# CD4 Count Slope and Mortality in HIV-Infected Patients on Antiretroviral Therapy: Multicohort Analysis From South Africa

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**Background:** In many resource-limited settings monitoring of combination antiretroviral therapy (cART) is based on the current CD4 count, with limited access to HIV RNA tests or laboratory diagnostics. We examined whether the CD4 count slope over 6 months could provide additional prognostic information.

**Methods:** We analyzed data from a large multicohort study in South Africa, where HIV RNA is routinely monitored. Adult HIV-positive patients initiating cART between 2003 and 2010 were included. Mortality was analyzed in Cox models; CD4 count slope by HIV RNA level was assessed using linear mixed models.

**Results:** About 44,829 patients (median age: 35 years, 58% female, median CD4 count at cART initiation: 116 cells/mm$^3$) were followed up for a median of 1.9 years, with 3706 deaths. Mean CD4 count slopes per week ranged from 1.4 [95% confidence interval (CI): 1.2 to 1.6] cells per cubic millimeter when HIV RNA was <400 copies per milliliter to −0.32 (95% CI: −0.47 to −0.18) cells per cubic millimeter with >100,000 copies per milliliter. The association of CD4 slope with mortality depended on current CD4 count: the adjusted hazard ratio (aHRs) comparing a >25% increase over 6 months with a >25% decrease was 0.68 (95% CI: 0.58 to 0.79) at <100 cells per cubic millimeter but 1.11 (95% CI: 0.78 to 1.58) at 201–350 cells per cubic millimeter. In contrast, the aHR for current CD4 count, comparing >350 with <100 cells per cubic millimeter, was 0.10 (95% CI: 0.05 to 0.20).

**Conclusions:** Absolute CD4 count remains a strong risk for mortality with a stable effect size over the first 4 years of cART. However, CD4 count slope and HIV RNA provide independently added to the model.

**Key Words:** HIV, resource-limited setting, CD4 count slope, mortality, HIV RNA, antiretroviral therapy

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## BACKGROUND

Monitoring changes in CD4 T-cell lymphocyte count (CD4 count) is a cornerstone of managing patients before combination antiretroviral therapy (cART) initiation and during cART. It is used for guiding timing of cART initiation and assessing risk of opportunistic illnesses and evaluating for possible treatment failure.[1] However, CD4 count decline before cART initiation is highly variable and depends on multiple hosts and viral factors. For example, HIV RNA level and current CD4 count both help to predict CD4 count decline before cART initiation.[2,3] Likewise, CD4 count increase after starting cART is highly heterogeneous and is also influenced by multiple factors.[4–6] This mismatch between CD4 count change and HIV RNA level limits the clinical value of using CD4 counts as a surrogate for HIV RNA suppression.[7–9] As a result, where the resources are available, both CD4 count and HIV RNA are typically monitored and are used in concert for making clinical decisions.

Absolute CD4 count is valuable for patient management because of the strong association between CD4 count and opportunistic illness and death, prior to cART use and during cART.[10–12] Although HIV RNA value may be associated with mortality given other variables, including CD4 count,[13] it is frequently not routinely available in resource-limited settings. A better understanding of the information provided by routine

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CD4 count testing may help with developing prognostic tools and provide further insight into mortality in resource-limited settings. For example, during the pre–antiretroviral therapy (ART) period, beyond the current CD4 count and HIV RNA level, CD4 slope has been reported to provide additional information regarding mortality risk.[14] However, during cART, it is unclear what additional prognostic information is provided by the CD4 count slope beyond that of the current CD4 count. In addition, although the association between risk of death and CD4 count around cART initiation is well documented, it is unclear if the effect size persists over time on cART and data are lacking from a resource-limited setting.[15,16] Thus, several questions remain regarding CD4 count slope and survival during cART, particularly in resource-limited settings.

We analyzed the South African cohorts from a large multicohort collaboration with extensive longitudinal CD4 count and HIV RNA data to assess change in CD4 count by HIV RNA level on cART and the associations between mortality and current CD4 count, CD4 count slope, and HIV RNA.

