poor. When the TLC cutoff was lowered, specificity increased, but sensitivity decreased substantially. Similar findings were made by Kassa et al. [16] among Ethiopian patients, and the study concluded that TLC could not be a general surrogate marker for CD4 cell count, given the variability in sensitivity and specificity with different TLC cutoff points.

MONITORING RESPONSE TO ART

As summarized above, the majority of existing data focuses on the correlation between TLC and CD4 cell count in patients for the purpose of determining the stage of HIV disease. However, the use of the TLC to monitor patients receiving ART is also of importance, given the increased access to ART in resource-poor settings and the long-term treatment and monitoring needs once therapy has been initiated. At this point, the data regarding this issue are quite limited.

Flanigan et al. [17] performed a preliminary analysis of 177 paired TLCs and CD4 cell counts from a subset of patients receiving ART in the study cohort described in Kumarasamy et al. [2] and found that patients with a statistically significant increase in CD4 cell count also had a parallel increase in mean TLC ($P < .001$). Similar results were reported by Spacek et al. [18] in a retrospective analysis evaluating the change in CD4 cell count as predicted by a change in TLC during a 24-month follow-up period in a subset of patients receiving ART (total cohort, 3269 patients). A similar observational cohort study by Badri and Wood [19] examined the use of TLC to monitor change in viral load or response to ART among 266 South African patients evaluated at 5 time points (baseline and weeks 4, 8, 12, and 48 of therapy). A significant correlation between the change in TLC and change in CD4 cell count ($r = 0.61; P < .0001$) was found at all time points. Furthermore, the sensitivity and specificity of an increase or decrease from baseline
in TLC for similar trend in CD4 cell count during follow-up were 83.4% and 87.3%, respectively.

**Implications and summary of studies.** TLC may have a role both in decisions about the initiation of ART and in the monitoring of immunologic response to ART in resource-constrained settings. There have been a wide range of findings in published studies, many of which have included small numbers of patients. These studies are outlined in table 1. To summarize, a total of 15,102 patients enrolled in 15 different studies have been followed up to determine the ability of the TLC to predict the CD4 cell count and HIV disease stage. Eleven of these studies (which included a total of 11,713 patients) contained data that, overall, indicated support for the predictive ability of the TLC, whereas 4 have concluded that the TLC was not a reliable predictor of the CD4 cell count. In contrast, only 3 different studies, with a total of 443 patients, have attempted to evaluate the use of TLC in monitoring the response to ART. All of these studies have produced data that, overall, support the use of TLC as a surrogate marker for CD4 cell count in monitoring patients receiving ART.

**TLC cutoff point.** The TLC cutoff point has a major impact on the sensitivity and specificity of this marker in predicting CD4 cell counts. The use of a TLC of $\leq 2000$ cells/mm$^3$ maximized sensitivity, but lowered specificity. The advantage of the use of a higher TLC cutoff point would be to reduce the likelihood of failing to identify those patients who might benefit from ART or opportunistic-infection prophylaxis. The disadvantages of such an approach include institution of ART prematurely in patients who may not yet need treatment, with resultant increased expenses and unnecessary cases of drug toxicity. In this scenario, the CD4 cell count would actually be higher than predicted by the TLC.

As the TLC decreases to 1000 cells/mm$^3$, sensitivity decreases and specificity increases. With the lower TLC cutoff point, fewer patients with CD4 cell counts of $\geq 200$ cells/mm$^3$ are incorrectly identified, but a greater percentage of persons with CD4 cell counts of $<200$ cells/mm$^3$ are not identified. This approach carries the advantage of reduced costs and avoids the problem of unnecessary cases of toxicity, but has the disadvantage of potentially failing to identify patients in need of treatment (i.e., CD4 counts are actually lower than predicted by TLCs).

Ideally, a TLC cutoff point of 2000 cells/mm$^3$ could be used in settings where resources such as ART and laboratory monitoring are affordable and readily available. However, in resource-limited settings where this is not the case, the advantages and disadvantages of each approach argue for use of a lower TLC cutoff point (e.g., 1000 cells/mm$^3$) with maximal specificity as the most appropriate and practical strategy for determining eligibility for the initiation of ART in asymptomatic HIV-infected patients. The TLC appears to be equivalent to the CD4 cell count in monitoring response to therapy, and, in the absence of viral load testing, available information suggests that it may serve as an inexpensive substitute for the CD4 cell count. Alternatively, an algorithm could be developed that, by using multiple measurements of the TLC in conjunction with occasional measurement of the CD4 cell count, could provide a less expensive way to guide HIV therapy and management.

