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Evaluating correlation between total lymphocyte counts and CD4 counts in monitoring HIV patients

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ABSTRACT

Monitoring individuals with HIV infection/AIDS requires the use of expensive tools, which are not readily available in resource-limited settings. The high cost of CD4 count estimation in resource-limited countries is a major challenge in initiating patients on highly active antiretroviral therapy (HAART). This study was initiated to ascertain the reliability of total lymphocyte count as a substitute for CD4 cell count in indigenous areas. 451 patients who had visited the clinic in FMC, Owo (HIV counseling and testing (HCT) unit and the global HIV/AIDS Initiative Nigeria (GHAIN)-Supported laboratory), between July 2014 and August 2015 were randomly recruited in this prospective cross sectional study. The recruited subjects were analyzed for CD4 counts & TLC to determine the correlation between TLC and CD4 cell counts. Spearman correlation between TLC and CD4 cell count were determined. Sensitivity, specificity, positive and negative predictive values of different TLC values was computed for CD4 count ≤200 and ≤350 cell/mm^3. This study showed positively significant correlation between TLC and CD4 counts and also it revealed reasonably adequate sensitivity and specificity to consider TLC as a substitute for CD4 count. Thus, TLC may be helpful in deciding when to initiate antiretroviral therapy and thus acts as a surrogate marker for CD4 counts in resource-poor settings.

Keywords: Total lymphocyte count, CD4 counts, HIV/AIDS, HAART, Surrogate marker

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INTRODUCTION

For years now, the entire world has been grappling with the rapid spread of HIV/AIDS. It has been estimated that over 90% of people living with HIV live in developing countries. The AIDS epidemic has resulted in a terrific cost in terms of loss of lives and life-quality worldwide, especially in Africa, where 70% of deaths from HIV-1 infection have been documented [1, 2]. Monitoring individuals with HIV infection/AIDS requires the use of expensive tools, which are not readily available in resource-limited settings [3]. The high cost of CD4 count estimation in resource-limited countries is a major challenge in initiating patients on highly active antiretroviral therapy (HAART) [4]. Due to this, the World Health Organization (WHO) specifies that CD4 count testing is suitable but not essential for HAART use in resource-limited settings [5]. Several studies have demonstrated the importance of absolute lymphocyte count (ALC) or total lymphocyte count (TLC) in identifying patients who would benefit from initiating

prophylaxis for acquired immunodeficiency syndrome (AIDS) and related opportunistic infections [5, 6, 7]. The identification of laboratory tests that help the clinician to predict progression is useful not only to monitor the patients' disease evolution but also to define the right time to initiate treatment [2, 8].

In April 2002, the WHO recommended the use of absolute lymphocyte count as an alternative marker when a CD4+ cell count is not available or is not affordable: a total lymphocyte count of less than 1,000-1,200 lymphocytes/mm^3 could be used as a threshold value to initiate antiretroviral therapy [6, 9]. WHO has suggested that total lymphocyte counts (TLC) could work as a potential marker for immunosuppression whenever CD4 counts are unavailable [5], because TLC could be easily obtained from routine complete blood cell (CBC) counts by multiplying the percentage of lymphocytes by the white-blood-cell count [2, 9]. Patients with low CD4+ T lymphocyte cell count have been reported as long-time infected patients than those with higher CD4 count [10]. Thus, it is obvious that late starters of highly active antiretroviral therapy with CD4 count <200 cells/µl have significantly poor response to therapy and a worse prognosis when compared with early starters with higher CD4+ T cell count [11, 12].

On the other hand, there is a report which showed that TLC has a low sensitivity and specificity, was not optimal for identifying patients requiring HAART [4], while another says TLC has a high specificity to identify patients for prophylaxis, but a quite low sensitivity as a surrogate marker to CD4+ T-cell counts in HIV-infected patients [2]. This showed that the available evidence regarding this issue is still controversial. In addition, most of the previous studies in different settings were used small sample sizes which were even one of the major limitations of Srirangaraj and Venkatesha's work in 2011. This study was initiated to ascertain the reliability of total lymphocyte count as a substitute for CD4 cell count in indigenous areas using relatively large sample size.