## METHODS

### Cohorts and Eligibility Criteria

The International epidemiological Databases to Evaluate AIDS in Southern Africa (IeDEA-SA, www.iedea-sa.org) is a collaboration of HIV treatment cohorts in southern Africa.[17,18] Each cohort regularly provides data to data centers at the Universities of Cape Town, South Africa, and Bern, Switzerland, through a specific standardized data sharing system. This study only included cohorts from South Africa, where regular assessment of CD4 cell count and HIV RNA is part of routine clinical care. All laboratory tests are performed by accredited clinical laboratories.[19]

The included cohorts were from the Aurum Institute, Africa Centre for Health and Population Studies, Themba Lethu Clinic, Khayelitsha, Gugulethu, Tygerberg, and Masiphumelele, and represented urban, periurban, and rural populations.[20] Patients were eligible if they were HIV positive ART naive at the time of entry into the HIV care programs, aged ≥16 years and <85 years, initiated ART between 2003 and 2010, and had at least 6 months of potential follow-up time. In addition, we excluded women known to be pregnant at the time of ART initiation due to potential effects of pregnancy on CD4 count and on ART response. Patients with no CD4 data were excluded from analysis. All IeDEA-SA sites obtained ethical approval from relevant local institutions before contributing anonymized patient data to IeDEA-SA.

### Definitions

Baseline characteristics (measured within 6 months before ART initiation) included age, sex, CD4 count, HIV RNA, calendar year of cART initiation, and cohort. Longitudinal characteristics included absolute CD4 counts and HIV RNA values during the course of ART. We stratified current CD4 count into 4 following levels: ≤100, 100–200, 201–350, >350 cells per cubic millimeter. Current CD4 count or current HIV RNA was defined as the most recent value, which was carried forward until the next value became available for a maximum of 180 days. We calculated CD4 count slope as the change in CD4 count between 2 consecutive CD4 values. The slope was expressed as the change in CD4 cells per week or the percentage change over 6 months in 3 categories as follows: >25% decrease, 25% decrease to 25% increase, and >25% increase. We used a 25% increase or decrease because the intertest variability of CD4 count measures is approximately 20%[21]; we referred to the <25% decline and <25% increase category as "no change." We considered HIV RNA as suppressed (<400 copies/mL), unsuppressed (≥400 copies/mL) and in 4 levels as follows: <400, 400–999, 1000–39,999, 50,000–100,000, and >100,000 copies per milliliter. The cutoff of 400 copies per milliliter was used, as this was the upper bound of the limit of detection for HIV RNA for assays used by some of the cohorts. The 4 levels were selected for convenience and consistency with the literature. Immunologic failure was defined as failure to achieve a CD4 count increase >50 cells per cubic millimeter by 6 months and >100 cells per cubic millimeter by 12 months on cART.

### Ascertainment of Deaths

Deaths were ascertained as per clinic protocols. To address the underascertainment of deaths from routine clinic data,[22–25] we linked civil identification (ID) numbers, when available, to the South African Department of Home Affairs vital status registry. To address incomplete data on ID numbers, we used inverse probability weighting to correct for missing deaths among those defined as lost to follow-up.[24,26] Briefly, patients who were lost to follow-up and who had ID numbers were upweighted to represent all patients lost to follow-up, enabling more accurate estimates of mortality. The weights were based on the inverse modeled probabilities of having an ID given cohort, gender, age, and CD4 count addressing potential differences between patients with and without ID numbers among those lost to follow-up.

