Research Article

Correlation of Flow Cytometry–Based CD4+ T-Cell Counts With Hematological Abnormalities Among People Living With HIV: A Cross-Sectional Study

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Abstract

Background: Hematological abnormalities are common manifestations of Human Immunodeficiency Virus (HIV) infection and serve as strong predictors of morbidity and mortality. Evaluating these changes alongside flow cytometry-based CD4+ T-cell counts provides key insights into disease progression.
Objective: To investigate the prevalence of hematological abnormalities and evaluate their correlation with CD4+ T-cell counts in treatment-naive People Living with HIV (PLWH).
Methods: A hospital-based cross-sectional study was conducted among 200 newly diagnosed, antiretroviral therapy (ART)-naive HIV-positive adults. Complete blood counts (CBC) were performed using an automated hematology analyzer, and absolute CD4+ T-cell counts were determined via multi-color flow cytometry. Statistical associations were evaluated using Pearson's correlation coefficient and the Chi-square test.
Results: The overall prevalence of anemia, leukopenia, and thrombocytopenia was 72.5%, 26.0%, and 14.5% respectively. A strong, statistically significant positive correlation was observed between CD4+ T-cell counts and both hemoglobin levels ($r = 0.64$), ($p < 0.001$) and absolute lymphocyte counts ($r = 0.71$), ($p < 0.001$). Cytopenias were progressively more severe in patients with CD4+ counts (< 200 cells/microL).
Conclusion: Hematological derangements are inversely proportional to CD4+ T-cell counts in ART-naive patients. Routine CBC parameters present a reliable, cost-effective tool to complement flow cytometry for tracking severe immunosuppression in resource-constrained environments[1].

Introduction

Human Immunodeficiency Virus (HIV) causes a progressive depletion of helper T-lymphocytes bearing the CD4 receptor (CD4+), compromising cell-mediated immunity and exposing the host to opportunistic infections and malignancies. Immunophenotyping via flow cytometry remains the gold standard for monitoring this immune decline, guiding the initiation of prophylaxis, and staging disease progression[2]

Beyond direct immunological failure, HIV targets the hematological system through direct bone marrow suppression, opportunistic infections, altered cytokine environments, and immune-mediated destruction. Peripheral cytopenias most notably anemia, leukopenia, and thrombocytopenia frequently complicate clinical management. While flow cytometry services may face financial or logistical barriers in developing or remote communities, basic hematological panels are highly accessible. Quantifying the exact operational relationship between peripheral cytopenias and absolute CD4+ counts establishes whether routine blood counts can act as reliable clinical red flags for advanced immune failure[3]

Materials and Methods

Study Design and Population

This descriptive, observational, cross-sectional study was conducted at a tertiary healthcare facility over a 12-month period. The study cohort comprised 200 newly diagnosed, adult PLWH who were completely naive to antiretroviral therapy (ART). Patients presenting with underlying primary hematological disorders, chronic kidney disease, chronic liver disease, concurrent malignancies, or those undergoing active chemotherapy were excluded from the study.

Laboratory Analysis

- **Hematological Profiling:** Venous blood samples collected in EDTA tubes were processed within two hours using a calibrated 5-part automated hematology analyzer to determine hemoglobin (Hb), total leukocyte count (TLC), absolute lymphocyte count (ALC), and platelet count.

- **Immunophenotyping:** Absolute CD4+ T-cell counts were determined using a multi-color flow cytometer utilizing direct immunofluorescence with conjugated monoclonal antibodies (CD45, CD3, and CD4) under standard lyse-no-wash protocols.