MATERIALS AND METHODS

Study design and setting

After obtaining an approval from the Federal Medical Centre (FMC) Joint Ethics Review Committee (FMC/EC/102014) and written informed consent (approved by the FMC Ethics committee) from each subject, we randomly recruited 451 patients who had visited the clinic in FMC, Owo (HIV counseling and testing (HCT) unit and the global HIV/AIDS Initiative Nigeria (GHAIN)-Supported laboratory), between July 2014 and August 2015 in this prospective observational cohort study. Demographic data, such as age and gender, were recorded. The patients were further divided into two groups: HIV-infected patients on treatment (n=246) and HIV-infected patients without previous antiretroviral therapy as naive (n=205).

Selection and description of participants

All recruited subjects were both newly diagnosed for HIV (naïve) and HIV-infected patients on ART treatment at FMC, Owo HIV counseling and testing (HCT) unit and the global HIV/AIDS Initiative Nigeria (GHAIN)-Supported laboratory.

After taking an informed consent for HIV testing, these individuals, voluntarily attending the clinic underwent pre-test counseling, followed by HIV testing as per the strategy III of the NACO guidelines (for HIV testing) [13]. After post-test counseling, those found HIV positive were referred to the ART Centre, where they underwent pre-ART counseling. After clinical evaluation, informed consent was taken from these patients and they were enrolled into the study if they satisfied the inclusion criteria. Those found eligible for ART as per the WHO guidelines [14] were started on anti-retroviral therapy.

Inclusion Criteria

All the volunteer subjects above 18 years of age, tested to be HIV-positive and either are on prior anti-retroviral therapy (ART) or naïve are recruited for the study.

Exclusion criteria

All HIV-seronegative subjects, HIV-infected subjects with pregnancy or breastfeeding and those below 18 years of age were excluded from the study.

Blood Collection

Five (5) ml of venous blood was collected using ethylene diamine - tetra acetic acid (EDTA) vacutainer tubes for CD4 and complete blood count between 9.00am to 12.00 noon with all bio-safety precautions [15]. Blood samples

were transported in cold chain boxes to the global HIV/AIDS Initiative Nigeria (GHAIN) supported laboratory for CD4 absolute counts and haematology assays, done within six hours of sample collection.

Haematology Assay and TLC Calculation

Haematology parameters including packed cell volume (PCV), white blood cell (WBC), lymphocyte counts were determined using the Automated Haematologic Analyzer, Sysmex, KX-N21 (Japan) as described by Olaniyi et al. [16] and Akinbo et al. [17]. TLC was derived from CBC by multiplying the percentage of lymphocytes with total white-blood cell count [2, 9].

Immunologic Indices Analysis

The samples for CD4 count were prepared and assayed on the Partec cyflow counter (Partec flow cytometer, GMBH, Germany) according to the manufacturer's instructions. Flow cytometry (cell measurement) is a process used to count, identify, and sort various types of cells. This technique is based on adding monoclonal antibodies (MAb) to a blood sample and running the fluid through a light source, usually a laser beam [18, 19, 20]. % CD4 count was calculated by dividing CD4 counts with TLC and multiplying it by 100 [2].

Statistical analysis

The continuous variables were presented as means and standard deviation using independent student t-test. Spearman correlations between TLC and CD4 cell count were assessed. Sensitivity, specificity, positive predictive value, and negative predictive values of various %CD4 count and TLC cut-offs were computed for CD4 count ≤200, 200-500 and >500 cells/cu.mm thresholds. The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant. All statistical analyses were performed using SPSS software (version 17.0, SPSS, Chicago, USA).

RESULTS

A total of 451 HIV infected subjects, which were further divided into two groups: HIV-infected patients with previous antiretroviral therapy (ART) (n=246) and HIV-infected subjects without previous antiretroviral therapy as naive (n=205), were recruited for the study. The recruited subjects were analyzed to determine the correlation between TLC and CD4 cell counts and to find out whether TLC can be used as a substitute for CD4 cell counts in resource-limited setting in both groups.

