The interplay between CD4 cell count, viral load suppression and duration of antiretroviral therapy on mortality in a resource-limited setting

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Abstract

OBJECTIVE To examine the interaction between CD4 cell count, viral load suppression and duration of antiretroviral therapy (ART) on mortality.

METHODS Cohort analysis of HIV-infected patients initiating ART between April 2004 and June 2011 at a large public sector clinic in Johannesburg, South Africa. A log-linear model with Poisson distribution was used to estimate risk of death as a function of the interaction between current CD4 count, current viral load suppression and duration on ART in 12-month intervals. We calculated predicted mortality using estimated coefficients within combinations of predictors.

RESULTS Amongst 14 932 ART patients, 1985 (13.3%) died. Current CD4 was the strongest predictor of death (<50 *vs.* ≥550 cells/mm^3 – RR: 46.3; 95% CI: 26.8–80), while unsuppressed current viral load *vs.* suppressed (RR: 1.8; 95% CI: 1.5–2.1) and short duration of ART (0–11.9 *vs.* 66–71.9 months RR: 1.7; 95% CI: 1.2–2.3) also predicted death. Our interaction model showed that mortality was highest in the first 12 months on treatment across all CD4 and viral load strata. As current CD4 and duration on ART increased and viral load suppression occurred, mortality dropped. CD4 count was the strongest predictor of death. The relative effect of current CD4 count varied strongly by viral load and duration of ART (from 1.3 to 55). Lack of suppression increased the risk of mortality upwards of six-fold depending on time on ART and current CD4.

CONCLUSIONS Our findings show that while CD4 count is the strongest predictor of death, the effect is modified by viral load and the duration of ART. Assessment of risk should take into account all three factors.

keywords current CD4 count, current viral load, antiretroviral therapy, mortality, resource-limited setting

Introduction

Antiretroviral therapy (ART) is highly effective at reducing morbidity and mortality through virologic suppression and immune function restoration in treatment-naive HIV-positive patients (Bartlett *et al.* 2001; Mocroft *et al.* 2003; Pérez-Hoyos *et al.* 2003; Sterne *et al.* 2005). However, in low-income countries, including those in sub-Saharan Africa, an estimated 6.5–8.9% of HIV-positive patients receiving ART die within the first 12 months on treatment, with the majority of those deaths taking place in the first 3–6 months of treatment (Braitstein *et al.* 2006; Keiser *et al.* 2008; May *et al.* 2010). Most of these deaths are attributable to late presentation for care characterised by low CD4 cell counts at the start of ART (median CD4 100–150 cells/mm^3 in programmes in sub-Saharan Africa (Lawn *et al.* 2008, 2005; Etard *et al.* 2006; Ferradini *et al.* 2006; Stringer *et al.* 2006; Zachariah *et al.* 2006; Moh *et al.* 2007; May *et al.* 2010), advanced World Health Organization (WHO) clinical disease stage, low body mass index and anaemia (Egger *et al.* 2002; Chene *et al.* 2003; van Sighem *et al.* 2003; Lawn *et al.* 2006a; Cornell *et al.* 2010; Rosen & Fox 2011).

Globally, as ART patients begin to age and remain on treatment longer, baseline predictors of poor outcomes, though valuable, may not provide as much information about long-term risk of HIV-related morbidity and

mortality as measures updated over the course of treatment. A study examining the association between a patient's response to treatment and risk of death found that the current updated CD4 cell count is a more relevant and stronger predictor of mortality over time than the baseline CD4 cell count (Lanoy *et al.* 2009; Lawn *et al.* 2009). Other measures of sustained immunosuppression, such as cumulative person-time with low CD4 cell counts (e.g. <100 cells/mm^3), also are strong predictors of mortality (Lawn *et al.* 2009), supporting the notion that baseline CD4 count alone may not be the most appropriate way to assess risk over time (Viard *et al.* 2001; Lawn *et al.* 2006a,b; Moh *et al.* 2007).

While CD4 cell count is the strongest determinant of mortality in HIV-positive patients who adhere to ART, few studies conducted in rich settings have directly explored the interactive relationship between CD4 count, viral load and time on treatment (Egger *et al.* 2009; The Opportunistic Infections Project Team of the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord 2012). One study suggests that long-term changes in CD4 cell count after ART initiation depend on interactions between CD4 cell count at treatment initiation, viral load response and time on treatment. Although this study did not assess the impact of these factors on mortality, it provides some insight into the interplay between these three crucial factors in determining overall patient risk for mortality during treatment. As none of these studies addressed the interaction between CD4 count and viraemia, we set out to accurately measure the risk of mortality of patients on ART over time as a function of the interaction between current CD4 count, viral suppression and time on ART over the first 6 years of treatment using data from one of the largest HIV treatment clinics in South Africa (Themba Lethu Clinic in Johannesburg).

Methods

Ethics statement

The University of the Witwatersrand and Boston University provided ethical approval of the study. The study was conducted as an unlinked, prospective analysis of a data set that did not contain any individual identifiers.

Study site

Themba Lethu Clinic was opened at the Helen Joseph Hospital in April 2004 and has enrolled nearly 31 000 patients in care (Fox *et al.* 2012). More than 22 000 of these patients have stated ART. Clinic staff provides HIV care according to South African National Department of Health guidelines (National Department of Health, Republic of South Africa 2004, 2010). All laboratory work is processed by the National Health Laboratory Service. First-line ART regimens before April 2010 consisted of stavudine or zidovudine with lamivudine and either efavirenz or nevirapine (National Department of Health, Republic of South Africa 2004). Tenofovir was substituted for stavudine after April 2010 (National Department of Health, Republic of South Africa 2010). After treatment has begun, patients are seen for follow-up visits and antiretroviral drug pickups monthly for the first 6–12 months on treatment, then every 2 months thereafter if stable. Patients have their first laboratory monitoring after 4 months to assess viral load suppression (NucliSENS EasyQ® HIV-1 assay; bioMérieux Clinical Diagnostics, France) and changes in CD4 count (PanLeucogated CD4+ Flowcount®; Beckman Coulter-Immunotech, France); they are monitored annually thereafter.