### Multiple Imputation of Missing CD4 Count and HIV RNA Values

To address gaps in CD4 count and HIV RNA data due to missed clinic visits or laboratory values, we created "expected visits" for any gap of more than 300 days. We then used multiple imputation to create complete longitudinal data for these "expected visit days" and also imputed baseline CD4 count and HIV RNA values, when missing. We created 10 imputed datasets using a specialized software package.[27] The imputation model included sex, age, CD4 count, HIV RNA, year of cART initiation, duration on cART, and mortality.[28,29] All results of our multivariable analyses are based on the imputed datasets and were combined with Rubin rules.[30]

### Statistical Analyses

Baseline characteristics were summarized using medians with interquartile ranges (IQR) or proportions. Age, CD4 count, and HIV RNA were described as continuous and categorical variables and used as categorical variables in

modeling. CD4 count change by HIV RNA level was assessed using a linear mixed model. Because our primary interest was immune reconstitution and not the initial rise in CD4 count, we excluded CD4 counts from the first 6 months of cART.[31] We included sex and age as fixed effects and cohort, patient, and each time interval within a given HIV RNA category as random effects. We displayed the distributions of CD4 count slope graphically by HIV RNA level, plotting the distribution of CD4 count slopes (cells/mm$^3$ per week) within each HIV RNA category. We also described the CD4 count slope strata of >25% decrease over 6 months, no change, and >25% increase in relation to the HIV RNA level.

Cox proportional hazards regression models were used to assess crude and adjusted associations between baseline and time-updated characteristics and mortality. Time was measured from the start of cART to the first of death, loss to follow-up, analysis closure, or 4 years on cART. Loss to follow-up was defined as at least 6 months without a visit (and no subsequent visits) with time censored at the last clinic visit. Analysis closure was at 6 months before database closure to allow for full loss to follow-up assignment. For patients who started ART but had no further contact, we added 1 day of follow-up to allow for their inclusion in survival analyses.

All available variables were considered potential confounders and were included in initial multivariable models. We examined interactions between duration on cART (split at 12 months) and all statistically significant variables to test the proportional hazard assumption. We also assessed for interactions between current CD4 count and CD4 count slope, current CD4 count and current HIV RNA, and CD4 count slope and sex. The final Cox model included statistically significant covariates and CD4 count, CD4 count slope, and the interaction between current CD4 count and CD4 count slope. Stratification by cohort was used to adjust for potential cohort differences. We included CD4 count slope and CD4 count in a nonlinear manner using polynomials. The hazard by current CD4 count, CD4 count slope, and interaction between the 2 was visualized by means of a 3-dimensional plot. In an attempt to assess whether the added value of CD4 count slope in the model was independent of absolute CD4 count, or may have provided a more precise estimate of CD4 count at the time of death, we repeated the analysis, limiting follow-up time to 30 days after the last CD4 count. In addition, to evaluate the effect size of an association between CD4 count slope and mortality in the absence of HIV RNA data, we repeated the analysis without including HIV RNA. Finally, we assessed mortality after 6 months on cART or 12 months on cART using immunologic failure at those 2 time points as one of the covariates. For this analysis, entry occurred at 6 or 12 months on cART and patients were censored 6 months after entry (at 12 or 18 months on cART).

Data were analyzed using STATA 12.0 (STATA Corporation, College Station, TX) and R 2.12 (The R Development Core Team).

## RESULTS

The cohorts included a total of 47,320 patients receiving cART. Thirty-nine (0.1%) patients were excluded because their age was <16 or >85 years at cART initiation; 547 (1.2%) were excluded because they initiated cART before 2002 or after 2010; 660 (1.4%) were excluded because they lacked a potential for 6 months follow-up on cART; and 1245 (2.6%) were excluded because they were pregnant. This left a total of 44,829 patients meeting eligibility criteria. The number of patients per cohort ranged from 517 to 16,415. Of these patients, 26,171 (58%) were female, the median age was 35 years (IQR: 30–42), the median CD4 count at cART initiation was 116 cells per cubic millimeter (IQR: 52–178; Table 1). Median duration of follow-up was 1.9 (IQR: 1.2–3.0) years. The median number of CD4 count measurements on cART was 3 (IQR: 2–5), obtained a median of 166 (IQR: 106–196) days apart. There were 293,192 actual CD4 count values present and 34,463 (10%) time points with missing CD4 counts; and 270,832 actual HIV RNA results present and 56,823 (17%) time points with missing HIV RNA values. During follow-up, there were 3706 (8.3%) deaths in the weighted analysis.

