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# Predictors of immune recovery and the association with late mortality while on antiretroviral treatment in Cambodia

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ABSTRACT

The objectives of this study were to examine the association of the on-treatment CD4 cell count with late mortality (after >6 months of antiretroviral treatment [ART]) and to identify the determinants of the long-term CD4 cell count evolution after treatment initiation. We conducted a retrospective analysis including all antiretroviral (ARV)-naïve adults initiating ART in a tertiary hospital in Phnom Penh, Cambodia from 2003–2010. We used Cox proportional hazards modelling (mortality analysis), including time-updated CD4 counts, and mixed-effects modelling (CD4 response over time). Overall, 2840 patients were included (47% male, median age: 34 years, median baseline CD4 count: 78 cells/μL). The median time on ART was 2.5 years (IQR 1.1-4.3); 71 patients died after >6 months of ART. The baseline CD4 count was the main determinant of the on-treatment CD4 cell count. Time-updated CD4 cell counts was the strongest determinant of late mortality with a HR of 0.32 (95% CI 0.16–0.63) and 0.29 (95% CI 0.11–0.71) for CD4 values of 200–350 cells/μL and 350–500 cells/μL respectively. We conclude that baseline CD4 counts strongly determine the long-term immune recovery, which critically affects late mortality. This calls for increased efforts for early ART initiation and availability of CD4 count testing in low-income countries.



## 1. Introduction

Significant progress has been made in the scaling-up of antiretroviral treatment (ART) in low and middle income countries (LMIC) over the last few years, with currently over 5 million individuals initiated on ART. A substantial part of these, close to 15%, live in Asia.[1] Although overall good treatment outcomes have been reported in LMIC, especially with regards to retention[2,3] and adherence,[4,5] mortality while on ART remains substantial.[6–9] Particularly within the first six months of treatment, mortality remains high ('early mortality').[10] A number of studies, including studies from Asia, have aimed to identify risk factors for early mortality in LMIC.[7,10–15]

Now that ART programs in LMIC countries are maturing, the majority of patients in these programs have been taking treatment for prolonged periods, with up to seven years of follow-up on treatment reported.[16,17] Hence, research should now also focus on the determinants of the long term treatment outcomes, after the first 6–12 months of treatment. However, few studies have been conducted, particularly in Asian countries.

Whereas baseline patient characteristics have been found highly predictive of early mortality, there are indications from high income countries that the risk of late mortality (after >6 months of ART) critically depends on the response to ART.[18–22] In these studies, the CD4 cell count and viral load were identified as important prognostic factors. Whereas viral load monitoring is not routinely

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available in LMIC, most ART programs currently perform CD4 cell count monitoring after ART initiation. However, to what extent the on-treatment CD4 cell count evolution, applied in routine settings, is a useful prognostic measure in terms of predicting late mortality remains poorly explored in LMIC, even more so within treatment programs in Asia. If on-treatment CD4 cell count were indeed predictive of mortality in these settings, this would help identify those at risk and target them for closer medical follow-up. Moreover, this would also indicate the need to understand the factors associated with a CD4 response after treatment initiation. The identification of predictive factors would assist in designing preventive strategies that could help reduce on-treatment mortality. The objectives of this study were to examine the association of the on-treatment CD4 cell count with late mortality and to describe and identify the determinants of the CD4 cell count evolution after treatment initiation in a large HIV treatment program in Cambodia.

## 2. Methods

### 2.1. Study setting

This study was conducted at the Sihanouk Hospital Center of Hope (SHCH), a tertiary-level hospital in Phnom Penh, Cambodia. Since 1 March 2003 ART has been provided in SHCH at no cost, as part of the national ART program.

### 2.2. Study design and population

A retrospective cohort analysis was conducted including all antiretroviral (ARV)-naïve adult patients initiating lifelong ART at the SHCH between 1 March 2003 and 1 September 2010. Patients initiating ART at another ART facility with subsequent referral to SHCH were excluded from analysis. Those without baseline and any follow-up CD4 cell count measurement after ART initiation were also excluded.

### 2.3. Antiretroviral therapy initiation and follow-up

Patients were initiated on ART following WHO recommendations: all patients with CD4 cell count <200 cells/μL, WHO stage III with CD4 cell count <350 cells/μL or WHO stage IV (irrespective of CD4 cell counts) were eligible for ART.[23,24] The preferential first line regimen consisted of a generic fixed-dose combination containing stavudine, lamivudine and nevirapine. Zidovudine, efavirenz and tenofovir were alternative drugs available for first line regimens, given in cases of contraindication or toxicity. The preferential second line regimen consisted of boosted lopinavir, lamivudine and tenofovir.

