PREDICTING CD4 COUNT USING TOTAL LYMPHOCYTE COUNT: A SUSTAINABLE TOOL FOR CLINICAL DECISIONS DURING HAART USE

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Abstract. Understanding the total lymphocyte count (TLC)-CD4 count relationship could aide design predictive instruments for making clinical decisions during antiretroviral therapy, especially in underserved resource-poor settings. We performed multiple regression analyses to assess the prediction of CD4 count using TLC on 771 participants with 4,836 visits. In linear and logistic regression TLC, hemoglobin, gender, history of AIDS, and weight predicted CD4 count and CD4 < 200, respectively, before and after highly active antiretroviral therapy (HAART) use. On HAART, the adjusted odds ratios (OR) for TLC < 1500 (optimal TLC cutoff) were 5.1 (95%CI 4.0, 6.5; P < 0.001), and off HAART, 4.6 (95%CI 3.4, 6.2; P < 0.001) with high predictive power. TLC predicts CD4 count and CD4 < 200 cells/μL well during HAART. Including the additional factors improves performance. TLC is simple and inexpensive and can be used in many ways to develop clinical decision-making tools in underserved resource-poor settings during HAART therapy.

INTRODUCTION

The burden of HIV in resource-poor countries is extensive, and a large proportion of HIV patients rely on accessing health care services in rural and underserved areas that do not have the capacity or capability to determine CD4 cell counts. In the absence of viral loads and CD4 counts for monitoring HIV disease, the value of total lymphocyte count (TLC) as a surrogate for CD4 has been argued.1,2 Viral loads and CD4 counts demand highly skilled laboratory personnel and costly maintenance of sophisticated equipment.3 The focus of research regarding TLC has been on its use in determining when to start therapy.4 Current World Health Organization (WHO) guidelines only commit to using TLC in conjunction with clinical data as a criteria to initiate highly active antiretroviral therapy (HAART) in resource poor settings.5 WHO provides no guidelines on the specific use of TLC in making clinical decisions after patients are started on HAART. TLC is inexpensive and simple to perform and has been shown to be reliable in the HAART-naïve population, but its value may not only be in its ability to identify the need to start HAART but to monitor patients’ progress and immune status during HAART as well.6 Because effective treatment with HAART would tend to stabilize CD4 count and TLC, the smaller variation in these parameters observed during therapy may be insufficient to adequately allow CD4 count prediction using TLC. Understanding TLC could aide designing useful and appropriate predictive instruments for use in making clinical decisions regarding ongoing antiretroviral therapy, especially in rural and underserved areas.

To the best of our knowledge, few studies have compared the utility of TLC as a predictor for CD4 count during and in the absence of HAART or have examined the collective impact of using hemoglobin, weight, presence of an AIDS defining illness (ADI), and gender to improve the TLC’s predictive performance.3,7 There are no large data sets of HAART-experienced populations in resource-poor settings.

METHODS

Participants. Study participants were those enrolled in the ongoing Nutrition for Healthy Living Study (NFHL) to examine the performance of TLC in predicting CD4 counts to demonstrate the possibility and to generate hypotheses for future studies when appropriate data are available in resource-poor settings. A secondary goal was to assess how including data on readily available factors may improve the utility of TLC in monitoring patients before and during antiretroviral therapy.

Measurements. History of ADI was obtained through interviews using a standardized questionnaire. Body weight was measured using a digital scale after a 5-hour fast to the nearest 0.1 kg. Complete blood counts with differential counts were performed using the Sysmex SE analyzer (Long Grove, IL). T-cell subsets, including CD4 cells, were determined using a fluorescent monoclonal antibody labeled cell sorter and expressed as cells/μL. HAART use was defined as receipt of any of the following regimens: 2 protease inhibitors (PI), 1 PI and 2 nucleotide reverse transcriptase inhibitors (NRTI), or 1 non-nucleotide reverse transcriptase inhibitor (NNRTI) and 2 NRTIs.3

Statistical analysis. Baseline analysis. We performed comparisons of baseline demographic and clinical characteristics...
according to HAART use using the Student’s *t* test for normally distributed variables, Wilcoxon rank sum test for non-normal variables, and χ² test for categorical variables.

