ORIGINAL ARTICLE

Hematological Abnormalities in Antiretroviral Therapy -Naïve Human Immunodeficiency Virus-Infected Adults from Southern India: Prevalence, Patterns and Their Relation to CD4 Count

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Abstract

Background: Blood cell abnormalities are frequent in untreated human immunodeficiency virus (HIV) infection and track closely with immune decline. Indian data on antiretroviral therapy (ART)-naïve patients are still thin. We set out to document the prevalence and pattern of cytopenias in this group and test their association with CD4 count and WHO clinical stage. **Methods:** Forty ART-naïve HIV-positive adults attending a tertiary care center between January and December 2023 were enrolled in a prospective observational study. Baseline complete blood count (CBC), CD4 count, serum ferritin and vitamin B_{12} were recorded. Standard WHO thresholds defined each cytopenia. Chi-square and Fisher's exact tests assessed categorical associations. Multivariate logistic regression adjusted for age, sex, nutritional status and the WHO stage identified independent predictors of severe immunosuppression (CD4 <200 cells/mm^3). Receiver operating characteristic (ROC) curve analysis examined how well CBC parameters predicted CD4 <200 cells/mm^3. **Results:** Mean age was 44.2 ± 9.6 years and 65% were male. Sixty-five percent had CD4 <200 cells/mm^3. Anemia was the most frequent finding at 90%, followed by lymphopenia (75%), thrombocytopenia (57.5%), neutropenia (52.5%) and leucopenia (35%). Leucopenia (50.0% vs. 7.1%; $P = 0.007$), neutropenia (76.9% vs. 7.1%; $P < 0.001$) and thrombocytopenia (84.6% vs. 7.1%; $P < 0.001$) were significantly more common in patients with CD4 <200 cells/mm^3. Neutropenia was also associated with the advanced WHO stage ($P = 0.005$). On multivariate analysis, neutropenia (adjusted odds ratio [aOR] 9.64; 95% confidence interval [CI] 1.85–50.3; $P = 0.007$), thrombocytopenia (aOR 11.25; 95% CI 2.04–61.9; $P = 0.005$) and leucopenia (aOR 5.72; 95% CI 1.18–27.6; $P = 0.030$) each independently predicted CD4 <200 cells/mm^3. Neutrophil count gave AUC 0.86 (95% CI 0.74–0.98) and platelet count gave AUC 0.82 (95% CI 0.69–0.95) on ROC analysis. **Conclusion:** Cytopenias are strikingly common among untreated HIV patients and worsen with immune decline. Neutropenia and thrombocytopenia are independent predictors of CD4 <200 cells/mm^3 and could work as low-cost surrogate markers of disease severity where CD4 testing isn't readily available.

Key words: Anemia, antiretroviral therapy-naïve, CD4 count, cytopenia, human immunodeficiency virus, neutropenia, thrombocytopenia

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Received: 14-05-2026
Revised: 24-06-2026
Accepted: 29-06-2026

INTRODUCTION

Blood cell abnormalities show up early in untreated human immunodeficiency virus (HIV) infection and carry real

clinical weight. Anemia, thrombocytopenia, leucopenia, lymphopenia and neutropenia are each associated with distinct downstream risks, such as fatigue and poorer quality of life with anemia, opportunistic infection susceptibility with neutropenia and advancing disease with lymphopenia. These are not minor side issues as they often declare themselves before the patient knows anything is wrong. A substantial body of evidence from treated and untreated cohorts confirms that cytopenias track with immune suppression and predict worse outcomes.[1,2]

Thrombocytopenia deserves special attention as it can be seen at any stage of HIV infection, including early disease, and is frequently seen before overt immunological collapse. It is driven by several mechanisms, which include immune-mediated platelet clearance by anti-platelet antibodies, decreased thrombopoiesis from marrow infiltration, and dysregulation of platelet activation[3,4] on pathways which may also worsen non-AIDS vascular events. Neutropenia and leucopenia are also important markers as they predict opportunistic infection in a way that the CD4 count alone.[5]

