



ABSOLUTE LYMPHOCYTE COUNT AS A COMPLEMENTARY MARKER OF IMMUNOLOGICAL STATUS IN PEOPLE LIVING WITH HIV/AIDS

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ABSTRACT

Monitoring HIV/AIDS disease progression primarily relies on CD4 cell count and viral load testing. Limited access to these assessments in resource-constrained settings highlights the need for simple and accessible biomarkers. Absolute lymphocyte count (ALC) is routinely measured and may provide additional information on immune status. To assess the association between ALC and CD4 cell count and evaluate its ability to identify advanced immunosuppression among people living with HIV/AIDS. A cross-sectional analytical observational study was conducted among people living with HIV/AIDS receiving care at Dr. M. Djamil Hospital, Padang, Indonesia, from May to September 2026. A total of 196 participants were recruited consecutively. ALC was measured using an automated haematology analyser and CD4 count by flow cytometry. Correlation, multiple linear regression, and receiver operating characteristic (ROC) analyses were performed. Of 196 participants, 20.4% had CD4 counts <200 cells/ μ L. Mean CD4 and ALC were 316.95 and 1,954.38 cells/ μ L, respectively. ALC was positively correlated with CD4 count ($r = 0.567$; $p < 0.001$) and remained significantly associated after adjustment ($B = 0.051$; $\beta = 0.315$; $p = 0.008$). ROC analysis showed good discrimination ($AUC = 0.925$; 95% CI, 0.845–1.00; $p = 0.001$). The optimal cut-off was 2,072.9 cells/ μ L, with 54.8% sensitivity and 95.2% specificity. ALC may serve as a complementary indicator of immunological status, particularly where CD4 testing is limited.

Keywords: absolute lymphocyte count; biomarker; cd4 count; disease progression; HIV/AIDS

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INTRODUCTION

Human Immunodeficiency Virus (HIV) infection remains a major global health challenge because it causes progressive immune dysfunction and chronic inflammation despite the increasing availability and coverage of antiretroviral therapy (ART) worldwide (Handayani & Susanti, 2024) (Heriana et al., 2018) (Global & Update, 2023). The progression of HIV disease is routinely monitored using CD4 cell count and viral load measurements (WHO, 2024b) (UNESCO, 2025). However, these examinations require relatively sophisticated laboratory facilities, trained personnel, and considerable financial resources, and their availability remains uneven, particularly in primary healthcare facilities and resource-constrained settings (Gao et al., 2022) (Daud et al., 2025) (Ernest et al., 2025). These limitations highlight the need to explore alternative biomarkers that are simple, affordable, and readily accessible to support the monitoring of HIV/AIDS disease progression (Huseynov et al., 2023) (James, 2025). Absolute Lymphocyte Count (ALC) is a routinely available hematological parameter that reflects the patient's immune status. Several recent studies have reported a significant association between ALC and the degree of immunosuppression among people living with HIV (Mabhida et al., 2024) (Waritu et al., 2024) (Chen et al., 2023). As an indicator of immune status, ALC may provide a simple and accessible measure for assessing changes in immune function and the clinical course of HIV/AIDS (Xiao et al., 2022) (Ramana, 2023). Nevertheless, the evidence regarding the effectiveness of ALC as an alternative marker of

HIV/AIDS disease progression remains inconsistent and has not yet been sufficiently integrated into clinical practice, particularly in resource-limited settings (AGRAWAL et al., 2023).

The rationale for conducting this study is based on the limited accessibility of CD4 cell count and viral load testing, which remain the standard laboratory parameters for monitoring HIV infection, particularly in primary healthcare facilities and resource-constrained areas (Piran et al., 2025) (AGRAWAL et al., 2023) (Abugah & Yabelang, 2025) (WHO, 2024a). Therefore, further investigation of routinely available and inexpensive hematological parameters such as ALC is warranted. The findings are expected to provide scientific evidence supporting the potential use of ALC as a simple, accessible, and cost-effective complementary biomarker for HIV/AIDS monitoring, particularly in resource-constrained healthcare settings. This study aimed to analyze the association between ALC and immune status among people living with HIV/AIDS and to evaluate the ability of ALC to identify patients with advanced immunosuppression, particularly those with a CD4 cell count below 200 cells/ μ L.

METHOD

Study Design and Setting

This study employed an analytical observational cross-sectional design to investigate the association between ALC and CD4 cell count among people living with HIV (PLHIV). A cross-sectional design was chosen because ALC and CD4 cell count were assessed during the same observation period, allowing the study to evaluate their relationship and the discriminative ability of ALC to identify patients with advanced immunosuppression. The study was conducted at Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia. Data collection was carried out from May to September 2026. Participants were recruited from the hospital's HIV care and treatment services during the study period.