**Statistical approach.** The main predictor variable was TLC and the outcome was CD4 count. We performed both linear and logistic regression modeling to explore the versatility of TLC in predicting CD4 count. We used generalized estimation equations (GEE) to adjust for the multiple visits per individual to obtain beta coefficients and odds ratios with robust standard errors. For the linear regression analysis, both TLC and CD4 were continuous variables. For the logistic regression, the outcome was a dichotomous variable representing CD4 < 200 cells/μL. For calibration of the TLC cutoff, we compared a range of cutoffs of TLC between 1,100 and 1,900 cells/μL to the CD4 < 200 cells/μL to obtain the optimal level for dichotomization for each subset analysis.

**Multivariate analyses.** We constructed separate multivariable linear and logistic regression models for those visits in which patients were on HAART and those off HAART. We selected factors that would be readily available in underserved areas in resource poor countries. These included gender, body weight, hemoglobin level, and presence of an ADI. We specifically sought to use each of these factors measured or obtained alongside CD4 count in each study visit to develop time-insensitive models. We used the C-statistic to compare the predictive powers of the various models.

Sensitivity analyses testing the influence of a) those categorized as non-HAART but on some form of antiretroviral therapy, and b) categorization of HAART use using data from the two consecutive visits (i.e., HAART use and non-HAART would be those who reported HAART use and those who reported no HAART use at both the study visit and the preceding visit, respectively).

All analyses were performed on SAS version 8.02 SE (SAS, Cary, NC).

**RESULTS**

There were 771 participants who contributed 4,836 visits, during which 3,177 visits were on HAART and 1,659 were not on HAART.

**Baseline characteristics.** Overall, of 771 participants whose average age was 40 years, 200 (26%) of the participants were female, 339 (47%) were non-white, and 389 (51%) were on HAART. Of those 382 (49%) participants categorized as off-HAART, 177 (46%) were not taking any antiretroviral medications, and 205 (54%) were on non-HAART regimens. The mean body weight was 76 kg (77 kg for men and 71 kg for women), and the mean hemoglobin was 13.8 g/dL (14.2 g/dL for men and 12.6 g/dL for women). The median CD4 count was 330 cells/μL, and 28% of the sample (216) had CD4 < 200 cells/μL. The median TLC was 1,692 cells/μL and 288 (37%) had TLC < 1,500 cells/μL. The baseline characteristics of the 771 study participants according to HAART use are shown in Table 1.

**Linear regression analyses.** The factors found to predict CD4 count and their associated beta coefficients are shown in Table 2. For those not on HAART, for each cell/μL increase in TLC (*P* < 0.001), being female (*P* < 0.001), having a history of ADI (*P* = 0.06), and every kg increase in body weight (*P* = 0.02), there was a mean increase in 0.19, 87.1, 12.9, and 1.14 cells/μL in the CD4 count, respectively, after adjusting for hemoglobin, year of recruitment, and non-HAART antiretroviral usage. R² for this model was 0.51. For those on HAART, for each cell/μL increase in TLC (*P* < 0.001), being female (*P* = 0.09), having a history of ADI (*P* = 0.84), and body weight (*P* = 0.008), there was a mean increase in 0.18,
29.4, 0.91, and 1.12 cells/μL in the CD4 count, respectively, after adjusting for hemoglobin and year of recruitment. R² for this model was 0.44. In both these linear regression models, hemoglobin lost its ability to predict CD4 count when adjusted for TLC.