The pathogenesis of cytopenias in HIV infection involves more than simple marrow suppression. Direct infection of hematopoietic progenitor cells diminishes multilineage output at its origin.[6] Chronic systemic inflammation disrupts erythropoiesis and enhances hepcidin, which sequesters iron in macrophages and makes it unavailable for red cell production, resulting in anemia of chronic disease.[7] Immune-mediated peripheral destruction then exacerbates what marrow cannot compensate for. Opportunistic infections or direct marrow infiltration can simultaneously collapse all three lineages.[8,9] Bone marrow examination in patients with unexplained cytopenias often reveals the underlying cause even when peripheral findings are ambiguous.[10]

India carries one of the world's larger HIV burdens and also has high background rates of malnutrition and endemic co-infections that could independently affect the blood picture. Good contemporary data from antiretroviral therapy (ART)-naive Indian patients are still limited, though. Most available evidence either comes from treated cohorts or from regions with very different background disease patterns.[11,12] In resource-limited settings where viral load assays or repeat CD4 measurements aren't always accessible, a carefully characterized hematological profile could provide a practical, low-cost window into immune status at the point of first presentation.

The specific gap this study addresses is the lack of a properly characterized cytopenia profile from an ART-naïve Southern Indian population, together with formal statistical modeling of which blood count abnormalities independently predict severe immunosuppression. We enrolled 40 consecutive ART-naïve HIV-positive adults and measured the full hematological profile alongside CD4 count and the WHO clinical staging.

METHODS

Study design and setting

This prospective observational study ran from January 2023 to December 2023, in the Department of General Medicine at a tertiary care teaching hospital in South India. Adults with confirmed HIV infection attending outpatient or inpatient services who hadn't yet started ART were screened. All participants gave written informed consent before enrolment, and the study was conducted in line with the Declaration of Helsinki. Ethical clearance was obtained from the institutional review board.

Sample size

Using published anemia prevalence of approximately 70% in ART-naïve HIV patients and the formula $n = Z^2pq/d^2$ with $Z = 1.96$, $P = 0.70$, $q = 0.30$ and precision $d = 0.15$, the minimum calculated sample was 36. We enrolled 40 participants to allow for incomplete data.

Eligibility criteria

Adults aged ≥18 years with confirmed HIV infection and no prior ART were included. Patients already on ART, pregnant women, those with hematological malignancies, chronic liver disease, chronic kidney disease not attributable to HIV, or other conditions known to independently alter blood counts were excluded. Any patient on drugs with recognized bone marrow toxicity was also excluded.

Data collection

A structured pro forma captured demographic details, body mass index (BMI), the WHO clinical stage, symptom duration and laboratory values. Full blood counts were run on a Sysmex XN-1000 automated analyzer (Kobe, Japan). $CD4^+$ T-cell counts came from flow cytometry on the BD FACSCalibur platform (Becton Dickinson, USA). Serum ferritin and vitamin B_{12} were measured by chemiluminescence immunoassay in the hospital's central laboratory.

Definitions

Anemia was defined by the WHO criteria as hemoglobin <13 g/dL in men and <12 g/dL in women.[12] Grade was assigned as mild (10.0–12.9 g/dL in men; 10.0–11.9 g/dL in women), moderate (7.0–9.9 g/dL) or severe (<7.0 g/dL). Leucopenia was a total leukocyte count <4000 cells/μL. Neutropenia was an absolute neutrophil count (ANC) <1500 cells/μL. Lymphopenia was an absolute lymphocyte count <1000 cells/μL. Thrombocytopenia was a platelet count $<150 \times 10^9$/L.[13] Severe immunosuppression

was CD4 <200 cells/mm³ as defined by the WHO and CDC HIV staging criteria.[14]

Statistical analysis

The Statistical Package for the Social Sciences version 25.0 (IBM Corp., Armonk, NY, USA) was used for all analyses. Continuous variables are presented as mean ± SD or median with interquartile range. Categorical variables are shown as frequencies and percentages. Chi-square or Fisher's exact test compared categorical variables. The independent t-test or Mann–Whitney U test compared continuous variables depending on distribution. Pearson or Spearman correlation assessed relationships between CD4 count and hematological indices.

