Retrovirology: Research and Treatment

ORIGINAL RESEARCH

Evaluating Total Lymphocyte Counts and Other Hematological Parameters as a Substitute for CD4 Counts in the Management of HIV Patients in Northeastern Nigeria

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Abstract: This study was designed to validate or refute the reliability of total lymphocyte count (TLC) and other hematological parameters as a substitute for CD4 cell counts. Participants consisted of two groups, including 416 antiretroviral naive (G1) and 328 antiretroviral experienced (G2) patients. $CD4^+$ T cell counts were performed using a Cyflow machine. Hematological parameters were analyzed using a hematology analyzer. The median ± SEM CD4 count (range) of participants in G1 was 199 ± 10.9 (5–1840 cells/μL) and the median ± SEM TLC (range) was 1. 61 ± 0.05 (0.07–6.63 × 10^3/μL). The corresponding values among G2 were 421 ± 15.8 (13–1801) and 2.13 ± 0.04 (0.06–5.58), respectively. Using a threshold value of 1.2 × 10^3/μL for TLC alone, the sensitivity of G1 was 88.4% (specificity (SP) 67.4%, the positive predictive value (PPV) 53.5% and negative predictive value (NPV) of 93.2% for CD4 < 200 cells/μL, the sensitivity for G2 was 83.3%, SP 85.3%, PPV 23.8%, and NPV of 93.2%. Using multiple parameters, including TLC < 1.2 × 10^3/μL, hemoglobin < 10 g/dL, and platelets < 150 × 10^3/L, the sensitivity increased to 96.0% (SP, 82.7%; PPV, 80%; NPV, 96.7%) among G1, while no change was observed in the G2 cohort. TLC < 1.2 × 10^3/μL alone is an insensitive predictor of CD4 count of < 200 cells/μL. Incorporating hemoglobin < 10 g/dL, and platelets < 150 × 10^3/L enhances the ability of TLC < 1.2 × 10^3/μL to predict CD4 count < 200 cells/μL among the antiretroviral-naïve cohort. We recommend the use of multiple, inexpensively measured hematological parameters in the form of an algorithm for predicting CD4 count level.

Keywords: CD4 count, total lymphocyte count, human immunodeficiency virus, sensitivity

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Introduction

The $CD4^+$ lymphocytes are a primary target of the human immunodeficiency virus (HIV). Reduction in the absolute number of CD4 T cells occurs as one of the earliest immunologic abnormalities of HIV infection and is the most important prognostic indicator for risk of developing opportunistic infections.[1] It is essential for assessing immune status in HIV-infected persons as the pathogenesis of acquired immunodeficiency syndrome (AIDS) is largely attributed to a decrease in absolute CD4 cell counts. In addition, it predicts host susceptibility to specific opportunistic infections, selected drug toxicities, and mortality.[2,3]

The absolute $CD4^+$ T cell count in HIV-infected adults compliment clinical history and physical examination to inform decisions about initiating antiretroviral therapy (ART).

However, few HIV treatment centers in sub-Saharan Africa have access to reliable CD4 T cell enumeration. Indeed, local resources for health care are already overstretched by HIV/AIDS and overlapping high-impact endemic diseases such as malaria, tuberculosis, and infant diarrheal illnesses.[4]

Against this background, the World Health Organization (WHO) recommended the use of total lymphocyte count of less than $1.0–1.2 \times 10^3/\mu L$ in individuals with stage II or III disease as an indication to initiate antiretroviral therapy in the absence of knowing the CD4 count.[5] This recommendation was exclusively derived from studies conducted in developed countries.[6–8] There is limited information regarding the relationship between CD4 cell counts and total lymphocyte count and other hematological indices in resource-limited settings. Studies from Southwestern Nigeria by Akanmu et al[9] and Akinola et al[10] indicated that total lymphocyte count is not a reliable surrogate for CD4 cell count in monitoring response to antiretroviral therapy.

Information on hematological and immunological profile of HIV/AIDS patients in Northeastern Nigeria is limited despite their socioeconomic and demographic characteristics. Therefore, this study was designed to validate or refute the reliability of total lymphocyte count as a substitute for CD4 cell count and determine its relationship with other hematological indices, including CD4 cell count.

Patients and Methods

This cross-sectional analytical study included HIV clients accessing care in the HIV clinic at the University of Maiduguri Teaching Hospital, a tertiary health institution designated as a center of excellence in for infectious diseases and immunology. Maiduguri, the capital of Borno State, is located in Northeastern Nigeria and is the largest settlement near Lake Chad. Permission for this study was obtained from the University of Maiduguri Teaching Hospital (UMTH) Ethical Committee.

