Immune recovery in HIV-1 infected patients with sustained viral suppression under long-term antiretroviral therapy in Ethiopia

Dawit Wolday¹*, Dorsisa Legesse², Yazezew Kebede¹, Dawd S. Siraj³, Joseph A. McBride³, Robert Striker³

¹ Mekelle University College of Health Sciences, Mekelle, Ethiopia, ² Hayat General Hospital, Addis Ababa, Ethiopia, ³ Division of Infectious Diseases, Department of Medicine, University of Wisconsin, Madison, Wisconsin, United States of America

* dawwol@gmail.com

Abstract

Background

There is very little data on long-term immune recovery responses in patients on suppressive antiretroviral therapy (ART) in the setting of sub-Saharan Africa (SSA). Thus, we sought to determine CD4+ T-cell, CD8+ T-cell and CD4/CD8 ratio responses in a cohort of HIV infected individuals on sustained suppressive ART followed up for more than a decade.

Methods

The cohort comprised adult patients who started ART between 2001 and 2007 and followed for up to 14 years. Trends in median CD4+ T-cells, CD8+ T-cells and CD4/CD8 ratio were reviewed retrospectively. Poisson regression models were used to identify factors associated with achieving normalized T-cell biomarkers. Kaplan-Meier curves were used to estimate the probability of attaining normalized counts while on suppressive ART.

Results

A total of 227 patients with a median duration of follow-up on ART of 12 (IQR: 10.5–13.0) years were included. CD4 cell count increased from baseline median of 138 cells (IQR: 70–202) to 555 cells (IQR: 417–830). CD4 cell increased continuously up until 5 years, after which it plateaued up until 14 years of follow up. Only 69.6% normalized their CD4 cell count within a median of 6.5 (IQR: 3.0–10.5) years. In addition, only 15.9% of the cohort were able to achieve the median reference CD4+ T-cell threshold count in Ethiopians (≥760 cells/μL). CD8+ T-cell counts increased initially until year 1, after which continuous decrease was ascertained. CD4/CD8 ratio trend revealed continuous increase throughout the course of ART, and increased from a median baseline of 0.14 (IQR: 0.09–0.22) to a median of 0.70 (IQR: 0.42–0.95). However, only 12.3% normalized their ratio (≥ 1.0) after a median of 11.5 years. In addition, only 8.8% of the cohort were able to achieve the median reference ratio of healthy Ethiopians.
Conclusion
Determination of both CD4+ and CD8+ T-cells, along with CD4/CD8 ratio is highly relevant in long-term follow-up of patients to assess immune recovery. Monitoring ratio levels may serve as a better biomarker risk for disease progression among patients on long-term ART. In addition, the findings emphasize the relevance of initiation of ART at the early stage of HIV-1 infection.

Introduction
Effective antiretroviral therapy (ART) suppresses HIV replication allowing progressive immune recovery which is used commonly to assess the status of disease progression [1, 2]. ART programs in sub-Saharan Africa (SSA) have achieved outcomes comparable to those in developed countries, especially in terms of immunological and virological responses despite differences in HIV-1 genetic diversity [3–6].

Reports on outcomes to ART in patients followed up for more than a decade are documented mostly from high-income countries (HICs) [7–12]. On the other hand, reports from SSA on outcomes of ART are those of short duration and long-term treatment outcomes, by large, are hardly known despite the fact that patients are living longer [13, 14]. In addition, there are no data with respect to long-term CD4/CD8 ratio responses among patients taking ART in SSA. Despite effective viral suppression, however, immune recovery is often incomplete, which very often remains below the normal threshold levels [15–24]. Incomplete immune recovery despite ART has been linked with increased mortality and morbidity, both due to AIDS-related and non-AIDS-related or non-communicable diseases [24–34], and monitoring the ratio appears to be both relatively simple and comprehensive way of evaluating changing risks for clinical events.

We have previously shown that apparently healthy HIV-negative adult Ethiopians exhibit low CD4+ T-cell count and CD4/CD8 ratio compared to Caucasians [35–39]. Despite low baseline CD4+ T-cell count or CD4/CD8 ratio among Ethiopians, HIV disease progression during pre-ART era remained the same in the two populations [38]. In settings, like ours, very low CD4+ T-cell count and low CD4/CD8 ratio in apparently healthy HIV-seronegative individuals poses challenge for ART guidelines based on normal reference threshold values. Hence, we sought to determine immune recovery among Ethiopian patients initiated with ART followed up for more than a decade. In addition, we determined factors associated with immune recovery in this patient population.

Methods
Study population
The cohort was enrolled at Hayat General Hospital’s ART Clinic in Addis Ababa, Ethiopia, which delivers HIV treatment and care [40]. The cohort consists of adult patients followed between 2001 and 2017. Patients with an AIDS defining illness or a CD4 count <200 cell/μL were started on with a combination of ART, based on National ART Guidelines [41]. First-line ART comprised of zidovudine (AZT) or stavudine (d4T) or tenofovir (TDF) in combination with lamivudine (3TC) plus non-nucleoside reverse transcriptase inhibitors, nevirapine (NVP) or efavirenz (EFV). The participants had clinic visits every 6 months and/or as needed for...
monitoring treatment outcomes. Laboratory tests performed during each visit included complete blood cell count, clinical chemistry, urine analysis, and immune-profiles (as measured by CD4 cell count, CD8 cell count and CD4/CD8 ratio). CD4, CD8 and CD4/CD8 ratio was measured using FACSCount (BD Biosciences, San Jose, USA). HIV-1 plasma viral load determination was done by real-time nucleic-acid sequence-based amplification (NASBA) using the NUCLISENSE EASYQ technology (BioMérieux, Boxtel, The Netherlands). The lower limit of detection (LDL) of the assay is 50 RNA copies/mL blood. HIV-1 viral load was done for patients in the event of clinical or immunological failure, as per National Guidelines [41]. Clinical failure is suspected when new or recurring WHO clinical stage III or IV event occurs, and immunological failure is considered when the CD4 count is persistently < 250 cells/μL, or there is persistent CD4 count levels < 100 cells/μL.