Study population

We performed a cohort analysis of data collected prospectively as part of routine care at the Themba Lethu clinic. We included all non-pregnant, ART-naïve, HIV-positive patients $\geq$18 years of age who started a standard public sector first-line ART regimen at the clinic between April 2004 and June 2011. Patients included contributed both a CD4 count and a viral load measure during at least one 12-month time period. We excluded pregnant women as they are initiated on ART at higher average CD4 counts and have variable CD4 counts compared to the general population (Smith *et al.* 2004).

Study variables

The primary outcome for this study was death. At Themba Lethu, mortality is ascertained via family or hospital report, active tracing and linkage with the South African National Vital Registration Infrastructure Initiative, a system estimated to have 90% sensitivity for adults (Timaeus *et al.* 2002; Statistics South Africa 2005). Eligible patients contributed person-time from the date of ART initiation until the date of the earliest of: (i) death; (ii) loss to follow-up (defined as not having attended the clinic for 4 months); (iii) transfer; or (iv) June 1, 2012. For analysis, we divided person-time for each subject into 12-month periods starting from ART initiation. For each 12-month period, a patient contributed one observation indicating whether death occurred, current viral load and current CD4 cell count. Subjects could contribute multiple observations to the analysis but not in the same

time period. CD4 count and viral load were the only two variables in our analysis that were time dependent, and therefore, 'current' refers to the most recent value of that variable. All other covariates were fixed at ART initiation in this analysis.

Data analyses

Patient baseline characteristics were summarised with descriptive statistics stratified by vital status. To estimate the risk of death as a function of current viral load status, current CD4 count and time on ART, we included multiple observations for each patient indicating their updated exposures and outcome status. Because we are interested in estimating absolute risks and not survival, we modelled 1-year and overall risk of death as a log-linear function of these covariates using two different models, each using a Poisson distribution (Zou 2004).

First, we looked for predictors of overall mortality on ART by modelling death as a function of current CD4 count (categorised as: <50, 50–99, 100–149, 150–249, 250–349, 350–449, 450–549 and ≥ 550 cells/mm^3), current viral load (<400 *vs.* ≥ 400 copies/ml) and duration on ART in yearly intervals referred to by the month the interval began with no interaction terms between predictors. Because this is a predictive model, we included covariates at ART initiation a priori deemed important or which had a P-value < 0.2 (age, gender, tuberculosis, body mass index, haemoglobin level and WHO stage).

To assess the interaction between the three predictors, we next fitted a model of the risk of death over 1 year as a function of the square root of current CD4 count, current viral load (<400 *vs.* ≥ 400 copies/ml) and the square root of duration on ART. In this model, we also included all possible two- and three-way interactions' terms between these three variables to allow the associations between the predictors and the outcome to vary within levels of each of the other covariates. Because we used a log-linear model assessing relative risks, our approach allowed for effect measure modification on the relative, not the absolute scale (Rothman 1998). We used the estimated coefficients from this model to calculate predicted mortality within each combination of current viral load, CD4 count and time on treatment (Appendix 1). This model was also adjusted for previously mentioned covariates. To show the predicted values, we estimated mortality for subjects who were women, 25–29.9 years of age, WHO stage I/II, body mass index ≥ 18.5 kg/m^2, haemoglobin ≥ 10 g/dl and no tuberculosis at ART initiation.

Data for current CD4 count (22.9%) and current viral load (21.4%) were not available for all patients. We employed multiple imputation by chained equations method using the PROC MI command in SAS to deal with the missingness (SAS). To use this method, we are assuming that the data in our cohort are missing at random, because the missingness is most likely associated with the outcome (death) (Rubin 1987). All prediction equations included log age at initiation of treatment, gender, square root of CD4 count (baseline and updated), square root of time period, log of viral load, haemoglobin at ART initiation (continuous), body mass index at ART initiation (continuous), WHO stage (I/II, III and IV) and tuberculosis at ART initiation. Indicator variables for death and loss to follow-up were also added to the equations but were not imputed. All models were fitted using 25 imputed data sets and estimated coefficients combined by averaging with the MIANALYZE procedure in SAS (SAS). Appropriate standard errors were calculated using the within and between imputation standard errors of the estimates using Rubin's rules (Rubin 1987). The analysis of the interaction between current CD4 count, current viral load and time on ART on mortality was also performed on the original data set prior to multiple imputation with complete cases only (Appendix 2).

Results

Cohort characteristics

A total of 14 932 patients were included in our analysis. Patients had a median follow-up time on ART of 28.9 months [interquartile range (IQR): 12.5–54.8]. During follow-up, 1985 (13.3%) patients died in a median of 5.8 months (IQR: 1.6–18.1). Of the remainder, 7072 (47.4%) were alive, and in care, 3288 (22.0%) were lost to follow-up, and 2587 (17.3%) had transferred to another treatment facility. Patients who died were slightly older, presented for treatment with a substantially lower median CD4 cell count and at a more advanced stage of their disease (higher proportion with a WHO clinical stage III/IV condition) than those who did not (Table 1).

Patients who were lost to follow-up were on treatment for a median time of 11.8 months (IQR: 5.0–27.9). They were predominantly men (43.3% *vs.* 37.3%) and younger at ART initiation (35.6 *vs.* 37.0 years) than those not lost. However, they were similar to those not lost in terms of clinical factors at ART initiation: CD4 count (88 *vs.* 93 cells/mm^3), body mass index (21.0 *vs.* 21.7 kg/m^2), haemoglobin (11.3 *vs.* 11.5 g/dl) and ART regimen (79.0% *vs.* 73.9% on lamivudine–stavudine–efavirenz). Patients who transferred to another facility during follow-up were similar to those included in the analysis, except that a higher proportion of them were women (67% *vs.* 62%).

