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Correlation of CD4 Cell Count with Absolute Lymphocyte Count, Haemoglobin Level, and Platelet Count in HIV-Positive Patients: A Cross-Sectional Study

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ABSTRACT

Background and aim: CD4+ T-lymphocyte count is the reference standard for assessing immune status and monitoring HIV progression, but its measurement is often unavailable in resource-limited settings. This study evaluated routine haematological parameters as potential surrogate markers for identifying severe immunosuppression in HIV-infected adults.

Material and methods: This descriptive cross-sectional study included 245 HIV-positive adults attending the ART Centre at Maharana Bhupal Government Hospital, Udaipur, India. Absolute lymphocyte count (ALC), haemoglobin (Hb), total leucocyte count (TLC), and platelet count were measured using an automated haematology analyser, while CD4 counts were determined by flow cytometry. Pearson's correlation assessed associations between CD4 count and haematological parameters, and diagnostic performance was evaluated using a CD4 threshold of ⩽200 cells/mm³ .

Results: The mean participant age was 40.06 ± 12.9 years, and 60.8% were male. Severe immunosuppression was identified in 42.1% of patients. ALC showed the strongest positive correlation with CD4 count ($r=0.482$, $p<0.05$) and demonstrated high specificity (86.6%) but modest sensitivity (43.7%) for detecting severe immunosuppression. Hb showed a weaker yet significant positive correlation with CD4 count ($r=0.272$, $p=0.002$), whereas platelet count and TLC were not significantly associated with CD4 levels.

Conclusions: ALC is a simple, inexpensive, and readily available marker that may assist in identifying severe immunosuppression when CD4 testing is inaccessible. However, because of its limited sensitivity, it should complement rather than replace CD4 estimation. Hb may provide additional clinical information, whereas platelet count and TLC have limited utility as surrogate markers.

1. Introduction

Human immunodeficiency virus (HIV) infection remains a major global public health challenge, characterized by progressive depletion of CD4+ T lymphocytes, leading to impaired cellular immunity and increased susceptibility to opportunistic infections.[1] According to the UNAIDS Global AIDS Update 2021, approximately 37.6 million people were living with HIV worldwide, with an estimated 1.5 million new infections and nearly 690,000 AIDS-related deaths reported in 2020.[2] Although India's adult HIV prevalence has declined substantially over the past two decades, the disease continues to impose a considerable healthcare burden, particularly in high-prevalence regions.[3] CD4+ T-cell enumeration remains the laboratory gold standard for assessing immune status, staging HIV infection, monitoring immune recovery following antiretroviral therapy (ART), and guiding prophylaxis against opportunistic infections.[4] A CD4 count below 200 cells/mm³ defines advanced immunosuppression and remains a clinically important threshold for AIDS and opportunistic infection risk.[5] Although the National AIDS Control Organization (NACO) adopted the "Treat All" strategy in 2017, recommending ART irrespective of CD4 count, periodic CD4 monitoring continues to play an essential role in evaluating immune reconstitution and guiding clinical management.[6] Despite its well-established clinical value, CD4 estimation by flow cytometry requires specialized equipment, trained personnel, and substantial financial resources, limiting its availability in many rural and resource-constrained healthcare settings.[7] Consequently, there is continuing interest in identifying inexpensive and widely available laboratory parameters that could complement or temporarily substitute CD4 testing where access to flow cytometry is limited. HIV

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infection is frequently associated with a wide spectrum of haematological abnormalities, including anemia, lymphopenia, neutropenia, leucopenia, and thrombocytopenia, resulting from direct viral bone marrow suppression, immune-mediated mechanisms, opportunistic infections, nutritional deficiencies, and adverse effects of antiretroviral therapy.[8, 9] Because these abnormalities are routinely measured as part of a complete blood count (CBC), they may provide practical and cost-effective surrogate indicators of immune status in resource-limited settings. Among these parameters, the absolute lymphocyte count (ALC) has received particular attention because CD4+ T lymphocytes constitute a major subset of circulating lymphocytes. Previous investigations have demonstrated variable correlations between ALC and CD4 count, and the World Health Organization (WHO) has acknowledged the potential role of total lymphocyte count in supporting clinical decision-making when CD4 testing is unavailable.[10] In addition, haemoglobin concentration and platelet count have also been explored as potential indicators of HIV disease progression, although published findings remain inconsistent across different populations and clinical settings.[11] Despite these observations, comparative evidence evaluating the diagnostic performance of ALC, haemoglobin, and platelet count simultaneously remains limited, particularly in tertiary-care settings in India. Furthermore, regional differences in disease stage, nutritional status, and ART exposure may influence these relationships. Therefore, the present study was undertaken to evaluate the correlation between CD4 cell count and routinely available haematological parameters, including absolute lymphocyte count, haemoglobin level, and platelet count, among HIV-positive patients attending a tertiary-care ART centre in Udaipur, Rajasthan. We hypothesized that ALC would show the strongest correlation with CD4 count and serve as the most clinically useful surrogate marker among routinely available haematological parameters in settings where CD4 testing is unavailable.

