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Original Article

TH17/TREG AND IL-17/IL-10 ratio profiles in people living with HIV on long-term cART in Western Kenya

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Abstract

Background: Despite sustained viral suppression following combination antiretroviral therapy (cART), a substantial proportion of people living with HIV (PLWHIV) on cART fail to achieve adequate immune reconstitution. Increasing evidence suggests that immune regulatory imbalance involving $CD3^+CD4^+IL\text{-}17^+$ T helper 17 (Th17) cells, $CD4^+CD25^{high}CD127^{low}$ Treg cells, and corresponding cytokines interleukin-17 (IL-17) and interleukin-10 (IL-10), may contribute to persistent dysfunction in immune recovery.

Objective: To evaluate $CD3^+CD4^+IL\text{-}17^+$ T helper 17 (Th17) and $CD4^+CD25^{high}CD127^{low}$ Treg cells dynamics and IL-17/IL-10 balance as associated with immune reconstitution among PLWHIV on long-term cART in western Kenya.

Methods: A cross-sectional study with retrospective clinical data abstraction was conducted at Moi Teaching and Referral Hospital (MTRH). Sixty-four virologically suppressed PLWHIV on cART for 1-6 years were categorized as 32 immunological responders (IRs) or 32 non-responders (INRs) based on $CD4^+$ T cell counts and compared with 32 HIV-negative individuals. $CD3^+CD4^+IL\text{-}17^+$ T helper 17 (Th17) cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells were quantified by flow cytometry, while IL-17 and IL-10 were measured using enzyme-linked immunosorbent assay. Data were analyzed using non-parametric tests, Spearman's correlation, and multivariable logistic regression.

Results: Ninety-six participants (32 per group) were enrolled. Age differed significantly across groups ($p=0.026$), while other demographics were comparable. Significant differences were observed in $CD3^+CD4^+IL\text{-}17^+$ T helper 17 (Th17) cells, $CD4^+CD25^{high}CD127^{low}$ Treg cells, Th17/Treg ratio, IL-17, IL-10, and IL-17/IL-10 ratio (all $p<0.0001$). INRs were characterized with lower $CD3^+CD4^+IL\text{-}17^+$ T helper 17 (Th17) cells, IL-17 levels, Th17/Treg and IL-17/IL-10 ratios and but higher

$CD4^+CD25^{high}CD127^{low}$ Treg cells and IL-10 levels. Th17/Treg and IL-17/IL-10 ratios positively correlated with $CD4^+$ T cell counts. In logistic regression analysis, higher Th17/Treg ratio (AOR = 0.05) and IL-17/IL-10 ratio (AOR = 0.60) were independently associated with lower odds of immunological non-response. Age (AOR = 1.05) and cART duration (AOR = 0.55) were not significantly associated.

Conclusion: Inadequate immune reconstitution is associated with reduced Th17/Treg ratio and IL-17/IL-10 imbalances.

Key words: HIV, immune reconstitution, Th17 cells, regulatory T cells, IL-7, IL-10, Kenya

Introduction

The introduction of combination antiretroviral therapy (cART) has markedly reduced HIV-related morbidity and mortality by achieving sustained viral suppression and immune reconstitution among people living with HIV (PLWHIV) (1). In most PLWHIV, viral suppression is accompanied by gradual immune reconstitution, evidenced by absolute $CD4^+$ T cell counts. However, immune reconstitution is usually heterogeneous, and approximately 7-40% of PLWHIV fail to achieve adequate absolute $CD4^+$ T cell count reconstitution despite viral suppression, a phenomenon referred to as immunological non-response (INR) (2).

Immunological non-responders (INRs) are at a higher risk of opportunistic infections, non-AIDS-defining illnesses, chronic inflammation, and increased all-cause mortality compared with immunological responders (IRs), even in the absence of detectable viremia (3). Persistent immune activation driven by microbial translocation from a compromised gut mucosal barrier, residual viral replication from latent reservoirs, and co-infections, and dysregulated immune homeostasis brought about by an imbalance between $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17) cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells, immune exhaustion, and impaired thymic output**,** are thought to underlie this impaired immune reconstitution despite effective cART in PLWHIV (4). Together, these mechanisms create a self-perpetuating cycle of immune activation, inflammation, and immunosuppression, ultimately resulting in inadequate absolute $CD4^+$ T cell count reconstitution despite sustained viral suppression (5).

Among $CD4^+$ T cell subsets, $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17) cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells play a central role in maintaining immune balance. $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17) cells are critical for mucosal immunity, particularly within the gastrointestinal tract, where they promote epithelial barrier integrity and protect against microbial translocation (6). In contrast, $CD4^+CD25^{high}CD127^{low}$ Treg cells suppress immune activation and inflammation, primarily through cell-contact mechanisms and secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10) (7).

During HIV infection, $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17) cells are preferentially depleted early, especially at mucosal sites, leading to disruption of the gut barrier and increased microbial translocation, which perpetuates systemic immune activation (8). Although cART partially

reconstitutes absolute $CD4^+$ T cell counts, $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cells recovery is often incomplete, particularly among INRs (9). Concurrently, expansion or functional dominance of $CD4^+CD25^{high}CD127^{low}$ Treg cells may suppress effective immune regeneration and T cell proliferation (10).

Signature cytokine profiles can provide functional insight into $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells dynamics. Interleukin-17 (IL-17), produced mainly by $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cells, promotes antimicrobial defense and tissue repair (11), while IL-10, produced by $CD4^+CD25^{high}CD127^{low}$ Treg cells and other immune cells, limits excessive immune activation (12). Although IL-10 plays a protective role during acute inflammation, persistently elevated IL-10 levels during chronic HIV infection may impair immune reconstitution by suppressing T cell activation and proliferation (13). The IL-17/IL-10 ratio therefore reflects the balance between pro-inflammatory and regulatory immune responses and may serve as an important immunological biomarker to assess immune reconstitution.

Despite growing evidence linking $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio imbalance to immune non-response, data from sub-Saharan Africa remain limited. Given the high HIV burden and frequent late initiation of cART in this region, understanding $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cells/immune regulatory mechanisms underlying suboptimal immune reconstitution is critical in providing effective care. This study aimed to evaluate $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cells, $CD4^+CD25^{high}CD127^{low}$ Treg cells and IL-17/IL-10 ratios as factors associated with immune reconstitution failure among PLWHIV on long-term cART at Academic Model Providing Access to Healthcare (AMPATH), MTRH, Eldoret, Kenya. We hypothesized that INRs would demonstrate an imbalance in $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells homeostasis, characterized by reduced $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cell frequencies, increased $CD4^+CD25^{high}CD127^{low}$ Treg cell proportions, and lower $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratios, as well as cytokine dysregulation characterized by decreased IL-17 levels, increased IL-10 levels, and reduced IL-17/IL-10 ratios compared to IRs and healthy volunteers despite effective virological suppression with cART.