Table 1 Baseline characteristics of patients on ART at the Themba Lethu Clinic in Johannesburg, South Africa, by vital status (n = 14 932)

	Vital status		
Characteristics	Died (n = 1985) n (%)	Alive (n = 12 947) n (%)	Total (n = 14 932) n (%)
Gender			
Male	906 (45.6)	4864 (37.6)	5770 (38.6)
Female	1079 (54.4)	8083 (62.43)	9162 (61.4)
Age (years)			
18–24.9	87 (4.4)	583 (4.5)	670 (4.5)
25–29.9	230 (11.6)	1883 (14.5)	2113 (14.2)
30–39.9	839 (42.3)	5884 (45.5)	6723 (45.0)
40–49.9	538 (27.1)	3363 (26.0)	3901 (26.1)
≥ 50	291 (14.7)	1234 (9.5)	1525 (10.2)
Age at ART initiation			
Median (IQR)	37.7 (32.3–45.2)	36.6 (31.3–43.0)	36.7 (31.4–43.2)
CD4 at ART initiation			
0–50 cells/mm^3	982 (49.5)	3869 (29.9)	4851 (23.5)
51–100 cells/mm^3	430 (21.7)	2685 (20.7)	3115 (20.9)
101–200 cells/mm^3	455 (22.9)	4676 (36.1)	5131 (34.4)
201–350 cells/mm^3	100 (5.0)	1553 (12.0)	1653 (11.1)
>350 cells/mm^3	18 (0.9)	164 (1.3)	182 (1.2)
CD4 at ART initiation (cells/mm^3)			
Median (IQR)	51 (16–112)	99 (39–168)	92 (35–162)
WHO stage at ART initiation			
I/II	898 (45.2)	7816 (60.4)	8714 (58.4)
III	882 (44.4)	4322 (33.4)	5204 (34.9)
IV	205 (10.3)	809 (6.3)	1014 (6.8)
Tuberculosis at ART initiation			
Yes	380 (19.1)	1831 (14.1)	2211 (14.8)
No	1605 (80.9)	11 116 (85.9)	12 721 (85.9)
First-line ART regimen			
d4T/3TC/EFV	1696 (85.4)	9504 (73.4)	11 200 (75.0)
d4T/3TC/NVP	89 (4.5)	800 (6.2)	889 (6.0)
TDF/3TC/EFV	142 (7.2)	2093 (16.2)	2235 (15.0)
TDF/3TC/NVP	6 (0.3)	134 (1.0)	140 (0.9)
AZT/3TC/EFV	50 (2.5)	368 (2.8)	418 (2.8)
AZT/3TC/NVP	2 (0.1)	48 (0.4)	50 (0.3)
Time on ART (months)			
Median (IQR)	5.8 (1.6–18.1)	33.1 (16.0–58.1)	28.9 (12.5–54.8)
Haemoglobin at ART initiation (g/dl)			
Median (IQR)	10.6 (9.1–12.2)	11.6 (10.1–13.1)	11.5 (9.9–13.0)
Body mass index at ART initiation			
Median (IQR)	20.1 (17.5–24.2)	21.8 (19.1–25.4)	21.6 (18.9–25.3)

ART, antiretroviral therapy; WHO, World Health Organization; d4T, stavudine; 3TC, lamivudine; EFV, efavirenz; TDF, tenofovir; AZT, zidovudine.

Mortality by duration on ART, viral load suppression and CD4 count

Model 1 in Table 2 shows the results of our analysis of time updated measures of CD4 count, viral load and duration on ART with no interactions. The adjusted model showed that shorter duration on treatment, lower current CD4 count and an unsuppressed current viral load were all associated with an increased risk of death. Compared to a current CD4 cell count ≥ 550 cells/mm^3, current CD4 count <50 cells/mm^3 (RR: 41.6; 95% CI: 24.4–71.0 cells/mm^3) and 50–99 cells/mm^3 (RR: 27.3; 95%CI: 16.5–45.2) carried a substantially higher risk of death. Having an unsuppressed viral load *vs.* suppressed (RR: 1.8; 95% CI: 1.5–2.1) and shorter duration of ART

Table 2 Crude and adjusted predictors of mortality amongst patients on ART at the Themba Lethu Clinic in Johannesburg, South Africa (n = 14 932)