### CD4 Count Change by HIV RNA Suppression

CD4 count increased on an average of 1.3 [95% confidence interval (CI): 1.1 to 1.5] cells per cubic millimeter

**TABLE 1.** Baseline Characteristics (Total Population: 44,829)

| | n (%) or Median (IQR) |
|---|---|
| Sex | |
| Men | 18,658 (42) |
| Women | 26,171 (58) |
| Age | |
| 16-24 | 2771 (6) |
| 25-34 | 17,833 (40) |
| 35-44 | 15,419 (34) |
| >44 | 8806 (20) |
| CD4 count at cART initiation | |
| Median | 116 (52–178) |
| HIV RNA at cART initiation | |
| Median | 51,000 (13,000–170,000) |
| Year of cART initiation | |
| 2003 | 1317 (3) |
| 2004 | 4614 (10) |
| 2005 | 7988 (18) |
| 2006 | 11,405 (25) |
| 2007 | 9606 (21) |
| 2008 | 6677 (15) |
| 2009 | 3222 (7) |
| Civil identification number present | |
| Yes | 21,033 (46) |
| No | 23,796 (53) |
| Cohort | |
| Aurum | 16,415 (37) |
| Gugulethu | 2198 (5) |
| Hlabisa | 7411 (17) |
| Khayelitsha | 5845 (13) |
| Masiphumelele | 517 (1) |
| McCord | 2791 (6) |
| Thembalethu | 8563 (19) |
| Tygerberg | 1089 (2) |

per week between 6 and 48 months on cART. The steepest rise was among those with HIV RNA <400 copies per milliliter with 1.4 (95% CI: 1.2 to 1.6) cells per cubic millimeter per week, followed by 0.41 (95% CI: 0.18 to 0.60) cells per cubic millimeter per week for HIV RNA 400–1000 copies per milliliter; 0 to 0.012 (95% CI: −0.17 to 0.14) cells per cubic millimeter per week for HIV RNA of 1000–50,000 copies per milliliter; −1.12 (95% CI: −3.6 to 1.4) cells per cubic millimeter per week for HIV RNA 50,000–100,000 copies per milliliter; and −0.48 (95% CI: −0.96, 0.007) cells per cubic millimeter per week for HIV RNA >100,000 copies per milliliter (Fig. 1).

However, even amongst individuals with evidence of persistent HIV RNA suppression, the CD4 count response was heterogeneous with both increases and marked decreases in CD4 count. During the 6-month period with HIV RNA <400 copies per milliliter, there was a decline in CD4 count of more than 25% among 9.0% of patients compared with such a decline among 14% of patients when HIV RNA was 400–1000 copies per milliliter, 24% of patients when HIV RNA was 1000–50,000 copies per milliliter, and 44% of patients when HIV RNA was >50,000 copies per milliliter.

## Mortality

All-cause mortality during the 48 months of follow-up was 4.1 per 100 person-years (95% CI: 3.9 to 4.2). Mortality risk was associated with current CD4 count and with the slope in CD4 count (Table 2). In assessing the proportional hazards assumption, we found that there was no interaction between either current CD4 count or CD4 count slope and duration on cART ($P > 0.1$). We also found no interaction between sex and CD4 count slope ($P = 0.9$). However, CD4 count slope interacted with time-updated absolute CD4 count, losing association with mortality when the time-updated CD4 count was >200 cells per cubic millimeter ($P < 0.001$ from test of interaction). This is graphically represented in Figure 2. In multivariable modeling, we included sex, age, current CD4 count, CD4 count trend, and HIV RNA category. In this model, compared with a 25% decline, the mortality hazard was lower for a nondecreasing CD4 count with a current CD4 count between 100 and 200 cells per cubic millimeter (0.76, 95% CI: 0.62 to 0.94 for no change and 0.77, 95% CI: 0.63 to 0.94 for a 25% increase). Thus, the risk of dying was lower with an increasing CD4 count, independent of current CD4 count and HIV RNA. HIV RNA level was also associated with mortality independent of CD4 count and CD4 count slope, with mortality hazard increasing consistently with increasing HIV RNA level (Table 2).