Patients were censored at the time of CD4, CD8 or CD4/CD8 ratio normalization, at the time of death, transfer to other clinic, loss to follow-up, or the last visit date before December 2017. CD4 count, CD8 count and CD4/CD8 ratio are considered normalized if ≥ 500 cells, between 500 and 1000 cells, and ≥ 1.0, respectively (based on values of healthy adult Ethiopians [35–39]). Viral suppression was defined as the attainment of HIV-1 plasma viral load of <400 copies/mL on at least two subsequent measurements. Poor adherence was defined as intake of prescribed medications <95%, based on National ART Guidelines [41]. Adherence was monitored based on pill counts during clinic visits, self-report as well as by checking pharmacy refill records.

Statistical analysis
Baseline characteristics for continuous variables were expressed as the median with inter-quartile range (IQR), and for categorical variables as proportions. Chi-square test was used to compare categorical variables and Wilcoxon rank-sum test for not normally distributed variables.

The rate of T-cell marker normalization was calculated per 100 person years at risk. Factors associated with probability of T-cell marker normalization were analyzed using Poisson regression analysis. Using univariate analysis, we initially analyzed the relative risk (RR) of not achieving CD4+ T-cell count normalization with respect to baseline demographic and clinical characteristics (age, gender and HIV disease stage, TDF-based regimen), presence of tuberculosis (TB) at time of ART initiation and baseline laboratory biomarkers including CD4+ T-cell count, CD8+ T-cell count, CD4/CD8 ratio and plasma viral load. In addition, time-dependent variables including drug toxicity, ART adherence, ART switching, as well as updated T-cell markers were included in the initial bivariate analysis. Then a multivariate analysis of probability [adjusted relative risk (ARR)] of achieving CD4 count ≥ 500 cells was done including all variables that were significant in univariate analysis.

Baseline CD4+ T-cell counts were built in to two categories whether normalized (≥500) or not normalized (<500) cells/μL, the CD8 cell counts in three categories (<500, between 500 and 1000, and >1000 cells/μL), and the CD4/CD8 ratio in two categories (<0.30 and ≥0.30). The cut-offs were chosen based on previous reports showing association of the biomarkers with AIDS-related events [31, 32, 40].

Kaplan-Meier curves were used to estimate the probability of CD4+ T-cell count normalization in patients on ART across explanatory variables and statistical differences were tested using log-rank test. P values <0.05 were considered statistically significant. Data was entered on excel and exported for analysis by STATA (Statistical package v. 14.0, StataCorp, Texas, USA).
**Ethical considerations**

The study was reviewed and approved by the Addis Ababa City Administration Health Bureau Research Ethics Review Committee, Addis Ababa, Ethiopia and the Institutional Review Board (IRB) of the University of Wisconsin, Madison, USA. As the nature of the study was retrospective, individual study participant consent was not obtained and approved by the ethics review committee. Nonetheless, all personal identifiers were de-linked from the original sources.

**Results**

**Characteristics of study participants**

Socio-demographic and baseline clinical features are shown in Table 1. A total of 227 patients receiving ART and with sustained virological suppression were included in the study. The median age at ART initiation was 39 years (IQR: 33–46) years. The majority were male (63.4%) with more than half (56.8%) being in Stage III and IV WHO clinical stage.

**Trends of T-cell biomarkers**

Trends of the CD4+ T-cell, CD8+ T-cell and CD4/CD8 ratio is shown in Fig 1. The CD4 cell count increased from baseline median of 138 (IQR: 70–202) to 555 (IQR: 417–830) cells/μL after a median of 12 (IQR: 10.5–13.0) years of follow up on ART (p<0.0001) (Fig 1A). The increase of CD4 T cells exhibited a biphasic pattern. It increased continuously from baseline median of 138 (IQR: 70–202) to 510 (IQR: 385–653) cells/μL after a median of 7.5 years of follow up on ART (p<0.00001). However, beyond 7.5 years of ART, the CD4+ T-cell count plateaued and there is no significant change when the median CD4 cell count at year 5 was compared to the median CD4 cell count at year 14 of follow up on ART (p = 0.8588). Whereas the median increase of CD4 count per year (slope) was 38 (IQR: 30–57) cells/μL in phase one, it plateaued at around -1 (-13–25) cells/μL per year in phase two of the follow-up (p = 0.0109). Continuous increases in the median CD4+ T-cell counts in both groups with baseline CD4/CD8 ratio of less than 0.30 and ≥0.30 were observed, though increases were more significant in the group with baseline CD4/CD8 ratio of ≥0.30 (Fig 1B). The median increase of CD4 count per year in those with baseline CD4/CD8 ratio ≥0.30 was 43 (IQR: 28–53) cells/μL up until 8.5 years compared to only 7 (IQR: -18–33) cells/μL after 8.5 years of follow-up. In addition, the median increase of CD4 count per year in those with baseline ratio <0.30 was 33 (IQR: 16–55) cells/μL up until 5 years, but only 4 (IQR: -18–10) cells/μL after 5 years of follow-up. Overall, only 69.6% of the cohort has normalized CD4 cell count (≥500 cells) during the 2631.6 person-years of follow-up, and the median time to CD4 count normalization was 6.5 (IQR: 3.0–10.5) years. In addition, only 15.9% of the cohort were able to achieve the median reference CD4+ T-cell threshold count in Ethiopians, which is ≈760 cells [35]. The median time to reach the reference CD4 cell count threshold of Ethiopians was 12 (IQR: 10.8–13.3) years.