Study procedure

Participants consecutively recruited for this study consisted of both antiretroviral-naïve group (G1) and antiretroviral-experienced; the second group consisted of those who had been on HAART for at least 6 months group (G2). All participants gave their informed consent to participate in the study. Blood samples from the participants were collected by venipuncture after scrubbing the area with sterile cotton soaked in methylated spirit from the antecubital vein into 10 mL of EDTA and fluoride and placed in vacutainer tubes bottles.

Blood samples analysis

Sample for $CD4^+$ T cell count was collected between 9:00–10:00 am and assayed within 6 hours of collection of whole blood using a standardized flow cytometric Cyflow machine (Cytec, Partec, Germany). Hemoglobin (Hb), white blood count (WBC), lymphocyte count and percentage, neutrophil count and percentage, and platelets numbers, were analyzed using a Haematology analyzer (manufactured by Sysmex®, CorporationKobe, Japan).

Statistical analysis

Data was analyzed in SPSS 11.5. The correlation coefficient established correlation and Kappa coefficient showed agreement between CD4 count and these parameters. Sensitivity, specificity and positive and negative predictive values for using direction on TLC hemoglobin (Hb), white blood count (WBC), lymphocyte count and percentage, neutrophil count and percentage, and platelets numbers, changes as a marker for direction of CD4 changes were calculated. $P < 0.05$ was considered to be statistically significant for all tests.

Table 1. Mean, median and range of TLC and other haematological parameters.

Marker					
HAART naive	**Mean**	**Median**	**SE mean**	**SD**	**Range**
TLC ($\times 10^3/\mu l$)	1.77	1.61	0.05	0.94	0.07–6.43
CD4 count (cells/μl)	249.83	199	10.90	222.09	5.0–1840
Hb (g/dl)	10.00	9.90	0.13	2.67	3.9–35.30
WBC ($\times 10^3/\mu l$)	5.74	5.1	0.15	2.98	1.0–28.80
Platelets $\times 10^3/l$	298.72	295.5	6.07	123.78	38–837
Lymphocyte %	32.72	32.95	0.63	12.84	1.70–81.50
Neutrophil count	18.72	3.60	1.23	24.95	0.40–90.10
Neutrophil %	38.98	44.70	1.31	26.61	0.10–93.30
HAART experienced					
TLC ($\times 10^3/\mu l$)	2.22	2.13	0.039	0.74	0.06–5.58
CD4 count (cells/μl)	455.62	421.00	15.78	292.70	13.00–1801.00
Hb (g/dl)	11.29	11.50	0.11	2.07	3.60–16.50
WBC ($\times 10^3/\mu l$)	5.30	5.20	0.08	1.54	2.3–15.00
Platelets $\times 10^3/l$	271.13	261.00	5.24	97.13	46.00–760.00
Lymphocyte %	42.61	43.00	0.57	10.54	9.30–67.30
Neutrophil count	2.63	1.20	0.07	1.20	0.70–12.30
Neutrophil %	48.47	48.05	0.59	10.93	23.70–86.80

Results

Of the 824 participants recruited into this study, 744 had complete records that were analyzed, including 416 HAART-naïve patients with mean ages of 35.44 ± 11.30 years (19–57) and 328 HAART-experienced patients with mean ages of 38.39 ± 10.01 years (18–62). The sex distribution was 60.5% female and 39.5% male in the HAART-naïve group and 57.7% and 42.3%, respectively, among HAART-experienced subjects. The mean and other descriptive parameters of TLC and other hematological parameters are as presented in Table 1. Among HAART-naïve subjects, a moderately strong positive correlation was observed between TLC and CD4 count, WBC, and lymphocyte %, with a similar correlation observed in HAART-experienced subjects. A weak positive correlation was observed between TLC and Hb in both groups, while no correlation between TLC and neutrophil count. A weak and strong negative correlation was observed between TLC and neutrophil % among HAART-naïve and– experienced, respectively. The correlation between TLC, CD4 count, and other hematological parameters is depicted in Table 2.

Sensitivity, specificity, PPV, and NPV for TLC cut-off values $< 1.2 \times 10^3/\mu L$ as compared to a CD4 count of < 200 cells/μL, Hb < 10 g/dL, WBC $< 4.5 \times 10^3/L$ platelets $< 150 \times 10^3/L$ lymphocyte % < 15 neutrophil

Table 2. Correlation between TLC and some haematological parameters.