Methods and materials

Study setting

The study was conducted at the AMPATH MTRH HIV adult Module III clinic, Eldoret, Kenya.

Study design and period

This was a cross-sectional design with retrospective abstraction of clinical records. Participants were assessed at a single time point to evaluated the association between immune profiles and immunological response status and therefore could not establish causal or temporal changes in immune reconstitution pattern over time. IRs, INRs, and healthy HIV-negative individuals were compared between and within groups.

Population and criteria

A total of 64 adults PLWHIV aged 18-65 years were recruited from the AMPATH MTRH HIV Adult Module III Clinic. Eligible participants were receiving routine HIV care, had been maintained on the same first-line cART regimen consisting of dolutegravir (DTG), abacavir (ABC), and lamivudine (3TC) for 1-6 years, demonstrated sustained virological suppression (<50 copies/mL) for at least one year prior to recruitment (2019-2024), had baseline absolute $CD4^+$ T cell counts <500 cells/µL, exhibited good

treatment adherence, and had no documented co-infections or severe acute illness at the time of enrollment. Participants were recruited using a consecutive sampling approach, whereby all eligible PLWHIV attending scheduled clinic visits during the study period were invited to participate until the target sample size was achieved. The inclusion of all consecutively eligible PLWHIV minimized selection bias and enhanced the representativeness of virologically suppressed PLWHIV receiving long-term cART at the study site.

The study included 32 healthy HIV-negative volunteers recruited from hospital employees, patient caretakers, and community blood donors. To broaden the recruitment base and reduce potential selection bias associated with recruiting exclusively from healthcare workers, participants were drawn from both hospital-based and community-based populations. Prior to enrollment, all healthy volunteers underwent a clinical assessment by a trained Clinical Officer, which included clinical observation and a review of self-reported health status. Individuals reporting a history of chronic inflammatory conditions, autoimmune diseases, chronic infections, acute illness, or ongoing medical treatment were not enrolled. In addition, participants were informed that their blood samples would undergo HIV screening, and only individuals confirmed to be HIV-negative were included in the study. These measures were implemented to ensure that the healthy control group was free from known conditions that could significantly influence immunological parameters and cytokine profiles.

IRs had absolute $CD4^+$ T cell counts ≥500 cells/µl, and INRs had absolute $CD4^+$ T cell counts <500 cells/µl after 1-6 years of cART. The inclusion of IRs and INRs with 1-6 years of cART exposure was intended to reflect reflects real-world treatment heterogeneity of long-term treated PLWHIV in routine clinical care and was evaluated as a potential covariate in subsequent analyses. All participants had been maintained on the same first-line cART regimen consisting of dolutegravir (DTG), abacavir (ABC), and lamivudine (3TC), had sustained viral suppression and were classified based on immunological response rather than treatment duration.

To minimize potential confounding and enhance internal validity, healthy HIV-negative participants were sex-matched to PLWHIV participants (IRs and INRs). This approach ensured a balanced distribution of these demographic characteristics between groups and reduced potential confounding arising from sex-related differences in immune parameters. Although healthy HIV-negative participants were recruited partly from hospital staff and blood donors, the inclusion of caregivers and community blood donors broadened the recruitment base and helped mitigate potential health worker bias. This matching strategy improved comparability between study groups while maintaining feasibility of recruitment within the study setting. Nevertheless, residual confounding from unmeasured factors such as nutritional status, behavioral factors, psychological stress, mental health status, host genetic factors, physical activity and lifestyle factors, could not be completely excluded.

Identified participants who met the inclusion criteria were invited to sign an informed consent form before recruitment after explaining the study protocol. Patient's clinical and demographic characteristics including baseline absolute $CD4^+$ T cell counts, age, gender, cART treatment duration, marital and employment status were obtained by interviewer administered questionnaires. The data obtained was verified against participants' medical records using a pre-tested data abstraction form to ensure the accuracy and validity of the collected data. A total of 96 participants were enrolled.

Sample size determination

Sample size was estimated for a one-way analysis of variance (ANOVA) comparing three

independent groups: INRs, IRs, and healthy HIV-negative controls. In the absence of reliable locally derived estimates and to avoid overestimation of effect magnitude from small prior studies, a moderate effect size (Cohen's f = 0.25) was assumed, consistent with Cohen's recommendations for ANOVA (14, 15). The significance level was set at $\alpha = 0.05$ and statistical power at 80% ($1-\beta = 0.80$).

Using standard ANOVA power analysis implemented in G*Power version 3.1, assuming three independent groups, a moderate effect size (Cohen's $f = 0.25$), $\alpha = 0.05$, and 80% power, the minimum required total sample size was estimated at 159 participants, corresponding to 53 participants per group. To account for an anticipated 10% non-response or incomplete data rate, the sample size was adjusted as follows:

$$n_{adjusted} = \frac{53}{0.90} = 58.9 \approx 59$$

Thus, the targeted sample size was 59×3=177 participants comprising 59 participants per group. However, due to financial constraints, limited reagent availability, and the high costs associated with flow cytometric immunophenotyping and cytokine assays, the final analyzed sample comprised 96 participants (32 participants per group). Although the final sample size was smaller than the estimated target sample size, statistically significant differences were observed for several primary immunological outcomes. Nevertheless, the reduced sample size may have decreased the statistical power to detect small-to-moderate differences between groups, thereby increasing the risk of Type II error. Therefore, nonsignificant findings should be interpreted with caution.

Laboratory procedures

Approximately 4ml of peripheral blood samples was drawn by a phlebotomist from each recruited study participant in each study group by venipuncture to the antecubital vein and collected into sterile ethylenediamine tetracetic acid (EDTA) BD vacutainer blood collection tubes. Median peripheral blood percentages of $CD3^{+}CD4^{+}$ $IL\text{-}17^{+}$ T helper 17 (Th17) cells were determined using BD FACSCalibur flow cytometer (BD Biosciences, San Diego, CA, USA) according to manufacturer's recommendation using BD anti-CD3 FITC, anti-CD4 APC and anti-Human IL-17A PE monoclonal antibodies. Median peripheral blood percentages of $CD4^{+}CD25^{high}CD127^{low}$ Treg cells were determined using CytoFLEX flow cytometer (Beckman coulter, USA) according to manufacturer's recommendation using BD Human Regulatory T Cell cocktail (CD4-FITC/CD25-PE/CD127-Alexa) monoclonal antibodies.

To minimize inter-instrument variability between the BD FACSCalibur and CytoFLEX flow cytometers, standardized sample preparation, staining protocols, and consistent gating strategies were applied across all analyses. Both instruments were routinely calibrated using manufacturer-recommended quality control procedures, and results were expressed as percentages to reduce platform-related variability. Plasma IL-17 levels were quantified using Human IL-17A Enzyme-linked Immunosorbent Assay (ELISA) kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Plasma IL-10 levels were quantified using Human IL-10 High Sensitivity Enzyme-linked Immunosorbent Assay (ELISA) kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA. CAT NO. BMS215HS). Quality control measures included daily instrument calibration and performance verification using manufacturer-recommended calibration beads. All assays were conducted in accordance with standardized protocols provided by BD Biosciences, Thermo Fisher Scientific, and internal standard operating procedures at AMPATH Reference Laboratory.