When we repeated the analysis excluding HIV RNA from the model, the effect size of the association between CD4 count slope increased slightly as follows: for CD4 count ≤100 cells per cubic millimeter with >25% decrease as the referent group, the hazard ratio for no change was 0.65 (95% CI: 0.55 to 0.78) and for >25% increase was 0.51 (95% CI: 0.44 to 0.60); for CD4 count 100–200 cells per cubic millimeter, the hazard ratios were 0.70 (95% CI: 0.57 to 0.86) and 0.65 (95% CI: 0.53 to 0.80), respectively; for CD4 counts 201–350 cells per cubic millimeter, the hazard ratios were 0.84 (95% CI: 0.56 to 1.09) and 1.0 (95% CI: 0.70 to 1.4), respectively; and finally for CD4 counts >350 cells per cubic millimeter, the hazard ratios were 0.87 (95% CI: 0.41 to 1.9) and 1.2 (95% CI: 0.59 to 2.6), respectively.

To further assess the role of CD4 count trend, we undertook an analysis censoring time 30 days after the last CD4 count and repeating the adjusted analysis. In this analysis, the association between CD4 count trend and mortality remained with a slight but nonsignificant increase in effect sizes.

We also assessed for an association between CD4 count response/failure at 6 and 12 months and mortality. In multivariable modeling, adjusting for current CD4 count, current HIV RNA, sex, and age, both 6 and 12 months CD4 count response was associated independently with reduced mortality with an adjusted hazard ratio of 0.63 (95% CI: 0.51 to 0.80, $P < 0.001$) for CD4 count response by 6 months and

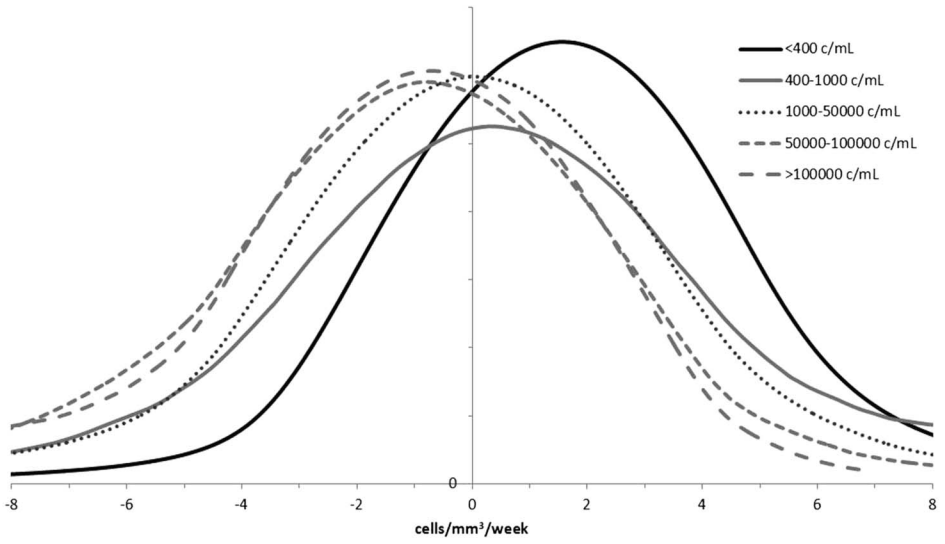


**FIGURE 1.** CD4 count slope distribution density by HIV RNA level. The *y* axis represents density of CD4 count slopes.

