AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 35 NUMBER 1 MARCH 2006

ISSN 1116—4077

Afr. J. Med. med. Sci. (2006) 35, 9-13

Should absolute lymphocyte count be used as a surrogate marker for CD4+ count in patients with HIV/AIDS?

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Summary

The World Health Organization (WHO) has recommended the use of absolute lymphocyte count (ALC) as a potential marker for immunosuppression where CD4+ count is unavailable. However, there are conflicting reports on the usefulness of ALC as a surrogate marker for CD4+ counts in patients with HIV/AIDS, more so, in patients with HIV-associated tuberculosis (TB). To evaluate the usefulness of ALC as an alternative to CD4 + counts and to see whether TB affects the correlation of ALC with CD4+ counts in patients with HIV-associated TB. A total of 66 consecutive patients (33 with and 33 without TB) with a diagnosis of HIV infection were recruited into the study as cases. Another group of 66 subjects (33 subjects each) age- and sex-matched HIV-negative controls were recruited as controls and stratified in to two: a) HIV-negative PTB patients. b) apparently healthy HIV and PTB negative individuals. The age range was from 15-60 years (median: 32 years). The highest percentage (39%) of subjects fell in the age range of 25-29 years. The mean ALC for HIV-associated PTB was 3906 ± 1092 cells/μl and for patients with HIV infection only, 4755 ± 1049 cells/μl. There was no significant difference in mean ALC between males and females in both groups ($P>0.05$). Patients with dual infection by *M. tuberculosis* and HIV had the lowest mean ALC (3906 ± 1092cells/μl). Healthy controls had mean ALC (±SD) of 5249±101 cells//μl. There was significant difference between the healthy controls and the other three groups. The observed difference was more in patients with HIV/TB co-infection ($P<0.005$) compared with patients with HIV alone ($P<0.05$). No significant correlation was observed between CD4+ cell counts and ALC in all the age groups of the study population. When the CD4+ counts were divided into <200 and ≥200 cells/μl and the ALC into <2000 and ≥2000 cells/μl, the sensitivity, specificity and positive predictive values of the diagnostic usefulness of ALC in HIV-associated PTB were 52%, 56.3% and 78.8% while for HIV only patients the same values were 56.3%, 55.9% and 54.5%, respectively. We cannot recommend the use of ALC as a surrogate for CD4+ count in our environment as this study has clearly shown that the correlation between the two is weak. Patients with dual infection by HIV and *M. tuberculosis* are more likely to have lower CD4+ cell and AL counts than those with HIV infection occurring alone.

Keywords: *CD4+ cells, ALC, surrogate marker*

Résumé

L'organisation mondiale de la sante a recommende l'usage des valeurs des taux de lymphocytes absolues (ALC) comme potentiel marqueur d'immunosuppressif ou le taux des CD4+ n'est pas disponible.Cependant il y a des rapprots contradictoires sur l'utilite du ALC comme markeur pour les taux de CD4+ aux patients ayant le VIH/SIDA, ainsi aux patients ayant le VIH associé a la tuberculose (TB). Cette étude avait pour but d'évaluer l'utilité du ALC comme alternative au taux des cellules CD4+ et observer si La tuberculose affecte la corrélation entre ALC et les taux des CD4+ aux patients ayant le VIH associes a la tuberculose. Au total 66 patients (33 avec et 33 sans TB) ayant le VIH étaient recrutés. Un autre groupe de 66 sujets d'age et sexe semblable sans VIH étaient recrutés comme contrôle et stratifie en deux groupes :a)personne sain sans VIH, ni TB pulmonaire (PTB).b) séropositif apparemment sain sans TB pulmonaire. L'age variait entre 25-60 ans (médiane : 32 ans). Le taux le plus élevé (39%) était dans l'age de 25-29 ans. Le ALC moyen aux séropositif ayant la PTB était de 3906 ±1092 cellules/μl et 4755 ±1049cellules/μl au séropositif seulement. Il n'avait pas de différence significative entre le ALC moyen entre les males et les femelles des 2 groupes ($P>0.05$). Les patients ayant la co-infection du *M. tuberculose* et du VIH avaient le plus bas ALC (3906±1092 cellules/μl) et taux des CD4+. Les contrôles sains avaient une ALC moyen de 5249 ±101 cellules/μl. Il avait une différence significative entre les contrôles sains et les autres groupes. Aucune corrélation significative était observe entre le taux des CD4+ et celui des lymphocytes absolus parmi les groups d'age étudie. Lorsque les taux des CD$+ étaient divide en <200 et ≥200 cellules/μl et ALC<2000 et ≥2000 cellules /μl, la sensitivité, la spécificité et les valeurs de prédiction positive de l'utilité du diagnostic étaient de 56.3%,55.9% et 54.5% respectivement. A base de la corrélation faible entre ALC et CD4+ obervée dans cette étude, nous ne recommandons pas l'usage des taux des lymphocytes absolus comme marqueur alternative du taux des cellules CD4+ dans notre environnement.