Study Population and Participant Selection

The study population comprised PLHIV receiving routine HIV care at Dr. M. Djamil Hospital during the study period. A total of 196 participants were included in the analysis. Participants were recruited using consecutive sampling, whereby eligible patients who met the predefined inclusion and exclusion criteria were enrolled sequentially until the required sample size was reached. The inclusion criteria were: (a) confirmed HIV infection documented in the medical record in accordance with applicable national diagnostic criteria; (b) receiving HIV care and treatment at the study site; (c) availability of CD4 cell count data; (d) willingness to undergo venous blood sampling for ALC and CD4 cell count assessment; and (e) clinically stable condition at the time of blood collection. The exclusion criteria were: (a) fever ($>38^{\circ}\text{C}$) at the time of sample collection; (b) current chemotherapy or radiotherapy; (c) use of immunosuppressive medications; (d) a history of hematological malignancies, including leukemia or lymphoma; and (e) pregnancy.

Variables and Operational Definitions

The primary outcome was immune status, operationalized as a CD4 cell count of <200 cells/ μ L, representing advanced immunosuppression. CD4 cell count was also analyzed as a continuous variable in the correlation and linear regression analyses. The primary predictor of interest was ALC, defined as the absolute number of circulating lymphocytes and expressed in cells/ μ L. ALC was analyzed as a continuous variable and, where applicable, categorized according to the optimal cut-off value derived from receiver operating characteristic (ROC) curve analysis. Potential confounding variables included age, sex, antiretroviral therapy (ART) adherence, duration of ART, time since HIV diagnosis, and history of opportunistic infections. These variables were obtained from medical records and considered in the multivariable analysis based on their clinical relevance and availability in the study dataset.

Data Sources and Laboratory Measurements

Demographic and clinical data were obtained from the hospital's clinical care system and medical records. The variables collected included age, sex, ART status and adherence, duration of ART, time since HIV diagnosis, viral load, and history of opportunistic infections. ART status was determined based on the documented treatment status at the time of laboratory assessment. ART adherence was determined from adherence information recorded in the clinical care system. Duration of ART was calculated from the documented date of ART initiation to the date of data collection. Time since HIV diagnosis was calculated from the date of confirmed HIV diagnosis to the date of data collection. For laboratory assessment, venous blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. CD4 cell counts were measured using flow cytometry with anti-CD3 and anti-CD4 monoclonal antibodies and expressed as cells/ μ L. ALC was obtained from the complete blood count performed using an automated hematology analyzer and expressed as cells/ μ L.

Ethical Approval

This study was approved by the Health Research Ethics Committee of Dr. M. Djamil Hospital, Padang, Indonesia (Approval No. DP.04.03/D.XVI.10.1/73/2026). All procedures involving human participants were conducted in accordance with applicable ethical standards and regulations. Written informed consent was obtained from all participants before data collection and venous blood sampling, as applicable.

Potential Sources of Bias

Several measures were implemented to minimize potential sources of bias. Participant selection was based on predefined inclusion and exclusion criteria, and participants were recruited consecutively. Laboratory measurements of ALC and CD4 cell count were performed using standardized laboratory procedures. Clinical and laboratory data were obtained from the hospital's clinical and laboratory information systems using predefined variables. Potential confounding factors, including age, ART adherence, duration of ART, time since HIV diagnosis, and history of opportunistic infections, were considered in the multivariable analysis.

Sample Size

The required sample size was determined using the Lemeshow formula. Based on the assumptions used in the sample size calculation, the minimum required sample size was 196 participants. Accordingly, 196 eligible PLHIV were included in the study.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for their distributional characteristics. Normally distributed variables were summarized as means and standard deviations (SDs), whereas non-normally distributed variables were summarized as medians and interquartile ranges (IQRs). Categorical variables were presented as frequencies and percentages. The association between ALC and CD4 cell count was assessed using Pearson's or Spearman's correlation coefficient, as appropriate based on the distribution of the data. Multiple linear regression analysis was performed to evaluate the independent association between ALC and CD4 cell count after adjustment for relevant demographic and clinical variables. Regression coefficients (B), standardized coefficients (β), standard errors, t statistics, p-values, tolerance values, and variance inflation factors (VIFs) were reported. Multicollinearity was assessed based on tolerance and VIF values.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminative ability of ALC for identifying participants with a CD4 cell count of <200 cells/ μ L. The area under the curve (AUC) with its 95% confidence interval (CI), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden index were

calculated. The optimal ALC cut-off value was determined based on the maximum Youden index. A two-sided p-value of <0.05 was considered statistically significant. Before statistical analysis, the dataset underwent data cleaning and editing to identify and resolve inconsistencies and ensure data completeness. No missing data were identified in the variables included in the final analysis. No subgroup analyses or interaction analyses were performed.