	N (%) mortality	Crude RR (95% CI)	Adjusted model * RR (95% CI)
Current viral load (copies/ml)			
<400	1195 (11.0)	Reference	Reference
≥400	790 (19.6)	3.6 (3.3–3.9)	1.8 (1.5–2.1)
Current CD4 count (cells/mm^3)			
≥550	36 (1.2)	Reference	Reference
450–549	61 (3.4)	2.6 (1.7–3.9)	2.2 (1.2–4.0)
350–449	92 (4.0)	2.7 (1.8–4.0)	2.9 (1.7–4.9)
250–349	240 (9.3)	6.0 (4.1–8.6)	5.0 (3.0–8.2)
150–249	456 (18.4)	12.7 (8.8–18.1)	8.6 (5.3–13.9)
100–149	332 (31.6)	27.8 (19.3–39.8)	16.8 (10.1–27.8)
50–99	360 (39.2)	48.8 (34.1–69.9)	27.3 (16.5–45.2)
<50	408 (54.7)	96.5 (67.0–138.9)	41.6 (24.4–71.0)
Time (months)			
0–11.9	1328 (36.9)	5.9 (4.4–7.9)	1.7 (1.2–2.3)
12–23.9	290 (10.0)	1.7 (1.3–2.3)	0.9 (0.7–1.3)
24–35.9	143 (6.4)	1.1 (0.8–1.6)	0.8 (0.6–1.1)
36–47.9	91 (5.3)	1.0 (0.7–1.4)	0.8 (0.6–1.1)
≥48	116 (5.0)	Reference	Reference
Age (years) at ART initiation			
18–24.9	57 (13.0)	1.2 (1.0–1.6)	1.2 (0.9–1.5)
25–29.9	230 (10.9)	Reference	Reference
30–39.9	839 (12.5)	1.1 (0.9–1.3)	1.1 (1.0–1.3)
40–49.9	538 (13.8)	1.3 (1.1–1.5)	1.2 (1.0–1.4)
≥50	291 (19.1)	1.9 (1.6–2.3)	1.7 (1.4–2.0)
Gender			
Female	1079 (11.8)	Reference	Reference
Male	906 (15.7)	1.4 (1.3–1.6)	1.0 (0.9–1.1)
Tuberculosis at ART initiation			
No	1605 (12.6)	Reference	Reference
Yes	380 (17.2)	1.3 (1.2–1.5)	1.0 (0.9–1.2)
WHO stage at ART initiation			
I/II	898 (10.3)	Reference	Reference
III	882 (17.0)	1.6 (1.4–1.8)	1.2 (1.1–1.4)
IV	205 (20.2)	2.0 (1.7–2.4)	1.3 (1.1–1.5
Body mass index at ART initiation			
≥18.5 kg/m^2	1313 (11.2)	Reference	Reference
<18.5 kg/m^2	672 (20.7)	2.1 (1.9–2.3)	1.3 (1.2–1.5)
Haemoglobin at ART initiation			
≥10.0 g/dl	1212 (10.9)	Reference	Reference
<10.0 g/dl	773 (20.3)	2.1 (1.9–2.3)	1.5 (1.4–1.7)

ART, antiretroviral therapy; WHO, World Health Organization.

*Model is adjusted for current CD4 count, current viral load, time on ART, female gender, years of age (18–24.9, 30–39.9, 40–49.9 ≥50 *vs.* 25–29.9), WHO stage III/IV *vs.* I/II, body mass index <18.5 kg/m^2 *vs.* ≥18.5 kg/m^2, haemoglobin <10 g/dl *vs.* ≥10 g/dl and tuberculosis *vs.* no tuberculosis at ART initiation. Model was not adjusted for CD4 count at ART initiation because of the collinear relationship between current CD4 count and CD4 count at ART initiation as described in the methods.

(e.g. 0–11.9 *vs.* ≥48 months RR: 1.7; 95% CI: 1.2–2.3) were also predictive of mortality.

The previous model ignores the interactions between duration on treatment, current CD4 count and viral load. Table 3 and Figure 1 show predicted mortality from a model including current CD4 count, current viral load and duration on ART as well as all two- and three-way interactions between them (Appendix 1 – parameter estimates and *P*-values). Predicted 1-year mortality ranged from 0.3% to 24.8%. For those with a CD4 cell count of 350 cells (current recommended WHO threshold), mortality was below 5% for all time periods for

Table 3 Predicted risk of 1-year mortality by current viral load status, current CD4 count and time on antiretroviral therapy (ART) amongst patients on ART at the Themba Lethu Clinic in Johannesburg, South Africa

Viral load (copies/ml)	Months on ART	Current CD4 count % Mortality							
		25 cells/mm^3	50 cells/mm^3	100 cells/mm^3	200 cells/mm^3	250 cells/mm^3	350 cells/mm^3	450 cells/mm^3	550 cells/mm^3
≥400	0	24.8 (20.1–29.4)	19.0 (15.8–22.3)	13.1 (10.9–15.3)	7.76 (6.22–9.30)	6.28 (4.90–7.66)	4.36 (3.20–5.51)	3.17 (2.19–4.16)	2.40 (1.55–3.24)
	12	15.6 (11.317.8)	11.3 (8.99–13.6)	7.84 (6.39–9.28)	4.69 (3.82–5.55)	3.81 (3.08–4.54)	2.66 (2.09–3.24)	1.95 (1.48–2.43)	1.48 (1.08–1.88)
	24	11.7 (8.57–14.9)	9.09 (6.88–11.3)	6.34 (4.96–7.71)	3.81 (3.00–4.61)	3.10 (2.42–3.78)	2.17 (1.63–2.71)	1.60 (1.15–2.05)	1.21 (0.83–1.60)
	36	9.92 (6.83–13.0)	7.70 (5.55–9.84)	5.38 (4.05–6.71)	3.25 (2.46–4.03)	2.65 (1.98–3.32)	1.86 (1.32–2.40)	1.37 (0.92–1.83)	1.05 (0.65–1.44)
	48	8.61 (5.61–11.6)	6.69 (4.61–8.78)	4.69 (3.40–5.98)	2.84 (2.07–3.61)	2.32 (1.66–2.98)	1.63 (1.10–2.17)	1.21 (0.75–1.67)	0.92 (0.52–1.32)
	60	7.61 (4.69–10.5)	5.92 (3.90–7.94)	4.16 (2.91–5.40)	2.52 (1.77–3.28)	2.06 (1.41–2.72)	1.46 (0.92–2.00)	1.08 (0.62–1.54)	0.82 (0.42–1.23)
<400	0	23.0 (18.1–28.0)	14.7 (12.0–17.4)	7.75 (6.52–8.99)	3.15 (2.64–3.66)	2.19 (1.80–2.58)	1.17 (0.92–1.42)	0.68 (0.51–0.85)	0.42 (0.29–0.54)
	12	13.1 (9.49–16.8)	8.74 (6.60–10.9)	4.92 (3.92–5.92)	2.18 (1.82–2.55)	1.58 (1.32–1.83)	0.89 (0.74–1.04)	0.55 (0.44–0.65)	0.35 (0.28–0.43)
	24	10.4 (6.81–14.0)	7.06 (4.92–9.20)	4.08 (3.07–5.09)	1.88 (1.52–2.23)	1.37 (1.13–1.62)	0.80 (0.65–0.95)	0.50 (0.40–0.61)	0.33 (0.25–0.41)
	36	8.72 (2.19–12.3)	6.00 (3.88–8.11)	3.53 (2.53–4.53)	1.67 (1.32–2.03)	1.24 (0.99–1.48)	0.74 (0.59–0.88)	0.47 (0.36–0.58)	0.31 (0.23–0.40)
	48	7.52 (7.08–11.0)	5.23 (3.15–7.30)	3.13 (2.13–4.12)	1.52 (1.16–1.87)	1.13 (0.89–1.38)	0.68 (0.53–0.84)	0.44 (0.32–0.56)	0.30 (0.20–0.40)
	60	6.59 (3.26–9.93)	4.63 (2.61–6.66)	2.81 (1.93–3.79)	1.39 (1.04–1.75)	1.05 (0.80–1.30)	0.64 (0.48–0.80)	0.42 (0.30–0.55)	0.29 (0.18–0.39)