During the first six months of ART, patients were seen at monthly visits; subsequently, clinically stable patients were scheduled to attend less frequently (every 2–3 months). All medical care was provided by physicians, supported by a team of nurses, social assistants and adherence counsellors. At each clinical visit, a number of issues were systematically addressed, including treatment adherence, the clinical evolution and ART-related toxicity. Baseline laboratory monitoring included haematology and CD4 cell count determination (FACSCount, Becton Dickinson, Franklin Lakes, NJ, USA). The CD4 cell count test was repeated every six months. In suspected cases of treatment failure, a viral load test was requested. Prophylactic treatment with cotrimoxazole was given if CD4 count was <200 cells/μL or presenting with symptomatic HIV disease; all patients with WHO stage 4 disease or a CD4 count <100 cells/μL were given fluconazole primary prophylaxis. Patients not presenting at their scheduled visit were contacted by telephone, or visited at home if living in the hospital neighbourhood. Those not presenting at the hospital for a period of six months without additional information were defined lost to follow-up (LTFU). All HIV care (including treatment for opportunistic infections) was provided free of charge. Details of the ART program in SHCH have been published previously.[13,25]

### 2.4. Data collection system and statistical analysis

At the launch of the ART program, a database and data collection tools were developed. On a daily basis, clinical and laboratory data were collected and entered in the database. Quality control of the stored data was done at regular intervals.

#### 2.4.1. Mortality rates and risk factors for mortality

The primary outcome was time to death while on ART. For each patient, person-time at risk was calculated, starting from the date of ART initiation up to either the date of death, date of last visit for those LTFU or transferred-out, and 31 December 2010 for the remainder. Cumulative mortality was estimated using Kaplan-Meier methods. We constructed two separate Cox proportional hazard models to determine the risk factors for early mortality (<6 months after ART initiation) and late mortality (after ≥6 months of treatment). Based on the literature, including a study on the determinants of early ART mortality from this program,[13] the following baseline characteristics were considered a priori as risk factors for mortality: age, sex, WHO clinical stage, body mass index (BMI), hemoglobin and CD4 cell count, year of ART initiation. Initiation of cotrimoxazole was not included in the model, since this is close to universal in this HIV treatment program. On-treatment (time-updated) CD4 cell counts were included as well in the analysis of late mortality, using all available on-treatment CD4 cell count measurements (total of 16 882 results). On average, 6.0 (interquartile range [IQR] 3.1–11.2) CD4 cell samples after ART initiation were available per patient, with an average of 2.0 (IQR 1.7–2.3) samples per patient/year. Starting from the full model including all co-variates, a backward selection process was performed by observing the effect on the outcome (mortality) of removing every individual predictor (besides the main exposure) one by one, starting with the variable with the weakest association with the outcome. Subsequently, all co-variates were added again in the model in a forward selection process to observe whether joint effects of co-variates existed. Co-variates were retained in the model if their removal/inclusion induced a change

of >10% in the measure of effect of the main exposure (baseline and time-updated CD4 cell count). A number of alternative modelling strategies were explored in sensitivity analysis. Additionally, we explored the effect of LTFU on the mortality analysis by repeating analysis with those LTFU defined as failure. The proportional-hazards assumption was tested graphically and formally using Schoenfeld residuals.

### 2.5. CD4 cell count evolution and risk factor analysis

To describe the CD4 cell count evolution after ART initiation, we determined the observed mean CD4 cell count at baseline (within 12 weeks prior to ART initiation) and at every 6-month interval (within 12 weeks) up to five years of treatment. Additionally, to visualize the overall CD4 cell count trajectory after ART initiation, a non-parametric method was used called LOWESS smoothing (for locally weighted scatterplot smoothing, 'lowess' command in Stata [StataCorp LP, College Station, TX, USA]). This provides a representative smooth curve through data using robust local regression. To examine the association of CD4 cell count on treatment and change over time, a mixed-effects linear regression model was constructed including the following covariates, a priori identified from the literature: age, sex, baseline BMI and WHO stage, baseline CD4 cell count and hemoglobin, use of zidovudine vs stavudine in the initial ART regimen, use of nevirapine vs efavirenz in the initial ART regimen. Given the non-linear association of time on ART and CD4 cell count evolution (as visualized in the descriptive analysis, and in line with reported studies[26–28]) polynomials for time on ART were included as fixed effects in the linear regression model. Random intercepts and slopes were included to allow for variation between individuals. Different models were compared based on the likelihood ratio test and the Akaike information criterion. In addition, the model fit was visually assessed by comparing predicted with observed CD4 cell count trajectories. The final model contained time on ART transformed to the power (−0.5), to the power (0.5) and the natural logarithm of time – fractional polynomial [3,−0.5, ln, 0.5)] – and a random intercept and coefficient for each patient. Interaction terms were included to identify determinants of the CD4 cell count trajectories, i.e., whether any effect changed over time. The coefficients of the main effects in the model output represented the vertical shift of the CD4 cell count trajectories relative to the baseline category, the interaction terms assessed the effect of the covariate on the slope of the CD4 count (interaction with time). The robustness of the findings to the specific model selected for analysis was assessed in sensitivity analysis. All analysis was done using Stata version 11. All statistical tests were two-sided, statistical significance was defined as $P < 0.05$.