Calibration for optimal TLC cutoff. For those not on HAART, the C-statistic peaked at a TLC cutoff (TLC1500) of 1,500 cells/μL with C = 0.778 when the sensitivity and specificity were 78.8 and 76.9, respectively. For those on HAART, the C-statistic also peaked at a TLC cutoff (TLC1500) of 1,500 cells/μL with C = 0.764 when the sensitivity and specificity were 70.6 and 82.3, respectively. In contrast, the C-statistics for predicting CD4 < 200 cells/μL using TLC as a continuous variable were 0.862 and 0.843 for those not on HAART and those on HAART, respectively. The sensitivities, specificities, and the C-statistics for each cutoff from 1,100 to 1,900 cells/μL models and the C-statistics for the continuous-TLC model are shown in Table 3.

Logistic regression analyses. In the absence of HAART, the associated adjusted odds ratios (OR) were 4.6 (95% CI 3.4, 6.2) for TLC1500, 0.73 (0.67, 0.80) for each g/dL higher in hemoglobin, 0.32 (0.23, 0.47) for female gender, 1.9 (1.4, 2.6) for having history of an ADI, and 0.98 (0.97, 0.99) for each kg heavier in body weight (all P values < 0.02). The C-statistic was 0.840. When the continuous TLC variable was used in this off-HAART model instead of the cutoff of 1,500 cells/μL, the C-statistic was 0.88. During HAART, the TLC < 1,500 was associated with an adjusted odds ratio (OR) of 5.1 (95% CI 3.4, 6.2) for TLC1500, 0.73 (0.67, 0.80) for each g/dL higher in hemoglobin, 0.32 (0.23, 0.47) for female gender, 1.9 (1.4, 2.6) for having history of an ADI, and 0.98 (0.97, 0.99) for each kg heavier in body weight (all P values < 0.02). The C-statistic was 0.852. The factors found to predict CD4 < 200 cells/μL and their associated odds ratios are shown in Table 4.

Sensitivity analyses. Adjusting for the use of non-HAART antiretroviral regimens, adjusting for the era or year of recruitment, or using two consecutive visits to categorize HAART use did not change the model outcomes.

DISCUSSION

We found that the total lymphocyte count (TLC) performed well in predicting CD4 count or CD4 < 200 cells/μL. Although better in those off HAART, the performance of TLC was good for both during and in the absence of HAART. The inclusion of hemoglobin, gender, history of AIDS defining illnesses, and weight in the model improved the performance significantly. Our findings suggest TLC, which is relatively inexpensive and available, is a reasonably accurate tool that can be used for monitoring the patients’ immune status during therapy in addition to determining when patients should start antiretroviral therapy. These findings also imply that the possibilities for modifying the models to suit specific needs exist such as employing simple scores, algorithms, or risk calculators for use in clinics in underserved areas.

TLC versus CD4. Studies have described the relationship between TLC and CD4 count previously that support our

<table>
<thead>
<tr>
<th>Effect</th>
<th>Off-HAART (N = 1,659; R² = 0.55)</th>
<th>On-HAART (N = 3,177; R² = 0.44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corresponding increase in CD4 count (cell/μL)</td>
<td>P value</td>
<td>Corresponding increase in CD4 count (cell/μL)</td>
</tr>
<tr>
<td>Increase in TLC (cell/μL)</td>
<td>0.19 &lt; 0.001</td>
<td>0.18 &lt; 0.001</td>
</tr>
<tr>
<td>Being female</td>
<td>87.1 &lt; 0.001</td>
<td>29.4 0.09</td>
</tr>
<tr>
<td>Having history of ADI</td>
<td>12.9 0.06</td>
<td>0.91 0.84</td>
</tr>
<tr>
<td>Increase in body weight (kg)</td>
<td>1.14 0.02</td>
<td>1.12 0.008</td>
</tr>
</tbody>
</table>

Both models were adjusted for hemoglobin level, non-HAART antiretroviral use, and year of recruitment. The predictive influence of gender and history of ADI during HAART is diminished.
To the best of our knowledge, few studies have examined the utility of TLC in predicting CD4 count. A relatively high positive correlation has been established between absolute values of TLC and CD4 count or between changes in TLC and CD4 cell count. A range of TLC cutoffs have been used and reported as predictors of CD4 < 200 cells/µL. These cutoffs range from 1000 cells/µL with a specificity of 98% and a sensitivity of 53% to 1,400 cells/µL with a specificity of 73% and a specificity of 88%. In another study, a TLC cutoff of 1,200 cells/µL was modeled with hemoglobin to improve sensitivity. Our study explored the development of a simple time-insensitive model for predicting CD4 count or having a CD4 < 200 cells/µL using TLC and other measures that would be readily available even in underserved resource-poor settings. Our study demonstrated that TLC could be used to predict CD4 count, in various formats, both before and during HAART use.