Model also adjusted for female gender, years of age (18–24.9, 30–39.9, 40–49.9 ≥50 *vs.* 25–29.9), WHO stage I/II and III *vs.* IV, body mass index ≥18.5 kg/m^2 *vs.* <18.5 kg/m^2, haemoglobin ≥10 g/dl *vs.* <10 g/dl and no tuberculosis *vs.* tuberculosis at ART initiation. Model parameters are given in Appendix 1.

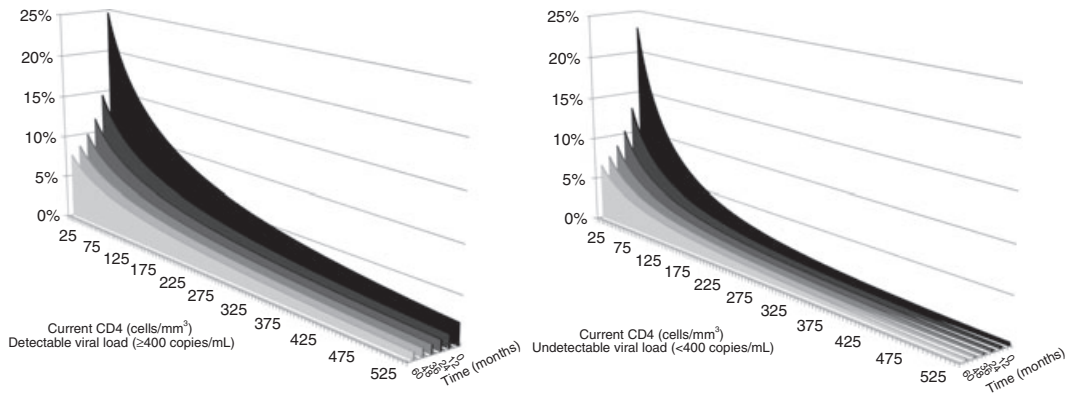

Figure 1 Predicted 1-year mortality by current viral load, current CD4 cell count and time on antiretroviral therapy (ART) amongst patients on antiretroviral therapy at the Themba Lethu Clinic in Johannesburg, South Africa. Model also adjusted for female gender, years of age (18–24.9, 30–39.9, 40–49.9 ≥ 50 *vs.* 25–29.9), WHO stage I/II and III *vs.* IV, body mass index ≥ 18.5 kg/m^2 *vs.* <18.5 kg/m^2, haemoglobin ≥ 10 g/dl *vs.* <10 g/dl and no tuberculosis *vs.* tuberculosis at ART initiation. Model parameters are given in Appendix 1.

unsuppressed patients and $\leq 1\%$ for suppressed patients. For those with a CD4 cell count of 200 cells (previous WHO threshold), mortality was below 8% for all time periods for unsuppressed patients and below $\leq 3\%$ for suppressed patients. One-year mortality was consistently higher amongst those with lower CD4 counts and amongst those virally unsuppressed at all time points. Mortality was highest in the first year on treatment across all current CD4 and viral load strata and consistently declined over time.

Despite being the strongest predictor of death, the relative effect of CD4 count was strongly modified by viral suppression and time on treatment (Appendix 3 – relative risks for mortality by current CD4 count, viral load and duration on ART). The effect of CD4 count was strongest amongst those virally suppressed and in their first year on treatment when mortality was at its highest. When comparing a CD4 cell count of 25–550 cells/mm^3, we found a 10–55-fold increased risk of death in patients across all time periods and viral load strata. In the first 12 months on ART for patients with a detectable viral load, those with a CD4 count of 25 cells/mm^3 had over 10 times the risk of 1-year mortality compared to those with a CD4 count 550 cells/mm^3 (24.8% *vs.* 2.4%). For comparable patients (in the first 12 months on ART) but with a undetectable current viral load where overall mortality is slightly lower, those with a current CD4 of 25 cells/mm^3 had roughly a 55-fold mortality risk of those with a CD4 cell count of 550 cells/mm^3. With increasing duration on ART, however, the relative effect of a CD4 cell count on 1-year mortality shows strong modification by viral suppression. Over time, amongst virally unsuppressed patients, the relative effect of CD4 count on 1-year mortality stayed steady (e.g. the effect of a 25 *vs.* 550 cells/mm^3 decreases from a RR of 10.4 at 0 months to a RR of 9.3 at 60 months) but fell over time amongst virally suppressed patients (e.g. from a RR of 55.4 at 0 month to a RR of 22.6 at 60 months).