**TABLE 2.** Univariable and Multivariable Associations With Mortality up to 3 Years on cART

| | Mortality per 100 Person-Years | HR | *P* | aHR | *P* |
|---|---|---|---|---|---|
| Sex | | | | | |
| Men | 5.1 (4.8 to 5.4) | Referent | <0.001 | Referent | 0.009 |
| Women | 3.4 (3.2 to 3.5) | 0.75 (0.69 to 0.81) | | 0.89 (0.82 to 0.97) | |
| Age (yrs) | | | | | |
| 16–24 | 3.3 (2.8 to 3.9) | Referent | <0.001 | Referent | <0.001 |
| 25–34 | 3.3 (3.1 to 3.5) | 0.96 (0.80 to 1.15) | | 1.02 (0.85 to 1.22) | |
| 35–44 | 4.3 (4.0 to 4.6) | 1.16 (0.97 to 1.39) | | 1.26 (1.05 to 1.52) | |
| >44 | 5.4 (5.0 to 5.8) | 1.43 (1.18 to 1.72) | | 1.58 (1.30 to 1.91) | |
| Weight at ART initiation (kg) | | | | | |
| <50 | 7.1 (6.6 to 7.7) | Referent | — | Referent | — |
| 50–80 | 3.7 (3.5 to 3.8) | 0.52 (0.48 to 0.57) | | 0.55 (0.50 to 0.61) | |
| >80 | 2.9 (2.5 to 3.4) | 0.37 (0.30 to 0.46) | | 0.46 (0.38 to 0.57) | |
| Current CD4 count (cells/mm$^3$) | | | | | |
| ≤100 | 14 (14 to 15) | Referent | <0.001 | Referent | <0.001 |
| 100–200 | 5.0 (4.7 to 5.4) | 0.32 (0.29 to 0.35) | | 0.38 (0.32 to 0.46) | |
| 201–350 | 2.5 (2.3 to 2.8) | 0.15 (0.14 to 0.17) | | 0.18 (0.13 to 0.25) | |
| >350 | 1.4 (1.2 to 1.6) | 0.09 (0.07 to 0.10) | | 0.10 (0.05 to 0.20) | |
| CD4 count trend* | | | | | |
| CD4 count: <100 | | | | | |
| >25% Decrease | 30 (27 to 33) | Referent | <0.001 | Referent | <0.001 |
| No change | 20 (17 to 23) | 0.66 (0.56 to 0.79) | | 0.75 (0.63 to 0.89) | |
| >25% Increase | 16 (14 to 18) | 0.52 (0.45 to 0.60) | | 0.68 (0.58 to 0.79) | |
| CD4 count: 100–200 | | | | | |
| >25% Decrease | 8.2 (6.9 to 9.7) | Referent | — | Referent | — |
| No change | 5.2 (4.4 to 6.0) | 0.70 (0.57 to 0.86) | | 0.79 (0.64 to 0.98) | |
| >25% Increase | 4.3 (3.7 to 5.0) | 0.65 (0.53 to 0.80) | | 0.79 (0.63 to 0.98) | |
| CD4 count: 201–350 | | | | | |
| >25% Decrease | 2.4 (1.8 to 3.4) | Referent | — | Referent | — |
| No change | 2.1 (1.8 to 2.5) | 0.79 (0.56 to 1.09) | | 0.87 (0.63 to 1.21) | |
| >25% Increase | 2.8 (2.5 to 3.2) | 1.00 (0.71 to 1.43) | | 1.11 (0.78 to 1.58) | |
| CD4 count: >350 | | | | | |
| >25% Decrease | 0.91 (0.46 to 2.0) | Referent | — | Referent | — |
| No change | 1.0 (0.83 to 1.3) | 0.88 (0.41 to 1.88) | | 0.95 (0.45 to 2.03) | |
| >25% increase | 1.6 (1.3 to 1.8) | 1.25 (0.60 to 2.62) | | 1.31 (0.63 to 2.76) | |
| HIV RNA (copies/mL) | | | | | |
| <400 | 2.8 (2.7 to 3.0) | Referent | <0.001 | Referent | <0.001 |
| 400–4999 | 3.6 (3.3 to 4.0) | 1.95 (1.69 to 2.26) | | 1.63 (1.41 to 1.89) | |
| 5000–50,000 | 9.7 (8.8 to 11) | 3.34 (2.94 to 3.80) | | 2.18 (1.92 to 2.48) | |
| >50,000 | 22 (20 to 23) | 6.51 (5.89 to 7.20) | | 3.16 (2.82 to 3.55) | |