Each of the groups is comprising (61 males and 185 females) and (61 males and 144 females) respectively with the overall age ranged from 18 to 70 years, and age mean of 39 years. Mean, standard deviation (SD), median and range values for the total lymphocyte counts and CD4 absolute counts are presented in Table 1 for each of the groups.

Table 2 shows comparison between %CD4 of < 15%, < 20% and >20%, and CD4 cell counts of ≤200, 200-500 and >500 cells/mm^3 thresholds respectively among overall recruited subjects. Thus, 79.1% of subjects with < 200 CD4$^+$ cells/mm^3 also had <15% CD4$^+$ and 89.5% of subjects with ≥ 500 cells/mm^3 also had > 20% CD4 cells.

Correlations of total Lymphocyte count (TLC) with CD4 cell counts and %CD4 cells are shown in Table 3. There was highly significant correlation between TLC and CD4 cell counts within the two groups, but it weakened considerably when the subjects were stratified into groups based on their respective CD4 cell counts. TLC also showed positive significant correlation within the groups, but significant inverse correlation was observed with %CD4 cell counts only within the stratified groups.

As shown in table 4 when we used a threshold value of 1, 500 cell/mm^3, we has maximal combination of sensitivity (91.9%), PPV (67.0%) and NPV (84.1%), but with specificity of only 48.7% for a CD4 cell counts <200 cells/mm^3. The same limit also gave maximal combined sensitivity (72.8%), specificity (86.1%), PPV (80.8%) and NPV (79.7%) for CD4 cell counts ≤350 cells/mm^3 (Table 5).

In table 6, a TLC of ≤1, 500 cells/mm^3 had sensitivity of only 48.6% to detect subjects with a %CD4 <20% and a specificity of 81.5%. Also, the same threshold value provided a sensitivity of 62.6% and a specificity of 76.1% to predict subjects with %CD4 <15%. Figure 1 showed distribution of TLC and CD4 T-cell counts of HIV-infected patients in both groups.

Table 1: Mean and Range of CD4 counts and TLC between both sexes among HIV infected patients for both groups

Markers	Sex (n)	CD4 (cells/mm^3)			TLC (cells/mm^3)		
		Median	Mean±SD	Range	Median	Mean±SD	Range
With previous ART	Male (n=61)	423.0	432.9±248.6	85-1198	1700.0	1899.0±833.0	875-4845
	Female (n=185)	443.0	461.7±234.0	91-1490	1815.0	1877.0±577.7	868-3933
	Combine (n=246)	434.5	454.6±237.5	85-1490	1803.0	1882.5.0±648.6	868-4845
Without previous ART	Male (n=61)	338.0	401.3±254.9	74-1064	1824.0	1979.7±888.3	616-5544
	Female (n=144)	427.0	440.2±237.4	84-1121	1942.5	2022.2±734.4	784-4500
	Combine (n=205)	415.0	428.6±242.8	73-1121	1920.0	2009.6±781.4	616-5544

Table 2: Comparison between different groups of %CD4 and CD4 cell counts among overall recruited subjects

P = 0.00		CLUSTER OF DIFFERENTIATION (cells/mm^3)			Total
		< 200	200 – 500	≥500	
%CD4	< 15% CD4	68 (79.1%)	27 (13.3%)	4 (2.5%)	99 (22.0%)
	< 20% CD4	18 (20.9%)	78 (38.4%)	13 (8.0%)	109 (24.2%)
	>20% CD4	-	98 (48.3%)	145 (89.5%)	243 (53.9%)
Total		**86 (100.0%)**	**203 (100.0%)**	**162 (100.0%)**	**451 (100.0%)**

79.1% of patients with less than 200 CD4$^+$ cells/mm^3 also had less than 15% CD4$^+$ cells and 89.5% of subjects with ≥ 500 cells/mm^3 also had > 20% CD4 cells.