**Other parameters.** Many studies have used multiple parameters (including hemoglobin levels, body mass index, platelet count, and clinical symptoms) to predict CD4 cell count and disease stage [9, 11, 12, 21]. Hemoglobin level is an inexpensive marker that has been shown to correlate with progression to AIDS and to decline around the time of the development of AIDS [12]. A recently published study by Spacek et al. [18] retrospectively evaluated the ability of TLC to predict CD4 cell count in a cohort of 3269 patients. They found that the TLC alone (with a cutoff point of $<1200$ cells/mm$^3$) had a relatively low sensitivity (71%) and specificity (82%) for predicting a CD4 count of $<200$ cells/mm$^3$. However, when both the TLC and the hemoglobin level (with a cutoff point of $<12$ g/dL) were used as predictors, this sensitivity was increased to 78% for men (specificity, 80%; PPV, 84%; NPV, 72%) and to 86% for women (specificity, 73%; PPV, 75%; NPV, 84%) (as calculated by an average of 2 algorithmic methods used in this study). Chen et al. [21] also used a multivariate model with similar parameters (TLC, hemoglobin level, platelet count, and sex) for predicting CD4 cell counts of $<200$ cells/mm$^3$. Their study of 1189 patients with HIV disease found this model to have 91% sensitivity, 73% specificity, and 88% PPV for predicting a CD4 cell count of $<200$ cells/mm$^3$. The use of multiple, inexpensively measured parameters in the form of an algorithm for determining disease stage thus appears to be useful in resource-constrained settings.

### OTHER FACTORS INFLUENCING CD4 CELL COUNT

**HIV-TB coinfection.** TLC can be influenced by factors independent of disease progression, including the presence of endemic diseases. In many resource-constrained countries, TB is the most common opportunistic infection associated with HIV disease, and both TB-related immune activation and anti-TB therapy may lead to fluctuations in CD4 cell count. Three published studies have examined the effect that TB coinfection and anti-TB therapy have on the CD4 cell count in HIV-infected patients [2, 20, 22]. Studies performed by Martin et al. [20] and Kumarasamy et al. [2] both found a positive correlation (correlation coefficients of 0.7 and 0.74, respectively) between CD4 cell count and TLC in patients coinfected with HIV and TB. A more recent study measured CD4 cell counts in coinfected patients treated for active TB alone (note: patients did not receive ART). The authors found a median increase in
<table>
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<th>No. of patients</th>
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<th>Study design</th>
<th>Correlation coefficient</th>
<th>Sens, spec, and PPV of TLC for prediction of CD4 cell count of &lt;200 cells/mm³ in %, by TLC cutoff point</th>
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<td>1993</td>
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<td>Sens, 95; spec, 60; Sens, 53; spec, 98</td>
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<td>2044</td>
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<td>Receiver-operator characteristic curve study</td>
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<td>India</td>
<td>405</td>
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<td>Cross-sectional</td>
<td>Spearman, 0.74</td>
<td>Sens, 92; spec, 62; PPV, 56; Sens, 46; spec, 97; PPV, 90</td>
<td>For TLC of &lt;1900 cells/mm³ to predict CD4 cell count of &lt;350 cells/mm³: sens, 74%; spec, 73%</td>
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<td>Kohreunudom [8]</td>
<td>2002</td>
<td>Thailand</td>
<td>525</td>
<td>Unknown</td>
<td>Linear regression analysis</td>
<td>0.23 × TLC−130 (Swilk R &lt; 0.01)</td>
<td>...</td>
<td>...</td>
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<td>Malawi</td>
<td>208</td>
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<td>Pearson, 0.52</td>
<td>Sens, 89; spec, 41; Sens, 44; spec, 91</td>
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<td>Pearson, 0.49</td>
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<td>Study of HIV-infected children (average age, 28 months)</td>
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<td>Ethiopia</td>
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<td>2003</td>
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<td>US</td>
<td>3269</td>
<td>Some</td>
<td>Retrospective</td>
<td>Spearman, 0.72</td>
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<td>PPV, 93; NPV, 69</td>
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<td>Initiation of ART: less supportive data</td>
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<td>149</td>
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<td>South Africa</td>
<td>2777</td>
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<td>$R = 0.7$</td>
<td>Sens, 90; spec, 54; Sens, 48; spec, 96</td>
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<td>Kassa et al. [16]</td>
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<td>Ethiopia</td>
<td>86</td>
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<td>Sens, 52; spec, 97; PPV, 90</td>
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<td>Monitoring response to ART</td>
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<td>177</td>
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<td>...</td>
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<td>266</td>
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<td>US</td>
<td>3269</td>
<td>Some</td>
<td>Retrospective</td>
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<td>...</td>
<td>Increase in TLC predicts increase in CD4 cell count; Large decrease in TLC predicts decrease in CD4 cell count.</td>
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NOTE. ART, antiretroviral therapy; NPV, negative predictive value; PPV, positive predictive value; sens, sensitivity; spec, specificity.
the CD4 cell count of 239 cells/mm$^3$ over a 6-month period [22].