*Interaction between updated CD4 count and CD4 count slope; $P < 0.001$.
HR, crude hazard ratio; aHR, adjusted hazard ratio.

an adjusted hazard ratio of 0.37 (95% CI: 0.20 to 0.69, $P = 0.002$) for CD4 count response by 12 months.

## DISCUSSION

Using a large multicohort ART population with robust mortality estimates and availability of routine laboratory monitoring, we have demonstrated that a lower current CD4 count, CD4 count slope with >25% decline averaged over 6 months, and a higher current HIV RNA level each are independently associated with increased mortality among people receiving cART. Furthermore, although mortality declined with time from cART initiation, the effect size between CD4 count and mortality remained constant over time on cART, consistent with a study from Northern cohorts.[15] Thus, we reconfirm the strong association between CD4 count and mortality, demonstrating that it remains constant for up to 4 years on ART, and have added findings on the associations both of current HIV RNA level and CD4 count slope in estimating mortality hazard. In addition, patients with a poor initial CD4 response despite virological suppression were at an even greater hazard of death than suggested by the other parameters, including current CD4 count.

Our findings of an association between CD4 count slope and mortality build on the overall understanding of mortality risk during cART, adding the independent value of

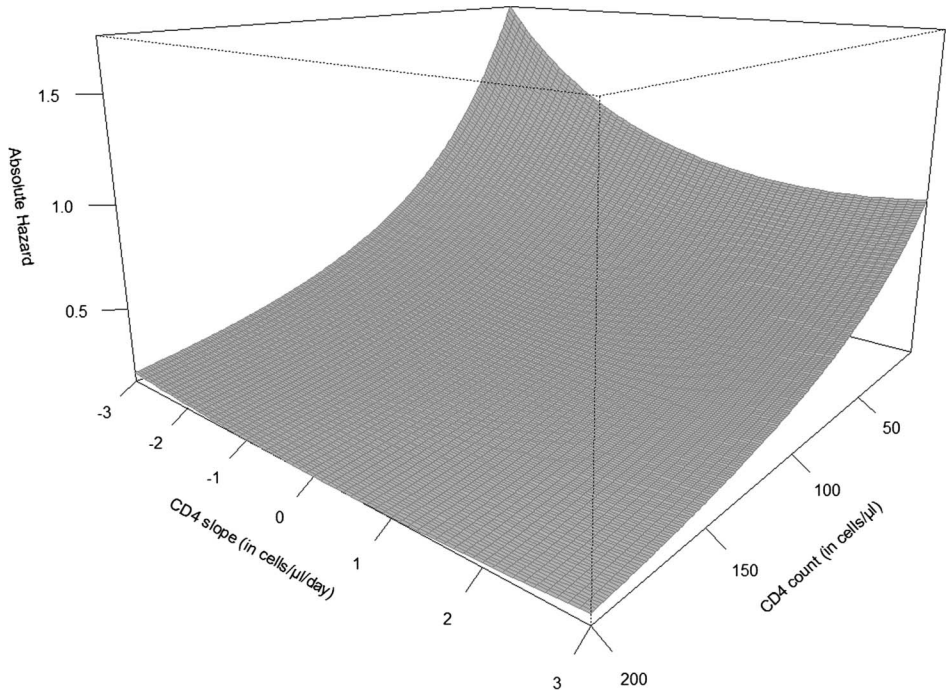


**FIGURE 2.** Absolute mortality hazard from a Cox proportional hazards model of CD4 count slope, (cells/mm$^3$per day), current CD4 count, and the interaction between the 2 for a reference population of men, aged <24 years, with HIV RNA <400 copies per milliliter.