The relative effect of viral suppression consistently increased with higher CD4 counts but declined over time for those with a current CD4 count >200 cells/mm^3 (Appendix 3). The largest effect of viral suppression (*vs.* non-suppression) is for patients with low 1-year predicted mortality. For those with a CD4 count of 550 (the highest CD4 for which we estimated mortality risk) in the first year of ART, the relative reduction in risk for suppressed patients was six-fold (0.42% *vs.* 2.4% for suppressed and unsuppressed patients, respectively) with a smaller effect in lower CD4 count strata.

The effect of duration on ART also was modified by current viral load and CD4 count. For both suppressed and unsuppressed patients, time was an important predictor of 1-year mortality (Appendix 3). The effect consistently declines over time but is always strongest amongst those with low CD4 cell counts (ranging from a RR of 3.3 to 2.9 for unsuppressed patients and 3.5 to 1.4 for suppressed patients comparing month 0–60 amongst those with a CD4 count of 25 and 550 cells, respectively). The effect of time on mortality holds fairly steady after 36 months on ART for all CD4 count and viral load strata.

Discussion

Despite the clear survival benefits of ART, mortality in the first 24 months of treatment amongst HIV-positive

patients is substantially higher than in the general uninfected population, particularly amongst patients who present for treatment severely immunocompromised (Brinkhof *et al.* 2009). Therefore, the main goals of ART are to get patients onto treatment early, achieve viral suppression in the shortest time possible and then sustain suppression allowing patients' CD4 count to increase. These targets lead to a marked reduction in poor clinical outcomes and an increase in life expectancy (Ledergerber *et al.* 1999; Miller *et al.* 1999; Deeks *et al.* 2000; Gange *et al.* 2001; Lima *et al.* 2009). All three of these factors are critical to survival, yet the interplay between these factors remains unclear as this requires large sample sizes and long follow-up. In this analysis, we assessed the role of current CD4 count on mortality while accounting for current viral suppression and duration of ART. We found that current CD4 count was the largest relative predictor of death on ART regardless of how long patients had been on treatment or whether or not they currently had a detectable viral load. Relative rises in mortality comparing patients with a CD4 count of 25–550 cells/mm^3 ranged from to 10- to 55-fold increases depending on the duration on ART and viral load status. These results support previous findings that CD4 cell count is the main driver of a patient's risk of mortality on ART (Egger *et al.* 2002; Chene *et al.* 2003; Zachariah *et al.* 2006; Lawn *et al.* 2009; Cornell *et al.* 2010; Rosen & Fox 2011; The Opportunistic Infections Project Team of the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord (2012), Philips *et al.* (2010).

We also modelled interactions between these three critical factors that drive the risk of mortality and demonstrate that the effect of CD4 count on death varies by current viral load status, consistent with recent findings (The Opportunistic Infections Project Team of the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord (Phillips *et al.* 2010). Not only was predictive mortality higher amongst patients who did not achieve viral load suppression, but they were at upwards of a six-fold increased risk of death in the first 12 months on ART compared to patients who did achieve viral load suppression, depending on the patients CD4 count. While still a strong driver of mortality amongst virally suppressed patients, the effect of CD4 count was reduced, as overall risk of death is greater in unsuppressed patients.

In our cohort, the majority of patients (85.8%) achieved viral load suppression in the first 12 months on ART. However, not all patients on ART are able to achieve virologic suppression, either as a result of poor adherence to treatment (Brennan *et al.* 2010) or due to resistance (Sethi *et al.* 2003). Several studies have shown that while not as strong a predictor as CD4 count, circulating virus remains an important prognostic indicator of HIV disease progression (Miller *et al.* 1999; Deeks *et al.* 2000; Brinkhof *et al.* 2009) and that rapid and consistent viral suppression is essential to maintaining positive clinical outcomes (Brinkhof *et al.* 2009). Our results show that the effect of viral load, while more stable than the effect of CD4, is not constant over time and appears strongest early on in treatment amongst patients with higher CD4 counts, when the overall risk of death is lower and viral suppression plays a stronger role.

Our results are comparable with an analysis of predicted mortality in cohorts throughout sub-Saharan Africa (May *et al.* 2010). The general similarity of our findings confirms the very high mortality amongst patients with low CD4 counts but further develops predictive models based on viral suppression. Our findings emphasise the interaction between time, viral load and CD4 count and demonstrate how relative effects are modified by each of the other predictors.

In rich environments, HIV treatment monitoring typically includes frequent viral load monitoring, viral resistance testing and regular CD4 count measurements. However, due to the prohibitive cost of HIV RNA monitoring, standard care in many settings in sub-Saharan Africa consists of clinical monitoring, coupled with routine CD4 measurements when possible (World Health Organization 2007). Our results confirm that lack of viral suppression continues to predict mortality. Because clinical deterioration and CD4 decline can occur well after virologic failure, viral load measures allow for faster and more appropriate use of second-line ART (ART-LINC of IeDEA Study Group *et al.* 2009).

Our findings should be considered alongside their limitations. First, as regards our model, the preferred approach to analysing the interactive relationship between CD4 count, viral load and time on ART would have been on the additive scale (Rothman 1998), which we tried to do, but failed when our linear model broke down. Second, while the South African National Vital Registration is highly sensitive (Timaeus *et al.* 2002; SAS), there is a 6-month delay in updating the registry, which could result in underascertainment of deaths. However, patients (n = 193) who were lost to follow-up <6 months prior to the linkage (June 2012) were removed from the analysis as they may not have been included in the registry if they had died because of delays in reporting. Also, because this misclassification is likely unrelated to any of our three primary exposures, the expectation is that this would reduce the size of estimated comparisons. Third, loss in our analysis refers to those

patients that do not have an observable outcome. More than 20% of patients in our cohort were considered lost to follow-up and 17% transferred out. Compared to those included in the analysis, patients lost from care were predominantly men and younger, while the majority of transferred patients were women. Although we are less concerned with those transferred because women are at lower risk of mortality than men (Fox *et al.* 2010), it is important to acknowledge that there is likely some selection bias, potentially making our results underestimates of mortality because male patients and those patients who leave care are more likely to stop treatment and increase their risk of death (Fox *et al.* 2010; Druyts *et al.* 2013). Fourth, multiple imputation helps make it possible to handle missing data routinely and improve the validity of research. However, the procedure requires the user to model the distribution of each variable with missing values, in terms of the observed data (SAS). The validity of results from multiple imputation depends on such modelling being performed carefully and is based on the assumption that our data are missing at random. Deviations from this could have led to unpredictable biases in our parameter estimates.