2. Material and methods

Study design and setting

A hospital-based descriptive cross-sectional study was conducted at the Antiretroviral Therapy (ART) Centre, Maharana Bhupal Government Hospital, in collaboration with the Departments of Pathology and Microbiology at RNT Medical College, Udaipur, Rajasthan, India. Consecutive sampling was employed, with all eligible HIV-positive patients attending the ART Centre during the study period recruited until the predetermined sample size was achieved. Ethical approval was obtained from the Institutional Review Board of RNT Medical College before study initiation, and written informed consent was obtained from all participants.

Sample Size

The required sample size was calculated using the Pearson correlation coefficient formula:

$$N = (Z\alpha + Z\beta)^2 / [0.5 \times \ln((1 + r)/(1 - r))]^2 + 3$$

Assuming an expected correlation coefficient (r = 0.187) reported by Alam et al.[12], a two-sided α error of 0.05, and 80% statistical power. The minimum required sample size was 223 participants. After allowing for a 10% non-response rate, the final target sample size was set at 245 participants.

Eligibility criteria

Adults (⩾18 years) with confirmed HIV infection diagnosed by ELISA and/or Western blot who attended the ART Centre and provided written informed consent were included. Both newly diagnosed and previously diagnosed patients receiving follow-up care were eligible. Patients with primary haematological disorders (including haemoglobinopathies, leukaemia, and aplastic anaemia), those receiving cytotoxic chemotherapy or immunosuppressive therapy other than ART, pregnant women, and patients with incomplete clinical or laboratory data were excluded. ART status (ART-naïve or ART-experienced) was recorded due to its potential influence on haematological parameters and CD4 cell count.

Laboratory procedures

Two 5-mL peripheral venous blood samples were collected in EDTA tubes under aseptic conditions. Complete blood count (CBC) parameters were measured using a five-part automated haematology analyser (Yumizen H550). Haemoglobin concentration was determined using the sodium lauryl sulfate method, while total leucocyte count and differential counts were obtained using the impedance (Colter) principle. Absolute lymphocyte count (ALC) was calculated as:

ALC = Total leucocyte count × Percentage lymphocytes / 100

CD4+ T-cell counts were determined by flow cytometry using a Partec flow cytometer with phycoerythrin (PE)-conjugated anti-CD4 monoclonal antibodies. Daily internal quality control of the haematology analyser was performed using manufacturer-supplied three-level control materials, and external quality assurance was maintained through participation in the national External Quality Assurance Scheme (EQAS). Flow cytometry performance was verified before each analytical batch using fluorosphere-based quality-control materials.[13, 14]

Statistical analysis

Data were analyzed using descriptive and inferential statistics. Continuous variables were summarised as mean ± standard deviation (SD), whereas categorical variables were expressed as frequencies and percentages. Normality of continuous variables (CD4 count, ALC, haemoglobin, platelet count, and total leucocyte count) was assessed using the Shapiro–Wilk test. Since all variables approximated a normal distribution ($p > 0.05$), Pearson's correlation coefficient was prespecified as the primary statistical method to evaluate the linear relationship between CD4 cell count (primary outcome) and each haematological parameter (ALC, haemoglobin, platelet count, and TLC). For clinical applicability, CD4 ⩽200 cells/mm³ was used as the reference definition of severe immunosuppression, as per international HIV treatment guidelines. The diagnostic performance of each haematological parameter was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), along with true-positive, false-positive, true-negative, and false-negative values. Comparisons of continuous variables across CD4 categories were performed using one-way analysis of variance (ANOVA), while categorical variables were compared using the chi-square test. A two-sided p-value <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis and multivariable regression were not performed and are acknowledged as study limitations.