**Table 4** Predictors of CD4 < 200 in the absence of HAART and during HAART (N = 4,836 visits)

<table>
<thead>
<tr>
<th>Effect</th>
<th>OEE-HAART Adjusted OR [95% confidence interval]</th>
<th>P value</th>
<th>On-HAART Adjusted OR [95% confidence interval]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC &lt; 1,500 (cells/µL)</td>
<td>4.6 (3.4, 6.2)</td>
<td>&lt; 0.001</td>
<td>5.1 (4.0, 6.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Increase in hemoglobin (each g/dL)</td>
<td>0.73 (0.67, 0.80)</td>
<td>&lt; 0.001</td>
<td>0.84 (0.79, 0.91)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Being female</td>
<td>0.32 (0.23, 0.47)</td>
<td>&lt; 0.001</td>
<td>0.59 (0.41, 0.84)</td>
<td>0.004</td>
</tr>
<tr>
<td>Having history of AIDS defining illness</td>
<td>1.9 (1.4, 2.6)</td>
<td>0.002</td>
<td>1.3 (1.1, 1.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Increase in body weight (each kg)</td>
<td>0.98 (0.97, 0.99)</td>
<td>0.02</td>
<td>0.99 (0.98, 1.00)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

study findings. A relatively high positive correlation has been established between absolute values of TLC and CD4 count or between changes in TLC and CD4 cell count. A range of TLC cutoffs have been used and reported as predictors of CD4 < 200 cells/µL. These cutoffs range from 1000 cells/µL with a specificity of 98% and a sensitivity of 53% to 1,400 cells/µL with a specificity of 73% and a specificity of 88%. In another study, a TLC cutoff of 1,200 cells/µL was modeled with hemoglobin to improve sensitivity. Our study explored the development of a simple time-insensitive model for predicting CD4 count or having a CD4 < 200 cells/µL using TLC and other measures that would be readily available even in underserved resource-poor settings. Our study demonstrated that TLC could be used to predict CD4 count, in various formats, both before and during HAART use.

**Calibration to TLC cutoff of 1,500 cells/µL.** In trying to develop a model suitable for use in underserved resource-poor settings, employing simple factors in simple formats is crucial. Using cutoff of TLC simplifies its utility. In this study, TLC < 1,500 cells/µL was consistently the strongest predictor of CD4 < 200 cells/µL. The cutoff level is a balance between sensitivity and specificity in the relationship between TLC and CD4 count. A higher TLC cutoff improves sensitivity at the expense of specificity and a lower TLC cutoff improves specificity at the expense of sensitivity. Using the test with a low sensitivity would result in many patients who have a CD4 < 200 cells/µL being missed and not started on therapy. In contrast, using a test with a low specificity would result in many patients inappropriately started on therapy. In HIV treatment, the implications are that a low specificity is a public health concern, and a low specificity is a cost and cost-effectiveness issue.

Although we present 1,500 cells/µL as the best overall performing cutoff for TLC for these data, a cutoff should be selected depending on the local clinical and/or public health strategy so that the choice of a cutoff level fits the desired goals and is most cost effective. The disadvantages of the desirable simplification of the model by converting TLC into a dichotomous measure, namely loss of precision, can be overcome by the inclusion of the other simple and readily available measures, such as hemoglobin, gender, body weight and history of ADI.