Conclusion

Long-term virologic suppression helps to ensure the recovery of CD4 cells to levels that reduce the risk of opportunistic infections and increase life expectancy. Our findings show that while low current CD4 count is the largest predictor of 1-year mortality on treatment, this relative effect is modified by current viral suppression and time on ART and that all three are important to assess when evaluating patient risk. Future efforts to refine mortality predictions should assess the role of body mass index, anaemia, tuberculosis and other opportunistic infections.

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Appendix 1 Model results for predicted risk of death by current CD4, current viral load and duration on antiretroviral therapy and all possible two- and three-way interactions' terms between these three variables

Parameter	β	Standard error	95% confidence limits		*P*-value
Intercept	−0.381976	0.158555	−0.6955	−0.06843	0.0173
Age 18–24.9 *vs.* 25–29.9 years	0.148467	0.1287	−0.1038	0.40073	0.2487
Age 30–39.9 *vs.* 25–29.9 years	0.103535	0.076338	−0.0461	0.25316	0.175
Age 40–49.9 *vs.* 25–29.9 years	0.182987	0.081785	0.02267	0.3433	0.0253
Age $\geq$50 *vs.* 25–29.9 years	0.496879	0.091819	0.3169	0.67686	<0.0001
Male *vs.* female gender	−0.049724	0.049708	−0.1472	0.04774	0.3172
Tuberculosis *vs.* non-tuberculosis	0.028178	0.067554	−0.1042	0.16059	0.6766
Hb <10.0 g/dl *vs.* $\geq$10.0 g/dl	0.396034	0.051901	0.29425	0.49782	<0.0001
BMI <18.5 kg/m^2 *vs.* $\geq$18.5 kg/m^2	0.278673	0.053242	0.17424	0.38311	<0.0001
WHO stage III *vs.* WHO stage I/II	0.200955	0.05719	0.08885	0.31306	0.0004
WHO stage IV *vs.* WHO stage I/II	0.263487	0.081458	0.10381	0.42317	0.0012
Viral load $\geq$400 *vs.* <400 copies/ml	0.75909	0.167412	−0.7118	−0.04921	0.0247
Current CD4 count^{½}	−0.380526	0.011185	−0.2398	−0.1953	<0.0001
Time period^{½}	0.8235162	0.048822	−0.2907	−0.09764	0.0001
2-way interaction terms					
Current CD4 count^{½} * viral load $\geq$400	0.090611	0.014761	0.06145	0.11977	<0.0001
Time period^{½} * viral load $\geq$400	0.037235	0.065396	−0.092	0.16642	0.5699
Current CD4 count^{½} * time period^{½}	0.006264	0.003078	0.00018	0.01235	0.0437
3-way interaction terms					
Current CD4 count^{½} * time period^{½} * viral load $\geq$400	−0.005448	0.00443	−0.0142	0.00329	0.2203

BMI, body mass index; Hb, haemoglobin; WHO, World Health Organization.

Appendix 2 Use of original data set of complete cases to predict the risk of 1-year mortality by current viral load status, current CD4 count and time on antiretroviral therapy (ART) amongst patients on antiretroviral therapy at the Themba Lethu Clinic in Johannesburg, South Africa

Viral load (copies /ml)	Months on ART	Current CD4 count % Mortality							
		25 cells/mm^3	50 cells/mm^3	100 cells/mm^3	200 cells/mm^3	250 cells/mm^3	350 cells/mm^3	450 cells/mm^3	550 cells/mm^3
≥400	0	11.0 (7.14–16.9)	6.70 (4.46–10.1)	3.33 (2.16–5.15)	1.24 (0.71–2.18)	0.84 (0.44–0.16)	0.42 (0.19–0.90)	0.23 (0.09–0.56)	0.14 (0.05–0.37)
	12	7.16 (4.67–11.0)	4.56 (3.05–6.81)	2.40 (1.61–3.59)	0.97 (0.61–1.55)	0.68 (0.41–1.12)	0.36 (0.20–0.65)	0.21 (0.11–0.41)	0.13 (0.06–0.27)
	24	6.00 (3.62–9.96)	3.88 (2.44–6.18)	2.10 (1.34–3.29)	0.88 (0.52–1.48)	0.62 (0.35–1.10)	0.34 (0.17–0.67)	0.20 (0.09–0.44)	0.12 (0.05–0.31)
	36	5.24 (2.92–9.41)	3.44 (2.03–5.82)	1.89 (1.14–3.13)	0.81 (0.45–1.48)	0.58 (0.30–1.13)	0.32 (0.14–0.72)	0.19 (0.07–0.50)	0.12 (0.04–0.36)
	48	4.68 (2.42–9.04)	3.10 (1.72–5.58)	1.73 (0.99–3.03)	0.76 (0.39–1.49)	0.55 (0.26–1.17)	0.31 (0.12–0.78)	0.19 (0.06–0.56)	0.12 (0.03–0.42)
	60	4.04 (1.89–8.65)	2.71 (1.38–5.32)	1.55 (0.82–2.93)	0.70 (0.32–1.54)	0.51 (0.21–1.24)	0.29 (0.10–0.88)	0.18 (0.05–0.67)	0.12 (0.03–0.53)
<400	0	9.78 (6.17–15.5)	5.90 (3.89–8.95)	2.89 (1.99–4.20)	1.05 (0.73–1.51)	0.70 (0.48–1.02)	0.35 (0.23–0.53)	0.19 (0.12–0.30)	0.11 (0.06–0.19)
	12	5.24 (3.07–8.94)	3.48 (2.15–5.66)	1.96 (1.28–2.99)	0.86 (0.60–1.24)	0.62 (0.44–0.88)	0.35 (0.25–0.50)	0.21 (0.15–0.31)	0.14 (0.09–0.21)
	24	4.05 (2.13–7.69)	2.80 (1.58–4.97)	1.66 (1.02–2.71)	0.80 (0.54–1.18)	0.59 (0.41–0.86)	0.35 (0.25–0.51)	0.23 (0.15–0.51)	0.15 (0.10–0.23)
	36	3.32 (1.58–6.96)	2.37 (1.23–4.56)	1.47 (0.85–2.53)	0.75 (0.49–1.15)	0.57 (0.38–0.85)	0.36 (0.24–0.53)	0.24 (0.15–0.36)	0.16 (0.10–0.27)
	48	2.81 (1.23–6.45)	2.06 (0.99–4.27)	1.32 (0.73–2.41)	0.71 (0.45–1.12)	0.55 (0.36–0.85)	0.36 (0.23–0.55)	0.24 (0.15–0.39)	0.17 (0.10–0.30)
	60	2.43 (0.97–6.04)	1.82 (0.82–4.03)	1.21 (0.63–2.31)	0.68 (0.41–1.11)	0.54 (0.34–0.85)	0.36 (0.23–0.57)	0.25 (0.15–0.43)	0.18 (0.10–0.34)