Ethical approval

The study proposal was approved by the ethics committee of the Rabindranath Tagore Medical College Informed written consent was obtained from all the participants before sample collection.

3. Results

Sociodemographic profile

Of 245 participants, 149 (60.8%) were male and 96 (39.2%) female (M:F ratio 1.56:1). The mean age was 40.06 ± 12.9 years (range 18–75); 61.63% fell within the 31–50 years age group. The majority were married (80.4%). Among males, the predominant occupation was business/service (38.9%), while homemakers constituted 64.6% of females. Full details are presented in Table 1. Of note, no statistically significant association was observed between demographic variables (age group, sex, marital status, or occupation) and CD4 category (chi-square test, $p > 0.05$ for all), suggesting that immunosuppression was distributed across sociodemographic subgroups in this cohort.

Table 1. Sociodemographic profile of HIV-positive patients (n = 245).

Characteristic	Category	Males n (%)	Females n (%)	Total n (%)
Age group (years)	≤30	34 (22.8)	27 (28.1)	61 (24.9)
	31–40	54 (36.2)	33 (34.4)	87 (35.5)
	41–50	38 (25.5)	24 (25.0)	62 (25.3)
	>50	23 (15.4)	12 (12.5)	35 (14.3)
Marital status	Married	122 (81.9)	75 (78.1)	197 (80.4)
	Unmarried/Widowed	27 (18.1)	21 (21.9)	48 (19.6)
Occupation	Labourer	36 (24.2)	8 (8.3)	44 (18.0)
	Homemaker	0 (0)	62 (64.6)	62 (25.3)
	Business/Service	58 (38.9)	14 (14.6)	72 (29.4)
	Others	55 (36.9)	12 (12.5)	67 (27.3)
Total		149 (60.8)	96 (39.2)	245 (100)

Values are expressed as n (%). Business/Service includes government and private sector employees.

Haematological and Immunological Profile

Table 2 summarises the laboratory parameters of all 245 patients. CD4 counts ranged from 9 to 953 cells/mm³ (mean 232.4 ± 122.4). Of 245 patients, 103 (42.1%) had CD4 ⩽200 cells/mm³. Leucopenia (TLC <4000 cells/mm³) was present in 25 patients (10.2%). ALC ⩽1200 cells/mm³ was found in 64 (26.1%). Haemoglobin ⩽12 g/dL was recorded in 107 (43.7%). Thrombocytopenia (⩽150 × 10³/μL) occurred in 54 patients (22.0%).

Table 2. Laboratory Parameters of HIV-Positive Patients (n = 245)

Parameter	Min	Max	Mean ± SD	Median	Abnormal* n(%)
Haemoglobin (g/dL)	5.0	18.0	12.5 ± 2.68	12.7	107 (43.7%)
TLC (cells/mm³)	1600	13120	6041.9 ± 1909.2	5840	25 (10.2%)
ALC (cells/mm³)	105	3804	1557.3 ± 696.3	1540	64 (26.1%)
Platelet count (×10³/μL)	15	696	214.5 ± 97.9	202	54 (22.0%)
CD4 count (cells/mm³)	9	953	232.4 ± 122.4	232	103 (42.1%)

*Abnormal thresholds: Hb ⩽12 g/dL; TLC <4000 cells/mm³; ALC ⩽1200 cells/mm³; platelet ⩽150 × 10³/μL; CD4 ⩽200 cells/mm³. ALC = absolute lymphocyte count; TLC = total leucocyte count.

When stratified by CD4 category, mean ALC and mean haemoglobin decreased progressively with falling CD4 counts (Table 3), with statistically significant differences across groups ($p < 0.001$ for both). By contrast, mean platelet count and TLC did not differ significantly across CD4 strata ($p > 0.05$ for both). The prevalence of thrombocytopenia (22.0%) should be interpreted with caution, as thrombocytopenia in HIV arises through mechanisms largely independent of CD4 depletion and may reflect immune-mediated platelet destruction or medication effects rather than immunosuppression per se. This may explain the lack of statistically significant differences in mean platelet count across CD4 categories despite the relatively high prevalence of thrombocytopenia observed in the study. (Figs. 1 and 2)

Table 3. Haematological Parameters Stratified by CD4 Category.