**HIV-malaria coinfection.** Malaria is another prevalent endemic disease known to have specific interactions with HIV infection. For example, the impact of malaria infection on HIV viral replication has been well documented [1, 23] and has been reported to result in up to a 100-fold increase in viral replication. The effect on CD4 cell count is less well documented. However, patients with HIV infection are known to have an increased likelihood of developing malaria, as well as a decreased response to prophylaxis [24]. Further studies are needed that examine the impact of different endemic diseases (e.g., TB, malaria, and other parasitic infections) on both HIV disease progression and response to ART, as well as any related effects on the correlation between TLC and CD4 cell count.

**Demographic, geographic, and regional variance.** Variation in CD4 cell count may occur among patients of different racial, ethnic, and geographic backgrounds and among patients of different ages. For example, CD4 cell counts in HIV-infected Asian and Ugandan patients with HIV disease have been reported to be lower than those of HIV-infected white patients in Europe and the United States [2]. Other studies have shown that West African adults have a physiological lymphocytosis that leads to TLC and CD4 cell counts that are higher than those of European patients. Also, lymphocyte counts in children with HIV disease are generally higher than those in adults with HIV disease, and only 1 published study [10] has focused on the relationship of TLC and CD4 cell count in children. Thus, consideration must be given to demographic differences and their effect on CD4 cell counts when interpreting studies and choosing TLC cutoff points. Finally, additional observational studies are needed that examine the value of TLC and CD4 cell counts in predicting the development of HIV-related opportunistic infections and response to ART in different age groups, as well as in settings outside of North America and Europe.

**FUTURE DIRECTIONS**

More convenient and less expensive technologies are needed as alternatives to currently available CD4 cell assays in resource-limited settings. Political pressure has been successful in reducing the cost of ART, and it needs to be extended to advocacy for reducing the cost of determining HIV disease stage and monitoring therapeutic outcomes.

**CD4 cell assays.** Cheaper and simpler manual CD4 assays have been developed by Beckman Coulter (Manual CD4 Kit) and Dynal (Dynabeads CD4). Rodriguez et al. [25] developed microchip-based methods for rapidly measuring CD4:CD8 ratios and CD4 percentages at low cost. In addition, Becton Dickinson has reduced prices for resource-limited countries, and affordable reagents for conducting CD4 cell testing have been introduced by the organization Afford CD4 [1].

Other investigators have introduced alternative laboratory methods, such as combining volumetric flow cytometry with automatic gating protocols and autobiosamplers and have found these methods to be both efficient and accurate [26, 27]. This system, which uses generic monoclonal antibodies, costs less than $1–$2 per assay for reagents alone [1]. Furthermore, a standardized fixative (TransFix; NEQAS) has been developed that permits optimal fixation and transport to laboratories where affordable CD4 testing can be performed [28].

**Viral load monitoring.** It is unlikely that viral load monitoring will be cost-effective for resource-poor countries. Recent guidelines have omitted the use of viral load altogether in resource-limited settings [4]. HIV p24 antigen level is less costly to measure and is a potential alternative to measurement of viral load because it is strongly correlated with HIV RNA levels [1].

**CONCLUSIONS**

TLC has a potential role as a surrogate marker for CD4 cell count in resource-constrained settings. Study findings indicate that the sensitivity and specificity of the TLC are not sufficiently high to replace the CD4 cell count, but they may be useful in circumstances where this is unavailable. Furthermore, the use of a lower TLC cutoff point (e.g., 1000 cells/mm$^3$) may be the most practical and cost-effective public health approach in resource-limited settings, where the consequences of unnecessary or premature treatment costs and cases of drug toxicity may outweigh the risk of failing to identify patients potentially in need of ART. Further research is needed to determine the utility of this measurement, both for making decisions about the initiation of ART and for monitoring response to ART. In addition, further studies are needed to assess the utility of using the TLC in combination with other clinical and laboratory data for risk stratification and evaluating therapeutic response. Finally, the development of newer, equally precise, but less expensive technologies is essential if the benefits of ART are to be extended widely in resource-limited settings.

**References**


