ORIGINAL ARTICLE

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

Postgrad Med J 2005;81:586–588. doi: 10.1136/pgmj.2004.030841

See end of article for authors' affiliations

Correspondence to:
Dr J Stebbing, Department
of Immunology, Chelsea
and Westminster Hospital,
369 Fulham Road, London
SW10 9NH, UK;
j.stebbing@imperial.ac.uk

Submitted
18 November 2004
Accepted
14 December 2004

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 finer cut off value for TLC where false positive error rate is minimum and sensitivity maximum was 1500–2000 cells/mm³. Patients with TLC 1000–1500 cells/mm³ were estimated to be at 40% increased risk of developing an ADOI. The cut off value for CD4 counts measured 200 cells/mm³ above which the risk developing an ADOI decreased. Patients with a CD4 count of 150–200 cells/mm³ were at a 34% increased risk of developing an ADOI. The area under the ROC curve for TLC 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 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.^{1–5} However, flow cytometry to measure lymphocyte subsets requires trained personnel and perishable reagents, extensive specimen processing and infrastructure.^{6–8} There are data suggesting that in resource poor settings, the cost of monitoring HIV-1 treatment exceeds the cost of HAART.^{9–10} While new technologies are becoming available for CD4 count testing, they are not readily available and remain expensive.^{11–13}

Measurement of total lymphocyte count (TLC) is straightforward and does not rely on prohibitively complex systems. We therefore assessed the utility of TLC measurement as a predictive marker for the development of AIDS defining opportunistic infections (ADOI) in a large cohort of HIV-1 positive patients.

METHODS

Patients

The Chelsea and Westminster HIV cohort is one of the largest in Europe and CD4 counts are measured routinely. Patients who attended between 1988 and September 2003 were identified including those who were diagnosed with ADOIs as follows: Pneumonia pneumocystis carinii (PCP), cytomegalovirus (CMV) retinitis, toxoplasmosis (Toxo), Mycobacterium avium intracellulare (MAI), Cryptococcosis (Crypto), pulmonary tuberculosis (PTB), and extrapulmonary tuberculosis (EPTB). The patients identified included presumptive as well as definitive diagnoses; those with AIDS related malignancies were excluded.

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 these 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 $\times 10^6/l$ or 50 cells/mm³, 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

Figure 1 Receiver operating characteristic curve showing sensitivity and false positive error rate of ADOI using the TLC and CD4 count. (Based on 50 cells/mm³ and 500 grouped cut offs). The diagonal line displays ties.

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

Figure 2 Hazard ratio showing the risk of any first ADOI at different TLC counts.

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/mm³ (table 1 and fig 1). Figure 2 shows likelihood of ADOI at different lymphocyte count groupings below 1700 cells/mm³ compared with values above this.

People with a TLC measuring 1000–1500 cells/mm³ 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

Table 1 Likelihood of an ADOI as measured by TLC and CD4 count groups (counts are measured in cells/mm³)

Lymphocyte count			
Lymphocyte count stratified grouped into groups of 500	Any first ADOI % in categories	Likelihood ratio showing risk of "any first ADOI"	Hazard ratio (95% CI) showing likelihood of any ADOI relative to reference category
≤ 500	43.8	3.36	1
500–1000	40.4	2.92	0.79 (0.64 to 0.97)
1001–1500	24.5	1.40	0.36 (0.29 to 0.44)
1501–2000	12.3	0.60	0.17 (0.14 to 0.21)
2001–2500	9.3	0.44	0.13 (0.10 to 0.17)
2501–3000	5.7	0.26	0.08 (0.05 to 0.12)
3001–3500	7.1	0.33	0.10 (0.06 to 0.17)
>3500	12.5	0.62	0.19 (0.13 to 0.29)
CD4 count			
CD4 count stratified into groups of 50			
≤ 50	53.4	4.90	1
51–100	43.5	3.30	0.74 (0.63 to 0.88)
101–150	31.7	1.99	0.50 (0.41 to 0.62)
151–200	23.9	1.34	0.30 (0.24 to 0.37)
201–250	18.5	0.97	0.26 (0.21 to 0.33)
251–300	15.6	0.79	0.24 (0.19 to 0.31)
301–350	6.3	0.29	0.10 (0.07 to 0.14)
>350	4.2	0.19	0.07 (0.05 to 0.08)

grouped into 100s (fig 2) showed a gradation of increased risk of ADOI with decreasing TLC compared with those whose TLC >1700 cells/mm³.