Trends in CD8 T cell count exhibited a three phase pattern, with initial increase up until 1 year, followed by plateau until 6 years and decline after 6 years of ART follow-up (Fig 1C). CD8+ T-cell counts increased initially until year 1, after which continuous decrease in CD8 + T-cell count was ascertained. Overall, there was no significant change in CD8+ T-cells when baseline counts were compared to those at 14 years. However, there was statistically significant decrease in CD8+ T-cells when median count at year 1 [1063 (IQR: 754–1490) cells/μL] was compared to the median count at year 14 [911 (IQR: 788–1154) cells/μL] on ART (p = 0.005). Although decrease in CD8+ T-cell count between year 1 and 6 was not significant, significant
Table 1. Sociodemographic and clinical characteristics.

| Variable                                      | Overall  |
|-----------------------------------------------|----------|
| Total patients                                | 227 (100.0) |
| Age Median years, (IQR)                       | 39 (33–46) |
| Sex                                           |          |
| Male                                          | 144 (63.4) |
| Female                                        | 83 (36.6)  |
| WHO Clinical Stage                             |          |
| I/II                                          | 98 (43.2)  |
| III/IV                                        | 129 (56.8) |
| Adverse drug effects                          |          |
| No                                            | 203 (89.4) |
| Yes                                           | 24 (10.6)  |
| ART adherence                                 |          |
| Good                                          | 210 (92.5) |
| Poor                                          | 17 (7.5)   |
| ART switching                                 |          |
| No                                            | 86 (37.9)  |
| Yes                                           | 141 (62.1) |
| CD4 cell count (cells/μL) at baseline         |          |
| Median (IQR)                                  | 138 (70–202) |
| < 200                                         | 167 (74.6) |
| ≥ 200                                         | 57 (25.4)  |
| CD8 cell count (cells/μL) at baseline         |          |
| Median (IQR)                                  | 899 (645–1266) |
| < 500                                         | 25 (12.1)  |
| 500–1000                                      | 93 (45.2)  |
| >1000                                         | 88 (42.7)  |
| CD4/CD8 ratio at baseline                     |          |
| Median (IQR)                                  | 0.14 (0.09–0.22) |
| < 0.30                                        | 178 (86.4) |
| ≥ 0.30                                        | 28 (13.6)  |
| Viral load (RNA copies/mL) at baseline        |          |
| Median (IQR)                                  | 120 000 (27 000–280 000) |
| Follow-up [median (IQR) Years]                | 12.0 (10.5–13.0) |
| ART regimen                                   |          |
| AZT+3TC+ EFV                                  | 91 (40.1)  |
| ddT+3TC+ EFV                                  | 60 (26.4)  |
| ddT+3TC+ NVP                                  | 31 (13.7)  |
| TDF+3TC+EFV                                   | 18 (7.9)   |
| AZT+3TC+ NVP                                  | 16 (7.1)   |
| TDF+3TC+NVP                                   | 4 (1.8)    |
| PI containing                                 | 6 (2.6)    |
| Other                                         | 2 (0.9)    |

Data are numbers (%) unless otherwise stated. ART = antiretroviral therapy; IQR = interquartile range.

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A decrease was noted between year 6 and year 14 of follow up [986 (IQR: 730–1322 cells/μL) vs. 911 (IQR: 788–1154 cells/μL), p = 0.009]. In the early phase until year 1, the median increase of CD8 count per year was 81 (IQR: 0–162) cells/μL. The median change of CD8 count per year between the years 1 and 6 was -28 (IQR: -35–1) cells/μL, and between the years 7 and 14...
was 3 (IQR: -41–10) cells/μL. However, the changes were not statistically significant. Although, the median CD8 count decline per year was -23 (-41–10) cells/μL after 1 year of follow-up, it was not statistically significant compared to the slope noted up until 1 year (p = 0.2341). CD8 T cell trends stratified by CD4/CD8 ratio did not show any pattern (Fig 1D).