Marker	HAART naive		HAART experienced	
	Correlation coefficient (r)	**p-value**	**Correlation coefficient (r)**	**p-value**
CD4 count (cells/μl)	0.494	0.000	0.632	0.000
Hb (g/dl)	0.172	0.000	0.157	0.004
WBC ($\times 10^3/\mu l$)	0.446	0.000	0.560	0.000
Platelets $\times 10^3/l$	–0.006	0.901	0.142	0.009
Lymphocyte %	0.596	0.000	0.585	0.000
Neutrophil count	–0.048	0.330	0.032	0.551
Neutrophil %	–0.230	0.000	–0.556	0.000

Table 3. Validity and predictive value for surrogate markers of TLC in HIV patients.

Marker						
HAART naive	**No**	**P**	**SE**	**SP**	**PPV**	**NPV**
TLC $< 1.2 \times 10^3$/l						
CD4 count < 200 cells/μl	200	48.1	88.4	67.4	53.5	93.2
Hb < Hb, g/dl	209	50.2	70.3	58.0	40.7	82.6
WBC $< 4.5 \times 10^3$/l	147	35.3	62.0	75.3	51.0	83.0
Platelets $< 150 \times 10^3$/l	036	08.7	15.9	94.5	55.6	72.1
Lymphocyte % <15	040	09.6	28.1	98.0	85.0	76.9
Neutrophil count <1.5	033	07.9	06.8	90.8	45.5	46.2
Neutrophil % <50	179	43.0	15.7	46.8	10.6	58.0
HAART experienced						
TLC $< 1.2 \times 10^3$/L						
CD4 count cells/μl	063	18.3	83.3	85.3	23.8	98.9
Hb < Hb, g/dl	079	23.0	27.8	77.3	06.3	95.1
WBC $\times 10^3$/l	106	30.8	66.7	71.2	13.3	97.5
Platelets $\times 10^3$/l	048	14.0	20.0	86.6	12.5	91.9
Lymphocyte %	004	01.2	16.7	99.7	75.0	95.6
Neutrophil count % <15	034	09.9	05.6	89.9	02.3	94.5
Neutrophil % <50	198	57.6	05.6	39.6	00.5	88.3

Surrogate markers expressed as %, P = prevalence.

count < 1.5, neutrophil % < 50 for both groups are listed in Table 3. Among the HAART-naïve cohort, a TLC of $\leq 1.2 \times 10^3$/μL was found to be sensitive (88.4%) and specific (67.4%) for predicting a CD4 count of < 200 cells/μL with PPV with a concurrent decrease in CD4 count and TLC of (53.5%), and NPV for concurrent increase in CD4 count and TLC of (93.2%). The corresponding sensitivity and specificity among HAART-experienced participants was 83.3% and 85.3%, respectively, with PPV for concurrent decrease in CD4 count and TLC of 23.8% and NPV for concurrent increase in CD4 count and TLC of 98.9%. As shown in Table 4, when a TLC of $\leq 1.2 \times 10^3$/μL, hemoglobin level (with a cutoff point of < 10 g/dL) and platelets $< 150 \times 10^3$/L were used as predictors in the HAART-naïve cohort, this sensitivity was increased to 96.0% (specificity, 82.7%; PPV, 80%; NPV, 96.7%). However, this trend was not observed in the HAART-experienced cohort.

The kappa coefficient for agreement between TLC and CD4 count, Hb, WBC, platelets, lymphocyte percentage, and neutrophil count and percentage are shown in Table 5. Among HAART-naïve subjects, significant agreement was observed between TLC and CD4 count, including other hematological variables, except neutrophil count and percentage. Although significant agreement was observed between TLC and CD4 count, WBC, and lymphocyte %, with no linear relationship observed between TLC and other analyzed parameters among the cohort on HAART.

Table 4. Agreement between TLC and multiple parameters among HAART naive cohort.

	TLC $\times 10^3$/μl							
Multiple parameters	**<1.2**	**≥1.2**	**SE**	**SP**	**PPV**	**NPV**	**kappa**	**p-value**
Group A	167	42	96.00	82.70	80.00	96.70	0.48	0.000
Group B	07	200						

Multiple parameters = CD4 count, Haemoglobin and platelets count.
Group A = CD4 count < 200 cells/μl or Haemoglobin < 10 g/dl or platelets $< 150 \times 10^3$/l.
Group B = CD4 count ≥ 200 cells/μl or Haemoglobin ≥ 10 g/dl or platelets $\geq 150 \times 10^3$/l.
Abbreviations: SE, Sensitivity; SP, Specificity; PPV, Positive predictive value; NPV, Negative predictive value; Kappa, kappa coefficient for agreement.

