Assessment of the efficacy of total lymphocyte counts as predictors of AIDS defining infections in HIV-1 infected people

J Stebbing, S Sawleshwarkar, C Michailidis, R Jones, M Bower, S Mandalia, M Nelson, B Gazzard

Background: The CD4 count is a dominant prognostic and predictive factor in HIV infection. This study assessed the utility of the total lymphocyte count (TLC) in place of the CD4 count to predict the development of AIDS defining opportunistic infections (ADOI).

Methods: The Chelsea and Westminster cohort was used to identify those people with a first episode of an ADOI. Corresponding CD4 and TLCs were recorded before diagnosis or at the time of first prescribing prophylaxis; patients without an AIDS defining opportunistic infection were defined as being at “risk” and receiver operating characteristic (ROC) curves were used to display the results of sensitivity and the false positive error rate of total lymphocyte and CD4 count groups.

Results: A significant linear correlation was seen between the log_{10} CD4 count and log_{10} TLC (Pearson’s correlation coefficient = 0.70, p < 0.001). The cut off value for TLC was determined using the ROC curve for TLC, where the risk developing an ADOI was 10% lower than that for CD4 count.

Conclusions: The TLC is minimally less reliable than the CD4 count as a predictor of ADOIs. In the absence of expensive equipment for CD4 measurement, the TLC is a useful test.

In an established market economies, the CD4 lymphocyte count is measured in HIV-1 infected people every three months to guide decisions about prognosis, starting and changing prescriptions for highly active antiretroviral therapy (HAART), and opportunistic infection prophylaxis. However, flow cytometry to measure lymphocyte subsets requires trained personnel and perishable reagents, extensive specimen processing and infrastructure. There are data suggesting that in resource poor settings, the cost of monitoring HIV-1 treatment exceeds the cost of HAART. The TLC is minimally less reliable than the CD4 count as a predictor of ADOIs. In the absence of expensive equipment for CD4 measurement, the TLC is a useful test.

In patients with a first episode of CMV, PTB, ETB and/or Crypto, CD4 and TLC counts were defined as those available three months before diagnosis. For patients who developed PCP, Toxo, or MAI for the first time, TLC and CD4 counts were defined as those that were available closest to three months before either first exposure to relevant prophylaxis, if this was prescribed, or three months before development of particular ADOI in patients who had not been prescribed prophylaxis before diagnosis. Patients who did not develop ADOI were defined as the “at risk” group. The TLC and CD4 count for this group were defined as those available three months before first exposure to prophylaxis if this was prescribed, otherwise the one that was available three months before either their last HIV care visit or date of death.

**Statistical methods**

As there was a large amount of variability in these measurements, CD4 count and TLC were log_{10} transformed to stabilise the variance and Pearson’s correlation coefficient was used to test for association between these two variables.

Data were analysed as any first ADOI grouped into one category. Using clinical diagnosis as a gold standard, sensitivity, specificity, and likelihood ratio statistics for ADOI were calculated for TLC and CD4 count categories, which were first grouped into its respective quartiles and further investigated using finer cut off values as deemed clinically useful. These were categorised into groups of 500 cells × 10^6/l or 50 cells/mm^3, respectively. Where likelihood ratio statistics between successive categories were found to be greater than 1 showing increased risk of developing an ADOI.

**Abbreviations:** TLC, total lymphocyte count; ADOI, AIDS defining opportunistic infections; ROC, receiver operator curve
and lower than 1 showing decreased risk of an ADOI, more accurate cut off values of TLC in groups of 100 were investigated within those groups to obtain point estimates of patients' risks of acquiring any of the aforementioned ADOIs.

These were estimated using Cox’s proportional hazards method where the event time was defined as time since TLC/CD4 count result to either development of first ADOI, last HIV care clinic visit, or date of death. The results from this method are presented as hazard ratios (HR) with 95% confidence intervals (CI). Data were analysed using the software package SAS version 8.2.

Receiver operating characteristic (ROC) curves were used to display the results of sensitivity and the false positive error rate (1−specificity) of the TLC and CD4 count groups.

**RESULTS**

Since 1988, a total of 9820 patients have attended for HIV care and of these 60% were included in the analysis as these cases were found to have CD4 count and/or TLC available at least three months before an ADOI. We saw a significant linear correlation between the log transformed CD4 count and TLC ($r = 0.70; p<0.001$). Furthermore, 19% of included patients developed at least one of the above mentioned ADOIs.

Figure 1 shows the sensitivity and the false positive error rate of the analysis based on data that were grouped into quartiles. The point at which the likelihood ratio switched from greater than 1 and lower than 1 was found to be between groups of 1200 and 1201–1700 cells/mm$^3$ (table 1 and fig 1).

Figure 2 shows likelihood of ADOI at different TLC counts.