Parameter	CD4 ≤200 (n=103)	CD4 201–350 (n=84)	CD4 >350 (n=58)	P-value
Hb (g/dL) Mean±SD	11.3 ± 2.4	12.8 ± 2.5	13.6 ± 2.6	0.001*
Anaemia n(%)	61 (59.2%)	30 (35.7%)	16 (27.6%)	<0.001*
TLC (cells/mm³) Mean±SD	5612 ± 1783	6148 ± 1924	6487 ± 2012	0.09
Leucopenia n(%)	14 (13.6%)	7 (8.3%)	4 (6.9%)	0.28
ALC (cells/mm³) Mean±SD	1124 ± 538	1642 ± 668	2078 ± 714	<0.001*
ALC ≤1200 n(%)	44 (42.7%)	13 (15.5%)	7 (12.1%)	<0.001*
Platelet (×10³/µL) Mean±SD	208 ± 101	217 ± 94	221 ± 99	0.54
Thrombocytopenia n(%)	24 (23.3%)	18 (21.4%)	12 (20.7%)	0.93

*p < 0.05 (ANOVA or chi-square as appropriate). CD4 strata: ≤200, 201–350, >350 cells/mm³. Anaemia = Hb ≤12 g/dL; leucopenia = TLC <4000 cells/mm³; thrombocytopenia = platelet ≤150 × 10³/µL.

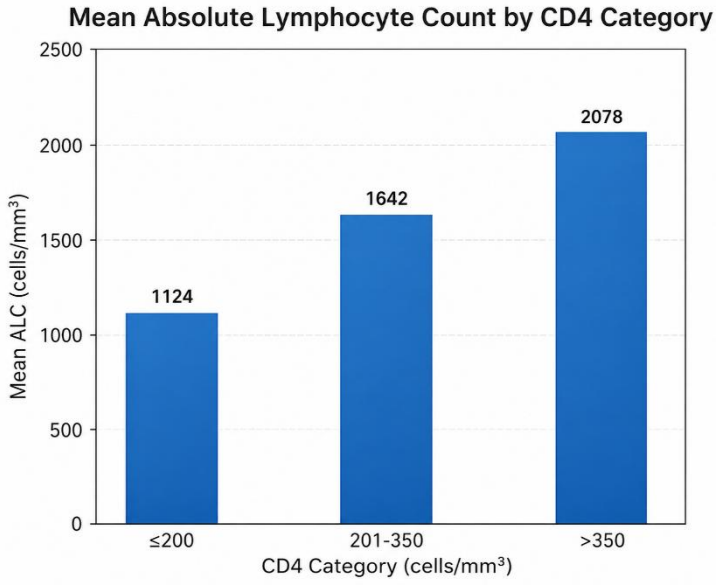

Fig. 1. Mean absolute Count (ALC) vs CD4 category.

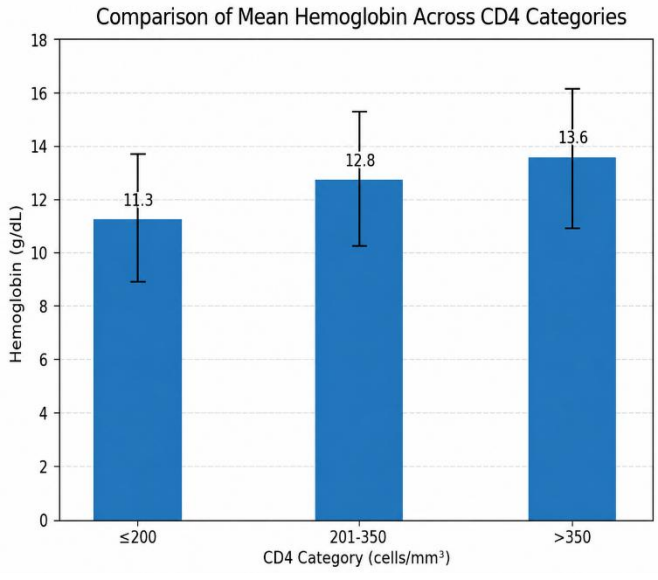

Fig. 2. Mean Hb vs CD4 category.