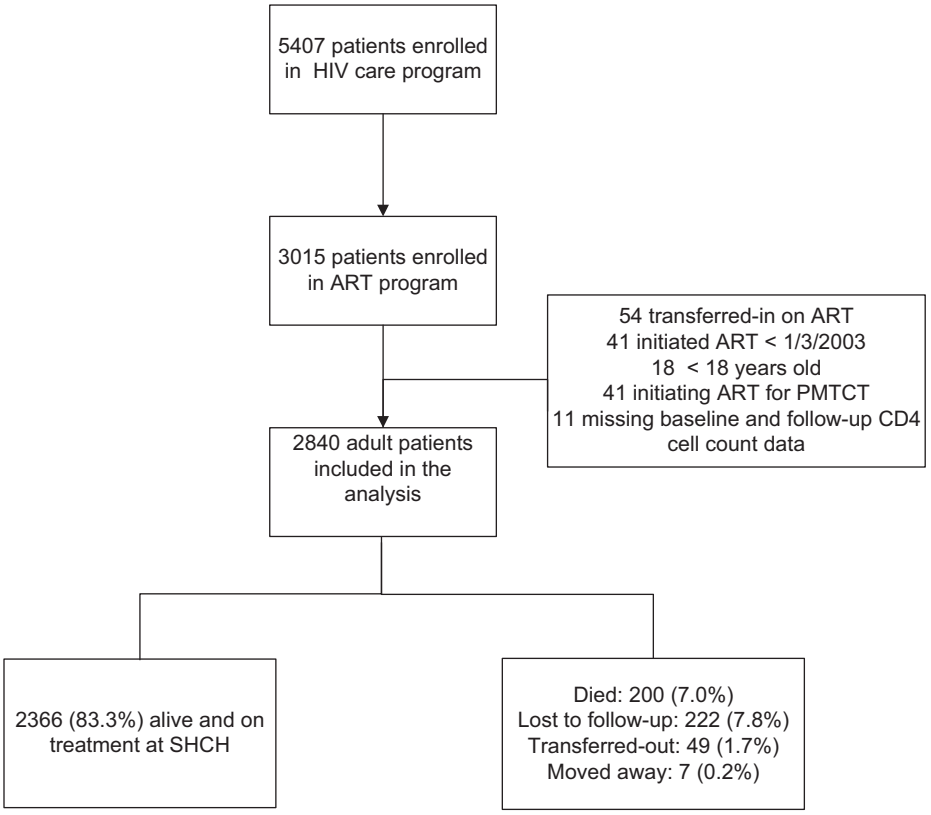


**Figure 1.** Flow-chart describing the flow from enrolment in the HIV care program up to inclusion in analysis. ART: antiretroviral treatment; PMTCT: prevention of mother-to-child transmission; SHCH: Sihanouk Hospital Center of Hope.

## 3. Results

### 3.1. Baseline characteristics

Of the 5407 adults registered into HIV care at SHCH, 3015 enrolled into the ART program. In total, 165 were excluded (see Figure 1), leaving 2840 for analysis. As seen in Table 1, 47% were male, the median age was 34 years. Most initiated with advanced HIV disease, the median baseline CD4 cell count was 78 cells/μL (IQR 23–196). The median time on ART at the time of analysis was 2.5 years (IQR 1.1–4.3).

Of the 2840 patients included in analysis, 117 (4.1%) individuals had missing data on a baseline co-variate. Ninety percent (105) of these were missing one single variable. Individuals with missing data were significantly more likely to initiate ART with WHO stage IV, had lower weight, hemoglobin and CD4 cell counts at baseline and were more likely to start a zidovudine-containing regimen.

### 3.2. Mortality rates and cumulative mortality

Of the 200 deaths, 129 (64.5%) consisted of cases of early mortality, occurring during the first six months of ART; the remaining 71 (35.5%) cases were defined as late mortality. This corresponded with an early mortality rate of 9.9/100 patient-years (95% CI 8.4–11.8), the late mortality rate was estimated at 1.1/100 patient-years (95% CI 0.8–1.4) (Table 1). Cumulative mortality, estimated using Kaplan-Meier analysis, was 4.7% (95% CI 4.0–5.6), 5.8% (95% CI 5.0–6.8) and 9.3% (95% CI 7.9–10.9) at 6, 12 and 60 months of ART respectively.

**Table 1**
Baseline characteristics and mortality rates of 2840 adult patients initiating antiretroviral treatment at Sihanouk Hospital Centre of Hope

| Characteristic | $n = 2840$ |
|---|---|
| Age, years; median (IQR) | 34 (29–40) |
| Male sex; *n* (%) | 1333 (46.9%) |
| Baseline WHO clinical stage; *n* (%) | |
| Stage 1 | 165 (5.8%) |
| Stage 2 | 422 (14.9%) |
| Stage 3 | 1144 (40.3%) |
| Stage 4 | 1109 (39.0%) |
| Baseline body weight, kg; median (IQR) | 49 (43–55) |
| Individuals with missing data; *n* (%) | 6 (0.2%) |
| Baseline body mass index, kg/m$^2$; median (IQR) | 19 (17–21) |
| Individuals with missing data; *n* (%) | 52 (1.8%) |
| Baseline CD4 count, cells/μL; median (IQR) | 78 (23–196) |
| Individuals with missing data; *n* (%) | 46 (1.6%) |
| Baseline hemoglobin, g/dL; median (IQR) | 11.2 (9.9–12.6) |
| Individuals with missing data; *n* (%) | 29 (1.0%) |
| NRTI in regimen at ART initiation; *n* (%) | |
| Stavudine | 2652 (93.4%) |
| Zidovudine | 188 (6.6%) |
| NNRTI in regimen at ART initiation; *n* (%) | |
| Nevirapine | 2057 (72.4%) |
| Efavirenz | 783 (27.6%) |
| Year of ART initiation; *n* (%) | |
| 2003–2004 | 268 (9.4%) |
| 2005–2006 | 974 (34.1%) |
| 2007–2008 | 911 (32.1%) |
| 2009–2010 | 691 (24.3%) |
| Follow-up time on ART, years; median (IQR) | 2.5 (1.1–4.3) |
| Mortality rate/100 patient-years (95% CI) | |
| 0–3 months of ART | 14.4 (11.5–17.3) |
| 3–6 months of ART | 5.4 (3.9–7.6) |
| 6–12 months of ART | 2.3 (1.5–3.3) |
| >1 year of ART | 0.8 (0.6–1.1) |

ART: antiretroviral treatment; IQR: interquartile range; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors.

