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B. GITURA, M.D. JOSHI, G.N. LULE and O. ANZALA

ABSTRACT

Objective: To evaluate the utility of Total Lymphocyte Count (TLC) as a surrogate marker for CD4 + T cell count in antiretroviral (ARV) treatment initiation in a Kenyan population of HIV sero-positive patients at Kenyatta National Hospital.

Design: Cross-sectional descriptive study.

Setting: Kenyatta National Hospital, HIV treatment and follow-up outpatient facility; Comprehensive Care Centre, Nairobi, Kenya.

Subjects: Two hundred and twenty five HIV Elisa positive, ARV naive patients visiting the Comprehensive Care Centre between january 2006 to March 2006.

Results: A significant linear correlation was found between TLC and CD4 cell count for the whole group with a Spearman rank correlation of 0.761 ($p < 0.01$); and was also independently observed in the four WHO clinical stages. The classification utility of TLC 1200 cells/mm^3 cut-off was sub-optimal; sensitivity 37% specificity of 99% and the NPV of 56%. The receiver operator characteristics (ROC) curve generated an optimal TLC cut-off of 1900cells/mm^3 cut-off to be of greatest utility with a sensitivity of 81.1%, specificity of 90.3%, PPV of 90.8% and NPV of 80.2%. This implies that a TLC cut-off of 1900cells/mm^3 correctly classify eight out of ten HIV positive patients as having a CD4 < 200 cells/mm^3 and only misclassify two such patients. Serial CD4 testing can then be performed on the minority of patients who despite a TLC≥1900 cells/mm^3 are, on basis of clinical data, suspect of more advanced disease warranting ARV therapy. This would reduce the number of patients tested for and focus the application of CD4 testing and thus reduce attendant cost in care provision in CD4 resource poor settings.

Conclusion: Our data showed a good positive correlation between TLC and CD4 cell count, however the WHO recommended TLC cuto-ff of 1200/mm^3 was found to be of low sensitivity in classifying patients as having a CD4 counts < 200 cells/mm^3. This would result in underestimation of advanced stage of disease and to withholding ARVs treatment to persons who need treatment. We recommend a TLC cut-off of 1900 cells/mm^3 for our population to classify patients as either above or below the CD4 count cut-off of 200 cells/mm^3 as an indicator of when to start antiretroviral therapy.

INTRODUCTION

The burden of HIV/AIDS in sub-Saharan Africa (SSA) is extensive with 27 mllion of the 40 million persons living with HIV/AIDS in the world living in SSA (1). This pandemic continues to be a source of significant morbidity and mortality despite the introduction of highly active antiretroviral therapy (HAART), which has been shown to be effective in reducing morbidity and mortality in clinical and observational studies and in practice in the western countries (2).

In Kenya the prevalence of HIV infected individuals has fallen from 10% in 1998 to 7% in 2005 with an estimated 1.25 million Kenyans currently living with HIV. By March 2006 approximately 200,000 Kenyans were eligible for antiretroviral therapy (ART), about 65,000 persons were on ART and 140,000 on follow-up without ART (3). Current approaches for HIV/AIDS care is based on models developed in resource-rich countries using the current extensive biological monitoring approaches, but ignoring the prohibitive patient load in the developing countries (4). A large proportion of our HIV patients rely on accessing health care services in rural and underserved areas that do not have the capacity or capability for CD4 evaluation. Addressing the global problem of HIV treatment in high prevalence/high caseload countries may require that treatment be monitored on the basis of clinical symptoms and possibly limited readily available laboratory tests. Indeed according to UNAIDS, even with the dramatic price reductions on HAART regimens, administration ofARV therapy will remain unaffordable for many resource-limited countries. The extent of funding required for the wide range of activities that make up ART programmes, including monitoring therapy, clinical management of complications, assessment and support of drug adherence, necessitate cost-effective solutions where appropriate and achievable (5).

Where CD4 count is not practically obtainable, current WHO guidelines recommends using TLC in conjunction with clinical data as criteria to initiate highly active antiretroviral therapy (HAART) in resource poor settings (6). However, despite this endorsement, the usefulness of TLC remains ambiguous and underutilised. Several studies have previously demonstrated a good correlation between CD4 T cell count and TLC in HIV infected patients, in the United Kingdom and United States (7, 8) and recently in Uganda (9). TLC has also been found to be an inexpensive and useful marker for staging disease, predicting progression to AIDS and death, and recently it has been shown that it can be used to monitor response to HAART (10-12). However this correlation has not been consistent with other investigators reporting conflicting results (13,14).

Understanding TLC-CD4 count relationship in our population will aid in designing useful and appropriate predictive instruments for use in clinical decisions making in HIV infected patients particularly in rural and undeserved areas. We therefore conducted a study to assess the utility of TLC to serve as a surrogate marker for CD4 count in the initiation of HAART in our Kenyan population.