Introduction

The CD4+ T-lymphocyte count is a major target cell for the human immunodeficiency virus (HIV). The number of CD4+ cells is the key index for evaluation of patients with HIV infection and is used for disease staging, prognosis and monitoring patients [1-6]. About 65% of the T-lympho-

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cytes express CD4 antigens [7] whilst 25-35% are CD8+ T-lymphocytes [8]. As such, CD4+ T-lymphocytes constitute at least 60% of total lymphocytes in the blood. It is therefore reasonable to say that if CD4+ cells fall significantly as occurs in HIV infection, it should reflect in concomitant fall in total lymphocyte count thereby making absolute lymphocyte count (ALC) a possible surrogate test for CD4+ cell count [9].

Although the WHO has suggested that ALC could serve as a potential marker for immunosupp-ression where CD4+ cell is unavailable [10], there are conflicting reports on the usefulness of ALC as a surrogate marker for CD4+ cell count in HIV/AIDS patients, more so, in patients with HIV-associated TB.

Although the CD4+ count is an integral test in HIV infection, its determination requires resource and technical expertise not routinely available in many settings in developing countries [4,11]. There is, therefore, the need to assess the usefulness of alternative tests such as the ALC, which are readily available, affordable and easy to carry out. This study was therefore aimed at evaluating the usefulness of ALC as an alternative to CD4+ count and to see whether TB affects the correlation of ALC with CD4+ count in patients with HIV-associated TB.

Materials and method

Sixty-six consecutive patients (33 with and 33 without TB) with a diagnosis of HIV infection as detected by two different Enzyme-Linked Immunosorbent Assay (ELISA), who consented to participate, were recruited into the study after obtaining an informed verbal consent. The study population was stratified into two groups, namely:

Cases: Adult patients with a first diagnosis of HIV infection as confirmed by double ELISA. This group was further divided into two a) patients with HIV and pulmonary TB (PTB), b) patients with HIV infection only.

Controls: Two groups, 33 each, of age- and sex- matched HIV-negative controls were used: a) HIV-negative PTB patients, b) apparently healthy HIV and PTB- negative individuals. Diagnosis of TB was based on the presence of Acid-Fast Bacilli (AFB) in the sputum smear.

Patients on antiretroviral, contraceptive, immunosuppressive, and antiTB drugs were excluded. Other patients excluded were pregnant women and those with sickle cell anaemia, congestive cardiac failure and connective tissue diseases.

A data sheet was used to document clinical and laboratory data. CD4+ cell count <200/μl used in CDC 1993 classification [6] were used in defining HIV infection and acquired immune deficiency syndrome (AIDS), respectively.

The ALC was calculated from the full blood count parameters (the product of total leucocyte and differential lymphocyte counts) obtained using standard techniques [12]. To get the total leucocyte count, 20μl of blood was diluted in 10ml of saline and 2 drops of specified counter reagents were added. 1:20 dilution of blood and diluting fluid was made and the neubauer counting chamber was filled. The diluting fluid lyses the red cells but the leucocytes remain intact, their nuclei staining deep violet-black. Visual counting was performed using both 4mm dry and 3.7mm oil immersion lens and the average of the two taken. The ALC, expressed as the percentage of the total leucocyte count, was reported in absolute numbers ($X10^9$/L). Blood specimen for CD4+ count was collected during morning hours (10 am-12 noon) from a free flowing venepuncture and added to containers with Tri-potassium EDTA (anticoagulant) with the ratio 1-1.5mg of K_3EDTA to 1ml of whole blood. Samples were adequately mixed to avoid clots. Blood samples were kept at ambient temperature avoiding refrigeration, and the analyses were carried out within 5 hours of specimen collection. Dynabeads CD4 (product No. 111 05), a Dynal Biotech product was used for the CD4+ count.