### 3.3. Risk factor analysis for early mortality while on antiretroviral therapy

In univariate analysis, all variables included in the model were associated with early mortality (Table 2), confirming that these factors have consistently been identified as risk factors for mortality in the literature. In multivariate analysis, the association with baseline CD4 cell count persisted with a hazard ratio (HR) of 0.48 (95% CI 0.30–0.78) for those with CD4 cell count 50–200 cells/μL and 0.22 (95%CI 0.09–0.57) for those with baseline CD4 counts >200 cells/μL. In addition, sex, baseline BMI and hemoglobin were retained in the model.

### 3.4. Risk factor analysis for late mortality while on antiretroviral therapy

High baseline CD4 cell count (>200 cells/μL) was associated with a reduced risk of late mortality in univariate analysis (Table 3). With time-updated CD4 cell counts <200 cells/μL as reference category, a lower mortality was seen with time-updated CD4 counts of 200–350 cells/μL. In addition, male sex, older age, and low baseline BMI and hemoglobin were identified as risk factors for late mortality.

In multivariate analysis, after adjusting for time-updated CD4 cell counts, no significant association with baseline CD4 cell count was seen. In contrast, the significant association with time-updated CD4 cell counts persisted with an HR of 0.32 (95% CI 0.16–0.63) for CD4 counts of 200–350 cells/μL and an HR of 0.29 (95% CI 0.11-0.71) for values of 350–500 cells/μL. The association with CD4 counts >500 cells/μL was not significant (HR 0.45, 95% CI 0.16–1.25). Age, sex, baseline BMI and hemoglobin were also retained in the model.

### 3.5. Description and determinants of CD4 cell count evolution after initiation of antiretroviral therapy

Predicted and observed CD4 cell count evolution is displayed in Figure 2. The observed CD4 cell count by 6, 12, 36 and 60 months was 247 (95% CI 241–253), 280 (95% CI 273–286), 364 (95% CI 354–374) and 420 (95% CI 404–437) cells/μL respectively. As shown in Figure 3, female sex, younger age (<35 years), higher baseline BMI ($\geq$18.5 kg/m$^2$) and less advanced WHO stage at baseline were associated with higher on-treatment CD4 cell counts. The most pronounced effects were seen with baseline CD4 cell counts: patients with lower baseline CD4 cell counts had clearly lower on-treatment CD4 counts throughout the observation period. Whereas a baseline hemoglobin $\geq$10 g/dL, and the use of zidovudine and nevirapine in the ART regimen were associated with higher CD4 cell counts at ART initiation, a clear interaction with time was seen, with

**Table 2**
Risk factors for early mortality (<6 months after initiation of antiretroviral treatment)

| Variable | Events $n = 97$ (%) | Rate/100 patient-years | Univariate analysis | | | Multivariate analysis[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value |
| Gender | | | | | | | | |
| Male | 60 (62) | 10.45 | 1 | | | 1 | | |
| Female | 37 (38) | 5.44 | 0.52 | 0.35–0.70 | <0.01 | 0.48 | 0.31–0.74 | <0.01 |
| Age | | | | | | | | |
| <35 years | 35 (36) | 6.02 | 1 | | | ND | | |
| ≥35 years | 62 (64) | 9.22 | 1.53 | 1.01–2.31 | 0.04 | ND | ND | ND |
| Baseline BMI | | | | | | | | |
| ≥18.5 kg/m$^2$ | 25 (26) | 3.37 | 1 | | | 1 | | |
| <18.5 kg/m$^2$ | 72 (74) | 14.08 | 4.15 | 2.63–6.55 | <0.01 | 2.70 | 1.67–4.35 | <0.01 |
| Baseline WHO stage | | | | | | | | |
| I/II | 7 (7) | 2.66 | 0.29 | 0.13–0.63 | <0.01 | ND | ND | ND |
| III/IV | 90 (93) | 9.08 | 1 | | | ND | | |
| Baseline hemoglobin | | | | | | | | |
| ≥10 g/dL | 46 (47) | 4.87 | 1 | | | 1 | | |
| <10 g/dL | 51 (53) | 16.54 | 3.36 | 2.26–5.01 | <0.01 | 2.38 | 1.56–3.64 | <0.01 |
| Baseline CD4 cell count | | | | | | | | |
| <50 cells/μL | 69 (71) | 15.16 | 1 | | | 1 | | |
| 50–200 cells/μL | 23 (24) | 4.90 | 0.32 | 0.20–0.52 | <0.01 | 0.48 | 0.30–0.78 | <0.01 |
| >200 cells/μL | 5 (5) | 1.52 | 0.10 | 0.04–0.25 | <0.01 | 0.22 | 0.09–0.57 | <0.01 |
| Year of ART initiation | | | | | | | | |
| <2004 | 7 (7) | 5.89 | 1.10 | 0.49–2.46 | NS | ND | ND | ND |
| 2005–2006 | 52 (54) | 11.93 | 2.22 | 1.46–3.37 | <0.01 | ND | ND | ND |
| >2006 | 38 (39) | 5.44 | 1 | | | ND | | |

ART: antiretroviral treatment; BMI: body mass index; HR: hazard ratio; ND: not determined; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NS: non-significant.