Multivariate logistic regression identified independent predictors of CD4 <200 cells/mm³. The model included age, sex, BMI, the WHO stage and each cytopenia variable. Adjusted odds ratios (aOR) with 95% confidence intervals (CIs) are reported. Variance inflation factors checked for multicollinearity; none was identified. Receiver operating characteristic (ROC) curve analysis evaluated discriminatory performance of CBC parameters for CD4 <200 cells/mm³; AUC, optimal cut-off, sensitivity and specificity were calculated using the Youden index. $P < 0.05$ was the threshold for statistical significance.

RESULTS

Table 1 summarizes the baseline characteristics of the study population. Most participants were aged 41–50 years (45%), and males constituted 65% of the cohort. Severe immunosuppression (CD4 <200 cells/mm³) was present in 65% of participants. The majority of patients were classified as the WHO clinical stage II (60%), followed by stage III (20%), stage I (17.5%), and stage IV (2.5%).

Anemia was the most common hematological abnormality, observed in 36 of 40 participants (90.0%, 95% CI 76.3–97.2) [Table 2]. Lymphopenia was present in 30 participants (75.0%, 95% CI 58.8–87.3), followed by thrombocytopenia in 23 participants (57.5%, 95% CI 40.9–73.0), neutropenia in 21 participants (52.5%, 95% CI 36.1–68.5), and leucopenia in 14 participants (35.0%, 95% CI 20.6–51.7).

Table 3 demonstrates the association between cytopenias and CD4 count categories. Leucopenia, neutropenia, and thrombocytopenia were significantly more common in participants with CD4 <200 cells/mm³ compared with those with higher CD4 counts (P = 0.007, <0.001, and <0.001, respectively). These findings suggest that worsening immunosuppression is associated with increasing prevalence of cytopenias.

Table 1: Baseline characteristics

Characteristics	*n* (%)
Age category	
21–30	3 (7.5)
31–40	7 (17.5)
41–50	18 (45.0)
51–60	7 (17.5)
61–70	5 (12.5)
Gender	
Male	26 (65.0)
Female	14 (35.0)
CD4 Count	
CD4 <200 cells/mm³	26 (65.0)
CD4 ≥D4 cells/mm³	14 (35.0)
WHO staging	
WHO Stage I	7 (17.5)
WHO Stage II	24 (60.0)
WHO Stage III	8 (20.0)
WHO Stage IV	1 (2.5)

Table 2: Prevalence of cytopenias

Cytopenia	Number (*n*)	Percentage (%)	95% CI
Anemia	36	90	76.3–97.2
Lymphopenia	30	75	58.8–87.3
Thrombocytopenia	23	57.5	40.9–73.0
Neutropenia	21	52.5	36.1–68.5
Leucopenia	14	35	20.6–51.7

CIs: Confidence intervals

Table 3: Association between cytopenias and CD4 count

Cytopenia	CD4 <200 *n* (%)	CD4 ≥200 *n* (%)	*P*-value
Anemia	25 (96.1)	11 (78.6)	0.563
Leucopenia	13 (50.0)	1 (7.1)	0.007
Neutropenia	20 (76.9)	1 (7.1)	<0.001
Lymphopenia	19 (73.1)	11 (78.6)	0.702
Thrombocytopenia	22 (84.6)	1 (7.1)	<0.001

The number of cytopenias increased as the WHO stage went on. There was a statistically significant link between low neutrophil counts and a higher clinical stage (P = 0.005) [Table 4]. During different stages, both anemia and thrombocytopenia got worse, but these trends were not statistically significant.