Table 3: The Spearman rank correlation between total Lymphocyte count (TLC) and CD4 cell counts and %CD4 cells

TLC (cells/mm^3)	N	CD4$^+$ cell count (cells/mm^3)		% CD4$^+$ (%)	
		r	p-Value	r	p-Value
HIV-infected patients in treatment group					
All subjects	246	0.761**	0.000	0.296**	0.000
<200	34	0.429*	0.011	-0.698	0.699
200 – 500	123	0.370**	0.000	-0.407**	0.000
≥500	89	0.396**	0.000	-0.562**	0.000
HIV-infected patients without previous antiretroviral therapy group					
All patients	205	0.646**	0.000	0.340**	0.000
<200	52	0.501**	0.000	0.196	0.591
200 – 500	123	0.370**	0.000	-0.407**	0.001
≥500	73	0.034	0.129	-0.350**	0.000

** correlation significant (p<0.05)*
*** correlation significant (p<0.01)*

Table 4: Combined sensitivity, specificity, PPV, NPV value of total lymphocyte counts for absolute CD4$^+$ T-Lymphocyte counts less than 200 cell/mm^3

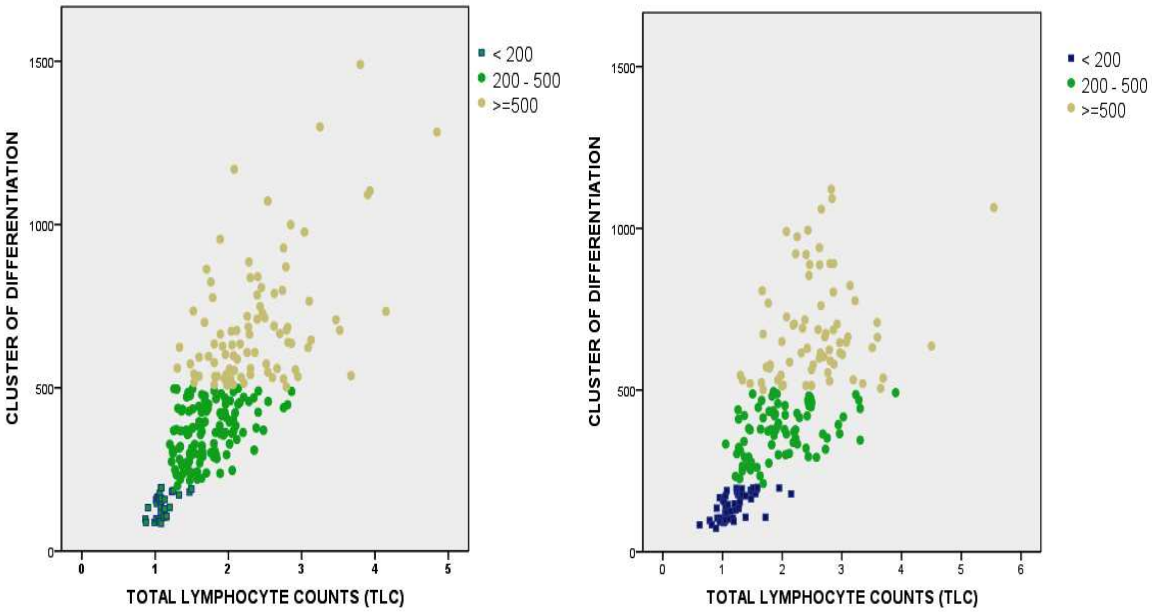
TLC (cells/mm^3)	N	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≤1,000	14	16.3	100.0	100.0	51.4
≤1,200	57	66.3	86.0	96.6	71.8
≤1,500	79	91.9	48.7	67.0	84.1
≤1,700	83	96.5	27.6	60.1	87.5
≤2,000	85	98.8	13.2	56.3	90.9
≤2,200	86	100.0	5.3	54.4	100.0
≤2,500	86	100.0	3.4	53.1	100.0

Table 5: Combined sensitivity, specificity, PPV, NPV value of total lymphocyte counts for absolute CD4$^+$ T-Lymphocyte counts ≤350 cell/mm^3