[a] All variables assessed in univariate analysis were included in the full model. Co-variates were retained in the model if their removal/inclusion induced a change of >10% in the measure of effect of the main exposure (baseline CD4 cell count). Only those with complete baseline data were included in analysis ($n = 2723$).

*P*-value for the likelihood ratio test comparing the model with/without the specific variable: in a) Univariate analysis: baseline CD4 cell count: <0.01; year of ART initiation: 0.03; b) Multivariate analysis: baseline CD4 cell count: <0.01.

more pronounced increase in CD4 cell counts associated with the use of stavudine, efavirenz and a low baseline hemoglobin (<10 g/dL). Data suggested a similar interaction with younger age, and with advanced WHO stage to a lesser extent. Similarly, a steeper CD4 cell count trajectory was suggested for those with baseline CD4 cell counts <50 cells/μL and female sex.

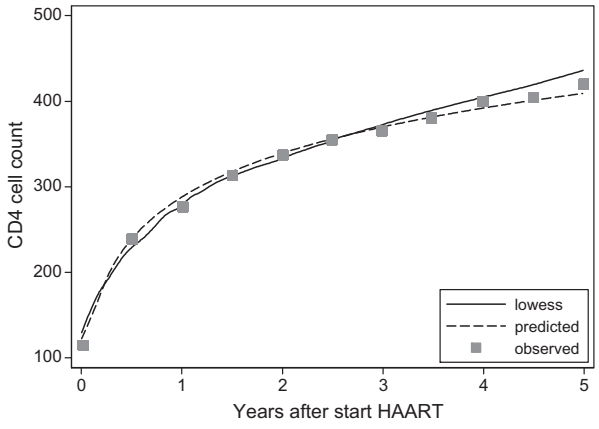


**Figure 2.** CD4 cell evolution after initiation of antiretroviral treatment. Data represented are: the observed mean CD4 cell count (cells/μL) at 6-monthly intervals, lowess-estimated CD4 cell count evolution, model-predicted CD4 cell counts (linear mixed-effects model). HAART: highly-active antiretroviral treatment.

The multivariate analysis is shown in Table 4. Female sex and younger age (<35 years) was associated with higher CD4 cell counts. Similarly, patients initiating zidovudine-based ART had higher CD4 cell counts at baseline. Pronounced effects were seen with baseline CD4 cell counts (+95 and 252 cells/μL for baseline CD4 count of 50–200 and >200 cells/μL relative to CD4 count <50 cells/μL). The slope of the CD4 cell count – the CD4 cell count trajectory – was determined by a number of factors. Whereas more pronounced CD4 cell increases on ART were seen for patients with a normal baseline BMI (≥18.5 kg/m$^2$) and younger age, lesser CD4 increases on treatment were observed with higher baseline CD4 cell counts (CD4 >200 cells/μL vs the reference category of CD4 cells <50 cells/μL). Similarly, those initiating zidovudine and nevirapine had lesser CD4 cell count recovery on-treatment. The main findings remained essentially unchanged during sensitivity analysis, evaluating different mixed-effects models.

## 4. Discussion

The robust and sustained CD4 cell count response up to five years after ART initiation demonstrates that successful immune recovery can be obtained with a public-health approach to ART provision, without routine viral load determination and with a limited choice of ARVs. Whereas early mortality was strongly associated with a number of baseline factors, including baseline CD4 cell count, late mortality was strongly determined by the on-treatment