Diagnostic Standards & Reference Values

Hematological abnormalities and immune status thresholds were defined according to established clinical reference ranges:[4]

Parameter	Standard Biological Reference Range	Definition of Abnormality (Abnormal Threshold)
CD4+ T-cell Count	500 - 1,500cells/microL	**Severe Immunosuppression (AIDS):**<200 cells/microL **Advanced Immunosuppression:** 200 - 349 cells/microL **Mild Immunosuppression:**350 - 499 cells/microL
Hemoglobin (Hb)	Males:13.5 - 17.5 g/dL Females:12.0 - 15.5 g/dL	**Anemia:**<13.0 g/dL (Males) / <12.0g/dL (Females)
Total Leukocyte Count (TLC)	4.0 - 11.0x 10^9 /L	**Leukopenia:**< 4.0x10^9/L
Platelet Count	150 - 450 x10^9 /L	**Thrombocytopenia:** < 150x 10^9/L

Statistical Analysis

Data were captured and analyzed using SPSS Version 26.0. Continuous variables were expressed as Mean+- Standard Deviation (SD). Categorical variables were expressed as frequencies and percentages. Patients were stratified into distinct immunological tiers based on their CD4+ counts. Pearson's correlation coefficient (r) evaluated linear associations between continuous parameters. Chi-square tests evaluated categorical distributions across immunological cohorts, with significance established at ($p < 0.05$)[5]

Results

Patient Baseline Characteristics

The study population had a mean age of 37.4+-7.1 years, with a baseline male distribution of 61.0%. The mean absolute baseline CD4+ T-cell count across the study cohort was 312 +- 46 cells/microL.

Prevalence of Hematological Cytopenias by Immune Status

Stratifying patients revealed a stark increase in peripheral blood abnormalities as immune function degraded:

Hematological Abnormality	Total Cohort (N=200)	CD4< 200 cells/microL (n=78)	CD4 200-499 cells/microL (n=84)	CD4 >or equal 500 cells/microL (n=38)	X^2 p-value
Anemia	145 (72.5%)	67 (85.9%)	61 (72.6%)	17 (44.7%)	<0.001
Leukopenia	52 (26.0%)	31 (39.7%)	17 (20.2%)	4 (10.5%)	0.003
Thrombocytopenia	29 (14.5%)	19 (24.4%)	8 (9.5%)	2 (5.3%)	0.008

Anemia emerged as the most dominant hematological complication, demonstrating a clinical prevalence of 85.9% among individuals entering care with severe immunosuppression (CD4< 200 cells/microL)

Correlation Analysis

Pearson correlation matrices established strong directional linearity between immune preservation and peripheral blood metrics:[3]

- **Hemoglobin vs. CD4+ Count:** A significant positive correlation ($r = 0.64$, $p < 0.001$) indicated that worsening anemia tracks directly with severe T-cell depletion.
- **Absolute Lymphocyte Count (ALC) vs. CD4+ Count:** Showed the tightest statistical linkage ($r = 0.71$, $p < 0.001$), acting as an explicit reflection of absolute lymphopenia.
- **Platelet Count vs. CD4+ Count:** Demonstrated a weak-to-moderate positive linear correlation ($r = 0.28$,$p = 0.014$).

4. Discussion

This cross-sectional investigation highlights the extensive structural impact of untreated HIV infection on hematopoietic lineages. Our structural data verifies that peripheral cytopenias scale predictably alongside the destruction of CD4+ T-cells.[5]

Anemia remains the principal hematological surrogate marker for clinical decline. The physiological drivers are multi-faceted: HIV-1 proteins directly induce cytokine-mediated suppression of erythroid progenitors in the bone marrow, while secondary opportunistic pathogens compromise nutritional absorption and accelerate peripheral destruction.[2]

Leukopenia and lymphopenia track alongside CD4+ loss due to both direct viral viral destruction and architectural damage to the bone marrow microenvironment. The tight, positive statistical correlation between absolute lymphocyte counts and CD4+ counts ($r = 0.71$) carries practical clinical utility. In rural or under-resourced medical facilities where flow cytometry platforms are absent or financially out of reach, a basic automated ALC can serve as an accessible proxy marker. A tumbling ALC combined with worsening anemia offers high diagnostic sensitivity for identifying individuals at immediate risk of opportunistic infections. [1,6,7]

Conclusion:

This study utilizes a cross-sectional approach, which prevents the determination of causal timelines. Furthermore, viral load parameters and specific micronutrient diagnostics (e.g., serum iron, B12 levels) were not evaluated, limiting the ability to rule out concurrent nutritional deficiencies.

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