The CD4/CD8 ratio trends revealed continuous increase throughout the course of ART, and increased from a median baseline of 0.14 (IQR: 0.09–0.22) to a median of 0.70 (IQR: 0.42–0.95) at the time of censoring the study (p = 0.0003) (Fig 1E). However, only 12.3% of the cohort has normalized ratio (≥ 1.0). The median time to ratio normalization was 11.5 (95% CI: 10.0–12.5) years. Continuous increases in the median CD4/CD8 ratio were more significant in the group with a baseline ratio of ≥ 0.30 than in those with less than 0.30. In the latter group, slight increase in ratio were noted up until year 4 on ART, then it plateaued (Fig 1F). The overall CD4/CD8 ratio increase per year (slope) was 0.04 (IQR: 0.02–0.05). The median increase of CD4/CD8 ratio per year in those with baseline ratio ≥ 0.30 was 0.03 (IQR: 0.02–0.06). On the other hand, the trend in ratio change for those with a baseline < 0.30 showed somewhat biphasic pattern. Whereas the median increase in CD4/CD8 ratio up until 9 years of follow-up was 0.02 (IQR: 0.01–0.04), it decreased by a median ratio of -0.01 (IQR: -0.02–0.03) beyond 9 years. However, the difference was not statistically significant (p = 0.0847). In addition, only 8.8% of the cohort were able to achieve the median reference ratio threshold value of healthy Ethiopians, which is ≈ 1.10 [35]. Overall, the proportion of patients with a CD4/CD8 ratio of ≥ 0.3, ≥ 0.5, ≥ 0.8, ≥ 1.0 and ≥ 1.2 were 85.5%, 57.3%, 26.9%, 12.3% and 6.6%, respectively (S1 Fig).

Factors associated with immune recovery

Predictors associated with failure to achieve CD4+ T-cell normalization ≥ 500 is shown in Table 2. On univariate analysis female sex (RR: 2.26, 95% CI: 1.26–4.07), CD4+ T-cell count < 200 cells at baseline (RR: 2.93, 95%CI 1.34–6.24), and CD4/CD8 ratio at baseline (per 0.1 point lower) (RR: 1.57, 95%CI 1.19–2.07) were all associated with failure to achieve CD4+ T-cell normalization. On the contrary, age, WHO clinical stage, adverse drug events, adherence, ART switching, TDF-based regimen, prevalent or incident TB, AIDS-defining events, non-AIDS-defining events, CD8+ T-cell count and baseline plasma viral load were all not associated with failure to achieve CD4 normalization.

In multivariate analysis, however, baseline CD4/CD8 ratio (per 0.1 point lower) (ARR: 1.36, 95% CI: 1.01–1.82, p = 041) was the only factor associated with increased probability of failure to achieve CD4+ T-cell normalization.

Discussion

Data on long-term outcomes of ART in the setting of SSA are limited [13, 14]. Here, we measured CD4+ T-cell count, CD8+ T-cell count and CD4/CD8 ratio during a median of 12 years with sustained virological suppression in HIV-1 infected patients on ART.

The CD4+ T-cell trends showed continuous increase up until year 5–7 of follow up, but plateaued thereafter. Median reference CD4+ T-cell count [35] was achieved within an average of 12 years follow-up in only 15% of the patients. In addition, using multivariate analysis, the likelihood of achieving CD4+ T-cell count normalization (≥ 500 cells) was significantly associated with time-updated CD4/CD8 ratio ≥ 0.30 at ART initiation. In addition, similar to previous studies undertaken in HICs [7–12] and SSA [13, 14], our findings showed that the majority of the patients (76%) started ART with a CD4+ T-cell lower than 200 cells. Only 4 patients were initiated ART with their CD4+ T-cell counts ≥ 350 cells. Therefore, we did not analyze the effect on CD4+ T-cell normalization when patients were initiated with ART when their CD4+ T-cell count was ≥ 350 cells. However, the changes were not statistically significant. Although, the median CD8 count decline per year was -23 (-41–10) cells/μL after 1 year of follow-up, it was not statistically significant compared to the slope noted up until 1 year (p = 0.2341). CD8 T cell trends stratified by CD4/CD8 ratio did not show any pattern (Fig 1D).
T-cell count was $\geq 350$ cells. Nonetheless, several studies conducted previously have demonstrated that baseline CD4+ T-cell count $\geq 350$ is a significant predictor of achieving CD4+ T-cell normalization [9, 12, 23, 24]. In our study, baseline CD4/CD8 ratio was the only factor associated with the likelihood of achieving normalized CD4+ T-cell count. Previous studies have demonstrated similar results [9, 10, 30, 31, 33, 42–48].

CD8+ T-cell counts increased during the first year, but remained persistently elevated up until year 6. However, after 6 years, it decreased continuously in subsequent years of follow up on ART. CD8+ T-cell count appears to play important role in mortality in patients on ART [33, 49] and need to be monitored for clinical assessment.

CD4+ T-cell recovery during long-term ART

Table 2. Factors associated with not achieving CD4+ T-cell count $\geq 500$ in HIV-infected patients on antiretroviral treatment despite sustained viral suppression.