MATERIALS AND METHODS

Study sites: Study subjects were recruited from the Comprehensive Care Centre (CCC) and Patient Support Centre (PSC) at Kenyatta National Hospital, Nairobi, Kenya. This centre provides comprehensive clinical care and counselling to patients with HIV including HAART, treatment of opportunistic infections and other HIV related complications and laboratory tests (excluding viral load and viral resistance testing). The majority of patients in this facility have advanced HIV infection and require antiretroviral treatment.

Study subjects and measurements: Between January and April 2006 two hundred and thirty one consecutive ARV naive HIV positive adults, 18 years or older, and stratified on WHO clinical stages were screened and recruited until the desired sample size of 50 in each clinical group was fulfilled. Exclusion criteria included pregnant mothers, use of immunomodulatory drugs including chemotherapy and malignancies other than Kaposis sarcoma, clinically identified ongoing acute opportunistic infections and non-consent. Each patient underwent a standardised comprehensive clinical evaluation by the PI including and in particular documenting current or past Aids defining illness utilising a standardised data sheet. Laboratory data included CD4 cell count measured by the automated flow cytometry analyser, FASCOUNT (Benedict Dick USA) and complete blood count and differential performed using the MS4 haematological analyser,

including haemoglobin (Hb), white blood cell count (WBC) and total lymphocyte count (TLC).The WBC differential count was confirmed for all samples by microscopic evaluation of the blood smear by a study haematologist. All samples were collected between 10.00 am and 12.00pm to avoid diurnal variation and all indices done on the same sample for each patient within 12 hours of the blood drawn.

Data analysis: Correlation between CD4 count and TLC was evaluated for the whole sample population and also in each of the four WHO clinical stages using spearman rank order correlation coefficient. The diagnostic test utility of a TLC of 1200cells/mm^3 was undertaken by calculating sensitivity, specificity, positive predictive value and negative predictive values. Receiver operating characteristic (ROC) curve was generated for various TLC cut-offs between 800 and 2400 cells/mm^3 to predict a CD4 < 200 cells/mm^3 to obtain the optimal cut-off for this sample population. Proportions were compared between groups using Chi-square for categorical variables and student's t-test for continuous variables. The mean and median, TLC, CD4 count, haemoglobin, age and weight were calculated .The Mann Whitney test was used to calculate statistical significance of the means and medians. A P-value of 0.05 or less was considered significant.

RESULTS

All 231 HIV sero-positive patients were recruited into the study with no exclusions at enrollment. Six patients were excluded from the analysis on account of missing complete blood counts; distributed evenly among WHO clinical stages. Data on 225 cases were analysed; 148 (65.8%) were females and age range was 20 - 75 years with a mean of 35 years. Mean body weight was 57.7kg (59kg for men and 56kg for women) and the mean haemoglobin was 12.3g/dl (12.3g/dl for men and 12.3g/dl for women). The median CD4 count was 180 cells/mm^3, and 121(54.2%) of the sample had CD4 < 200 cells/mm^3. The median TLC was 1942 cells/mm^3. The baseline characteristics of study participants are summarised in Table 1.

The median CD4 and TLC in the sample in WHO Stage I (n-56) was 444 cells/mm^3 and 2789 cells/mm^3 respectively; WHO stage II (n=51) 265 cells/mm^3 and of 2260 cells/mm^3; WHO stage III (n=54) 130 cells/mm^3 and 1604 cells/mm^3, and WHO stage IV (n=64) 25 cells/mm^3 and 1158 cells/mm^3. As expected, the median CD4 and TLC declined with advancing WHO clinical stages (Table 2).

A significant positive linear correlation was found between TLC and CD4 cell count for the entire sample group; Spearmans rho 0.76. P-value < 0.001 (Figure 1).

The WHO prescribed TLC cut-off of 1200 cells/mm^3 to predict a CD4 < 200 cells/mm^3 performed poorly with a low Sensitivity of 37%; Specificity 99%, positive predictive value (PPV) 98% and negative predictive value (NPV) 56%. This means that use of this TLC cut-off would correctly classify only 37% of individuals with a CD4 less than 200 cells/mm^3 resulting in a high rate of false negative results (Table 3). In order to increase sensitivity, a higher TLC cut-off is required but this sacrifices specificity.