Table 5. Agreement between CD4 count, TLC and other haematological parameters.

Marker		HAART naive				HAART experienced			
				Kappa (95% CI)	p-value			Kappa (95% CI)	p-value
HAART naive		TLC < 1.2	TLC ≥ 1.2			TLC < 1.2	TLC ≥ 1.2		
CD4 count	<200	107	93	0.47 (0.39–0.55)	0.000	15	48	0.31 (0.18–0.45)	0.000
	≥200	14	192			03	278		
Hb	<10	85	124	0.23 (0.15–0.32)	0.000	05	74	0.02 (–0.06–0.10)	0.309
	≥10	36	171			13	252		
WBC	<4.5	75	72	0.35 (0.26–0.45)	0.000	12	94	0.11 (0.03–0.20)	0.000
	≥4.5	46	223			06	232		
Platelets	<150	20	16	0.13 (0.04–0.21)	0.000	06	42	0.05 (–0.06–0.17)	0.159
	≥150	106	274			24	272		
Lymphocyte %	<15	34	06	0.32 (0.23–0.42)	0.000	03	01	0.26 (0.02–0.50)	0.000
	≥15	87	289			15	325		
Neutrophil count	<1.5	15	18	–0.02 (–0.07–0.03)	0.821	01	33	–0.03 (–0.11–0.05)	0.736
	≥1.5	206	177			17	293		
Neutrophil %	<50	19	158	–0.32 (0.40–0.25)	1.000	01	197	–0.10 (–0.14–0.05)	1.000
	≥50	100	139			17	129		

Discussion

Absolute CD4 count and CD4% measurement constitute the main criteria for monitoring disease progression and timing for prophylaxis against Pneumocystis jiroveci pneumonia or infection with other opportunistic pathogens. However, monitoring individuals with HIV infection/AIDS using CD4 count and CD4% parameters in dangerous and resource-limited settings is not realistic. As a result, the WHO recommends the use of less expensive TLC as an alternative marker when CD4 count is not available or affordable.[5]

We previously reported TLC as a suitable surrogate for CD4 count in healthy HIV-negative adults in our environment.[18] Several studies have suggested TLC as a substitute for CD4 count in HIV/AIDS patients.[6,7,11,12] In contrast to studies reporting TLC as suitable predictor of CD4 counts, we found that TLC alone is not a good predictor of CD4 count, which is similar to the results of Akinola[10] and Van Der Ryster et al.[11] In sub-Saharan Africa, a high incidence of opportunistic infections such as tuberculosis among HIV patients is associated with decline in CD4 count and exaggerated TLC. Another factor that can blunt the correlation between TLC and CD4 T cell count is that TLC captures both B and T cell subsets. Thus, a person with a low CD4 T cell count may show relatively high TLC if high amounts of B cells are expressed due to immune hyperactivation from exposure to the wide variety of circulating antigens consequent on varieties opportunistic infections in HIV patients with severe immunosuppression.[4] Our previous study indicated that most HIV patients in our setting present late with a varieties of opportunistic infections.[19]

We observed good correlation between TLC and CD4 count using the Spearman's rank test (r = 0.632) in HAART-experienced and r = 0.494 in HAART-naïve cohorts, similar to the value of 0.431 reported by Akinola.[10] The correlation in our study between TLC and CD4 count was weaker than that observed in India,[12] (r = 0.744), England (r = 0.76),[6] North America (r = 0.744),[7] and South Africa (r = 0.70).[11] We reported a sensitivity of 88.4%, specificity of 67.4%, PPV of 53.5%, and NPV of 93.2% among HAART-naïve participants. The corresponding values in HAART-experienced subjects were 83.3%, 85.3%, 23.8%, and 98.9%, respectively. We observed that despite high sensitivity, the PPV that shows a direct relationship between CD4 count and TLC in both HAART-naïve and experienced participants was low, indicating that TLC alone is not a suitable surrogate for CD4 count. In this study, we observed that 46.5% of patients had TLC $> 1.2 \times 10^3/\mu L$, despite that the CD4 count was < 200 cells/μL, higher than the 38% reported by Akinola.[10] However, when we incorporated hemoglobin < 10 g/dL and platelets $<150 \times 10^3/L$, the sensitivity, specificity, PPV, and NPV increased to 96.0%, 82.7%, 80.0%, and 96.7% among HAART-naïve participants.