Results

The age of the study subjects ranged from 15-60 years (median: 32 years). Twenty seven percent of the HIV-positive patients were between 30 and 34 years. The highest percentage (39%) of subjects in the other three groups fell in the same age range of 25-29 years. There was a female preponderance in the study population with 80 (60.6%) females and 52 (39.4%) males.

Table 1: Mean value (±SD) of ALC and correlation between CD4+ cell count and ALC in the study population.

Subjects	Mean ALC (±SD) (cells/μl)	Correlation coefficient (r-value)	P-value
HIV/PTB			
Male (n=13)	4715 ± 920	0.15	0.09
Female (n=20)	4780 ± 1147		
Combined (n=33)	4755 ± 1049		
HIV positive only			
Male (n=13)	4231 ± 784	0.29	0.08
Female (n=20)	3695 ± 1177		
Combined (n=33)	3906 ± 1092		
PTB positive only			
Male (n=13)	4046 ± 784	0.36	0.11
Female (n=20)	4460 ± 902		
Combined (n=33)	4297 ± 869		
Healthy controls			
Male (n=13)	5562 ± 1009	0.24	0.13
Female (n=20)	5047 ± 1043		
Combined (n=33)	5249 ± 1011		

Healthy subjects had the highest CD4+ cell mean value (±SD) of 818±189 cells/μl. Patients with HIV and PTB had the lowest mean (±SD) CD4+ cell count of 175 ± 55 cells/μl. Those with only HIV infection or PTB had mean CD4+ counts of 199±62 cells/μl, and 587±157 cells/μl, respectively.

As shown in table 2, the mean ALC for HIV-associated PTB was 3906 ±1092 cells/μl and for patients with HIV infection only it was 4755±1049 cells/μl. Patients with HIV infection alone had a higher mean value compared with patients infected by *M. tuberculosis* and HIV. The difference was, however, not statistically significant ($P>0.05$)

There was no significant difference in mean between males and females in both groups ($P>0.05$). Healthy controls had mean (±SD) ALC of 5249 ± 1011 cells/μl. There was significant difference between healthy controls and the other three groups ($P<0.05$). The observed difference was more in patients with HIV/PTB co-infection compared with patients with HIV alone ($P<0.005$).

Regression analysis showed that age did not significantly affect ALC in all the groups ($P>0.05$). Although there was a gross difference in ALC between males and females in the healthy controls, the difference was not statistically significant ($P=0.17$). No significant correlation was observed between CD4+ cell counts and ALC in all the groups of the study population as shown in table 2.

Tables 3 and 4 show the sensitivity, specificity and positive predictive values of the diagnostic usefulness of ALC in HIV-associated PTB and HIV infection only patients when CD4+ cell counts were divided into two groups: <200 cells/μl and ≥200 cells/μl and ALC also divided into two groups <2000 cells/μl (negative) and ≥2000 cells/μl (positive).

Table 2: Diagnostic usefulness of ALC in PTB patients and HIV-positive only patients with CD4+ counts <200 cells/μl and ≥200 cells/μl).

ALC (cells/μl)	PTB positive patients (No.) CD4+ counts < 200	CD4+ counts ≥ 200	HIV positive only patients (No.) CD4+ counts < 200	CD4+ counts ≥ 200
<2000	26	24	18	14
≥2000	7	9	15	19
Total	33	33	33	33

For PTB positive patients			*For HIV-positive only patients*	
Sensitivity	=	*52%*	*Sensitivity*	*56.3%*
Specificity	=	*56.3%*	*Specificity*	*55.9%*
Positive predictive value	=	*78.8%*	*Positive predictive value*	*54.5%*

Discussion

CD4+ cells appear to be the primary reservoir of HIV in infected persons. The number of CD4 cell count remains a useful marker of disease progression and widely used as indicators for starting antiretroviral treatment or prophylaxis for opportunistic infections. However, its determination requires resource and technical expertise not routinely available in many settings in developing countries [4, 10] and is limited by high cost in several large hospitals.

Patients with dual infection by *M. tuberculosis* and HIV had a higher mean value compared with patients infected by HIV alone. The difference was, however, not statistically significant ($P>0.05$) and may be explained by relative lymphocytosis seen in patients with PTB.