the CD4 count slope.[22,23,32–37] The finding raises the question of whether the CD4 count decline is a result of an ongoing illness or whether the declining CD4 count predisposes to a potentially life-threatening illness. Another possibility is that the CD4 count slope provides a better measure of the true CD4 count at the time of an event. However, our finding of association between CD4 count slope and mortality, even when limiting follow-up to 30 days after the last CD4 count, makes this a less likely explanation. Whether this finding can be translated into clinical use will require further study; however, seeing a decline in CD4 count should raise a clinician's concern for the potential of increased mortality risk.

HIV RNA category was independently associated with mortality with a consistent increase in hazard with increasing HIV RNA. It is notable that the mortality hazard was 64% higher for patients with even modestly elevated HIV RNA between 400 and 4999 copies per milliliter versus <400 copies per milliliter. Prior studies from resource-limited settings have focused on the association between suppressed versus nonsuppressed in assessing for associations with mortality and have not assessed multiple gradation of viremia.[23,32,33] Studies from high-income settings that have assessed for association between gradations of HIV RNA and mortality during cART have reported disparate findings. Several studies found no association between HIV RNA level if CD4 count was included in the model.[38–41] Although others reported an association between HIV RNA and either mortality, mortality and AIDS-defining illnesses, or AIDS-defining illness with an effect size of 1.0–1.8 for HIV RNA 1000–10,000 copies per milliliter and 1.8–3.9 for HIV RNA ≥10,000 copies per milliliter, each compared with either <80 or <500 copies per milliliter.[42–45] These studies did not specifically assess the 400–4999 copies per milliliter range and either identified no or minimal association among participants with HIV RNA between 1000 and 10,000 copies per milliliter. It is plausible that the larger size of our study, with 3848 deaths, allowed us to detect an association that was not found in some prior studies. Another plausible explanation is that causes of mortality in South Africa, such as tuberculosis, may be more closely associated with HIV RNA level than causes or behaviors associated with mortality in Europe.[13,46–48]

Our estimates of CD4 change by HIV RNA level build on prior reports. For example, studies from high-income countries have estimated CD4 count increase among individuals on cART with suppressed HIV RNA to range from 32 to 127 cells per cubic millimeter per year[4,49,50]; our slope for HIV RNA <400 copies per milliliter of 72 cells per cubic millimeter per year fits in the middle of this range. In addition, the variability in CD4 count change during cART is well described.[6,51]

Although this study has the strength of a large dataset generated from routine HIV care programs, it also has several limitations. First, because we used routinely collected data, missing data are inevitable. We used multiple imputations to address this limitation, generating datasets with imputed missing values for up to 17% of laboratory results. However, multiple imputations assume that missingness can be predicted from observed variables. It is possible that individuals were missing CD4 counts or HIV RNA values because they were sick and at increased risk for death, in excess of what can be predicted from other available covariates. This could introduce bias, attenuating the observed associations with mortality. In addition, although our dataset was large, the number of patients contributing outcomes to specific combinations of characteristics may be small and may have contributed to the wide CIs in our adjusted model as we observed with hazard ratio point estimates for CD4 count slope with higher time updated CD4 counts. Although the

effect of uncontrolled HIV viremia on the risk of opportunistic illnesses and other conditions is known, our finding of an independent association between viremia and mortality could be partially confounded by behaviors or circumstances which result in both poor medication adherence and increased risk of illness or death.

Measuring absolute CD4 count remains the cornerstone of ART management in resource-limited settings, and our study confirms the strong association between absolute CD4 count and mortality. We have, however, described a strong independent association between HIV RNA and mortality in a treatment context typical of many in southern Africa and have additionally highlighted the contribution of CD4 count trajectory in hazard of future mortality. The next steps are to better understand the relationships between each phenomenon and severe illness and to identify optimal clinical and monitoring interventions to reduce mortality among those at highest risk.

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