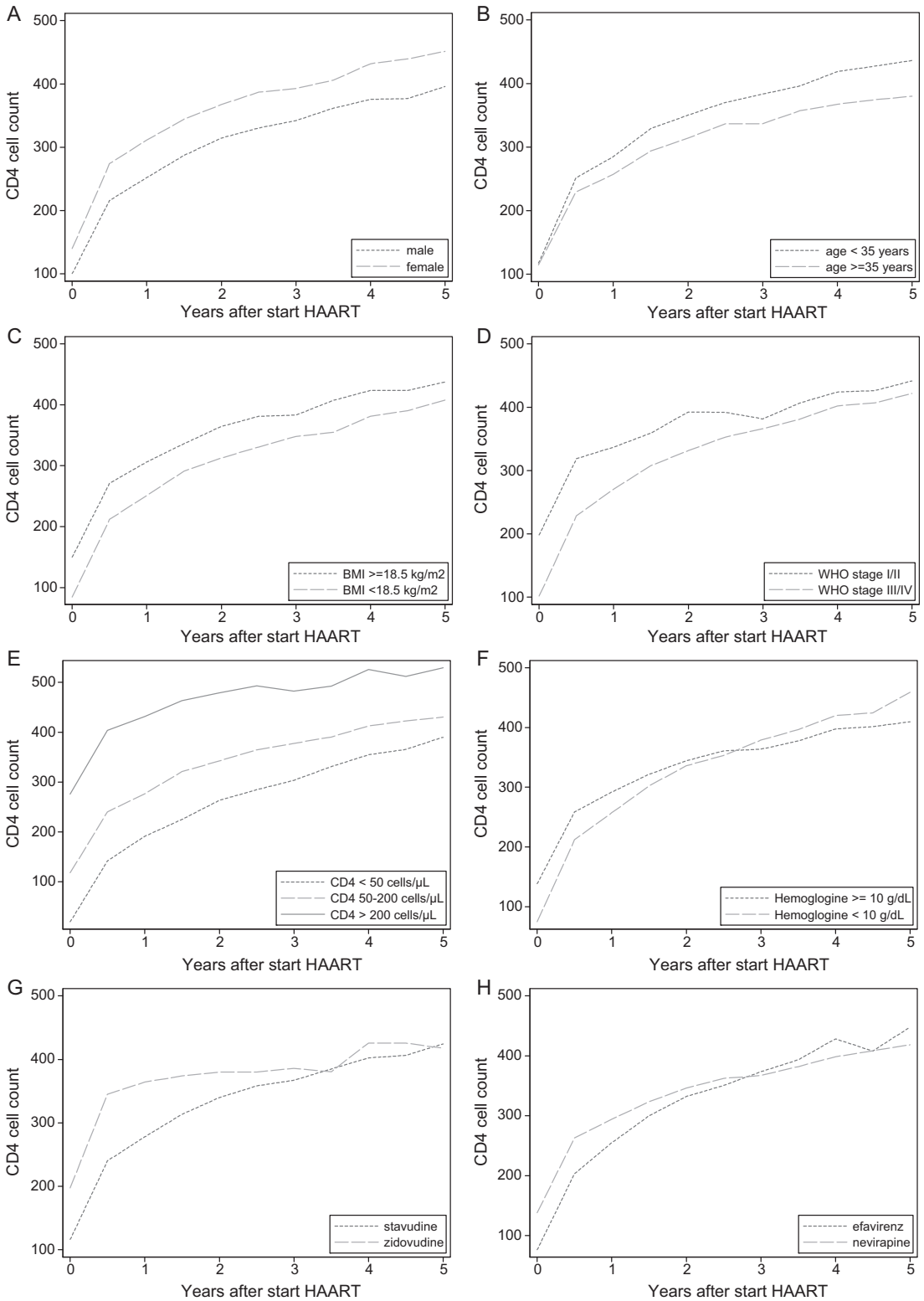


**Figure 3.** CD4 cell evolution after initiation of antiretroviral treatment. The CD4 cell count trajectory (cells/µL) is stratified by sex (A), age (B), baseline body mass index (BMI) (C), baseline WHO stage (D), baseline CD4 cell count (E), baseline hemoglobin (F), nucleoside reverse transcriptase inhibitor at initiation of highly-active antiretroviral treatment (HAART) (G), non-nucleoside reverse transcriptase inhibitor at initiation of HAART (H).

**Table 3**
Risk factors for late mortality (≥6 months after initiation of antiretroviral treatment)

| Variable | Events $n = 64$ (%) | Rate/100 patient-years | Univariate analysis | | | Multivariate analysis[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value |
| Gender | | | | | | | | |
| Male | 38 (59) | 1.27 | 1 | | | 1 | | |
| Female | 26 (41) | 0.77 | 0.60 | 0.36–0.99 | 0.04 | 0.58 | 0.34–0.99 | 0.05 |
| Age | | | | | | | | |
| <35 years | 20 (31) | 0.68 | 1 | | | 1 | | |
| ≥35 years | 44 (69) | 1.29 | 1.88 | 1.01–3.18 | 0.02 | 1.73 | 1.01–2.95 | 0.05 |
| Baseline BMI | | | | | | | | |
| ≥18.5 kg/m$^2$ | 25 (39) | 0.68 | 1 | | | 1 | | |
| <18.5 kg/m$^2$ | 39 (61) | 1.46 | 2.19 | 1.32–3.62 | <0.01 | 1.77 | 1.04–2.99 | 0.03 |
| Baseline WHO stage | | | | | | | | |
| I/II | 9 (14) | 0.86 | 0.75 | 0.37–1.52 | NS | ND | ND | ND |
| III/IV | 55 (86) | 1.04 | 1 | | | ND | | |
| Baseline hemoglobin | | | | | | | | |
| ≥10 g/dL | 35 (55) | 0.73 | 1 | | | 1 | | |
| <10 g/dL | 29 (45) | 1.87 | 2.60 | 1.59–4.25 | <0.01 | 2.60 | 1.54–4.40 | <0.01 |
| Baseline CD4 cell count | | | | | | | | |
| <50 cells/μL | 32 (50) | 1.24 | 1 | | | 1 | | |
| 50–200 cells/μL | 26 (41) | 1.08 | 0.83 | 0.50–1.40 | NS | 1.45 | 0.83–2.51 | NS |
| >200 cells/μL | 6 (9) | 0.44 | 0.32 | 0.13–0.77 | 0.01 | 1.08 | 0.38–3.00 | NS |
| Year or ART initiation | | | | | | | | |
| <2004 | 7 (11) | 0.55 | 0.93 | 0.37–2.32 | NS | ND | ND | ND |
| 2005–2006 | 39 (61) | 1.22 | 1.79 | 1.00–3.22 | 0.05 | ND | ND | ND |
| >2006 | 18 (28) | 0.95 | 1 | | | ND | | |
| Updated CD4 cell count | | | | | | | | |
| <200 cells/μL | 38 (60) | 2.27 | 1 | | | 1 | | |
| 200–350 cells/μL | 13 (20) | 0.61 | 0.29 | 0.15–0.56 | <0.01 | 0.32 | 0.16–0.63 | <0.01 |
| 350–500 cells/μL | 7 (11) | 0.44 | 0.23 | 0.10–0.53 | <0.01 | 0.29 | 0.11–0.71 | <0.01 |
| >500 cells/μL | 6 (9) | 0.59 | 0.32 | 0.13–0.79 | 0.01 | 0.45 | 0.16–1.25 | NS |

ART: antiretroviral treatment; BMI: body mass index; HR: hazard ratio; ND: not determined; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NS: non-significant.

[a] All variables assessed in univariate analysis were included in the full model. Co-variates were retained in the model if their removal/inclusion induced a change of >10% in the measure of effect of the main exposure (baseline and time-updated CD4 cell count). Only those with complete baseline data and >6 months of follow-up after ART initiation were included in analysis ($n = 2365$).

*P*-value for the likelihood ratio test comparing the model with/without the specific variable: in a) Univariate analysis: updated CD4 cell count: <0.01; baseline CD4 cell count: 0.02; year of ART initiation: 0.06; b) Multivariate analysis: updated CD4 cell count: <0.01; baseline CD4 cell count: 0.40.

CD4 cell counts. This highlights the importance of CD4 cell recovery on ART in terms of reducing mortality.

The observed association of on-treatment CD4 cell count evolution and mortality is in agreement with a study from South-Africa.[29] The authors equally observed that time-updated but not baseline CD4 cell counts were strongly associated with late mortality. The findings of this study on the determinants CD4 trajectories are remarkably similar to the data of a large multi-country study of ART programs in LMIC, mainly African countries.[26] In this and our study, more pronounced increases in CD4 cell count were seen for females and younger patients. Importantly, the baseline CD4 cell count was the principal determinant of the long-term CD4 cell response in both studies. Altogether, these findings suggest that early and timely ART initiation is the main way forward to increase on-treatment CD4 cell counts and reduce both early and late mortality.

However, many patients in LMIC have initiated ART at low CD4 cell counts, often below 50 cells/μL.[7,13,16] The recent modification of the WHO guidelines, increasing the CD4 cell count threshold for ART initiation from 200–250 cells/μL to 350 cells/μL, might help in that respect.[30] However, late HIV diagnosis and delays in ART initiation are also determinants of the CD4 values at treatment initiation. Whereas baseline CD4 cell counts at treatment start have increased in many ART programs over the last years,[13,16,31] many patients still initiate ART with values clearly below 200 cells/μL even today. Although increasing the CD4 threshold for ART initiation is key, this should go hand in hand with innovative approaches for early detection of HIV, enhanced pre-ART retention and timely ART initiation. Increased availability of CD4 cell count testing at point-of-care level would obviously be crucial.

Although the data from our study clearly suggest that on-treatment CD4 cell counts >200 cells/μL are associated with reduced mortality, the limited sample size precludes to determine whether there is an incremental protective effect of higher on-treatment CD4 cell count or whether there is a kind of threshold of CD4 cell count, above which patients would be relatively 'safe'. Similar to our findings, a recent publication from South Africa equally observed no statistically significant mortality reduction with updated CD4 cell count values >350 cell/μL.[29] On the other hand, several recent reports from high-income countries have demonstrated an incremental survival benefit with higher baseline CD4 cell counts, even for CD4 values >350–500 cells/μL.[32,33] A similar association with time-updated CD4 cell counts has been reported previously.[18,19,21,22]

The findings of this study underline the value of the CD4 cell count monitoring within routine ART care in LMIC. Although it has been clearly documented that the CD4 cell count evolution is poorly predictive of

**Table 4**
Determinants of CD4 cell count evolution after initiation of antiretroviral treatment[a]

| Variable | Main effect [a] | | Interaction with time[a] | |
|---|---|---|---|---|
| | Coefficient (95% CI) | *P*-value | Coefficient (95% CI) | *P*-value |
| Sex | | | | |
| Male | Ref | | Ref | |
| Female | 11.9 (6.1;17.7) | <0.01 | 2.1 (-2.0;7.8) | NS |
| Age | | | | |
| <35 years | Ref | | Ref | |
| ≥35 years | −11.4 (−16.9; −5.9) | <0.01 | −5.0 (−9.6; −0.3) | 0.03 |
| Baseline BMI | | | | |
| ≥18.5 kg/m$^2$ | Ref | | Ref | |
| <18.5 kg/m$^2$ | −4.9 (−11.8; 1.1) | NS | −6.4 (−11.3;−1.4) | 0.01 |
| Baseline WHO stage | | | | |
| I/II | Ref | | Ref | |
| III/IV | −1.0 (−8.5;6.4) | NS | 0.4 (−4.7;5.6) | NS |
| Baseline CD4 count | | | | |
| <50 cells/μL | Ref | | Ref | |
| 50–200 cells/μL | 94.9 (88.4;101.5) | <0.01 | −3.3 (−8.6;2.0) | NS |
| >200 cells/μL | 252.2 (244.4;260.0) | <0.01 | −12.5 (−19.2; −5.8) | <0.01 |
| Baseline hemoglobin | | | | |
| ≥10 g/dL | Ref | | Ref | |
| <10 g/dL | −4.6 (−11.4;2.2) | NS | 3.1 (−2.6;8.8) | NS |
| NRTI at ART initiation | | | | |
| Stavudine | Ref | | Ref | |
| Zidovudine | 39.4 (28.3;50.5) | <0.01 | −11.5 (−20.0; −2.9) | <0.01 |
| NNRTI at ART initiation | | | | |
| Efavirenz | Ref | | Ref | |
| Nevirapine | 2.6 (−4.1;9.2) | NS | −17.3 (−22.8; −11.8) | <0.01 |

ART: antiretroviral treatment; BMI: body mass index; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NS: non-significant.

[a] Multivariate linear mixed effects model including the factors listed. The coefficients of the main effects in the model output represent the vertical shift of the CD4 cell count trajectories relative to the baseline category; the interaction terms assess the effect of the covariate on the slope of the CD4 count. *P*-value for the likelihood ratio test comparing the model with/without the variable baseline CD4 cell count <0.01.

virological treatment failure,[34] the observed association with mortality suggests that, besides for the identification of ART eligibility, CD4 cell count monitoring after ART initiation could help identifying those at higher risk of mortality, requiring closer medical follow-up. Although the exact mechanism underlying this association remains to be assessed in future studies, a number of factors could be involved including virological failure, ongoing immune activation or subclinical infections. Improved understanding could help design preventive strategies.

The independent association of male sex with on-treatment mortality, adjusted for a number of confounders, has been observed previously.[8] This suggests that, besides the lower baseline CD4 cell count up on ART initiation and possibly the older age, other mechanisms should explain the increased mortality for males. Although males had a less pronounced CD4 cell response after ART initiation, the association was not significant in this study. Whereas increased immune recovery (hence steeper CD4 slope) in female patients was observed in a number of studies,[26,35,36] other studies have reported conflicting data.[37,38] Whether the associations of sex with ART outcomes relate to biological or behavioural factors, remains to be assessed.[36,39]

The association of older age, increased mortality and poor immune response has been reported in a number of studies.[8,26,40] Still, the underlying mechanism remains unclear. A number of factors could be involved, including immunosenescence and thymic dysfunction, poor adherence, increased toxicity and competing mortality risks.[41–43] With the aging of the HIV-infected population in low income countries, detailed studies are warranted. Whereas the reduced CD4 recovery associated with the use of zidovudine has been reported before,[44] the more pronounced CD4 increase associated with the use of efavirenz is in contrast with other reports.[45] However, a more recent paper from 2008 reported on the strong CD4 recovery with efavirenz containing regimens.[46] The reason for the conflicting findings remains to be assessed.

A number of limitations have to be pointed out. This is a retrospective analysis, using data from operational settings. However, data were collected prospectively using standardized data collection tools, data quality and completeness was verified throughout the program and retention in care was high. Data appeared robust in sensitivity analysis. In addition, data were missing for some parameters, and patients with missing data were significantly different on a number of characteristics, suggesting they were actually more 'sick'. Still, given the overall limited number of missing data, we think the overall impact on the study findings to be limited. Moreover, this compares favourably with other studies from similar settings reporting missing data for >30% of patients.[8,26] Data on adherence, viral load and opportunistic infections might have been of interest. Finally, detailed analysis of the causes of death might have been informative. In particular, with the increased awareness of the importance of non-AIDS related deaths for patients on ART, at least in high-income countries, this is an area for future research in LMIC.

## 5. Conclusion

With the maturing of ART programs in LMIC, increased understanding of rates and determinants of late mortality is important. In this HIV program, the on-treatment CD4 cell count was the main determinant of late mortality, and the on-treatment CD4 cell count was critically dependent on the baseline CD4 cell count. This calls for increased efforts for timely and early ART initiation in LMIC. Moreover, it confirms the value of CD4 cell count in monitoring of ART treatment in resource-constrained settings where viral load monitoring might be difficult to implement. Although increased access to viral load testing remains pivotal for early detection of treatment failure, wider access to CD4 cell count monitoring could allow timely ART initiation and could help identifying those at higher risk of mortality.

**Authors' contributions:** JvG conceived the study, performed data analysis and wrote the first draft of the paper. ST contributed by improving the intellectual content of the paper. Both authors read and approved the final version. JvG is guarantor of the paper.

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**Conflicts of interest:** None declared.

**Ethical approval:** Since the launch of the HIV care program, clinical data have been routinely collected for purposes of program monitoring and evaluation, and research activities. All patients signed informed consent to store and use the data. No linkage of these data with other sources (e.g. hospital data) was done. The data collection and informed consent procedure were approved by the institutional review board of the SHCH and Institute of Tropical Medicine, Antwerp, Belgium. No patient identifiers were included in the dataset used for this analysis.

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