Healthy controls in this study had a higher mean ALC compared with those reported among Caucasians.[13] Chronic parasitic infection in our environment is the likely contributing factor as a sizeable proportion of the study population were farmers who were probably repeatedly exposed to parasitic infections [14].

In this study, an insignificant ($P>0.05$) weak linear correlation ($r<0.29$) between CD4+ count and ALC was observed in all groups of the study population. This finding extends that of Akanmu *et al* [15] in Lagos, Nigeria who reported that out of 136 paired ALC and CD4+ count values, 51 had CD4+ count < 200 cells/μl. Of these, 29 measurements had corresponding values of ALC below 2000 cells/μl. They reported that ALC below 2000 cells/μl has sensitivity of 57% as a predictor of low CD4+ count and specificity of 72%. Our study also complements the findings of another study [16] which reported a poor correlation between ALC and CD4+ counts. The study concluded that it is not possible to predict CD4+ counts on the basis of ALC. This was also extended by the work of Van der Ryst and colleagues [3] who looked at the correlation between ALC, CD4+ count and CD4+ percentage in 2777 HIV-infected patients. They demonstrated that an ALC of ≥2000 cells/μl had a sensitivity of 90.3% to detect patients with a CD4+ count of <200 cells/μl, but a specificity of only 53.7%. When the ALC cut-off value was lowered specificity increased but sensitivity decreased. For the observation as a group, a correlation ($r=0.704$) between CD4+ count and ALC was demonstrated but if the patients were divided into three groups according to their CD4+ counts, this correlation weakened considerably. Therefore,

it was concluded that although ALC shows a correlation with CD4+ counts, it is not a good predictor of the latter. Although the predictive value among patients with HIV-related TB is high, the specificity and sensitivity are low to justify the use of ALC as a surrogate to CD4+ counts.

In contrast, Freeney and his colleagues [17] found a strong linear correlation between CD4+ counts and ALC (r=0.948). In 1996, Beck and co-workers [2] evaluated 1534 paired ALC and CD4+ counts in HIV infection and found the correlation to be high (r=0.76). When analyzed by stage of HIV infection, the correlation increased from r=0.64 for asymptomatic patients to r=0.72 for patients with symptomatic non-AIDS HIV infection and r=0.73 for AIDS patients. It was concluded that high correlation between ALC and CD4+ counts especially for patients with symptomatic HIV disease, demonstrates the suitability of the use of ALC in the absence of CD4+ counts, a finding not complemented by our study. The finding in our study is also in contrast to that of Ledru *et al.* [18] who, in a study to determine the use of clinical staging and haemogram data for basic management of HIV disease in West Africa showed that the lymphocyte count, clinical stage and platelet count predicted CD4+ counts below 350 cells/μl by regression analysis. Infectious agents abound in tropical countries like Nigeria, which may likely be responsible for the increased levels of ALC and subsequent poor correlation with CD4+ count.

Martins and co-workers [19] enrolled 345 patients in South Africa in a study to assess the usefulness of ALC as a surrogate of CD4+ count in patients dually infected by HIV and TB, and showed that the CD4+ count of the HIV/TB cohort were significantly lower than the TB group with medians of 230 and 630 cells/μl, respectively ($P<0.0001$). The finding in our study that HIV/TB patients had the lowest mean CD4+ count complements this work. However, they reported that an ALC of 1300-1500/μl was found to be predictive of a CD4+ count of ≥200 cells/μl, a finding that has not been corroborated by our study even though we used a cut-off value of 2000 cells/μl. Methodology may account for the differences observed in our study when compared with these studies as our CD4 + counts were done manually, which is associated with inherent errors (coefficient of variation 16%). It is however, unlikely that the ALC could have predicted low CD4+ counts in our study even if we reduced the cut-off value. In this study, at ALC ≥2000 cells/μl among patients with HIV-associated PTB the sensitivity, specificity and positive predictive values of CD4+ cell count were 52%, 56.3% and 78.8%, respectively while among those with HIV only the corresponding figures were 56.3%, 55.9% and 54.5%, respectively.

In conclusion, therefore, we cannot recommend the use of ALC as a surrogate for CD4+ count in our environment as this study has clearly shown that the correlation between the two is weak. Patients with dual infection by HIV and *M. tuberculosis* are more likely to have lower CD4+ cell and ALC than those with HIV infection occurring alone. The results of our study might have been affected by the inherent errors associated with manual counting used as opposed to flow cytometry and therefore, in view of this we recommend larger studies in developing countries to further elucidate the correlation between ALC and CD4+ counts as there are still conflicting reports, which led to the suggestion by some authors that the CD4+ count be always measured separately from the ALC [16].