RESULT

General Characteristics of the Patients

The study included 196 people living with HIV (PLHIV). The median age of the participants was 37 years (range, 11–83 years). Overall, 71.4% participants were classified as adherent to antiretroviral therapy (ART). Most participants 66.8% had been receiving ART for less than one year. All data were verified for completeness and validity before statistical analysis. The baseline characteristics of the participants are presented in table 1.

Table 1.

Baseline Characteristics of the Participants			
Variable	Overall, n = 63	CD4 <200 cells/ μ L	CD4 ≥ 200 cells/ μ L
Age, years	37 (11–83)	38.08 (19–52)	38.94 (11–83)
Sex (%)			
Male	74	92.3	70
Female	26	7.7	30
ART adherence, n (%)			
Adherent	71.4	61.5	74
Non-adherent	28.6	38.5	26
Duration of ART, n (%)			
<1 year	66.8	76.9	64
1–5 years	28.4	23.1	30
>5 years	4.8	0.1	6
Duration since HIV diagnosis, n (%)			
<1 year	44.5	46.2	44.0
1–5 years	46.0	46.2	46.0
>5 years	9.5	7.7	10.0
CD4 cell count, cells/ μ L	316.95 (33–823)	121.31 (33–198)	367.82 (217–823)
ALC, cells/ μ L	1,954.38	2,255.8 (915.2–4,110)	—

Note: Data are presented as n (%) or mean $\pm$ standard deviation (SD), median (range), as appropriate. ALC = absolute lymphocyte count; ART = antiretroviral therapy; CD4 = cluster of differentiation 4.

Correlation Between ALC and CD4 Cell Count

A statistically significant positive correlation was observed between ALC and CD4 cell count ($r = 0.567$, $p < 0.001$) (Table 2). The magnitude of the correlation indicated a moderate positive association. This finding suggests that higher ALC values were associated with higher CD4 cell counts. Conversely, lower ALC values tended to occur among participants with lower CD4 cell counts.

Table 2.

Correlation Between ALC and CD4 Cell Count			
Variable	CD4 cell count (cells/ μ L)	Correlation coefficient (r/p)	p-value
ALC	1	0.567	<0.001

Note: ALC = absolute lymphocyte count; CD4 = cluster of differentiation 4. The correlation coefficient (r) was calculated using the Pearson or Spearman correlation test, as appropriate.

ROC Analysis of ALC for Identifying CD4 <200 Cells/ μ L

Receiver operating characteristic (ROC) analysis was performed to evaluate the ability of ALC to discriminate participants with a CD4 cell count <200 cells/ μ L. The area under the ROC curve (AUC) was 0.925 (95% CI, 0.845–1.00; $p = 0.001$), indicating a statistically significant discriminatory ability of ALC for identifying participants with advanced immunosuppression. The optimal ALC cut-off identified by the ROC analysis was 2,072.9 cells/ μ L, with a sensitivity of 54.8% and specificity of 95.2%. (Table 3).

Table 3.
ROC Analysis of ALC for Identifying CD4 <200 Cells/ μ L

Parameter	P-Value (CI 95%)
AUC	0.925
95% CI	0.845–1.00
p-value	0.001
Optimal ALC cut-off, cells/ μ L	2,072.9
Sensitivity	54.8%
Specificity	95.2%
PPV	4.2%
NPV	48.7%
Youden index	0.500

Note: ALC = absolute lymphocyte count; AUC = area under the curve; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value. The optimal ALC cut-off was determined using the Youden index.

Multiple Linear Regression Analysis

Multiple linear regression analysis was performed to examine the associations of demographic and clinical variables with CD4 cell count (Table 4). The assessment of multicollinearity indicated that there was no substantial multicollinearity among the independent variables. Tolerance values ranged from 0.315 to 0.979, while variance inflation factor (VIF) values ranged from 1.022 to 3.174, all below the commonly used threshold of 10. In the multivariable model, age was positively associated with CD4 cell count ($B = 2.167$, $\beta = 0.194$, $t = 2.124$, $p = 0.038$). This indicates that, after adjustment for the other variables in the model, each one-year increase in age was associated with an estimated increase of 2.167 cells/ μ L in CD4 cell count. ALC was also positively and significantly associated with CD4 cell count ($B = 0.051$, $\beta = 0.315$, $t = 2.776$, $p = 0.008$). This finding indicates that higher ALC values were associated with higher CD4 cell counts after adjustment for the other variables included in the model. ART adherence showed a positive association with CD4 cell count ($B = 104.513$, $\beta = 0.310$, $t = 1.928$, $p = 0.049$). Duration since HIV diagnosis ($p = 0.530$) and duration of ART ($p = 0.426$) were not statistically significantly associated with CD4 cell count.

Table 4.
Multiple Linear Regression Analysis of Factors Associated with CD4 Cell Count

Variable	B	SE	β	t	p-value	Tolerance	VIF
ALC	0.051	0.018	0.315	2.776	0.008	0.635	1.575
Age	2.167	1.020	0.194	2.124	0.038	0.979	1.022
Duration since HIV diagnosis	-24.178	38.290	-0.072	-0.631	0.530	0.624	1.603
Duration of ART	-21.959	27.358	-0.077	-0.803	0.426	0.895	1.118
ART adherence	104.513	54.218	0.310	1.928	0.049	0.315	3.174
Opportunistic infection	37.770	49.343	0.113	0.765	0.047*	0.376	2.662

Note: B = unstandardized regression coefficient; SE = standard error; β = standardized regression coefficient; ALC = absolute lymphocyte count; ART = antiretroviral therapy; VIF = variance inflation factor. VIF values were used to assess multicollinearity among the independent variables.

DISCUSSION

Monitoring HIV disease status generally relies on CD4 cell count and viral load measurements. However, limited access to these examinations, particularly in resource-constrained healthcare settings, highlights the need for alternative indicators that are simpler, more affordable, and widely available. In the present study, absolute lymphocyte count (ALC) was evaluated as a potential indicator of immunological status among people living with HIV/AIDS through correlation, regression, and receiver operating characteristic (ROC) analyses. The findings showed that participants with lower ALC values tended to have lower CD4 cell counts. Correlation analysis demonstrated a significant positive association between ALC and CD4 cell count ($r = 0.567$; $p < 0.001$). This finding indicates that an increase in ALC tends to be accompanied by an increase in CD4 cell count. Biologically, this association may be explained by the fact that ALC represents the total number of circulating peripheral lymphocytes, which include CD4⁺ T lymphocytes as an important subpopulation. HIV infection causes progressive depletion of CD4⁺ T lymphocytes;

therefore, changes in the overall lymphocyte count may be associated with changes in immunological status.

Comparison with Previous Studies

The positive association between ALC and CD4 cell count observed in the present study is consistent with several previous studies. A study entitled *Immuno-Hematological Profiles of HIV-Positive Patients* reported a positive correlation between ALC and CD4 cell count ($r = 0.505$; $p < 0.001$) and showed that patients with severe immunosuppression had lower ALC values. The authors suggested that ALC could serve as an additional indicator of immunological status among people living with HIV, particularly in healthcare settings where access to CD4 testing is limited (Arbaeen et al., 2025).

These findings are also consistent with a 2024 study that reported an association between ALC and CD4 cell count among patients with HIV receiving antiretroviral therapy. However, that study also emphasized that the use of ALC as a substitute for CD4 testing requires further validation (Shashikumar S D, Bhargavi S K, Karthik N, Yashica Gowda R, Sai Sailesh Kumar Goothy & K, 2024). The positive association between absolute lymphocyte count (ALC) and CD4 cell count observed in the present study is consistent with several previous studies demonstrating that ALC may reflect immunological status in people living with HIV. Kakar et al. reported a strong positive correlation between ALC and CD4 cell count ($r = 0.714$) among 182 samples from HIV-infected patients, with an ALC threshold of $\leq 1,200$ cells/ μL showing a sensitivity of 64.4% and specificity of 91.1% for identifying patients with CD4 counts ≤ 200 cells/ μL (Kakar et al., 2011). Similar findings were reported by Sreenivasan and Dasegowda in 108 antiretroviral therapy-naïve patients with HIV, who found a positive correlation between ALC and CD4 count ($r = 0.5604$); an ALC threshold of 1,400 cells/ μL demonstrated a sensitivity of 71.08% and specificity of 78.26% for identifying patients with CD4 counts < 200 cells/ μL (Sreenivasan & Dasegowda, 2011). In a longitudinal study of 108 HIV-infected patients receiving highly active antiretroviral therapy (HAART), ALC was also positively correlated with CD4 count ($r = 0.553$), and an increase in ALC after six months of therapy showed reasonable ability to identify increases in CD4 count (Srirangaraj & Venkatesha, 2012).

Agrawal et al., in a prospective study involving 61 patients with HIV/AIDS, found a significant correlation between ALC and CD4 count ($r = 0.327$; $p < 0.05$), although the ability of ALC to identify CD4 counts < 200 cells/ μL varied according to the threshold applied (AGRAWAL et al., 2023). Another study among HIV-infected patients receiving antiretroviral therapy also demonstrated a positive correlation between ALC and CD4 count, although the correlation was relatively weak ($r = 0.25$); an ALC threshold of 2,000 cells/ μL had a sensitivity of 57% and specificity of 72% for predicting CD4 counts < 200 cells/ μL , highlighting that ALC cannot completely replace CD4 testing (Srirangaraj & Venkatesha, 2012). In Nigeria, a study of 100 antiretroviral therapy-naïve HIV-infected individuals reported a moderate correlation between ALC and CD4 count ($r = 0.51$), including among patients with CD4 counts < 200 cells/ μL , and suggested that ALC may provide a simple alternative in resource-limited settings (Akanmu et al., 2001). A retrospective study from Ethiopia also demonstrated a positive relationship between lymphocyte count and CD4 count and evaluated lymphocyte thresholds as a potentially useful approach when CD4 testing is unavailable (Erhabor et al., 2006). In addition, a large study of HIV-infected patients with virological suppression and immune reconstitution reported a moderate correlation between ALC and CD4 count ($r = 0.589$; $p < 0.0001$). Although most estimated CD4 values were within 25% of the actual values, the study emphasized that the ability of ALC to accurately estimate individual CD4 counts remained limited (Young et al., 2015).

Ability of ALC to Identify Advanced Immunosuppression

ROC analysis in the present study indicated that ALC had good discriminatory ability for distinguishing participants with CD4 counts <200 cells/ μL from those with CD4 counts ≥ 200 cells/ μL . Nevertheless, the ROC findings should be interpreted cautiously, particularly because the sensitivity at the selected threshold was relatively limited. Limited sensitivity indicates that some patients with advanced immunosuppression may remain unidentified if ALC is used as the sole indicator. Conversely, the relatively high specificity suggests that a particular ALC threshold may help identify individuals who are less likely to have severe immunosuppression. These findings are consistent with those of Sreenivasan et al., who reported a sensitivity of 34.48% and specificity of 67.50% when using an ALC threshold of $\leq 1,200$ cells/ μL . Differences in sensitivity, specificity, and cut-off values across studies may be attributable to differences in population characteristics, the prevalence of immunosuppression, antiretroviral therapy use, and methods used to determine the optimal threshold. Therefore, ALC may be more appropriately considered an initial or complementary indicator rather than a replacement for CD4 testing. A high ALC value cannot be used directly to rule out immunosuppression, particularly because its sensitivity is not perfect. CD4 testing remains necessary when available, particularly for assessing immunological status and supporting clinical decisions that require more specific immunological information.

Interpretation of the Association Between ALC and CD4

The multiple linear regression analysis further supported the positive association between ALC and CD4 cell count after adjustment for several covariates. In the present model, ALC showed a positive regression coefficient and a statistically significant association. This finding indicates that ALC remained associated with CD4 cell count after accounting for age, time since HIV diagnosis, duration of antiretroviral therapy, adherence to antiretroviral therapy, and opportunistic infections. Biologically, this association may be explained by the composition of peripheral lymphocytes. ALC reflects the total number of circulating lymphocytes, whereas CD4⁺ T lymphocytes constitute an important component of this lymphocyte population. HIV infection results in CD4⁺ T-cell depletion through several mechanisms, including infection and destruction of infected cells and disruption of immune-system homeostasis. Consequently, ALC may tend to change in the same direction as CD4 cell count. However, this relationship is not absolute because ALC is also influenced by various other factors. Several factors may affect lymphocyte counts, including opportunistic infections, inflammatory conditions, nutritional status, age, comorbidities, medication use, and other clinical conditions. Therefore, an increase or decrease in ALC cannot be assumed to directly reflect a corresponding change in CD4 cell count in every individual. Interpretation of ALC should consider the patient's clinical condition and other laboratory findings.

CONCLUSION

This study demonstrates that absolute lymphocyte count (ALC) exhibits a significant positive correlation with CD4 cell count ($r = 0.567$; $p < 0.001$) and strong diagnostic performance ($\text{AUC} = 0.925$) for identifying advanced immunosuppression ($\text{CD4} < 200$ cells/ μL) among people living with HIV/AIDS, with an optimal cut-off value of 2,072.9 cells/ μL (specificity 95.2%). Consequently, routine and cost-effective ALC measurement can serve as a reliable complementary indicator for monitoring immunological status, particularly in resource-constrained healthcare settings with limited access to standard CD4 testing.

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