Several previous reports have used multiple parameters (including hemoglobin levels, body mass index, platelet count, and clinical symptoms) to predict CD4 cell count and disease stage.[13–16] Hemoglobin level is an inexpensive marker that has been shown to correlate with progression to AIDS and to decline around the time of the development of AIDS.[12] Spacek et al[17] retrospectively evaluated 3269 patients to test the ability of TLC to predict CD4 cell. They found that the TLC alone (<1200 cells/mm^3) had a relatively low sensitivity (71%) and specificity (82%) for predicting a CD4 count of < 200 cells/mm^3. However, when both the TLC and the hemoglobin level (with a cutoff point of < 12 g/dL) were used as predictors, this sensitivity was increased to 78% for men (specificity, 80%; PPV, 84%; NPV, 72%) and to 86% for women (specificity, 73%; PPV, 75%; NPV, 84%) (as calculated by an average of 2 algorithmic methods used in the study). Similarly Chen et al[16] evaluated 1189 HIV patients, also using multivariate model with similar parameters (TLC, hemoglobin level, platelet count, and sex) for predicting CD4 cell counts of < 200 cells/mm^3. They reported 91% sensitivity, 73% specificity, and 88% PPV for predicting a CD4 cell count of < 200 cells/mm^3.

Clinical symptoms and body mass index were not considered in this study; however, unlike most previous studies that evaluated only TLC as a substitute for CD count, we considered multiple parameters.

Conclusion

The use of TLC $<1.2 \times 10^3$/μL alone is insensitive predictor of CD4 count of < 200 cells/μL; however, incorporating hemoglobin < 10 g/dL and platelets < 150×10^3/L enhancedthe ability of TLC $<1.2 \times 10^3$/μL to predict CD4 count < 200 cells/μL among our cohort. We recommend the use of multiple, inexpensively measured hematological parameters in the form of an algorithm for predicting CD4 count level for monitoring disease progression and timing for prophylaxis in resource-constrained settings.

Author Contributions

Conceived and designed the experiments: BAD. Analyzed the data: BAD, CBA. Wrote the first draft of the manuscript: BAD, AHG. Agree with manuscript results and conclusions: BAD, AUA, CBA, IMK, AHG, AAB. Made critical revisions and approved final version: BAD, CBA, AUA. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the author has provided signed confirmation of compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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Supplementary Data

Peculiarity of Northeastern Nigeria

The observations highlighted below make the Northeastern region of Nigeria unique or peculiar, as no other region shares any of the outlined characteristics with it.

Nigeria is divided into 6 geopolitical regions; the Northeastern region has 6 states. It is the least-developed of all regions, with very low literacy rate, and most of the population live in poverty and have resentment to western education and health care.

Study location and population

Maiduguri is the capital city of Borno state, located on the fringe of the Sahara desert between longitude 11°8E and 14°4E and latitudes 10°2N 13°4N.

The city has an estimated population of 0.63 million distributed as 0.34 million (54%) male and 0.29 million (46%) female.

Climate

The region has a semi-arid climate with savannah and prominent patterns of weather—the rainy season is between July and September with average annual rainfall below 1000 mm and environmental temperatures between 20°C and 26°C.

The dry season starts from October and ends in July. Within the dry season, however, two weather patterns exist. The period between October and February is characterized by the Harmmattan, which is a cold, dusty wind that blows in from the Sahara. During this period, environmental temperatures range between 20°C and 32°C. The period from March to June is dry, with environmental temperature ranging between 40°C and 43°C. However, temperatures may reach up to 50°C. Humidity is very low, while the evaporation rate is very high and averages about 2000 mm/year.

Environmental problems

The serious environmental problems of Maiduguri include desert encroachment, water shortage, human settlement, and soil erosion.

Nutrition

Presently, there is need for nutritional surveys which will focus on increasing urbanization and other changes in lifestyle, human diets, and methods of food preparation. Cereals account for a major part of the diet. There is a high preference for flours made from local grains, with millet being the most preferred. In a study carried out by Salami et al (1990) on students at the University of Maiduguri, although most of the meals were comparatively high in the nutrients analyzed, the level of protein and total energy content of meals were rather inadequate.[1]

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