Model also adjusted for female gender, years of age (18–24.9, 30–39.9, 40–49.9 ≥ 50 *vs.* 25–29.9), WHO stage I/II and III *vs.* IV, body mass index ≥ 18.5 kg/m^2 *vs.* <18.5 kg/m^2, haemoglobin ≥ 10 g/dl *vs.* <10 g/dl and no tuberculosis *vs.* tuberculosis at ART initiation. Model parameters are given in Appendix 1.

Appendix 3 Relative risks of mortality by current viral load status, current CD4 count and time on antiretroviral therapy (ART) amongst patients on antiretroviral therapy at the Themba Lethu Clinic in Johannesburg, South Africa

Relative risk of mortality by current CD4 counts

Viral load	Time on treatment (months)	25 cells/mm^3	50 cells/mm^3	100 cells/mm^3	200 cells/mm^3	250 cells/mm^3	350 cells/mm^3	450 cells/mm^3	550 cells/mm^3
≥400	0	10.40	8.00	5.52	3.26	2.64	1.83	1.33	Reference
	12	9.88	7.64	5.31	3.18	2.58	1.80	1.32	Reference
	24	9.66	7.49	5.23	3.14	2.56	1.79	1.32	Reference
	36	9.51	7.38	5.16	3.11	2.54	1.78	1.31	Reference
	48	9.37	7.29	5.11	3.09	2.53	1.78	1.31	Reference
	60	9.26	7.21	5.07	3.07	2.51	1.77	1.31	Reference
<400	0	55.38	35.29	18.66	7.58	5.27	2.81	1.63	Reference
	12	37.11	24.73	13.94	6.19	4.47	2.53	1.55	Reference
	24	31.43	21.35	12.35	5.70	4.17	2.43	1.52	Reference
	36	27.68	19.07	11.26	5.34	3.96	2.35	1.50	Reference
	48	24.86	17.33	10.41	5.06	3.78	2.28	1.48	Reference
	60	22.62	15.94	9.72	4.82	3.64	2.23	1.46	Reference

Relative risk of mortality by current viral load status

Time on treatment (months)	Viral load	25 cells/mm^3	50 cells/mm^3	100 cells/mm^3	200 cells/mm^3	250 cells/mm^3	350 cells/mm^3	450 cells/mm^3	550 cells/mm^3
0	<400	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
	≥400	1.08	1.30	1.69	2.46	2.86	3.72	4.67	5.72
12	<400	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
	≥400	1.11	1.29	1.59	2.14	2.42	2.98	3.56	4.18
24	<400	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
	≥400	1.13	1.29	1.55	2.03	2.25	2.71	3.18	3.67
36	<400	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
	≥400	1.14	1.29	1.53	1.94	2.14	2.53	2.92	3.32
48	<400	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
	≥400	1.15	1.29	1.50	1.87	2.04	2.38	2.72	3.06
60	<400	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
	≥400	1.16	1.28	1.48	1.81	1.96	2.26	2.55	2.84

Relative risk of mortality by time on treatment

Viral load	Time on treatment (months)	25 cells/mm^3	50 cells/mm^3	100 cells/mm^3	200 cells/mm^3	250 cells/mm^3	350 cells/mm^3	450 cells/mm^3	550 cells/mm^3
≥400	0	3.27	3.23	3.17	3.08	3.05	3.00	2.95	2.91
	12	1.92	1.91	1.89	1.86	1.85	1.83	1.82	1.80
	24	1.55	1.54	1.53	1.51	1.51	1.50	1.49	1.48
	36	1.31	1.30	1.30	1.29	1.29	1.28	1.28	1.27
	48	1.13	1.13	1.13	1.13	1.13	1.12	1.12	1.12
	60	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
<400	0	3.53	3.19	2.77	2.27	2.09	1.82	1.61	1.44
	12	2.01	1.90	1.76	1.57	1.50	1.39	1.30	1.22
	24	1.59	1.53	1.45	1.35	1.31	1.25	1.19	1.14
	36	1.33	1.30	1.26	1.20	1.18	1.14	1.11	1.09
	48	1.14	1.13	1.11	1.09	1.08	1.06	1.05	1.04
	60	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference