

Original article

Evaluating Total Lymphocyte Count as a Surrogate Marker for Immunological Monitoring in HIV Patients Receiving Antiretroviral Therapy: Evidence from a Libyan Cohort

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Abstract

Total lymphocyte count (TLC) is a simple and inexpensive laboratory parameter that may provide supportive information about immune status in people living with HIV, particularly in settings where access to CD4 testing is limited. This study evaluated the relationship between TLC and CD4 count and assessed the diagnostic performance of TLC for identifying immune suppression among HIV-infected patients receiving routine HIV care at Tripoli University Hospital, Libya. TLC was obtained from complete blood count analysis, while CD4 count was determined by flow cytometry. Pearson and Spearman correlation analyses were used to assess the relationship between TLC and CD4 count. Diagnostic performance was evaluated using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), receiver operating characteristic (ROC) analysis, and the Youden Index. Multivariate logistic regression was used to evaluate the association between lymphocyte count and immune suppression after accounting for age and sex. TLC showed a statistically significant moderate positive correlation with CD4 count (Pearson $r=0.52$ and Spearman $\rho=0.49$; both $p<0.001$). For identifying CD4 counts <200 cells/ μL , TLC demonstrated 62% sensitivity, 80% specificity, a PPV of 48%, and an NPV of 88%. At the CD4 <350 cells/ μL threshold, sensitivity was 66%, specificity was 74%, PPV was 71%, and NPV was 69%. ROC analysis showed an area under the curve of 0.87. In multivariate analysis, lymphocyte count remained significantly associated with immune suppression after accounting for age and sex, with adjusted odds ratios of 0.11 (95% CI: 0.04–0.31) for CD4 <200 cells/ μL and 0.20 (95% CI: 0.10–0.40) for CD4 <350 cells/ μL (both $p<0.001$). TLC was moderately correlated with CD4 count and remained independently associated with immune suppression. Although TLC may provide useful supportive information when access to CD4 testing is limited, its diagnostic performance is insufficient to replace direct CD4 measurement. TLC may therefore be more appropriate for preliminary immune assessment or patient triage in resource-limited settings.

Keywords. HIV, Total Lymphocyte Count, CD4 Count, Immune Suppression, Flow Cytometry.

Introduction

Human immunodeficiency virus (HIV) remains one of the leading global infectious diseases despite remarkable advances in antiretroviral therapy (ART). The widespread use of ART has substantially reduced HIV-related morbidity and mortality, transforming HIV infection into a manageable chronic condition for many patients. Nevertheless, continuous assessment of immune status remains essential for evaluating treatment response, monitoring disease progression, and identifying individuals at increased risk of opportunistic infections and HIV-related complications [1–3]. CD4⁺ T-cell measurement by flow cytometry remains the reference standard for immunological monitoring in HIV infection because it provides an accurate assessment of immune function [4]. More recently, additional immunological markers, including CD8⁺ T-cell counts and the CD4/CD8 ratio, have gained increasing attention owing to their value in evaluating immune recovery and predicting long-term clinical outcomes among patients receiving ART [5].

Despite its clinical value, flow cytometry is not universally accessible. The technique requires specialized instrumentation, trained personnel, regular quality assurance, and relatively high operational costs, which limit its routine implementation in many low- and middle-income countries. Consequently, there has been sustained interest in identifying simple, inexpensive, and widely available laboratory markers that could provide supportive information about immune status when flow cytometry is unavailable. Total lymphocyte count (TLC), derived directly from a routine complete blood count (CBC), has been proposed as one such marker because it is inexpensive, readily available, and can be measured using automated hematology analyzers commonly found in clinical laboratories. These practical advantages have made TLC an attractive candidate for HIV monitoring, particularly in resource-limited settings where access to CD4 testing remains constrained [3,6,7,8]. Several studies have investigated the relationship between total lymphocyte count (TLC) and CD4⁺ T-cell count as a potential alternative for monitoring HIV-infected patients. Most studies have reported a statistically significant positive correlation between TLC and CD4 count, suggesting that TLC may provide supportive information regarding immune status. However, the strength of this relationship has varied considerably across different populations and healthcare settings. Moderate correlations have been reported in studies conducted in Ghana and China, whereas weaker associations have been observed in Ethiopian and Nigerian

cohorts. These inconsistencies indicate that the predictive value of TLC is influenced by demographic characteristics, disease stage, coexisting infections, and differences in laboratory methods.

Consequently, although TLC may be useful as a supportive laboratory marker, current evidence does not support its use as a universal substitute for CD4 measurement. [9, 10]. Although immunological studies in Libyan HIV populations have reported CD4 profiles using flow cytometry, evidence specifically evaluating TLC as a surrogate or supportive marker for CD4 count remains limited [4]. Previous studies from other populations have demonstrated an association between TLC and CD4 count, but the strength and clinical usefulness of this relationship have varied across different populations and healthcare settings [5, 11]. These variations highlight the importance of local evaluation before TLC can be considered a useful supportive marker for HIV monitoring in routine clinical practice. The present study aimed to evaluate the usefulness of total lymphocyte count (TLC) as a supportive marker of immune status in HIV-positive patients receiving antiretroviral therapy in Libya. Specifically, the study examined the relationship between TLC and CD4 count and evaluated the diagnostic performance of TLC for identifying immune suppression at clinically relevant CD4 thresholds. The findings are expected to provide local evidence regarding the potential role of TLC in supporting HIV monitoring in resource-limited healthcare settings.

Methods

Study Design and Setting

This cross-sectional study was conducted to evaluate the relationship between total lymphocyte count (TLC) and CD4 count in HIV-positive patients receiving antiretroviral therapy (ART). The study was carried out at the HIV Department of Tripoli University Hospital, Tripoli, Libya, where HIV-positive patients were recruited, and clinical blood samples were collected for laboratory analysis. CD4 count was determined by flow cytometry, while haematological parameters were obtained from routine complete blood count (CBC) testing. The study further evaluated the diagnostic performance of TLC as a potential supportive marker for assessing immune status by comparing TLC with CD4 count measured by flow cytometry.

Study Population

A total of 150 blood samples were included in the study and analysed for total lymphocyte count (TLC) and CD4 cell count. Participants were recruited from the HIV Department at Tripoli University Hospital, Tripoli, Libya. Demographic characteristics, including age and sex, were recorded for all participants. The study population included 76 males and 74 females, with ages ranging from 15 to 76 years. Blood samples were collected for hematological and immunological analyses, including total lymphocyte count (TLC) and CD4 cell count, which were subsequently used for descriptive, correlation, and diagnostic performance analyses.

Blood Sample Collection

Venous blood samples were collected from all participants using standard aseptic techniques. Approximately 3 mL of whole blood was drawn into K₂-EDTA anticoagulant tubes for hematological and immunological analyses. Following collection, each sample was gently inverted several times to ensure proper mixing with the anticoagulant and transported to the laboratory under appropriate conditions for analysis. Complete blood count (CBC) testing and flow cytometric immunophenotyping were subsequently performed according to standard laboratory procedures. Samples that did not meet laboratory quality requirements were excluded from analysis.

Complete Blood Count Analysis

Complete blood count (CBC) analysis was performed using the Sysmex XQ-320 haematology analyser (Sysmex Corporation, Kobe, Japan). Whole blood samples collected in K₂-EDTA tubes were analysed according to the manufacturer's instructions following routine laboratory quality control procedures. Haematological parameters, including total white blood cell count, differential leukocyte count, and total lymphocyte count (TLC), were obtained directly from the analyser. TLC values were subsequently used for correlation and diagnostic performance analyses in relation to CD4 count measured by flow cytometry.

Flow Cytometry Analysis

Immunophenotypic analysis was performed using the Beckman Coulter DxFLEx flow cytometer (Beckman Coulter Inc., Brea, CA, USA). Whole blood samples collected in K₂-EDTA tubes were processed according to the manufacturer's recommended protocol for lymphocyte immunophenotyping. A fluorochrome-conjugated monoclonal antibody specific for CD4 was used for identification of CD4⁺ T lymphocytes, and absolute CD4 counts were determined by flow cytometry. Lymphocyte populations were identified using established flow-cytometric gating procedures based on light-scatter characteristics and immunophenotypic markers, consistent with previously described approaches for lymphocyte analysis [12, 13]. The resulting CD4 counts were used as the reference measurements for evaluating the relationship and diagnostic performance of total lymphocyte count.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics. Continuous variables are presented as mean $\pm$ standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables are presented as frequencies and percentages. The relationship between total lymphocyte count (TLC) and CD4 count was evaluated using Pearson and Spearman correlation analyses. The diagnostic performance of TLC for identifying immune suppression at CD4 thresholds of < 200 and < 350 cells/ μL was evaluated using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), receiver operating characteristic (ROC) analysis, and the Youden Index. A two-tailed p -value of less than 0.05 was considered statistically significant.

Ethical Approval

The study was approved by the Scientific Research and Ethics Committee of the University of Tripoli (SREC-UOT) under Approval Letter No. SREC/010/182. Permission to conduct the study was also obtained from the participating institutions. All procedures were performed in accordance with institutional ethical guidelines, and written informed consent was obtained from all participants before sample collection.

Results

Characteristics of the Study Population

A total of 150 HIV-infected patients were included in the study. The cohort comprised 76 (50.7%) males and 74 (49.3%) females, demonstrating a nearly equal sex distribution (Table 1; Figure 1). The age of the participants ranged from 15 to 76 years, with a mean age of 48.6 ± 12.8 years and a median age of 50 years (interquartile range [IQR]: 38–57 years). The largest proportion of patients (56.7%) belonged to the 40–59 years age group, followed by 21.3% aged 20–39 years and 16.7% aged 60 years or older. Only 5.3% of participants were younger than 20 years. Overall, the study population represented a heterogeneous adult HIV cohort with balanced sex distribution and a predominance of middle-aged individuals receiving routine HIV care.

Descriptive Statistics of Immunological Parameters

Table 2 summarizes the descriptive statistics of the study variables. The mean CD4 count was 594.5 ± 283.4 cells/ mm^3 , with a median of 611 cells/ mm^3 and a range of 105–1310 cells/ mm^3 , indicating considerable variability in immune status among the participants. The mean total lymphocyte count was $2.23 \pm 0.69 \times 10^9/\text{L}$, with a median of $2.15 \times 10^9/\text{L}$ and values ranging from 0.9 to $3.8 \times 10^9/\text{L}$. These findings demonstrate substantial heterogeneity in both immunological and haematological parameters within the study population.

Table 1. Demographic Characteristics of the Study Population (N = 150)

Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	76	50.7
	Female	74	49.3
Age (years)	Mean $\pm$ SD	48.6 ± 12.8	–
	Median (IQR)	50 (38–57)	–
	Range	15–76	–
Age Group	<20 years	8	5.3
	20–39 years	32	21.3
	40–59 years	85	56.7
	≥ 60 years	25	16.7

Correlation Between CD4 Count and Total Lymphocyte Count

The relationship between CD4 count and total lymphocyte count (TLC) was evaluated using both Pearson and Spearman correlation analyses. A statistically significant moderate positive correlation was observed between the two variables. Pearson correlation analysis yielded a correlation coefficient of $r = 0.52$ ($p < 0.001$), while Spearman rank correlation analysis demonstrated a similar association ($\rho = 0.49$, $p < 0.001$) (Table 3). The scatter plot (Figure 2) further illustrates the positive linear relationship between CD4 count and TLC. Although higher TLC values were generally associated with higher CD4 counts, considerable variability was observed across the study population, indicating that TLC alone cannot accurately predict individual CD4 counts.

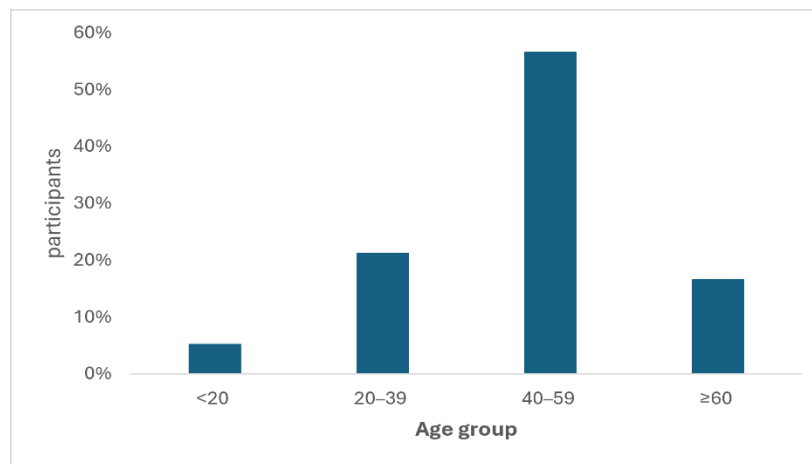


Figure 1. Age group distribution of the study population

Diagnostic Performance of Total Lymphocyte Count

The diagnostic performance of total lymphocyte count (TLC) for identifying immune suppression at CD4 thresholds of <200 and <350 cells/mm³ is presented in Table 4. For identifying patients

Table 2. Descriptive Statistics of Study Variables

Variable	Mean	Median	SD	Min	Max
CD4 (cells/mm ³)	594.5	611	283.4	105	1310
Lymphocytes (×10 ⁹ /L)	2.23	2.15	0.69	0.9	3.8

Table 3. Correlation Between CD4 Count and Total Lymphocyte Count

Variable Pair	Correlation Test	Coefficient	p-value	Strength
CD4 vs. Lymphocytes	Pearson (r)	0.52	<0.001	Moderate positive
CD4 vs. Lymphocytes	Spearman (ρ)	0.49	<0.001	Moderate positive

With CD4 counts < 200 cells/mm³, TLC demonstrated a sensitivity of 62% and a specificity of 80%, with a positive predictive value (PPV) of 48% and a negative predictive value (NPV) of 88%. At the CD4 threshold of <350 cells/mm³, sensitivity was 66%, and specificity was 74%, with corresponding PPV and NPV values of 71% and 69%, respectively. Overall, TLC exhibited moderate diagnostic performance for identifying immune suppression at these CD4 thresholds. Overall, TLC exhibited moderate diagnostic performance for identifying immune suppression. The relatively high negative predictive values suggest that TLC is more useful for excluding severe immunosuppression than for confirming it, although its overall diagnostic accuracy remains insufficient to replace direct CD4 measurement.

Table 4. Diagnostic Performance of Total Lymphocyte Count for Predicting CD4 Thresholds

Measure	Sensitivity	Specificity	PPV	NPV
CD4 <200 cells/mm ³	62%	80%	48%	88%
CD4 <350 cells/mm ³	66%	74%	71%	69%

Receiver Operating Characteristic (ROC) Analysis

Receiver operating characteristic (ROC) analysis was performed to evaluate the ab. Receiver operating characteristic (ROC) analysis demonstrated good discriminative performance of TLC, with an area under the curve (AUC) of 0.87. The optimal operating point identified using the Youden Index provided the best balance between sensitivity and specificity for identifying immune suppression. Nevertheless, TLC should be considered a supportive rather than a standalone marker for assessing immune status in HIV-infected patients.

Multivariate Logistic Regression Analysis

Multivariate logistic regression analysis was performed to evaluate factors associated with advanced and moderate/advanced HIV disease. For advanced HIV disease, defined as CD4 < 200 cells/μL, lymphocyte count was the only statistically significant predictor. Higher lymphocyte counts were associated with substantially lower odds of advanced disease, with an adjusted odds ratio (OR) of 0.11 (95% CI: 0.04–0.31, *p* < 0.001). In contrast, age and male sex were not significantly associated with advanced HIV disease (Table 5). A similar pattern was observed when moderate/advanced HIV disease was defined as CD4 < 350 cells/μL.

Lymphocyte count remained the only statistically significant predictor, with an adjusted OR of 0.20 (95% CI: 0.10–0.40, $p < 0.001$). Neither age nor male sex showed a statistically significant association with CD4 counts below 350 cells/ μ L (Table 6). These findings indicate that lymphocyte count was independently associated with the severity of immune suppression in this cohort.

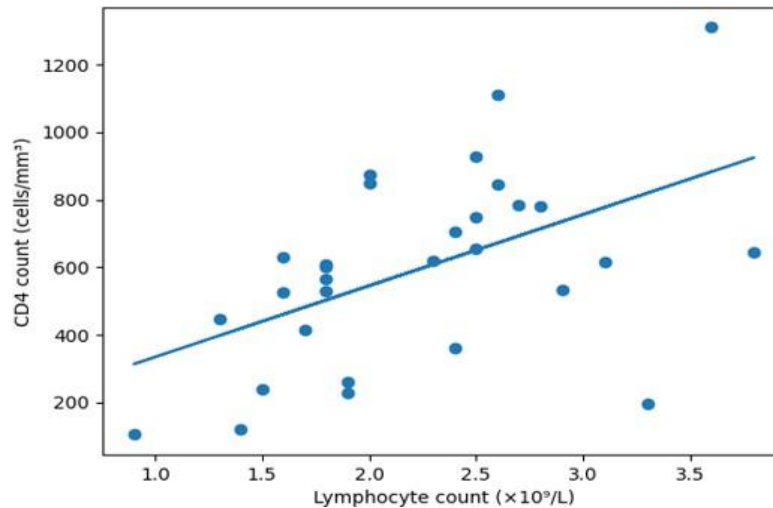


Figure 2. Scatter plot showing the relationship between CD4 count and total lymphocyte count.

Table 5. Multivariate Logistic Regression Analysis of Factors Associated with Advanced HIV Disease (CD4 < 200 cells/ μ L)

Variable	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Age (years)	1.01	0.96–1.05	0.809	1.01	0.96–1.05	0.809
Male sex	1.63	0.57–4.70	0.365	1.63	0.57–4.70	0.365
Lymphocytes	0.11	0.04–0.31	<0.001	0.11	0.04–0.31	<0.001

Table 6. Multivariate Logistic Regression Analysis of Factors Associated with Moderate/Advanced HIV Disease (CD4 < 350 cells/ μ L)

Variable	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Age (years)	1.01	0.98–1.04	0.549	1.01	0.98–1.04	0.549
Male sex	1.43	0.64–3.17	0.383	1.43	0.64–3.17	0.383
Lymphocytes	0.20	0.10–0.40	<0.001	0.20	0.10–0.40	<0.001

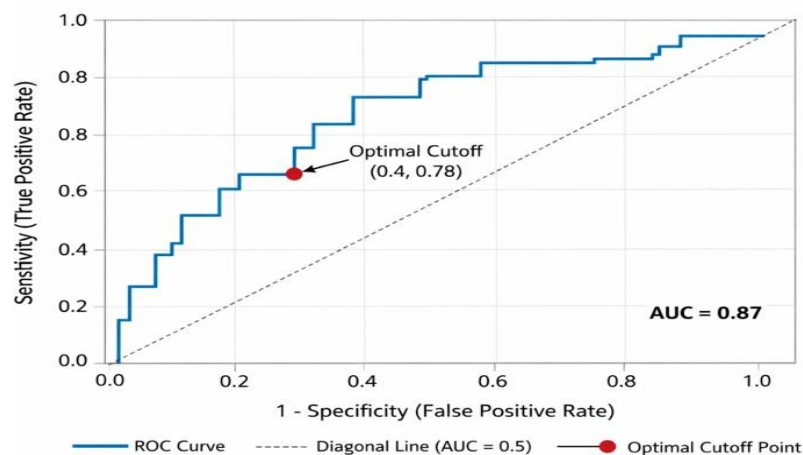


Figure 3. Receiver operating characteristic (ROC) curve showing the diagnostic performance of total lymphocyte count for identifying immune suppression.

Discussion

This study evaluated the usefulness of total lymphocyte count (TLC) as a supportive marker for immunological monitoring in HIV-infected patients receiving antiretroviral therapy in Libya. Overall, TLC demonstrated a statistically significant moderate positive correlation with CD4 count and showed fair diagnostic performance for identifying immune suppression. However, the observed variability in TLC across patients and the moderate discriminatory ability indicated that TLC cannot reliably replace CD4 measurement by flow cytometry. These findings suggest that although TLC may provide useful supportive information in resource-limited settings, it should not be considered a standalone marker for assessing immune status.

The moderate positive correlation observed between TLC and CD4 count in the present study is consistent with previous reports evaluating TLC as a surrogate marker for immune status in HIV-infected patients. Studies conducted in China, Ethiopia, and Ghana similarly reported positive associations between TLC and CD4 count, although the strength of the correlation varied across different populations and healthcare settings [11, 10, 14]. These variations may reflect differences in patient demographics, disease stage, antiretroviral treatment status, and laboratory methodologies. Despite these differences, the collective evidence indicates that TLC generally reflects changes in immune status but lacks the precision required to accurately estimate individual CD4 counts. Our findings therefore support previous studies suggesting that TLC may serve as a useful adjunctive marker in settings where flow cytometry is unavailable, but it should not be considered a direct substitute for CD4 measurement. The diagnostic evaluation further demonstrated that TLC has a moderate ability to identify immune suppression. Although the evaluated TLC thresholds provided reasonable specificity and negative predictive value, sensitivity remained insufficient for accurately identifying all patients with low CD4 counts.

Receiver operating characteristic (ROC) analysis further supported the diagnostic potential of TLC, with an area under the curve (AUC) of 0.87, indicating good overall discriminative performance. Despite this overall discrimination, the sensitivity and specificity observed at clinically relevant CD4 thresholds indicate that TLC should not be used as a standalone marker. These findings suggest that TLC may have value as a supportive screening tool in healthcare settings where flow cytometry is not readily available, but it should not be considered a replacement for direct CD4 testing. The multivariate analysis further strengthened the observed relationship between lymphocyte count and immune suppression. After accounting for age and sex, lymphocyte count remained significantly associated with both advanced HIV disease ($CD4 < 200$ cells/ μ L) and moderate/advanced disease ($CD4 < 350$ cells/ μ L), with adjusted odds ratios of 0.11 (95% CI: 0.04–0.31) and 0.20 (95% CI: 0.10–0.40), respectively ($p < 0.001$ for both). In contrast, age and sex were not independently associated with either CD4 threshold. These findings indicate that the association between lymphocyte count and immune suppression was not explained by differences in age or sex within this cohort. Nevertheless, the significant association observed in the regression analysis does not overcome the moderate diagnostic performance of TLC, and therefore does not support its use as a replacement for direct CD4 measurement. Despite its limitations, the findings of this study have important implications for HIV care in resource-limited settings. In many developing countries, including Libya, access to flow cytometry remains restricted by financial constraints, limited laboratory infrastructure, and the availability of specialised personnel.

In contrast, complete blood count (CBC) analysis is inexpensive, widely available, and routinely performed in most healthcare facilities. Under these circumstances, TLC may provide supportive information for the initial assessment of immune status or for identifying patients who require priority referral for CD4 testing. However, given its only moderate diagnostic accuracy, TLC should be regarded as an adjunct rather than a replacement for flow cytometry. Whenever CD4 testing is available, clinical decisions should continue to be based on direct immunophenotypic assessment. The present study has several strengths. To our knowledge, it is among the few studies evaluating the relationship between total lymphocyte count and immunological markers in HIV-infected patients from Libya. In addition to correlation analysis, the study employed multiple statistical approaches, including diagnostic performance assessment and receiver operating characteristic (ROC) analysis, providing a comprehensive evaluation of the clinical utility of TLC. Nevertheless, several limitations should be acknowledged. The study was conducted at a single centre, which may limit the generalizability of the findings to other populations. Furthermore, the cross-sectional study design precluded assessment of longitudinal changes in TLC and CD4 count during antiretroviral therapy. Future multicenter studies with larger sample sizes and prospective follow-up are warranted to further validate the clinical usefulness of TLC for HIV monitoring in resource-limited settings.

Overall, the findings of this study demonstrate that total lymphocyte count is associated with CD4 count and reflects general changes in immune status among HIV-infected patients receiving antiretroviral therapy. However, its moderate correlation and fair diagnostic performance indicate that TLC lacks the accuracy required to replace flow cytometry for routine immunological monitoring. While TLC may assist clinicians in resource-limited settings as a readily available supportive marker, direct CD4 measurement remains the preferred method for evaluating immune status and guiding clinical management.

Conclusion

This study evaluated the potential of total lymphocyte count (TLC) as a surrogate marker for immunological monitoring in HIV-infected patients receiving antiretroviral therapy in Libya. TLC demonstrated a statistically significant moderate positive correlation with CD4 count and remained independently associated with immune suppression after accounting for age and sex. However, its moderate sensitivity and fair discriminative performance indicate that TLC cannot reliably replace direct CD4

measurement for routine clinical assessment. Nevertheless, because TLC is inexpensive, readily available, and routinely obtained from complete blood count analysis, it may serve as a useful supportive marker for preliminary immune assessment or patient triage in resource-limited settings where access to CD4 testing is limited. Future multicenter prospective studies involving larger and more diverse patient populations are needed to validate these findings and determine whether clinically useful TLC thresholds can be established.

Conflict of interest. Nil

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