A ROC curve was generated by plotting the true positive (sensitivity) against false positive (1 -

Table 1

Participants clinical and laboratory characteristics data by gender

Variable	All (n = 225)	Male (n = 77)	Female (n = 148)	P-value
Mean age (year)	35.4	38.4	33.8	< 0.001
Mean weight (kg)	57.7	59	56	
Mean Hb (g/dl)	123	12.36	12.38	0.985
Median WBC/mm^3	5.2	5.32	5.15	0.272
Mean TLC/mm^3	2055	2063	2051	
Median TLC/mm^3	1942	2021	1941	0.810
Median CD4 (cells/mm^3)	180	153	191	0.415
Mean CD4 (cells/mm^3)	234	205	249	

Table 2

CD4 and TLC cell count by WHO Clinical Stages

WHO Stage	N	Median CD4	Mean CD4	IQR CD4	Median TLC	Mean TLC	IQR TLC
1	56	444	498	305-672	2789	2792	2230-3345
II	51	265	268	210-344	2260	2947	1851-3041
III	54	130	150	50-194	1604	1791	1253-2229
IV	64	25	47	11-69	1158	1321	893-1493

Figure 1

Scatter plot of TLC by CD4 cell counts

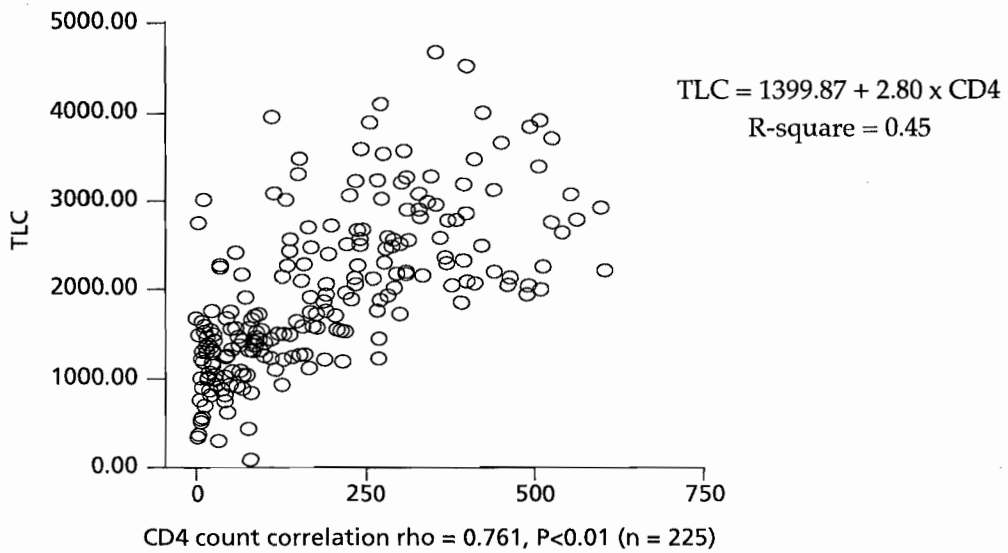

Figure 2

ROC curve

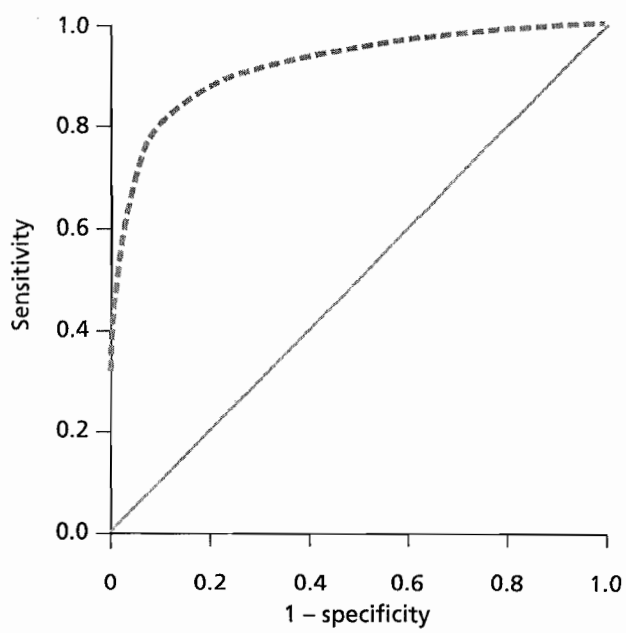

Table 3

Sensitivity/Specificity of the recommended WHO cut-off of TLC 1200

	CD4 < 200	CD4 ≥ 200	Total
TLC < 1200	44	1	45
TLC ≥ 1200	76	104	180
Total	120	105	225
	Sensitivity = 37%	Specificity = 99%	

Positive Predictive Value 98% and Negative Predictive Value 56%.