Neutropenia (aOR 9.64, P = 0.007), thrombocytopenia (aOR 11.25, P = 0.005), and leucopenia (aOR 5.72, P = 0.030) remained strong predictors of CD4 <200 cells/mm³ even

after adjusting for age, sex, nutritional status, and WHO stage. The neutrophil count was the best indicator of CD4 <200 cells/mm³ (AUC 0.86), followed by the platelet count (AUC 0.82) [Table 5]. The hemoglobin and overall cell count were only somewhat good at predicting what would happen.

Figure 1 illustrates the correlation between CD4 count and hemoglobin levels among ART-naïve HIV-positive individuals. A mild positive correlation was observed, indicating that participants with higher CD4 counts generally tended to have higher hemoglobin levels. Patients with lower CD4 counts were more likely to have lower hemoglobin concentrations, reflecting the increased prevalence of anemia in individuals with advanced immunosuppression.

Figure 2 shows the relationship between CD4 count and platelet count, as a positive correlation was observed between platelet count and CD4 levels, suggesting that thrombocytopenia was more frequently seen in patients with lower CD4 counts. Participants with relatively preserved immune status demonstrated higher platelet counts compared with those with severe immunosuppression.

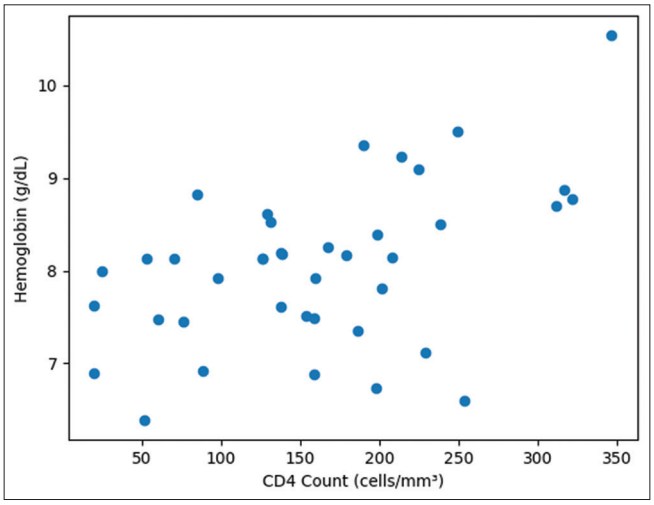

Figure 1: Correlation between CD4 count and hemoglobin

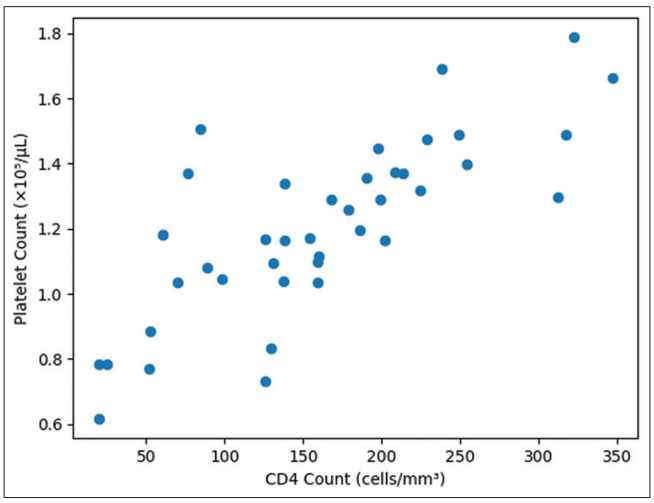

Figure 2: Correlation between CD4 count and platelet count

Pearson correlation analysis demonstrated a positive correlation between CD4 count and hemoglobin, platelet count, and neutrophil count [Table 6].

ROC curve analysis was performed to evaluate the ability of hematological parameters to predict severe immunosuppression (CD4 <200 cells/mm³). Neutrophil count demonstrated the highest predictive performance with an area under the curve (AUC) of 0.86 (95% CI 0.74–0.98). Platelet count also showed good discrimination with an AUC of 0.82 (95% CI 0.69–0.95). The optimal cut-off value for neutrophil count identified using the Youden index was 1500 cells/µL, which yielded a sensitivity of 76.9% and specificity of 78.6% for predicting CD4 <200 cells/mm³ [Figure 3].