TLC (cells/mm^3)	N	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≤1,000	14	8.6	100.0	100.0	57.6
≤1,200	59	36.4	100.0	100.0	66.1
≤1,500	118	72.8	86.1	80.8	79.7
≤1,700	138	85.2	70.1	69.7	85.5
≤2,000	151	93.2	42.2	56.6	88.5
≤2,200	158	97.5	24.9	51.1	92.6
≤2,500	162	98.2	29.7	44.6	95.6

Table 6: Ability of total lymphocyte count (TLC) to predict % $CD4^+$ cells at <20% and <15%

	<20% CD4		<15% CD4	
TLC (Cells/mm^2)	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
≤1000	6.7	100.0	12.1	99.4
≤1200	27.4	99.2	41.4	94.9
≤1500	48.6	81.5	62.6	76.1
≤1700	57.7	67.9	71.7	63.9
≤2000	67.3	47.7	75.8	54.0
≤2200	75.0	37.0	79.8	34.7
≤2500	84.1	22.6	84.8	20.7



(a) With ART treatment (graph above) **(b) Without previous antiretroviral (Naive)**

Figure 1: Distribution of TLC and CD4 T-cell counts of HIV-infected patients.

DISCUSSION

Monitoring individuals with HIV infection/AIDS requires the use of expensive tools, which are not readily available in resource-limited settings [3]. It had been established that an absolute CD4 threshold of 200 cells/mm^3 could define when prophylaxis treatment should be initiated [2]. The high cost of CD4 count estimation in resource-limited countries is a major challenge in initiating patients on highly active antiretroviral therapy (HAART) [4]. Due to this, the World Health Organization (WHO) specifies that CD4 count testing is suitable but not essential for HAART use in resource-limited settings [5]. Thus, there is a need to evaluate other less expensive surrogate markers like total lymphocyte count (TLC) in initiating patients on highly active antiretroviral therapy Several studies have demonstrated the importance of absolute lymphocyte count (ALC) or total lymphocyte count (TLC) in identifying patients who would benefit from initiating prophylaxis for acquired immunodeficiency syndrome (AIDS) and related opportunistic infections [5, 7].

In this study, we have demonstrated reliability of TLC as a surrogate measure for CD4 cell counts as a routine marker of immune status in indigenous areas. A model linear regression analysis was applied to the data and the sensitivity and specificity of the World Health Organization recommended TLC thresholds corresponding to CD4 count <200, 200-500 and >500 cells/mm^3 were determined.

Several studies have revealed that TLC can be used to predict the CD4 cell count in immune-compromised patients. Blatt et al. [6] discovered that TLC was a useful indicator of significant immunosuppression patients and also Kumarasamy et al. [9] and Srirangaraj and Venkatesha, [5] found that TLC could serve as a low-cost parameter to

determine when to initiate prophylaxis in resource-constrained settings. Whereas, some other studies found that TLC is not a good predictor of CD4 cell count [2, 3, 21].

This study revealed a good positive significant correlation between TLC and CD4 counts with spearman correlation (r=0.761 and r=0.646 for HIV-infected subjects with ART and previous without ART treatments respectively). Our observation corroborates what was observed in India [9], England [22], North American [23] and South African [21]. In contrast, Akinola et al. [3] demonstrated a poor correlation while Angelo et al. [2] reported weaker correlation, when comparing all data. We found a weak positive correlation between TLC and %CD4 cell count among the groups, but significant inverse correlation was observed with %CD4 cell counts only within the stratified groups. This is similar to what was reported by Blatt el al. [6]; Van Der Ryst et al. [21] and Angelo et al. [2].

When we used a threshold value of 1, 500 cell/mm^3, we has maximal combination of sensitivity (91.9%), PPV (67.0%) and NPV (84.1%), but with specificity of only 48.7% for a CD4 cell counts <200 cells/mm^3. We found that a better result was observed when the same limit also gave maximal combined sensitivity (72.8%), specificity (86.1%), PPV (80.8%) and NPV (79.7%) for CD4 cell counts ≤350 cells/mm^3. Our observation was in agreement with the report documented by Kumarasamy et al. [9] and Blatt et al. [6]. This indicates that such a limit could be used to safely detect immune-compromised patients and to initiate early prophylaxis against opportunistic infections.