Correlation and Diagnostic Performance

Table 4 presents full diagnostic performance metrics (TP, FP, FN, TN, sensitivity, specificity) and Pearson's for each parameter against the CD4 ⩽ 200 cells/mm³ threshold. ALC showed the strongest significant correlation ($r = 0.482$, $p < 0.05$) with the highest specificity (86.6%) but moderate sensitivity (43.7%). Haemoglobin showed a significant but weaker correlation ($r = 0.272$, $p = 0.002$). Neither TLC nor platelet count reached statistical significance.

Table 4. Diagnostic Performance of Haematological Parameters for CD4 ≤200 cells/mm³.

Parameter (cut-off)	TP	FP	FN	TN	Sens.(%)	Spec.(%)	r (p-value)
TLC (<4000/mm³)	14	11	89	131	13.59	92.25	0.170 (0.13)
ALC (≤1200/mm³)	45	19	58	123	43.68	86.61	0.482 (<0.05)*
Hb (≤12 g/dL)	57	50	46	92	55.33	64.79	0.272 (0.002)*
Platelet (≤150×10³/μL)	20	34	83	108	19.42	76.06	−0.039 (0.54)

TP = true positive; FP = false positive; FN = false negative; TN = true negative; Sens. = sensitivity; Spec. = specificity; r = Pearson correlation coefficient. *Significant at $p < 0.05$. PPV (ALC): 70.3%; NPV (ALC): 68.0%. PPV (Hb): 53.3%; NPV (Hb): 66.7%.

4. Discussion

The present study investigated the relationship between routinely available haematological parameters and CD4 cell count among HIV-positive patients attending a tertiary-care ART centre in Rajasthan. The principal finding was that absolute lymphocyte count (ALC) demonstrated the strongest positive correlation with CD4 count, whereas haemoglobin showed a weaker but statistically significant association. In contrast, platelet count and total leucocyte count (TLC) were not significantly associated with CD4 count. These findings support the potential role of ALC as the most useful surrogate marker of immune status among routinely available haematological parameters, particularly in resource-limited settings where access to flow cytometry remains limited. The demographic characteristics observed in this study are comparable to those reported in previous studies from India and other developing countries. Male predominance and the higher proportion of patients in the economically productive age group have been consistently reported, reflecting the continued burden of HIV among sexually active adults. Similarly, the predominance of laborers and homemakers corresponds with observations from Kumawat et al. and Singh et al., emphasizing the influence of socioeconomic factors on HIV transmission and healthcare access.[15-20] Anaemia, thrombocytopenia, and leucopenia remain among the most common haematological abnormalities in HIV infection. The prevalence of anaemia in the present study (43.7%) was lower than that reported in previous studies[15, 17, 21] but slightly higher than that reported in another study.[22] These differences may reflect improvements in antiretroviral therapy (ART) coverage, earlier diagnosis, nutritional status, and variations in disease severity across study populations. Likewise, the prevalence of thrombocytopenia (22.0%) was comparable to that reported in previous studies,[12, 15] although lower frequencies have been described elsewhere.[21–24] Among all haematological parameters evaluated, ALC showed the strongest correlation with CD4 cell count ($r = 0.482$, $p < 0.05$). This observation is biologically plausible because CD4-positive T lymphocytes constitute a substantial proportion of circulating lymphocytes. Progressive depletion of CD4 cells during HIV infection is therefore accompanied by a reduction in total lymphocyte count, particularly in advanced disease. Similar findings have been reported in previous studies; however, the strength of the correlation has varied considerably across studies.[16, 17, 23, 25–29] Such variability is likely attributable to differences in ART exposure, disease stage at enrolment, nutritional status, laboratory methodology, and demographic characteristics. Although ALC demonstrated excellent specificity (86.6%) for identifying patients with severe immunosuppression, its sensitivity remained relatively low (43.7%). Consequently, while a reduced ALC strongly suggests advanced immune deficiency, a normal ALC cannot reliably exclude patients with CD4 counts ⩽200 cells/mm³ . Similar observations have been reported in previous studies, which also highlighted the limitations of ALC as a standalone screening tool.[27, 28] Therefore, our findings support the World Health Organization's recommendation that ALC may be used as a triage or supportive marker when CD4 testing is unavailable, but should not replace direct CD4 measurement when flow cytometry is accessible. Haemoglobin concentration also demonstrated a significant positive correlation with CD4 count, although the association was considerably weaker than that observed for ALC. This finding is consistent with previous reports,[16, 25, 29] whereas no significant association has been reported in another study.[26] The relatively modest correlation observed in the present study may be explained by the multifactorial pathogenesis of anaemia in HIV infection. In addition to progressive immunosuppression, haemoglobin concentration is influenced by nutritional deficiencies, chronic inflammation, opportunistic infections, bone marrow suppression, renal dysfunction, and adverse effects of antiretroviral therapy. Consequently, haemoglobin reflects overall disease severity rather than immune status alone and should be considered a supportive rather than an independent surrogate marker of CD4 count. Neither platelet count nor TLC showed a significant correlation with CD4 count. Similar findings have been reported in previous studies.[21, 29] However, significant associations have been described in other studies.[24, 26] HIV-associated thrombocytopenia develops through several mechanisms, including immune-mediated platelet destruction, impaired megakaryocyte function, and direct viral effects on platelet production, many of which occur independently of CD4 depletion.[30] Likewise, because TLC includes multiple leucocyte subpopulations, changes in neutrophils and monocytes may obscure the effect of declining CD4-positive lymphocytes, explaining its limited value as an indicator of immune status. The heterogeneity observed across published studies highlights that several clinical and demographic factors, including ART status, duration of HIV infection, nutritional status, and regional differences, influence the relationship between routine haematological parameters and CD4 count. The inclusion of both ART-naïve and ART-experienced patients in the present study may partly explain why the observed ALC–CD4 correlation was moderate rather than strong. From a clinical perspective, the present findings suggest that ALC may represent the most practical and readily available surrogate marker for identifying patients at increased risk of severe immunosuppression in settings where CD4 testing cannot be performed. However, its limited sensitivity indicates that it should be used only as an adjunctive screening tool. Haemoglobin may provide additional supportive information regarding disease progression, whereas platelet count and TLC appear to have limited clinical utility for estimating immune status. Future multicentre prospective studies incorporating viral load measurements,