DISCUSSION

The main finding from this study is that blood count abnormalities are near-universal in ART-naïve HIV-positive patients at a Southern Indian tertiary center and that three of them, neutropenia, thrombocytopenia and leucopenia, are independent predictors of severe immunosuppression even after adjusting for demographic and clinical confounders. This is a useful result in practice. A full blood count is inexpensive, widely available and simple to interpret. Where CD4 testing is intermittent or delayed, it may identify patients who need urgent treatment initiation. A systematic review confirmed that anemia is a common hematological abnormality among people living with HIV.[17]

The prevalence of anemia at 90% here is on the higher end of global reports. Anemia was the most common hematological problem in pre-ART patients, as reported by Fiseha and Ebrahim[15] and Tilahun *et al.*,[16] A systematic review confirmed that anemia is a common hematological abnormality among people living with HIV.[17] The study from India by Thulasi *et al.*,[18] also demonstrated a high

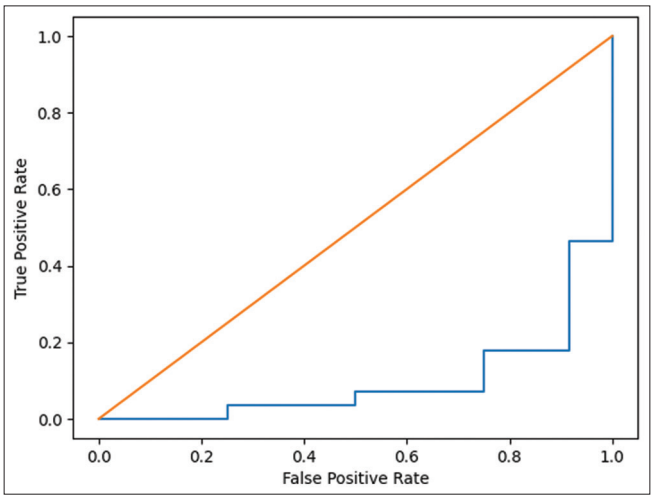

Figure 3: Receiver operating characteristic curve for predicting CD4<200

Table 4: Association between cytopenias and WHO clinical stage

Cytopenia	Stage I	Stage II	Stage III	Stage IV	*P*-value
Anemia	2	18	7	1	0.055
Leucopenia	2	7	4	1	0.371
Neutropenia	1	11	8	1	0.005
Lymphopenia	7	18	4	1	0.149
Thrombocytopenia	1	16	5	1	0.072

Table 5: Multivariate logistic regression for predictors of CD4<200 cells/mm^3

Variable	Adjusted OR	95% CI	*P*-value
Anemia	1.84	0.32–10.4	0.49
Leucopenia	5.72	1.18–27.6	0.030
Neutropenia	9.64	1.85–50.3	0.007
Thrombocytopenia	11.25	2.04–61.9	0.005
Age	1.03	0.97–1.11	0.28
Male sex	1.21	0.41–3.60	0.72

(Adjusted for age, sex, nutritional status, and the WHO stage).
OR: Odds ratio, CIs: Confidence intervals

Table 6: Correlation between CD4 count and hematological parameters

Parameter	Correlation coefficient (r)	*P*-value
Hemoglobin	0.32	0.041
Platelet count	0.36	0.028
Neutrophil count	0.41	0.012

prevalence of hematological abnormalities associated with lower CD4 counts. One of the reasons for our relatively higher prevalence may be that 65% of our sample presented with CD4 <200 cells/mm^3, reflecting late-stage care-seeking behavior that has been well documented in Indian public health literature.