Data entry and analysis

Data were entered into Excel before being analyzed using SPSS version 29.0. The normality of continuous variables was assessed using the Shapiro-Wilk test, which is appropriate for small sample sizes, and complemented by visual inspection of histograms and normal Q-Q plots. The data were found to be non-normally distributed; therefore, non-parametric statistical methods were applied. Continuous variables were summarized as medians with interquartile ranges (IQR), while categorical variables were expressed as frequencies and percentages. All statistical tests were two-tailed, and $p<0.05$ was considered statistically significant. Demographic and categorical variables, including gender, marital status, employment status, and cART treatment duration, were compared across the study groups using the Chi-square (χ^2) test. Fisher's exact test was used where expected cell counts were less than five. Participants' ages were compared among study groups using the Kruskal-Walli's test. The median peripheral blood percentages of $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17) cells, $CD4^+CD25^{high}CD127^{low}$ Treg cells, $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio, plasma levels of IL-17 and IL-10, and IL-17/IL-10 ratio were compared among study groups using the Kruskal-Walli's test. Pairwise intergroup comparisons were performed using Dunn's multiple comparisons post-hoc test. The correlation between $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio, IL-17/IL-10 ratio, and absolute $CD4^+$ T cell counts among INRs, IRs, and healthy HIV-negative individuals was evaluated using Spearman's rank correlation coefficient (ρ). $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells and IL-17/IL-10 ratios were pre-specified as composite variables to reflect the balance between pro-inflammatory and regulatory immune responses. These ratios were analyzed alongside their individual components to ensure that observed associations were not driven solely by ratio-based measures.

A multivariable logistic regression analysis was performed to determine factors independently associated with immunological response while adjusting for confounders. The dependent variable was coded as immunological non-response (INR) = 1 and immunological response (IR) = 0, with IR serving as the reference category. Variables entered into the regression model were selected based on biological plausibility, evidence from previous literature, and their clinical relevance to immune reconstitution. Because only 32 INR events were observed, inclusion of a large number of variables would have increased the risk of model overfitting and unstable parameter estimates. Therefore, the final model was intentionally restricted to four biologically relevant variables: treatment duration (years), age (years), $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio and IL-17/IL-10 ratio. These variables were selected because they represent complementary measures of treatment exposure, immune reconstitution, immune regulation, and inflammatory balance, which were central to the study hypothesis. Sociodemographic variables and other candidate variables were not included in the final model because they were not primary variables of interest and their inclusion would have exceeded the recommended events-per-variable ratio for reliable logistic regression modelling. Model diagnostics included assessment of goodness-of-fit using the likelihood ratio test and evaluation of multicollinearity. Model diagnostics were performed to assess goodness-of-fit using the likelihood ratio test and McFadden's pseudo-R^2 statistic. Potential multicollinearity among predictor variables was evaluated using variance inflation factors (VIFs), with VIF values less than 5 considered indicative of acceptable collinearity. Model convergence was also assessed to ensure reliable parameter estimation. No evidence of multicollinearity was observed. The assumptions of logistic regression, including independence of observations, absence of multicollinearity, linearity of continuous variables in the logit, and adequate events-per-(0

to 1 scale), in which the higher value indicated that the model predicts well.

Results

Demographic and clinical characteristics of the study population

Thirty-two (32) healthy HIV-negative individuals, 32 INRs, and 32 IRs were included in the analysis. There was no significant difference in gender distribution within and across the three groups (p=0.482). Females comprised 25.0% of healthy HIV-negative individuals, 65.6% of INRs, and 84.4% of IRs. Median age (years) differed significantly between groups (p=0.026). Immunological non-responders (INRs) had the highest median age 44 (IQR:26-63), IRs 38 (IQR:19-62) and healthy HIV-negative individuals 35 (IQR:21-62). Marital status did not significantly differ across groups (p=0.182). The majority of participants were married in the healthy HIV-negative comparison group (71.9%), IR group (68.8%), and INRs (78.1%). Employment status was comparable between groups (p=0.371), with formal employment reported in 53.1% of healthy HIV-negative comparison group, 28.1% of INRs, and 34.4% of IRs.

Baseline absolute CD4^{+} T cell count at cART initiation differed significantly between INRs and IRs (p=0.016). A majority of INRs (68.8%) initiated treatment with CD4^{+} T cell counts <200 cells/µL, whereas all IRs (100.0%) initiated treatment with CD4^{+} T cell counts ≥200 cells/µL. Treatment duration since cART initiation was also significantly different between INRs and IRs (p=0.002). Most participants in both groups had been on treatment for ≥2 years (81.2% of INRs and 87.5% of IRs), while a smaller proportion had been on treatment for <2 years (18.8% of INRs and 12.5% of IRs) (Table 1).

Table 1: Demographic and clinical characteristics of study participants

Characteristics	Healthy HIV-negative Individuals (n = 32)	Immunological non-responders (INRs) (n = 32)	Immunological Responders (IRs) (n = 32)	p-value
Gender, n (%)				0.482
Female	8 (25.00)	21 (65.60)	27 (84.40)	
Male	24 (75.00)	11 (34.40)	5 (15.60)	
Age (Years)				0.026
Median (IQR)	35 (21-62)	44 (26-63)	38 (19-62)	
Marital Status, n (%)				0.182
Single	9 (28.10)	25 (78.10)	10 (31.20)	
Married	23 (71.90)	7 (21.90)	22 (68.80)	
Employment status, n (%)				0.371
Employed	17 (53.10)	9 (28.10)	11 (34.40)	
Unemployed	15 (46.90)	23 (71.90)	21 (65.60)	
Treatment Duration since cART Initiation, n (%)				
< 2 years		6 (18.80)	4 (12.50)	0.002
≥ 2 years		26 (81.20)	28 (87.50)	

Peripheral blood percentage of $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17) cells among study participants

The percentage of $CD3^+CD4^+IL\text{-}17^+$ T helper 17 (Th17) cells differed significantly across study groups (Kruskal-Wallis $p<0.0001$). Median (IQR) values were lowest in INRs [0.52% (0.42- 0.73)], intermediate in IRs [1.41% (1.06-1.64)], and highest in healthy HIV-negative individuals [2.24% (1.83-2.62)]. Post-hoc analysis confirmed significant differences between all pairwise comparisons (IRs and INRs: $p=0.0017$; INRs and healthy: $p<0.0001$; IRs vs healthy: $p<0.0001$) (Fig. 1).