| Variable                        | Univariate RR (95% CI) | P-value | Multivariate ARR (95% CI) | P-value |
|---------------------------------|------------------------|---------|---------------------------|---------|
| Age (years) $\geq 50$ vs. < 50   | 1.75 (0.90–3.43)       | 0.100   |                           |         |
| Gender, male vs female          | 2.26 (1.26–4.07)       | 0.006   | 1.64 (0.88–3.06)          | 0.120   |
| WHO stage III/IV vs. I/II       | 1.11 (0.69–1.80)       | 0.664   |                           |         |
| Adverse drug events vs none     | 1.11 (0.53–2.32)       | 0.783   |                           |         |
| Poor vs good adherence          | 0.97 (0.39–2.40)       | 0.939   |                           |         |
| ART switch vs. no switch        | 0.95 (0.59–1.54)       | 0.831   |                           |         |
| TB at ART initiation vs. no TB  | 1.86 (0.85–4.06)       | 0.120   |                           |         |
| Incident TB vs. no TB           | 1.53 (0.80–2.92)       | 0.194   |                           |         |
| AIDS-defining events vs. none   | 1.29 (0.74–2.22)       | 0.367   |                           |         |
| Non-AIDS-defining events vs none| 1.29 (0.74–2.26)       | 0.369   |                           |         |
| CD4+ T-cell count at baseline $< 200$ vs. $\geq 200$ | 2.93 (1.34–6.40) | 0.007 | 1.82 (0.78–4.21) | 0.165 |
| CD8+ T-cell count at baseline $< 500$ vs. $\geq 500$ | 1.55 (0.21–1.40) | 0.208 |                 |         |
| $>1000$                         | 0.78 (0.46–1.30)       | 0.338   |                           |         |
| CD4/CD8 ratio at baseline (per 0.1 lower) | 1.57(1.19–2.06) | 0.002 | 1.36 (1.01–1.82) | 0.041 |
| Viral load at baseline (RNA copies/mL) $< 100,000$ vs. $\geq 100,000$ | 0.98 (0.41–2.33) | 0.964 |                 |         |

RR: relative risk; ARR: adjusted relative risk;
*Data available for only 59 patients at baseline.

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evaluation of patients with respect to immune recovery. Taken together, all T-cell biomarkers (i.e. CD4+ T-cell count, CD8+ T-cell count and CD4/CD8 ratio) failed to normalize to the levels of healthy individuals despite long-term virologically suppressive ART.

The mechanisms related to incomplete immune recovery in patients on ART despite suppressive ART remains to be elucidated. Several studies have demonstrated that chronic immune activation [10, 20, 21, 30], older age [24], male sex [50, 51], background genetic differences [22] might play significant role.

Our study has several strengths. First, the cohort included a long-term of follow-up of HIV-1 patients on sustained suppressive ART for more than a decade. Second, data on immune recovery biomarkers was availability both at initiation of ART and also time-dependent changes in CD4+ T-cell, CD8+ T-cell counts and CD4/CD8 ratio were obtained. Third, to the best of our knowledge, our study included long-term analysis of CD4/CD8 ratio in patients under suppressive ART in the setting of SSA. Nonetheless, limitations related to our study include that our findings cannot be generalized to the entire population as the data is generated from only one study site. Furthermore, the retrospective design of the study has limitations in respect to completeness of available data and the small sample size is a potential weakness of the study. In particular relevant data that are potentially confounders, such as parasitic infections and malnutrition were not available or incomplete. Finally, the number of patients during follow-up of such long duration is compounded by patient attrition. Thus, interpretation of our data after 10 years where a substantial number of patient followed is decreased showing unstable trends.

Conclusion

Our data demonstrate that the measurement of both CD4+ and CD8+ T-cells, along with the assessment of the CD4/CD8 ratio is highly relevant in long-term follow-up and evaluation of patients with respect to immune recovery. In addition, our findings show that the majority (76%) of the patients started ART with a CD4+ T-cell lower than 200 cells, and all the T-cell biomarkers (CD4+ T-cell count, CD8+ T-cell count and CD4/CD8 ratio) did not normalize to the levels of healthy individuals despite long-term virologically suppressive ART. The results emphasize the relevance of initiation of ART at the early stage of HIV-1 infection [51–53].

Whether current "treat-all" ART guidelines [54] will result in complete immunological recovery in the setting of SSA should be investigated. In addition, continuous increase in CD4/CD8 ratio while CD4+ T-cell count plateau after 5 years of ART initiation in our study suggests that monitoring ratio levels may serve as a better biomarker risk for disease progression monitoring among patients on long-term ART.

Supporting information

S1 Fig. Proportion of patients with different CD4/CD8 ratio cut-offs. (TIF)

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Author Contributions

**Conceptualization:** Dawit Wolday, Yazezew Kebede, Dawd S. Siraj, Joseph A. McBride, Robert Striker.

**Data curation:** Dawit Wolday, Dorsisa Legesse, Dawd S. Siraj, Joseph A. McBride, Robert Striker.

**Formal analysis:** Dawit Wolday, Yazezew Kebede, Dawd S. Siraj, Robert Striker.

**Funding acquisition:** Dawd S. Siraj, Robert Striker.

**Investigation:** Dawit Wolday, Dorsisa Legesse, Dawd S. Siraj, Joseph A. McBride, Robert Striker.

**Methodology:** Dawit Wolday, Dorsisa Legesse, Dawd S. Siraj, Robert Striker.

**Project administration:** Dawit Wolday, Dorsisa Legesse, Robert Striker.

**Resources:** Dawit Wolday, Robert Striker.

**Supervision:** Dawit Wolday, Dawd S. Siraj, Robert Striker.

**Validation:** Dawit Wolday, Robert Striker.

**Writing – original draft:** Dawit Wolday, Yazezew Kebede, Robert Striker.

**Writing – review & editing:** Dawit Wolday, Dorsisa Legesse, Yazezew Kebede, Dawd S. Siraj, Joseph A. McBride, Robert Striker.