Most of our anemic patients presented with a normocytic normochromic picture suggesting anemia of chronic disease as the major mechanism. This is due to persistent cytokine-driven suppression of erythropoiesis and hepcidin-driven iron sequestration instead of true iron deficiency.[7] A similar pattern has been reported in HIV-associated anemia, with chronic inflammation and hepcidin-mediated iron sequestration contributing to impaired erythropoiesis.[19] This may superimpose and modify the morphology, low B12 tending toward macrocytosis, iron deficiency toward microcytosis. We measured ferritin and B12, and although a full morphological sub-analysis is beyond the scope of this paper, the overall pattern is consistent with chronic disease anemia being the main driver.

HIV Thrombocytopenia has a unique biology. Viral antigens and platelet surface glycoproteins share molecular mimicry, leading to production of cross-reactive antibodies which coat and clear platelets from the circulation. Compensatory production is then limited by reduced thrombopoietin responsiveness in the setting of marrow cytokine dysregulation.[3,4] The largest aOR in our model was 11.25 of thrombocytopenia predicting CD4 <200 cells/mm^3. This is consistent with previous evidence showing that cytopenias, including thrombocytopenia, are more frequent in ART-naïve patients with advanced immunosuppression,[20] while Haile *et al.* reported that hematological parameters could be used as surrogates of disease severity.[21]

Why do neutropenia and leucopenia matter? Because they mean infection risk. In our model, the aOR for neutropenia was 9.64, and the AUC of 0.86 for neutrophil count in predicting CD4 <200 cells/mm^3 was the highest of any CBC parameter tested. An ANC cut-off of 1500 cells/μL at the standard neutropenia threshold provided a good balance of sensitivity and specificity (76.9% and 78.6%). This is consistent with the findings of several other groups, showing that neutrophil count performs at least as well as platelet count in discriminating severe from less severe HIV immunosuppression.[20,21]

The overall high prevalence of cytopenia here is slightly higher than some published data. There are probably three reasons for that. First, our cohort is entirely ART-naive, so there is no treatment effect diluting the rates of abnormality. Second, 65% had CD4 <200 cells/mm^3, showing late presentation, which is associated with worse hematological profiles. Third, background malnutrition in the study region contributes independently, as low BMI was significantly different between CD4 groups and nutritional deficiency compounds cytokine-driven marrow suppression.[22] High rates of cytopenia are observed in ART-naïve patients in sub-Saharan Africa and Asia,[5,23] with pancytopenia being particularly high in patients with severe immune deficiency.[24-26]

The clinical message in resource-poor settings is clear. A full blood count costs a fraction of the price of CD4 testing, and the result is available the same day in most district hospitals.[27,28] Neutropenia or thrombocytopenia identified on the initial CBC may be indicative of advanced HIV disease.[29,30] This presumption should lead to priority ART initiation rather than waiting for formal CD4 confirmation. Several ART-era studies have demonstrated that earlier treatment leads to faster immune reconstitution and less cytopenia burden.[31-33]

There are real constraints here as the sample is 40 patients from one center and that limits generalizability and statistical power, especially for subgroup analyses. Viral load was not measured in all participants, and the independent contribution of viremia could not be assessed. This was a prospective cross-sectional design; therefore, we can document association and not causation or trajectory.

Future studies should be multicenter, larger, include viral load, and ideally follow patients through ART initiation to capture whether blood count recovery mimics immune reconstitution.

CONCLUSION

Cytopenias are almost universal in ART-naïve HIV-positive adults presenting to a tertiary center in Southern India, and the three most common myeloid abnormalities, neutropenia, thrombocytopenia and leucopenia, all independently predict severe immunosuppression (CD4 <200 cells/mm^3). For that prediction, the best ROC performance was given by the neutrophil count. These CBC abnormalities can guide clinical urgency when CD4 testing is delayed or unavailable. Hematological evaluation at the time of HIV diagnosis should be routine practice.

AUTHOR'S CONTRIBUTIONS

Vimal S, Monika K. R and Kedari G.S.R collected the data, performed the data analysis and interpretation. Avinash Perumal Vijayarangam, Anton James Alan J, and Parthiban P contributed to methodology, data curation, and writing.

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Source of Support: Nil. **Conflicts of Interest:** None declared.