The cut off value for CD4 cell counts was 200 cells/mm³. Patients with a CD4 of 150–200 cells/mm³ 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/mm³, with further analysis identifying 1700 cells/mm³ 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/mm³, adjusted hazard ratios were 0.74 (95% CI 0.62 to 0.89) for 50–99 cells/mm³, 0.52 (0.44 to 0.63) for 100–199 cells/mm³, 0.24 (0.20 to 0.30) for 200–349 cells/mm³, and 0.18 (0.14 to 0.22) for 350 or more CD4 cells/mm³.¹⁴ 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.^{15 16}

In the developed world, early survival improvements occurred soon after the introduction of HAART in the mid-1990s and they have continued to increase.^{17 18} 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 receiving HAART, the number receiving HAART in developing countries is likely to increase.^{9 19}

We show that in the absence of sophisticated testing for CD4 counts, TLC can be used as a reliable predictor of ADOIs in HIV-1 infected people. 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.

Authors' affiliations

J Stebbing, S Sawleshwarkar, C Michailidis, R Jones, M Bower, S Mandalia, M Nelson, B Gazzard, St Stephen's Centre, Chelsea and Westminster Hospital, London, UK

Funding: none.

Conflicts of interest: none.

REFERENCES

- 1 Delta Coordinating Committee and Virology Group. An evaluation of HIV RNA and CD4 cell count as surrogates for clinical outcome. *AIDS* 1999;13:565–73.
- 2 Chaisson RE, Keruly JC, Moore RD. Association of initial CD4 cell count and viral load with response to highly active antiretroviral therapy. *JAMA* 2000;284:3128–9.
- 3 Yeni PG, Hammer SM, Carpenter CC, et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *JAMA* 2002;288:222–35.
- 4 BHIVA. British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy. *HIV Med* 2001;2:276–313.
- 5 Sabin CA, Lampe FC, Chaloner C, et al. An audit of antiretroviral treatment use in HIV-infected patients in a London clinic: the limitations of observational databases when auditing antiretroviral treatment use. *HIV Med* 2003;4:87–93.
- 6 Woods GL. Automation in clinical microbiology. *Am J Clin Pathol* 1992;98:S22–30.
- 7 Diagbouga S, Durand G, Sanou PT, et al. Evaluation of a quantitative determination of CD4 and CD8 molecules as an alternative to CD4+ and CD8+ T lymphocyte counts in Africans. *Trop Med Int Health* 1999;4:79–84.
- 8 Brown M, Wittwer C. Flow cytometry: principles and clinical applications in hematology. *Clin Chem* 2000;46:1221–9.
- 9 Rabkin M, El-Sadr W, Katzenstein DA, et al. Antiretroviral treatment in resource-poor settings: clinical research priorities. *Lancet* 2002;360:1503–5.
- 10 Hirschel B. Antiretroviral treatment and research in resource-poor countries. *Lancet* 2003;361:434–5.
- 11 Mwaba P, Cassol S, Pilon R, et al. Use of dried whole blood spots to measure CD4+ lymphocyte counts in HIV-1-infected patients. *Lancet* 2003;362:1459–60.
- 12 Shapiro HM, Mandy FF, Rinke de Wit TF, et al. Dried blood spot technology for CD4+ T-cell counting. *Lancet* 2004;363:164; author reply 164–5.
- 13 Janossy G. Dried blood spot technology for CD4+ T-cell counting. *Lancet* 2004;363:1074; author reply 1074–5.
- 14 Egger M, May M, Chene G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002;360:119–29.
- 15 Taha TE, Graham SM, Kumwenda NI, et al. Morbidity among human immunodeficiency virus-1-infected and -uninfected African children. *Pediatrics* 2000;106:E77.
- 16 Porter K, Babiker A, Bhaskaran K, et al. Determinants of survival following HIV-1 seroconversion after the introduction of HAART. *Lancet* 2003;362:1267–74.
- 17 Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998;338:853–60.
- 18 Hammer SM. Increasing choices for HIV therapy. *N Engl J Med* 2002;346:2022–3.
- 19 Stebbing J, Gazzard B, Douek DC. Where does HIV live? *N Engl J Med* 2004;350:1872–80.