References

1. Battegay M, Nüesch R, Hirschel B, Kaufmann GR. Immunological recovery and antiretroviral therapy in HIV-1 infection. Lancet Infect Dis 2006; 6:280–287. https://doi.org/10.1016/S1473-3099(06)70463-7 PMID: 16631548

2. Mocroft A, Phillips A, Gatell J, Ledergerber B, Fisher M, Clumeck N, et al. Normalisation of CD4 counts in patients with HIV infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. Lancet 2007; 370:407–413. https://doi.org/10.1016/S0140-6736(07)60948-9 PMID: 17659333

3. Ivers LC, Kendrick D, Doucette K. efficacy of antiretroviral therapy programs in resource-poor settings: a meta-analysis of the published literature. Clin Infect Dis 2005; 41:217–224. https://doi.org/10.1086/431199 PMID: 15983918

4. Geng EH, Neilands TB, Thiebaut R, Bwana MB, Nash D, Moore RD, et al. CD4+ T cell recovery during suppression of HIV replication: an international comparison of the immunological efficacy of antiretroviral therapy in North America, Asia and Africa. Int J Epidemiol. 2015; 44:251–263. https://doi.org/10.1093/ije/dyu271 PMID: 25859596

5. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients on antiretroviral treatment programs in Sub-Saharan Africa: a systematic review. Lancet Infect Dis 2010; 10:155–166. https://doi.org/10.1016/S1473-3099(09)70328-7 PMID: 20185094

6. Nash D, Katyal M, Brinkhof MWG, Keiser O, May M, Hughes R, et al. Long-term response to antiretroviral therapy in low-income countries. AIDS 2008; 22:2292–2302. https://doi.org/10.1097/QAD.0b013e3283121ca9 PMID: 18981768

7. Nakanjako D, Kiragga AN, Musick BS, Yiannoutsos CT, Wools-Kaloustian K, Diero L, et al. Frequency and impact of suboptimal immune recovery on first-line antiretroviral therapy within the international epidemiologic databases to evaluate AIDS in East Africa. AIDS. 2016; 30:1913–1922. https://doi.org/10.1097/QAD.0000000000001085 PMID: 26959510

8. Rönsholt FF, Ullum H, Katzenstein TL, Genstorf J, Ostrowski SR. T-cell subset distribution in HIV-1-infected patients after 12 years of treatment-induced virologic suppression. J Acquir Immune Defic Syndr 2012; 61:270–8. https://doi.org/10.1097/QAI.0b013e31825e7ac1 PMID: 22614900

9. Torti C, Prosperi M, Motta D, Digiambrunedetto S, Maggiolo F, Paraninfo G, et al. Factors influencing the normalization of CD4+ T-cell count, percentage and CD4+/CD8+ T-cell ratio in HIV-infected patients.
on long-term suppressive antiretroviral therapy. Clin Microbiol Infect 2012; 18:449–458. https://doi.org/10.1111/j.1469-0691.2011.03650.x PMID: 21919996

10. Zhang X, Hunt PW, Hammer SM, et al. Immune activation while on potent antiretroviral therapy can predict subsequent CD4+ T-cell increases through 15 years of treatment. HIV Clin Trials. 2013; 14:61–67. https://doi.org/10.1310/hc1402-61 PMID: 23611826

11. Hughes RA, May MT, Tilling K, Taylor N, Wittkop L, Reiss P, et al. Long-term trends in CD4+ cell counts, CD8+ cell counts, and the CD4+/CD8+ ratio. Clin Infect Dis 2018; 32: 1361–1367 https://doi.org/10.1097/QAD.0000000000001848 PMID: 29851663

12. Mutoh Y, Nishijsima T, Inaba Y, Tanaka N, Kikuchi Y, Gatanaga H, et al. Immune activation and poor recovery of CD4 T cell count, CD4 percentage, and CD4/CD8 ratio in patients with human immunodeficiency virus infection and suppressed viremia during long-term antiretroviral therapy. Clin Infect Dis 2018; 67:927–933. https://doi.org/10.1093/cid/ciy174 PMID: 29509894

13. Ndumbi P, Falutz J, Pant Pai N, Tsoukas CM. Delay in cART initiation results in persistent immune dysregulation and poor recovery of T-cell phenotype despite a decade of successful HIV suppression. PLoS One 2014; 9:e94018. https://doi.org/10.1371/journal.pone.0094018 PMID: 24710051

14. Castelnuovo B, Kiragga A, Musaazi J, Sempa J, Mubiru F, Wanyama J, et al. Outcomes in a cohort of patients started on antiretroviral treatment and followed up for a decade in an urban clinic in Uganda. PLoS ONE 2015; 10: e0142722. https://doi.org/10.1371/journal.pone.0142722 PMID: 26642214

15. Kaufmann GR, Furrer H, Ledergerber B, Perrin L, Opravil M, Vernazza P, et al. Characteristics, determinants, and clinical relevance of CD4+ T cell recovery to <500 cells/uL in HIV type 1-infected individuals receiving potent antiretroviral therapy. Clin Infect Dis 2008; 47:1093–1101. https://doi.org/10.1086/592113 PMID: 18783328

16. Khanna N, Opravil M, Furrer H, Cavassini M, Vernazza P, Bernasconi E, et al. CD4+ T cell count recovery in HIV type 1-infected patients is independent of class of antiretroviral therapy. Clin Infect Dis 2008; 47:1093. https://doi.org/10.1086/592113 PMID: 18783328

17. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. Clin Dev Immunol 2012; 2012:670957. https://doi.org/10.1155/2012/670957 PMID: 22474480

18. Emu B, Moretto WJ, Hoh R, Krone M, Martin JN, Nixon DF, et al. Composition and function of T cell subpopulations are slow to change despite effective antiretroviral treatment of HIV disease. PLoS One 2014;9:e85613. https://doi.org/10.1371/journal.pone.0085613 PMID: 24465619

19. Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, et al. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis 2003; 187:1534–1543. https://doi.org/10.1086/374766 PMID: 12721933

20. Massanella M, Negredo E, Perez-Alvarez N, Puig J, Hernandez RR, Bofill M, et al. CD4 T-cell hyperactivation and susceptibility to cell death determine poor CD4 T-cell recovery during suppressive HAART. AIDS. 2010; 24:959–968. https://doi.org/10.1097/QAD.0b013e3283397b95 PMID: 20177358

21. Kroese S, Wit FW, Rossouw TM, Steel HC, Kityo CM, Siwale M, et al. Plasma biomarkers of human immunodeficiency virus-related systemic inflammation and immune activation in Sub-Saharan Africa before and during suppressive antiretroviral therapy. J Infect Dis. 2019; 220:1029–1033. https://doi.org/10.1093/infdis/jiz252 PMID: 31086991

22. Seng R, Ghislain M, Girard PM, Cotte L, Meybeck A, Rafli F, et al. Sub-Saharan African migrants have slower initial CD4+ cell recovery after combined antiretroviral treatment initiation than French natives. AIDS. 2017; 31:1323–1332. https://doi.org/10.1097/QAD.0000000000001482 PMID: 28492394

23. Moore RD, Keruly JC. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virological failure. Clin Infect Dis 2007; 44:441–446. PMID: 17205456

24. Kroese S, Onoza P, Kitoy CM, Siwale M, Akanmu S, Wellington M, et al. Suboptimal immune recovery during antiretroviral therapy with sustained HIV suppression in Sub-Saharan Africa. AIDS. 2018; 32:1043–1051. https://doi.org/10.1097/QAD.0000000000001848 PMID: 29854745

25. Lawn SD, Myer L, Orrell C, Bekker LG, Wood R. Early mortality among adults accessing a community antiretroviral service in South Africa: implications for programme design. AIDS 2005; 19: 2141–2148. https://doi.org/10.1097/01.aids.0000194802.89540.e1 PMID: 16284464

26. Lawn SD, Little F, Bekker LG, Kaplan R, Campbel E, Orrell C, et al. Changing mortality risk associated with CD4 cell response to antiretroviral therapy in South Africa. AIDS 2009; 23: 335–342. https://doi.org/10.1097/QAD.0b013e3282821823 PMID: 19114870

27. Young J, Psychogiou M, Meyer L, Ayayi S, Grabar S, Rafli F, et al. CD4 cell count and the risk of AIDS or death in HIV-infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from COHERE. PLoS Med. 2012; 9: e1001194. https://doi.org/10.1371/journal.pmed.1001194 PMID: 22448150
28. Loutfy MR, Genebat M, Moore D, Raboud J, Chan K, Antoniou T, et al. A CD4+ cell count <200 cells per cubic millimeter at 2 years after initiation of combination antiretroviral therapy is associated with increased mortality in HIV-infected individuals with viral suppression. J Acquir Immune Defic Syndr 2010; 55: 451–459. https://doi.org/10.1097/qai.0b013e3181e2c8f PMID: 21105259

29. Takuva S, Maskew M, Brennan AT, Long L, Sanne I, Fox MP. Poor CD4 recovery and risk of subsequent progression to AIDS or death despite viral suppression in a South African cohort. J Int AIDS Soc. 2014; 17:18651. https://doi.org/10.7448/IAS.17.1.18651 PMID: 24594114

30. Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog 2014; 10: e1004078. https://doi.org/10.1371/journal.ppat.1004078 PMID: 24831517

31. Mussini C, Lorenzini P, Cozzi-Lepris A, Lapadula G, Marchetti G, Nicastri E, et al. CD4/CD8 ratio normalization and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. Lancet HIV 2015; 2:e96–106. https://doi.org/10.1016/S2352-3018(15)00006-5 PMID: 26424550

32. Serrano-Villar S, Perez-Elias MJ, Dronda F, Casado JL, Moreno A, Royuela A, et al. Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. PLoS One 2014; 9:e85798. https://doi.org/10.1371/journal.pone.0085798 PMID: 24497929

33. Trickey A, May MT, Schommers P, Tate J, Ingle SM, Guest JL, et al. CD4:CD8 ratio and CD8 count as prognostic markers for mortality in human immunodeficiency virus-infected patients on antiretroviral therapy: the antiretroviral therapy cohort collaboration (ART-CC). Clin Infect Dis. 2017; 65:959–966. https://doi.org/10.1093/cid/cix466 PMID: 28903507

34. Geltzeiler CB, Xu Y, Carchman E, Ghouse Y, Beczkiewicz J, Son J, et al. CD4/CD8 ratio as a novel marker of increased risk of high grade anal dysplasia and cancer in HIV+ patients: a retrospective cohort study. Dis Col Rec, 2020, In press.

35. Tsegaye A, Messele T, Tlahun T, Hailu E, Sahlu T, Doorly R, et al. Immunohematological reference ranges for adult Ethiopians. Clin Diag Lab Immunol 1999; 6:410–14.