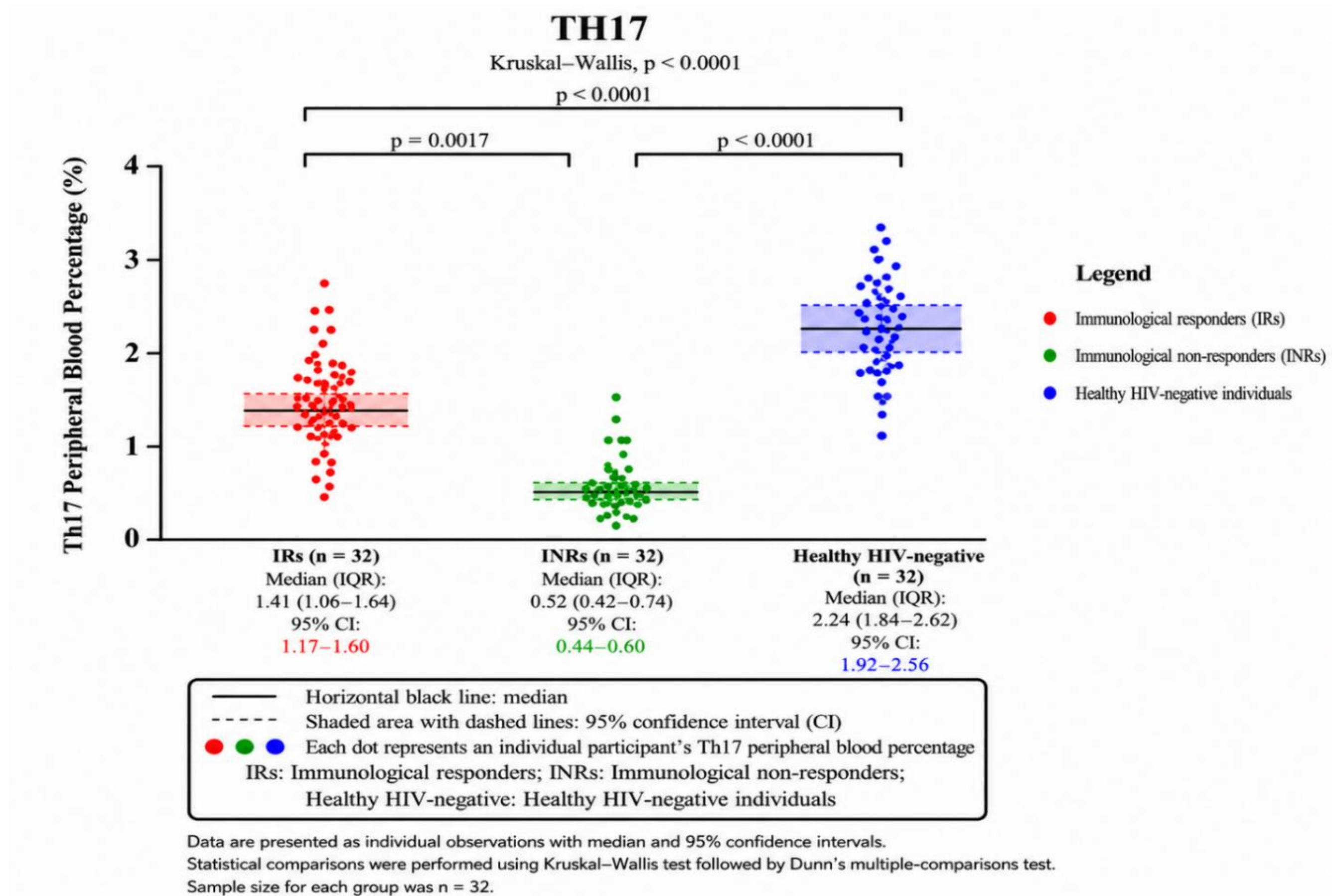

Figure 1: Peripheral blood percentage of CD3+CD4+ IL-17+ T helper 17 (Th17) cells among study participants

Peripheral blood percentage of $CD4^+CD25^{high}CD127^{low}$ Treg cells among study participants

A significant difference in $CD4^+CD25^{high}CD127^{low}$ Treg cells percentages was also observed across groups ($p<0.0001$). INRs had the highest levels [7.64% (6.67-8.86)],

followed by IRs [5.85% (5.28-6.68)], while healthy individuals had the lowest levels [3.91% (3.60-5.11)]. All pairwise comparisons were statistically significant [IRs and INRs (p=0.0002), INRs and healthy HIV-negative individuals (p<0.0001), and IRs and healthy HIV-negative individuals (p=0.0003)] (Fig. 2).

$CD3^+CD4^+$ IL-17^+ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg Cells Ratios Among Study Participants

The $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio differed significantly between groups (p<0.0001), with progressively increasing values from INRs [0.08 (0.06-0.10)] to IRs [0.24 (0.18-0.28)] and healthy individuals [0.51 (0.41-0.63)]. Pairwise comparisons were significant for all groups [IRs and INRs (p<0.001), INRs and healthy HIV-negative individuals (p<0.0001), and IRs and healthy HIV-negative individuals (p<0.01)].