People with a TLC measuring 1000–1500 cells/mm$^3$ were at 40% increased risk of experiencing any ADOI (sensitivity 69%, 95% CI 65.7 to 71.3 and specificity 66%, 95% CI 64.6 to 67.3). The hazard ratios of further finer cut off values of TLC were

<table>
<thead>
<tr>
<th>Lymphocyte count</th>
<th>Any first ADOI % in categories</th>
<th>Likelihood ratio showing risk of “any first ADOI”</th>
<th>Hazard ratio (95% CI) showing likelihood of any ADOI relative to reference category</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤500</td>
<td>43.8</td>
<td>3.36</td>
<td>1</td>
</tr>
<tr>
<td>501–1000</td>
<td>40.4</td>
<td>2.92</td>
<td>0.79 (0.64 to 0.97)</td>
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<tr>
<td>1001–1500</td>
<td>24.5</td>
<td>1.40</td>
<td>0.36 (0.29 to 0.44)</td>
</tr>
<tr>
<td>1501–2000</td>
<td>12.3</td>
<td>0.60</td>
<td>0.17 (0.14 to 0.21)</td>
</tr>
<tr>
<td>2001–2500</td>
<td>9.3</td>
<td>0.44</td>
<td>0.13 (0.10 to 0.17)</td>
</tr>
<tr>
<td>2501–3000</td>
<td>5.7</td>
<td>0.26</td>
<td>0.08 (0.05 to 0.12)</td>
</tr>
<tr>
<td>3001–3500</td>
<td>7.1</td>
<td>0.33</td>
<td>0.10 (0.06 to 0.17)</td>
</tr>
<tr>
<td>&gt;3500</td>
<td>12.5</td>
<td>0.62</td>
<td>0.19 (0.13 to 0.29)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CD4 count</th>
<th>Any first ADOI % in categories</th>
<th>Likelihood ratio showing risk of “any first ADOI”</th>
<th>Hazard ratio (95% CI) showing likelihood of any ADOI relative to reference category</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤50</td>
<td>53.4</td>
<td>4.90</td>
<td>1</td>
</tr>
<tr>
<td>51–100</td>
<td>43.5</td>
<td>3.30</td>
<td>0.74 (0.63 to 0.88)</td>
</tr>
<tr>
<td>101–150</td>
<td>31.7</td>
<td>1.99</td>
<td>0.50 (0.41 to 0.62)</td>
</tr>
<tr>
<td>151–200</td>
<td>23.9</td>
<td>1.34</td>
<td>0.30 (0.24 to 0.37)</td>
</tr>
<tr>
<td>201–250</td>
<td>18.5</td>
<td>0.97</td>
<td>0.26 (0.21 to 0.33)</td>
</tr>
<tr>
<td>251–300</td>
<td>15.6</td>
<td>0.79</td>
<td>0.24 (0.19 to 0.31)</td>
</tr>
<tr>
<td>301–350</td>
<td>6.3</td>
<td>0.29</td>
<td>0.10 (0.07 to 0.14)</td>
</tr>
<tr>
<td>&gt;350</td>
<td>4.2</td>
<td>0.19</td>
<td>0.07 (0.05 to 0.08)</td>
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</tbody>
</table>
grouped into 100s (fig 2) showed a gradation of increased risk of ADOI with decreasing TLC compared with those whose TLC >1700 cells/mm$^3$.

The cut off value for CD4 cell counts was 200 cells/mm$^3$. Patients with a CD4 of 150–200 cells/mm$^3$ were at 34% increased risk of developing an ADOI (sensitivity 73.8%, 95% CI 71.2 to 76.4 and specificity 75.6%, 95% CI 74.4 to 76.8). The area under the ROC curve for the TLC was 10% lower than that for the CD4 count showing that TLC is a useful prognostic marker at predicting the first ADOI.

DISCUSSION
We found a statistically significant linear correlation between the log transformed CD4 count and TLC showing the utility of TLC in this setting, as a reliable alternative to the CD4 count.

The value at which the error rate for TLC is at its lowest and the sensitivity maximal (fig 1) was 1500 cells/mm$^3$, with further analysis identifying 1700 cells/mm$^3$ being a more precise cut off.

In 2002, a large meta-analysis included data from 13 studies containing 12 574 patients and found that baseline CD4 count was strongly associated with the probability of progression to AIDS or death: compared with patients starting HAART with less than 50 CD4 cells/mm$^3$, adjusted hazard ratios were 0.74 (95% CI 0.62 to 0.89) for 50–99 cells/mm$^3$, 0.52 (0.44 to 0.63) for 100–199 cells/mm$^3$, 0.24 (0.20 to 0.30) for 200–349 cells/mm$^3$, and 0.18 (0.14 to 0.22) for 350 or more CD4 cells/mm$^3$. These values are similar to those seen here with use of the TLC. The CD4 count was also seen to be a more significant and dominant prognostic factor than others such as HIV-1 RNA viral load, advanced age, infection through intravenous drug use, and a previous diagnosis of AIDS, all prognostic factors reported in other studies.

In the developed world, early survival improvements occurred soon after the introduction of HAART in the mid-1990s and they have continued to increase. HAART itself is responsible for most of the improvement as before antiretrovirals, HIV caused an almost uniformly fatal illness. While fewer than one million people worldwide are currently infected with HIV caused an almost uniformly fatal illness. As such, TLC can also be used to facilitate decisions about timing of HAART and ADOI prophylaxis. Additional studies are required to determine the utility of TLC as a predictive marker in place of the CD4 count, in different settings.

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REFERENCES