References

1. Paxton H, Pius M, Delton G, *et al.* Comparison of CD4+ cell count by simple enzyme-linked immunosorbent assay using the TRX CD4 test kit and by flow cytometry and haematology. Clin Diagn lab Immunol. 1995; 2: 104-114.
2. Beck EJ, Kupe KE, Gompels MM and Pinching AJ. Correlation between total and CD4+ lymphocyte counts in HIV infection: not making an enemy of the not so perfect. Int *J STD AIDS* 1996; 7:422–428.
3. Van der Ryst E, Kotzt M, Joubert G *et al.* Correlation among total lymphocyte count, CD4+ count and CD4 percentage in a group of HIV-1-infected South African patients. J Acquir Immune Defic Synd Hum Retroviral 1998; 19: 238-244.
4. Post FA, Wood R, and Maartens. CD4+ and total lymphocyte counts as predictors of HIV disease progression. QJM. 1996; 89: 505-508.
5. Diagbouga S, Fumoux F, Ledru E, *et al.* Lack of direct correlation between CD4+ lymphocyte count and induration sizes of the tuberculin skin test in human immunodeficiency virus type I seropositive patients. Int J Tuberc Lung Dis. 1998; 2: 317-323.
6. Centres for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition of AIDS among adolescents and adults. MMWR. 1992; 41:1-19.
7. Terhorst C, Van Agthovern A, Reinher Z E and Sclossman S. Biochemical analysis of human T-lymphocyte Differentiation Antigens T4 and T5 Science. 1980; 209:520.
8. Snow PM and Terhorst C. The T8 antigen is a multimeric complex of two distinct subunits as human thymogens but consists of homomultimetric forms on peripheral blood T lymphocyte. J Bio Chem. 1983; 258: 146-175
9. Schechter M, Zaidenverg R, Machadol L, *et al* Predicting CD4 cell counts in HIV infected Brazilian individuals. A model based on the WHO staging system. J Acquired immune deficiency syndr 1994; 2: 163-168.
10. WHO. Acquired immune deficiency syndrome (AIDS). Interim proposal for a WHO staging sys-

tem for HIV infection and disease. Wkly Epidemiol *Rec.* 1990; 65: 221-228.

Lifson AR, Allison S, William Wolf BS and Serufilira A. Classification of HIV infection and disease in women from Rwanda. Ann Int Med 1995; S122: 262-270.

Bain BJ. Basic Haematological Techniques. In: Dacie JV, Lewis SM (eds). Practical Haematology. 8th ed. ELBS. Church Livingstone, London. 1995; 49-82.

Erice A. Comparism of ultra sensitive assays for quantification of HIV RNA in plasma samples. VI Conference on retroviruses and opportunistic infections. Chicago. Abstract 1999; 149.

Olugbile AO and Oyemade A. Health and the environment-a comparative study of agricultural and industrial workers in Nigeria. Afr J Med Med Sci. 1982; 10(3-4): 107-112.

Akanmu AS, Akinsete A, Eshefonie AO, *et al.* Absolute lymphocyte count as surrogate for CD4+ cell count in monitoring response to antiretroviral therapy. Nig. Postgrad. Med. J. 2002; 8 (3): 105-111.

16. Guarnrer J, Sanchez-Mejorada-Fernandez G del Rio-Chiriboga C and Mohar A. Simplified CD4+ T-lymphocyte counts in patients with HIV/AIDS in Mexico. Salud Publica Mex. 1996; 38: 207-211.

17. Freeny C, Bryzman S, Kong L, *et al.* T-lymphocyte subsets in acute illness. Critical Care Med. 1995; 23: 1680-1685.

18. Ledru E, Diagbouga S, Meda N, *et al.* A proposal of basic management of HIV disease in West Africa: use of clinical staging and haemogram data. Int. J STD AIDS. 1998; 9 (8): 463-470.

19. Martins DJ and Sim JG. CD4+ lymphocyte counts in African patients co-infected with HIV and tuberculosis. J Acquir Immune Defic Syndr. 1995; 8: 386-391.

Received: 17/12/04
Accepted: 07/12/05