36. Kassu A, Tsegaye A, Wolday D, Hailu E, Tlahun T, et al. Distribution of lymphocyte subsets in healthy human immunodeficiency virus-negative adult Ethiopians from two geographic locales. Clin Diag Lab Immunol 2001; 8: 1171–76. https://doi.org/10.1128/CDLI.8.6.1171-1176.2001

37. Kassu A, Tsegaye A, Wolday D, Petros B, Aklilu M, Sanders EJ, et al. Role of incident and/or cured intestinal parasitic infections on profile of CD4+ and CD8+ T cell subsets and activation status in HIV-infected and uninfected adult Ethiopians. Clin Exp Immunol 2003; 132:113–119. PMID: 12653845

38. Abuye C, Tsegaye A, West CE, Versloot P, Sanders EJ, Wolday D, et al. Determinants of CD4 counts among HIV-negative Ethiopians: Role of body mass index, gender, cigarette smoking, khat (Catha Edulis) chewing, and possibly altitude? J Clin Immunol 2005; 25: 127–133. https://doi.org/10.1007/s10875-005-2818-y PMID: 15821889

39. Mekonnen Y, Geskus RB, Hendriks JCM, Messele T, Borghans J, Miedema F, et al. Low CD4 T cell counts before HIV-1 seroconversion do not affect disease progression in Ethiopian factory workers. J Infect Dis 2005; 192: 739–748. https://doi.org/10.1086/432545 PMID: 16088823

40. Wolday D, Kebede Y, Legesse Y, Siraj DS, McBride JA, Kirsch MJ, et al. Role of CD4/CD8 ratio on the incidence of tuberculosis in HIV-infected patients on antiretroviral therapy followed up for more than a decade. PLoS One 2020; 15:e0233049. https://doi.org/10.1371/journal.pone.0233049 PMID: 32442166

41. Ministry of Health (MoH). HIV/AIDS Prevention and Control Office. National Guidelines for Use of Anti-retroviral Drugs in Ethiopia. MoH, 2001.

42. Sauter R, Huang R, Ledergerber B, Battegay M, Bernasconi E, Cavassini M, et al. CD4/CD8 ratio and CD8 counts predict CD4 response in HIV-1-infected drug naive and in patients on cART. Medicine (Baltimore) 2016; 95: e5094. https://doi.org/10.1097/MD.0000000000005094 PMID: 27759638

43. Lu W, Mehraj V, Vyboh K, Cao W, Routy JP. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. J Int AIDS Soc. 2015; 18:20046. PMID: 26130226

44. Serrano-Villar S, Gutiérrez C, Vallejo A, Hernández-Novoa B, Díaz L, Abad Fernández M, et al. The CD4/CD8 ratio in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression. J Infect 2013; 66:57–66. https://doi.org/10.1016/j.jinf.2012.09.013 PMID: 23046968

45. Tinago W, Coghlan E, Macken A, McAndrews J, Doak B, Prior-Fuller C, et al. Clinical, immunological and treatment-related factors associated with normalized CD4+/CD8+ T-cell ratio: effect of naive and
memory T-cell subsets. PLoS One 2014; 9: e97011. https://doi.org/10.1371/journal.pone.0097011 PMID: 24816636

46. Leung V, Gillis J, Raboud J, Cooper C, Hogg RS, Loutfy MR, et al. Predictors of CD4:CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. PLoS One 2013; 8:e77665. https://doi.org/10.1371/journal.pone.0077665 PMID: 24204912

47. Caby F, Guihot A, Lambert-Niclot S, Guiguet M, Boutolleau D, Agher R, et al. Determinants of a low CD4/CD8 ratio in HIV–infected individuals despite long-term viral suppression. Clin Infect Dis 2016; 62:1297–1303. https://doi.org/10.1093/cid/ciw076 PMID: 26908792

48. Sainz T, Serrano-Villar S, Diaz L, Tome MIG, Gurbindo MD, Jose MI, et al. The CD4/CD8 ratio as a marker of T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. AIDS 2013; 27:1513–1516. https://doi.org/10.1097/QAD.0b013e32835faa72 PMID: 23435292

49. Helleberg M, Kronborg G, Ullum H, Ryder LP, Obel N, Gerstoft J. Course and clinical significance of CD8 counts in a large HIV cohort. J Infect Dis 2015; 211:1726–1734. PMID: 25489001

50. Sempa JB, Kiragga AN, Castelnuovo B, Kamya MR, Manabe YC. Among patients with sustained viral suppression in a resource-limited setting, CD4 gains are continuous although gender-based differences occur. PLoS ONE 2013; 8: e73190. https://doi.org/10.1371/journal.pone.0073190 PMID: 24013838

51. Plana M, Garcia F, Gallart T, Tortajada C, Soriano A, Palou E, et al. Immunological benefits of antiretroviral therapy in very early stages of asymptomatic chronic HIV-1 infection. AIDS 2000; 14:1921–1933. https://doi.org/10.1097/00002030-200009080-00007 PMID: 10997396

52. Le T, Wright EJ, Smith DM, Catano G, Okulicz JF, Young JA, et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. N Engl J Med 2013; 368:218–230. https://doi.org/10.1056/NEJMoa1110187 PMID: 23323898

53. Thornhill J, Inshaw J, Kaleebu P, Cooper D, Ramjee G, Schechter M, et al. Enhanced normalization of CD4/CD8 ratio with earlier antiretroviral therapy at primary HIV infection. J Acquir Immune Defic Syndr 2016; 73:69–73. https://doi.org/10.1097/QAI.00000000000001013 PMID: 27070122

54. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. 2017, WHO, Geneva. http://wwwapps.who.int/iris/bitstream/10665/208825/1/9789241549684_eng.pdf?ua=1