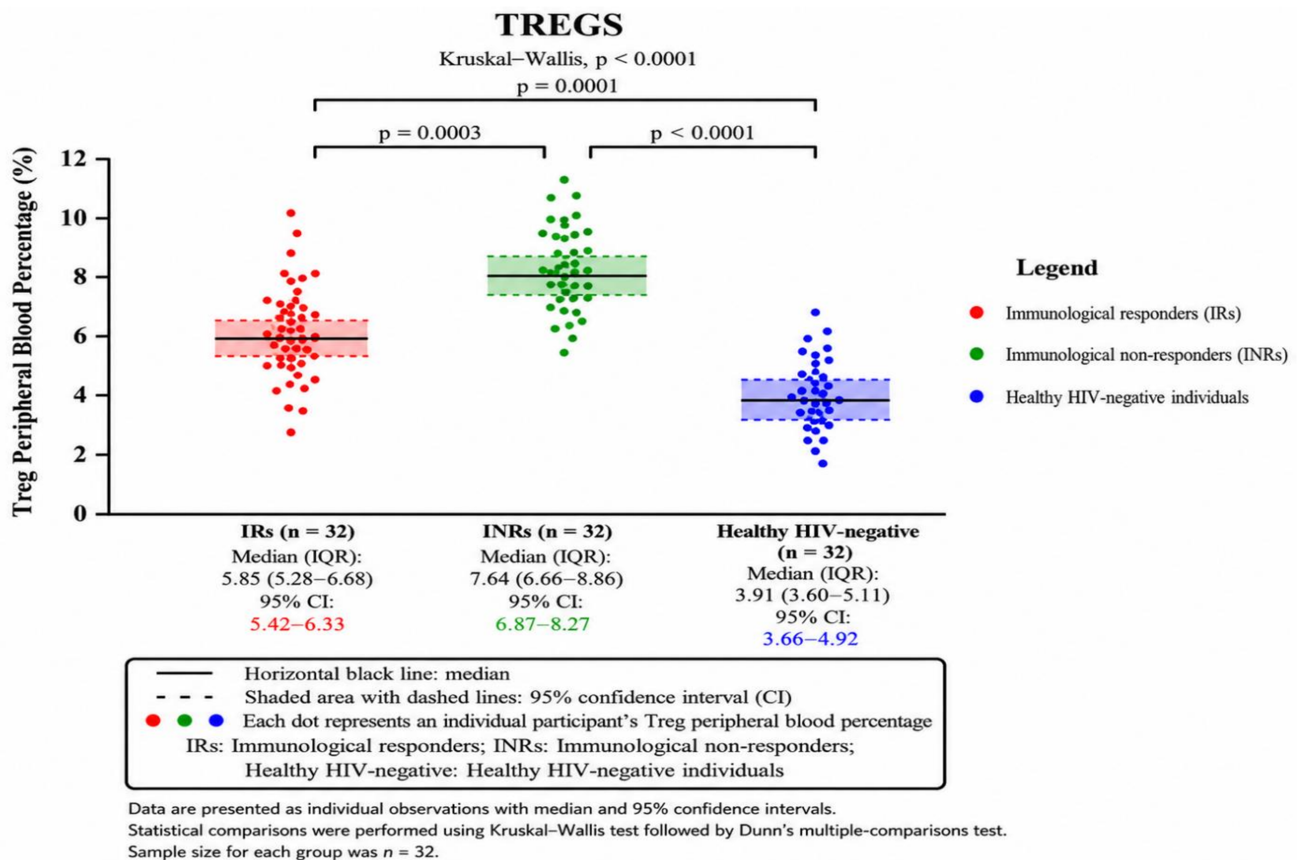

Figure 2: Peripheral blood percentage of CD4+CD25highCD127low Treg cells among study participants.

Correlation Between $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg Cells Ratio and Absolute $CD4^+$ T Cell Counts among study participants

Among IRs, the $CD3^+CD4^+$ IL-17^+ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio showed a strong positive correlation with absolute $CD4^+$ T cell counts (ρ=0.7483, p<0.0001) (Fig. 3A). A weaker but statistically significant correlation was observed in INRs (ρ=0.3567, p=0.0450) (Fig. 3B), while a very strong correlation was seen in healthy individuals

(ρ=0.957, p<0.0001) (Figure 3C).

Plasma Interleukin 10 (IL-10) and IL-17 Levels Among Study Participants

Significant differences in plasma IL-10 levels were observed across groups (p<0.0001), with highest levels in INRs [0.34 pg/ml (0.269-0.427)], followed by IRs [0.243 pg/ml (0.190-0.296)] and lowest levels in healthy individuals [0.097 pg/ml (0.072-0.120)].

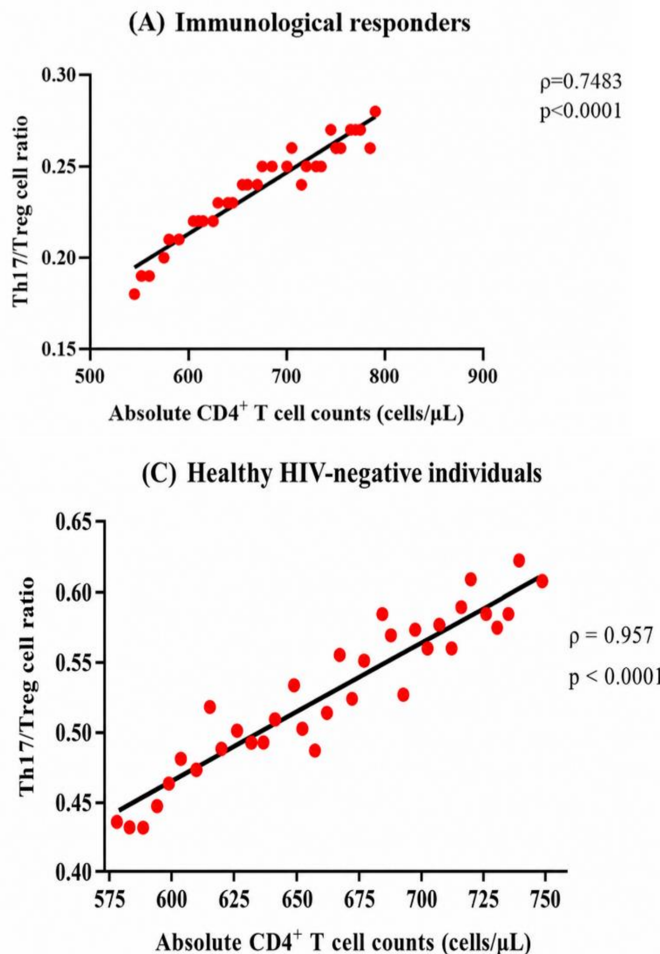

Fig. 3 (A, B and C): Correlation between $CD3^{+}CD4^{+}$ $IL-17^{+}$ T helper 17 (Th17)/$CD4^{+}CD25^{high}CD127^{low}$ Treg cells ratio and absolute $CD4^{+}$ T cell count among (A) Immunological responders (IRs), (B) Immunological non-responders (INRs), and (C) Healthy HIV-negative individuals. Red circles represent individual participants. The solid black line represents the linear regression fit. Correlations were assessed using Spearman's rank correlation test. Sample sizes were IRs (n=32), INRs (n=32), and healthy controls (n=32).

Pairwise comparisons were significant [IRs and INRs (p = 0.1245), IRs and healthy HIV-negative individuals (p<0.0001), and INRs and healthy HIV-negative individuals (p<0.0001)] (Figure 4). Similarly, plasma IL-17 levels differed significantly (p<0.0001), increasing from INRs [0.19 pg/ml (0.134-0.234)] to IRs [0.29 pg/ml (0.211-0.360)] and healthy individuals [0.42 pg/ml (0.311-0.480)]. All pairwise differences were statistically significant [IRs and INRs (p = 0.0042), IRs and healthy HIV-negative individuals (p = 0.0074), and INRs and healthy HIV-negative individuals (p<0.0001)] (Fig. 5).

Interleukin 17 (IL-17)/Interleukin 10 (IL-10) Ratios among study participants

The IL-17/IL-10 ratio varied significantly across groups (p<0.0001), with lowest values in INRs [0.53 (0.419-0.855)], intermediate in IRs [1.23

(0.949-1.606)], and highest in healthy individuals [3.71 (2.680-5.631)]. All pairwise comparisons were significant ($p<0.001$).

Correlation between Interleukin 17 (IL-17)/Interleukin 10 (IL-10) Ratios and Absolute $CD4^{+}$ T Cell Count among study participants

A strong positive correlation was observed between the IL-17/IL-10 ratio and absolute $CD4^{+}$ T cell counts in IRs ($\rho=0.9597$, $p<0.0001$) (Fig. 6A). Moderate but significant correlation was observed in INRs ($\rho=0.4469$, $p=0.0103$) (Fig. 6B), while a very strong correlation was found in healthy individuals ($\rho=0.983$, $p<0.0001$) (Fig. 6C).

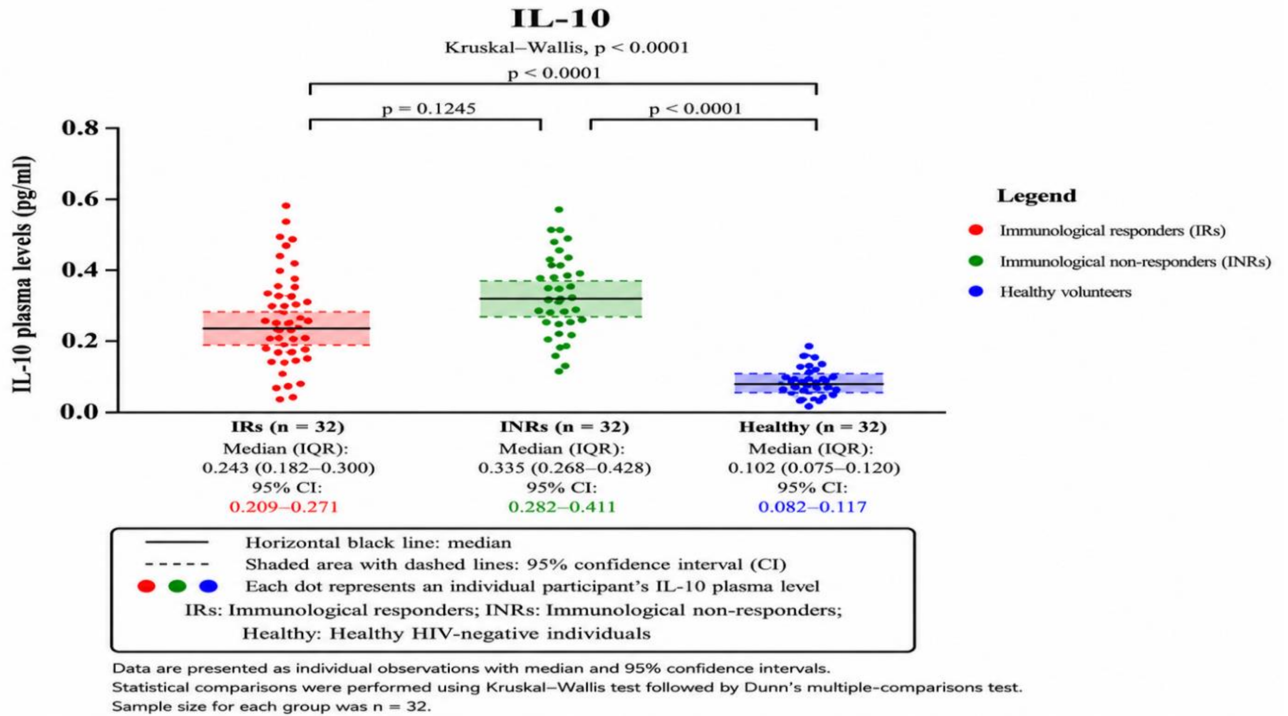

Figure 4: Plasma interleukin 10 (IL-10) levels among study participants

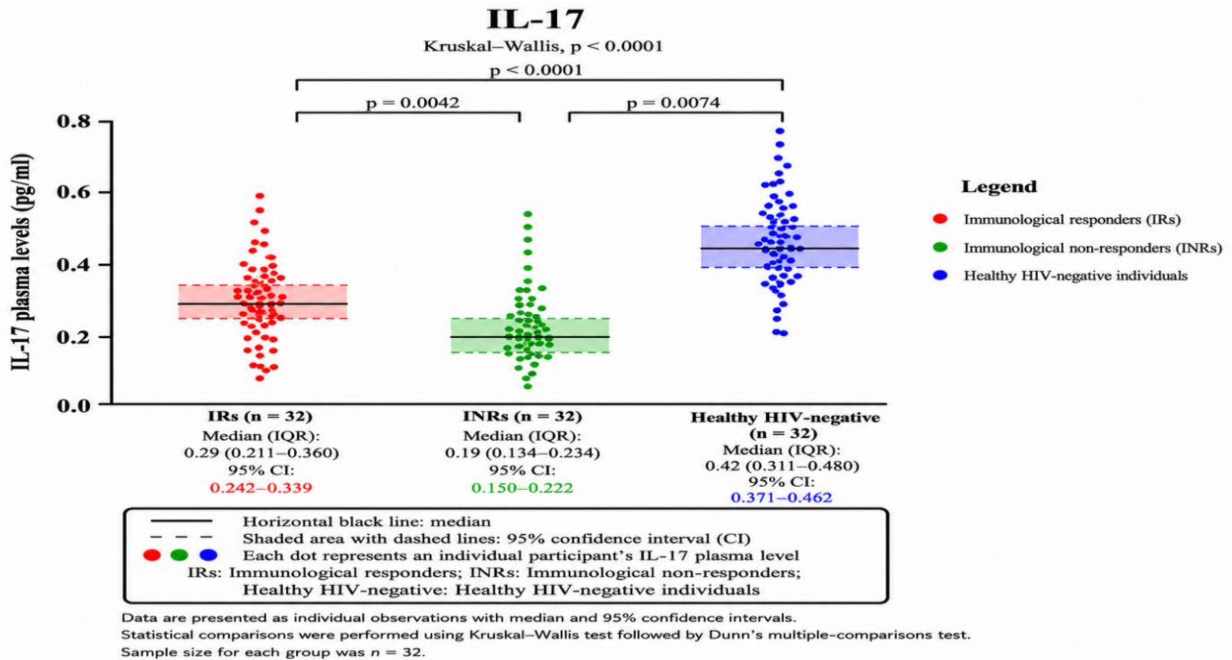

Figure 5: Plasma interleukin 17 (IL-17) levels among study participants

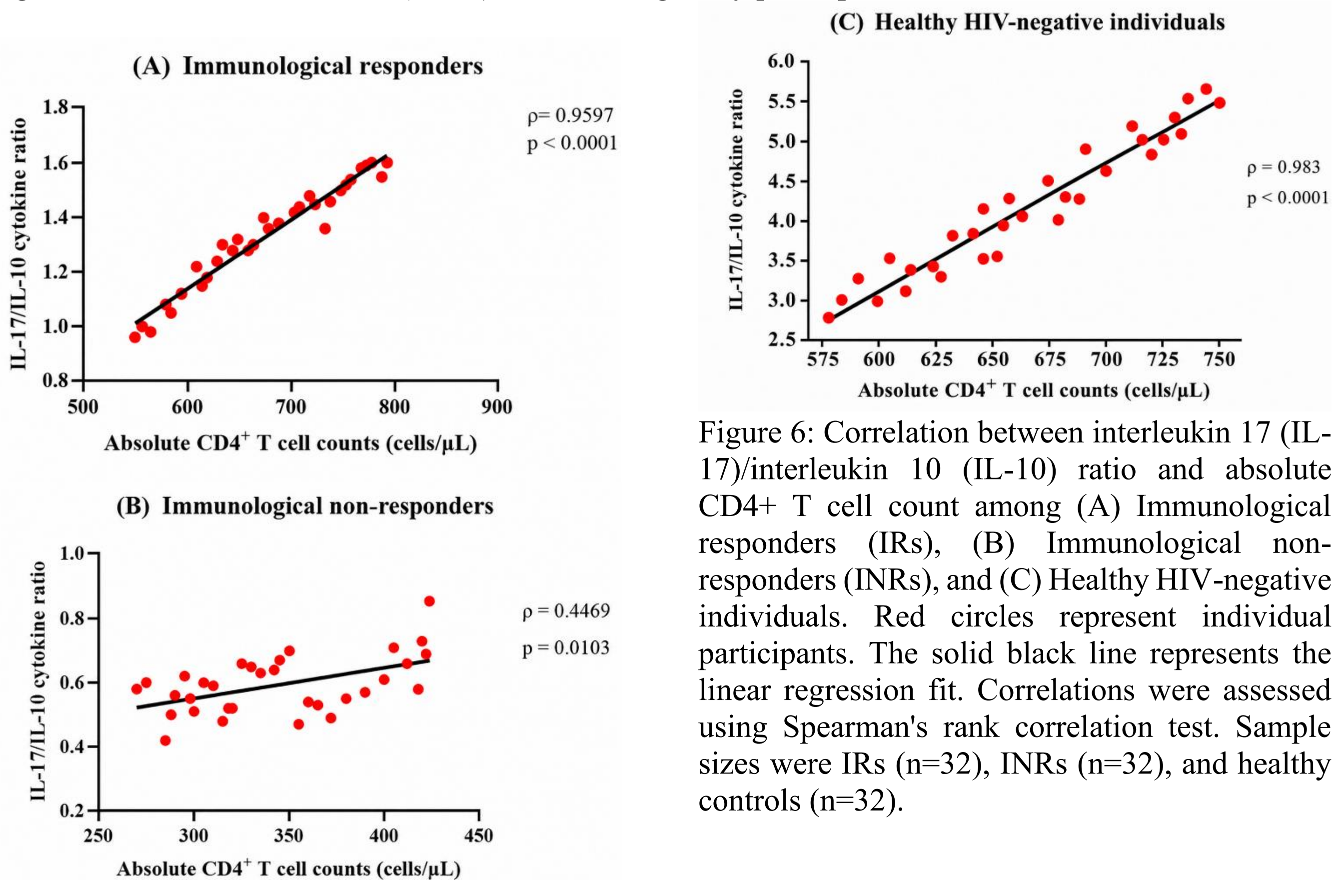

Figure 6: Correlation between interleukin 17 (IL-17)/interleukin 10 (IL-10) ratio and absolute CD4+ T cell count among (A) Immunological responders (IRs), (B) Immunological non-responders (INRs), and (C) Healthy HIV-negative individuals. Red circles represent individual participants. The solid black line represents the linear regression fit. Correlations were assessed using Spearman's rank correlation test. Sample sizes were IRs (n=32), INRs (n=32), and healthy controls (n=32).

Factors Independently associated with Immunological Response Among Immunological Responders (IRs) and immunological non-responders (INRs)

A multivariable logistic regression model was fitted to determine factors independently associated with immunological non-response (INR) among PLWHIV, with immunological response (IR) serving as the reference group. The overall model was statistically significant, indicating good explanatory power. The analysis demonstrated that both $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cell ratio and IL-17/IL-10 ratio were independently associated with immunological response status. Higher $CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cell ratio was associated with significantly reduced odds of immunological non-response (AOR = 0.05, 95% CI: 0.008-0.298; p = 0.001). Similarly, higher IL-17/IL-10 ratio was associated with significantly reduced odds of immunological analyses and supports the stability and reliability of the model estimates. A total of 64 PLWHIV were included in the analysis, with equal non-response (AOR = 0.60, 95% CI: 0.433-0.833; p = 0.002). In contrast, age (AOR = 1.05, 95% CI: 0.951-1.164; p = 0.321) and treatment duration (AOR = 0.55, 95% CI: 0.282-1.087; p = 0.086) were not independently associated with immunological non-response after adjustment for the other variables in the model (Table 2).

The multivariable regression model converged successfully and demonstrated good overall fit and stability (McFadden pseudo-R^2 = 0.723; likelihood-ratio test $p < 0.001$), indicating that the estimated regression coefficients were stable and that the model adequately explained variation in immunological response status. No evidence of problematic multicollinearity was observed among the independent variables (VIF range: 1.07-1.12). The final multivariable logistic regression model included four variables and 32 outcome events, yielding approximately eight events per variable, which is within the recommended range for logistic regression

distribution between INRs and IRs, each comprising 32 (50%) of the total PLWHIV that were enrolled in the study.

Table 2: Factors independently associated with immunological response among immunological responders (IRs) and immunological non-responders (INRs)

Variable	IRs n (%)	INRs n (%)	AOR (95% CI)	p-value
$CD3^+CD4^+$ $IL\text{-}17^+$ T helper 17 (Th17)/$CD4^+CD25^{high}CD127^{low}$ Treg cells ratio	32 (50.0)	32 (50.0)	0.05 (0.008-0.298)	0.001
IL-17/IL-10 ratio	32 (50.0)	32 (50.0)	0.60 (0.433-0.833)	0.002
Age (years)	32 (50.0)	32 (50.0)	1.05 (0.951-1.164)	0.321
Treatment duration (years)	32 (50.0)	32 (50.0)	0.55 (0.282-1.087)	0.086

Outcome: Immunological non-response (INR = 1) Reference category: Immunological responder (IR = 0); INR: Immunological non-response;IR: Immunological response;INRs were coded as the outcome event (INR = 1), whereas IR served as the reference category (IR = 0);IRs : Immunological responders;INRs: Immunological non-responders;CI:Confidence interval; AOR (Adjusted Odds ratios) <1 indicate lower odds of immunological non-response; Statistical significance was set at $p < 0.05$;Model diagnostics: McFadden pseudo-R^2 = 0.723;

likelihood-ratio test $p < 0.001$; variance inflation factor (VIF) range = 1.070-1.120, indicating no evidence of problematic multicollinearity