On the other hand, one of the previous reports had showed that TLC has a low sensitivity and specificity and was not thus optimal for identifying patients requiring HAART [4, 7], while another says TLC has a high specificity to identify patients for prophylaxis, but a quite low sensitivity as a surrogate marker to CD4+ T-cell counts in HIV-infected patients [2].

CONCLUSION

Our findings in this study revealed a good positive significant correlation between TLC and CD4 counts and also it revealed reasonably adequate sensitivity and specificity to consider TLC as a substitute for CD4 count. Thus, TLC may be helpful in deciding when to initiate antiretroviral therapy and thus acts as a surrogate marker for CD4 counts in resource-poor settings.

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REFERENCES

[1] UNAIDS/WHO. Global Report **2004**.
[2] ALD Angelo, CD Angelo, AJL Torres, AMC Ramos, M Lima, EM Netto and C Brites. *Brazilian Journal of Infectious Diseases*. **2007**; 11(5):466-470.
[3] OO Akinola, IA Adediran, O Onayemi, et al. *Clin Infect Dis.* **2004**; 39: 579-81.
[4] Y Wondimeneh, G Ferede, G Yismaw and D Muluye. *AIDS Research and Therapy*. **2012**; 9:21
[5] S Srirangaraj and D Venkatesha. *J Glob Infect Dis*. **2011**; 3(3): 265–268. doi: 10.4103/0974-777X.83533
[6] SP Blatt, CR Lucey, CA Butzin, CW Hendrix, and DR Lucey. *JAMA*. **1993**; 269:622–6. [PubMed: 8093628]
[7] D Daka and E Loha. *AIDS Res Ther*. **2008**; 5:26–31.
[8] JMI Pascale, P Contreras, B Gomez, et al. *Clin and Diagnostic Laboratory Immunol*. **1997**; 4(4): 474-7.
[9] NMA Kumarasamy, TP Flanigan, R Hemalatha, et al. *JAIDS*. **2002**; 31:378-83.
[10] SM Hammer, KE Squires, MD Hughes, JM Grimes, LM Demeter, JS Currier, JJ Eron, JE Feinberg, HH Balfour, LR Deyton, JA Chodakewitz, MA Fischl. *N. Engl. J. Med*. **1997**; 337:725-733.
[11] KR Kilaru, A Kumar and N Sippy. *Pan. Am. J. Public Health*. **2004**; 16:302-307.
[12] KA Fasakin, CT Omisakin, AJ Esan, IO Adebara, IS Owoseni, DP Omoniyi, OD Ajayi, RG Ogundare and MB Moronkeji. *Journal of Medical Laboratory and Diagnosis*. **2014**; 5(2): 22-28.
[13] National AIDS Control Organization**. 2001**; p. 162.
[14] World Health Organization. **2006**; [Last cited on **2010**, Mar 4]. Available from: http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf
[15] K Ray, SM Gupta, M Bala, S Muralidhar and J Kumar. *Indian J Med Res.* **2006**; 124:319-330.

[16] JA Olaniyi, KS Akinlade, AD Atere and OG Arinola. *International Journal of Tropical Disease and Health*. **2014**; 4(8):917-927.
[17] BD Akinbo, AD Atere and OO Eluwole. *International Journal of Enhanced Research in Medicines & Dental Care*. **2014**; 1(8):7-11.
[18] A Calman, et al. *BMJ*. **2007**; 335:190.
[19] S Ashwini, T Madhuri, RA Philip, and P Ramesh. *Indian J Med Res.* **2010**; 132(6):667-675.
[20] BD Akinbo, AD Atere, HB Fatunade and NO Iyabor. *BJMMR*. **2015**; 8(8): 717-723.
[21] KM Der Ryste, G Joubert, M Steyn, et al. *J Acquir Immune Defic Syndr and Human Retrovirology*. **1998**; 19(3): 238-44.
[22] MA Jacobson, H Khayam-Bashi, SG Deeks, et al. *AIDS*. **2003**; 17(6):917-919.
[23] AM Fournier and JM Sosenko. *Am J Med Sci*. **1992**; 304(2): 79-82.