**HAART versus non-HAART.** To the best of our knowledge, few studies have examined the utility of TLC in predicting CD4 measures during HAART. This is the first time the TLC/CD4 relationship has been compared between those on and off HAART in the same sample of patients. The similarity in the performance of TLC in predicting CD4 < 200 cells/µL by HAART use groups was in spite of the significant difference in viral load levels in the two groups. Our findings suggest physicians and auxiliary health care workers may readily be able to monitor patient after initiating HAART without having concerns that HAART use may affect the utility of TLC. Because, in our approach, study predictors (TLC, hemoglobin, ADI, female gender, and weight) and the outcome (CD4 count) were measured at the same time, our models are time-insensitive. Time-insensitive models are particularly important because, with limitations to health care access in underserved areas and other resource-poor settings, completing patient investigations and making clinical decisions in a single visit minimizes patient loss-to-follow up during care. Often, under these resource-limiting circumstances, only a nurse or clinical assistant manages the clinics. Usually these clinics are the first point of contact or sometimes the only point of care for the HIV/AIDS patient. Our study provides new evidence that for patients on HAART, TLC and other simple measures may be used in making clinical decisions, such as commencing prophylaxis for opportunistic infections, referral to a physician at a district hospital, and possibly even change in HAART regimen depending on the level of care; thus, a potential for improving the access to HIV/AIDS care and lessening the burden of HIV/AIDS care on secondary and tertiary care facilities.

**Possible models for prediction instruments.** One of the implications of our study is that user-friendly prediction instruments may be designed to ensure efficient and accurate exploitation of these predictive models in the field. Discussing the specific possible designs for the various predictive instruments in details is beyond the scope of this paper. However, our model is a demonstration that TLC may be used to predict CD4 count or CD4 < 200 cells/µL before and during HAART. For specific populations (and specific clinical/public health strategies), appropriate calibration with local (or applicable) data to obtain an optimal cutoff for TLC will be required in building the model and developing the predictive instrument. The range of predicted values of CD4 count or probabilities of CD4 < 200 cells/µL derived from the regression models can be converted into an intuitive scale (or a simple algorithm) so that the tools may be used even in primary care clinics in resource-poor settings. To make clinical decisions from such a scale, two strategies may be used in the field depending on availability of resources and population distributions. a) A single cutoff score can be determined by basing calculations on desired (or acceptable) levels of sensitivity and specificity and projected cost effectiveness of implementing such a cutoff. Specific clinical decisions can be made if the score is above or below the selected cutoff, or b) an interval range of scores between two strategically selected cutoff points that would ensure a high specificity below the lower cutoff (albeit with low sensitivity) and a high sensitivity.
above the upper cutoff (albeit with low specificity). Clinical decisions in the underserved clinic can be made if the score is above or below the selected interval. All patients presenting with scores within this interval, who strategically should represent a small and manageable proportion of all patients, can be referred for CD4 count and/or viral load determination. The performance and cost effectiveness of the strategy in this second example would depend on the level of the each cutoff and the size of the interval between them.

**Strengths and limitations.** The NFHL data offered two unique opportunities: the availability of data to permit the comparisons between off-HAART and on-HAART performance of TLC in predicting CD4 < 200 cells/μL, and the use of a large sample size, which also accounts for the within individual variation of measures. The limitations of these models are that the coefficient estimates may not necessarily be applicable to populations in resource-poor settings. Thus, the purpose of this study was to provide evidence that significant relationships between TLC and CD4 count exist during and in the absence of HAART and that this relationship can be exploited elsewhere. Appropriate models for specific populations may be developed using local data.

In summary, this study demonstrates the ability of TLC, whether used as continuous or dichotomous data, to predict CD4 count or CD4 < 200 cells/μL, respectively, TLC, which is an inexpensive and simple test to perform, is valuable for monitoring therapy and making clinical decisions regarding the therapy. The inclusion of readily available factors such as hemoglobin, gender, history of ADI, and body weight significantly improves the predictive performance of TLC. User-friendly predictive instruments based on these models can be developed for use in underserved resource-poor settings and can be tailored to specific HIV/AIDS care needs. With the expansion of the provision of HAART in developing countries, local data can be used to design area and strategy specific predictive models.

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