Table 4

Sensitivities, Specificities, Positive and Negative predictive values for various cut-offs of TLC to predict CD4 cell count below 200 cells/mm³

TLC cut off Cells/mm³	Sensitivity	Specificity	PPV	NPV	LR+	LR-
TLC <800	12.5	100	100	50		
TLC <900	19	100	100	52		
TLC <1000	20	100	100	52		
TLC <1100	33	99	100	56		
TLC <1200	37	99	98	56	37	0.63
TLC<1300	46	98	97	62	25	0.55
TLC <1400	54	98	97	65	27	0.47
TLC <1500	61	97	96	68	20	0.4
TLC <1600	67	95	94	71	13.4	0.34
TLC <1700	71	95	94	74	14	0.3
TLC <1800	79.2	92	92	80	9.8	0.22
TLC <1900	81	90	90	80	8	0.2
TLC <2000	82.5	85.7	86.8	81.1	5.7	0.2
TLC <2200	85.8	73.3	78.6	81.9	1.1	0.21
TLC <2300	89.2	66.7	75.4	84.3	2.67	0.16
TLC <2400	89.2	62.9	73.3	83.5	2.4	0.17

Table 5

Sensitivity and Specificity of a TLC cut-off of 1900 cells/mm³

	CD4 < 200	CD4 ≥ 200	Total
TLC < 1900	99	10	109
TLC ≥ 1900	23	93	116
Total	122	103	225
	Sensitivity = 81%	Specificity = 90%	

Positive Predictive Value 98% and Negative Predictive Value 80%.

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specificity), and all indices of laboratory utility test were optimised at a TLC cut-off of 1900 cells/mm³; Sensitivity 81% , Specificity 90%. PPV 90% , NPV 80% (Figure 2). The area under the curve was 0.901 (95% CI 0.86-0.92, $p < 0.001$). The utility parameters of a TLC cut-offs from 800 to 2400 cells/mm³ are depicted in Table 4.

Using this cut-off will correctly identify eight of ten individuals with a CD4 less than 200 cells/mm³, which is the threshold in practice for initiating ARV treatment. The equally high specificity results in a low rate of false positive results; implying that only one out of ten patients with a CD count of above 200 will be misclassified as warranting ARV therapy (Table 5).

DISCUSSION

Our study confirms the previously documented positive correlation between TLC and CD4 cells in western and African HIV positive populations (7,8,12). However our study patients had relatively low CD4 cell counts with median CD4 cell counts in stage I and II of 444 cells/mm³ and 265 cells/mm³ respectively. This is in contrast to what is reported in the WHO international study of the WHO staging system where patients in WHO stage I and II had median CD4 cell counts of > 500 and > 400 respectively (15).This aspect of our findings has been similarily reported in other African studies (16-18). In contrast however we report a high median TLC of 1942 cells/mm³. In advanced HIV disease, TLC is expected to decline as a result of progressive depletion of CD4 T lymphocytes. This discrepancy has however been reported in other African patients cohorts (9,17,18); and proposed explanations have included the endemic nature of parasitic and protozoal diseases and higher prevalence of tuberculosis; genetics may also play apart (19).

Our findings however do not support the WHO recommended TLC cut-off point of 1200/mm³ to initiate HAART, in the absence of CD4 count, on account of a dismally low sensitivity of 37%. Utilisation of such a cut-off would result in an unacceptably high false negative results leading to misclassification of 63% of individuals, and thus withholding ARVs to persons who need them. These findings confirm the low sensitivity of this cut-off reported by other studies in African populations; namely Uganda 39.7 % (9), Nigeria 45.5 %(14) and 48.9 % in Mozambique (20)

Our study sample finding recommends a TLC cut-off of 1900 cells/mm³ as a sensitive surrogate marker for CD4 less than 200cells/mm³. With a sensitivity of 81%, specificity of 90%, PPV of 90% and NPV of 80%, this implies that eight out of ten patients will be correctly classified as having depressed CD counts; with only two out of ten patients being missed and not recommended for initiation ofARV therapy. Our findings suggest that a TLC cut-off of 1900/mm³ combined with clinical data can be used to decide when to initiate antiretroviral therapy where CD4 cell count is not available. This will be particularly important in undeserved areas and other resource poor settings. It will also cut back on the number of patients needed to do CD4 count and hence reduce overall cost in the management of these patients. This has the potential for improving access to HIV/AIDS care. Serial testing of CD4 count can then be recommended for patients with TLC greater than 1900/mm³ who are symptomatic. Although we present 1900/mm³ as the best overall performing cut-off for TLC for this data, we recommend sub-region specific cut-offs to be ascertained for different regions of the world.

Our findings support the use of TLC, which is relatively inexpensive and readily available, as a reasonably accurate tool that can be utilised as an objective parameter in the clinical decision analysis of determining when patients should be considered for initiating antiretroviral therapy.

CONCLUSIONS

Our results indicate that the WHO recommended TLC cut-off of 1200 cells/mm³ to predict a CD4 count of 200 cells/mm³ has unacceptably low sensitivity and is not appropriate to determine eligibility for HAART in our population; and recommends instead that a cut-off of 1900cells/mm³ be utilised, with subsequent serial and selective CD4 evaluation.

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