Discussion

This study demonstrates that inadequate immune reconstitution among PLWHIV on long-term cART is characterized by a significant imbalance between CD3+CD4+ IL-17+ T helper 17 (Th17) cells and CD4+CD25highCD127low Treg cells, characterized by reduced CD3+CD4+ IL-17+ T helper 17 (Th17) cell frequencies, increased CD4+CD25highCD127low Treg cell proportions, and consequently a decreased CD3+CD4+ IL-17+ T helper 17 (Th17)/CD4+CD25highCD127low Treg cells ratio. This is further accompanied by cytokine imbalance, characterized by reduced IL-17 levels alongside elevated IL-10 levels, resulting in a decreased IL-17/IL-10 ratio. These alterations persist despite sustained virological suppression, highlighting apparent discordance between viral load control and effective immune reconstitution. Immunological non-responders (INRs) exhibited reduced CD3+CD4+ IL-17+ T helper 17 (Th17) cells frequencies, expanded CD4+CD25highCD127low Treg cells populations, and lower CD3+CD4+ IL-17+ T helper 17 (Th17)/CD4+CD25highCD127low Treg cells ratios compared with IRs and healthy HIV-negative individuals. The observed lower CD3+CD4+ IL-17+ T helper 17 (Th17) cells percent among INRs is consistent with previous studies showing preferential loss and incomplete restoration of this subset during HIV infection (13). Incomplete restoration of CD3+CD4+ IL-17+ T helper 17 (Th17) cells despite viral suppression may contribute to poor viral control, persistent microbial translocation and chronic immune activation, all of which are strongly associated with poor immune reconstitution (16).

In contrast, elevated CD4+CD25highCD127low Treg cells frequencies observed among INRs suggest enhanced immune regulatory activities. While CD4+CD25highCD127low Treg cells play a crucial role in limiting immune-mediated tissue damage, excessive or sustained CD4+CD25highCD127low Treg cells expansion may suppress beneficial immune responses necessary for effective $CD4^+$ T cell regeneration (10). This dual role of CD4+CD25highCD127low Treg cells may partially explain why immune activation persists alongside immune suppression in the studied INRs participants.

The cytokines in this study mirror CD3+CD4+ IL-17+ T helper 17 (Th17) cells and CD4+CD25highCD127low Treg cells and thus reinforce these cellular findings. Reduced IL-17 levels among INRs reflect impaired CD3+CD4+ IL-17+ T helper 17 (Th17) cells function, whereas elevated IL-10 levels indicate heightened immune regulation that limit absolute $CD4^+$ T cell count (17). Similar cytokine/cell patterns have been reported in previous studies of PLWHIV exhibiting poor immune reconstitution (13). Further, IL-17 and IL-10 have been identified as key immunological biomarkers reflecting disease progression and immune dysfunction in PLWHIV (18). Importantly, the IL-17/IL-10 ratio was significantly lower among INRs and independently associated with absolute $CD4^+$ T cell count reconstitution, suggesting that immune balance rather than absolute cytokine levels may be a more reliable predictor of immune reconstitution.

The unusually strong correlations observed among healthy HIV-negative individuals may reflect the intact and tightly regulated immune system in healthy individuals, where coordinated interactions between immune cells and cytokines are preserved. In contrast, HIV infection is associated with immune dysregulation, which may weaken these relationships. However, the strength of correlations in the control group may also be influenced by the relatively small sample size and limited variability, which can inflate correlation coefficients. Therefore, these findings should be interpreted with caution.

A key implication of these findings is that viral load monitoring alone may fail to capture the underlying functional immune dysregulation in some PLWHIV. Despite achieving virological suppression, INRs retained immunological abnormalities and risk of disease progression. This observation is supported by recent evidence demonstrating that immune dysfunction, inflammation, and immune reconstitution abnormalities persist even in virologically suppressed PLWHIV (19). Therefore, viral load suppression to below the detection limit by cART should not be equated to complete immune restoration.

The combined assessment of CD3+CD4+ IL-17+ T helper 17 (Th17) cells and CD4+CD25highCD127low Treg cells dynamics alongside IL-17 and IL-10 cytokine profiles, together with viral load monitoring, provides deeper insight into both the quantitative magnitude and qualitative functional aspects of immune reconstitution. These biomarkers capture immune dysfunction that remains undetected by conventional viral load monitoring and may serve as valuable tools for identifying PLWHIV at risk of persistent immunological failure. Furthermore, emerging studies suggest that addressing the CD3+CD4+ IL-17+ T helper 17 (Th17)/CD4+CD25highCD127low Treg cells ratio axis may offer a novel therapeutic opportunity to improve long-term outcomes in PLWHIV (20).

Unlike studies from high-income settings, this study provides evidence from a sub-Saharan African context, particularly Kenya, where late presentation and prolonged immune damage are common. These factors, including late presentation, are known to exacerbate immune dysregulation and limit the capacity for full immune reconstitution despite effective cART.

This study has several limitations that should be considered when interpreting the findings. The cross-sectional study design limits the ability to establish causal relationships between immunological profiles and immune reconstitution outcome. Additionally, the relatively small sample size may have reduced the precision of some estimates. It was also not possible to match participants across study groups for age and other variables, which may have influenced the observed outcomes. Furthermore, residual confounding from both measured and unmeasured variables cannot be completely excluded. Despite these limitations, statistically significant differences were detected across the primary immunological outcomes, and the findings are consistent with existing literature. Moreover, methodological approaches, including strict participant selection criteria and multivariable logistic regression analysis, were employed to enhance the validity and robustness of the results.

Conclusion

Immunological non-responders (INRs) are characterized by immune dysregulation, reflected by reduced CD3+CD4+ IL-17+ T helper 17 (Th17) cell frequencies, expanded CD4+CD25highCD127low Treg cell proportions, and a lower CD3+CD4+ IL-17+ T helper 17 (Th17)/CD4+CD25highCD127low Treg cells ratio, alongside elevated IL-10 levels, reduced IL-17 levels, and a diminished IL-17/IL-10 ratio despite effective virological suppression with cART. These findings indicate an association between altered immune profiles and incomplete immune reconstitution, highlighting persistent immunological imbalance in INRs. Therapeutic targeting of the immune balance may improve immune reconstitution among INRs. Longitudinal studies are required to validate the clinical utility of these biomarkers and establish standardized thresholds for identifying immunological non-response.

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Ethical considerations

Ethical clearance was obtained from MTRH/Moi University College of Health Sciences-Institutional Scientific and Ethics Review Committee (MTRH/MU- ISERC) (FAN 697/2023), permission from MTRH (ELD/MTRH/R&P/10/2/V.2/2010), National Commission for Science, Technology and Innovation in Kenya (NACOSTI) (NACOSTI/P/24/33717), and AMPATH, Eldoret, Kenya (RES/STUD/2/2024). Written informed consent was obtained from all participants. Confidentiality was maintained through coded identifiers and secure data storage. Participants received their immunological results, and those with abnormal findings were counseled and referred for appropriate clinical follow-up and management.

Data availability statement

The data are available upon reasonable request from the corresponding author

Conflicts of interest

The authors declare that there are no conflicts of interest.

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