receiver operating characteristic (ROC) analysis, and multivariable regression are warranted to establish clinically applicable prediction models for identifying advanced immunosuppression using routinely available laboratory parameters.

Limitation of the study

Several limitations of this study should be acknowledged. First, the cross-sectional design precludes causal inference and does not allow assessment of temporal changes in haematological parameters relative to CD4 trajectory. Second, this is a single-centre study from a tertiary care ART center in Udaipur, Rajasthan; findings may not be generalizable to other Indian settings or to community-based cohorts. Third, viral load was not measured, limiting the ability to contextualise immunosuppression within the broader HIV disease milieu. Fourth, ART status (naïve vs. ART-experienced) and therapy duration were recorded but not included as covariates in the correlation analysis, which represents a potential confounder; future studies should perform stratified analyses by ART status. Fifth, multivariable regression was not performed to adjust for potential confounders such as nutritional status, opportunistic infections, and comorbidities. Sixth, ROC curve analysis was not performed to determine data-derived optimal cut-off values, which limits the precision of diagnostic performance estimates.

5. Conclusion

This study demonstrated that among routinely available haematological parameters, absolute lymphocyte count (ALC) showed the strongest correlation with CD4 cell count in HIV-positive patients, whereas haemoglobin showed a weaker but statistically significant association. In contrast, platelet count and total leucocyte count were not significantly correlated with CD4 count. The findings suggest that ALC may serve as a practical adjunctive marker for identifying patients at increased risk of severe immunosuppression, particularly in resource-limited settings where CD4 flow cytometry testing is unavailable or delayed. However, the relatively low sensitivity observed in this study indicates that ALC should not be considered a replacement for CD4 measurement. Haemoglobin may provide additional supportive information regarding disease progression but has limited value as an independent indicator of immune status. Overall, routine haematological parameters, particularly ALC, may help clinicians prioritize patients for immunological assessment when access to CD4 testing is limited. Further multicentre prospective studies incorporating viral load assessment and receiver operating characteristic (ROC) analysis are warranted to validate optimal cut-off values and improve the predictive performance of these readily available biomarkers.

Conflict of Interest

The authors declared that there is no conflict of interest.

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