Response to antiretroviral therapy: improved survival associated with CD4 above 500 cells/μl

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Objective: We investigated the association between immune response and mortality in four HIV African programs supported by Médecins Sans Frontières.

Design: Multicentric retrospective cohort study.

Methods: All antiretroviral therapy (ART) naive adults (>15 years) who initiated therapy between March 2001 and November 2010 and receiving therapy for 9 months or more were included. We described the evolution of mortality over time. Mixed Poisson models were used to assess the effect of updated CD4 cell counts and other potential risk factors on mortality.

Findings: A total of 24 037 patients, of which 68% were women, contributed 69 516.2 person-years of follow-up. At ART initiation, 5718 patients (23.7%) were classified as WHO clinical stage 4, 1587 (6.6%) had a BMI below 16 kg/m^2 and 2568 (10.7%) had CD4 cell count below 50 cells/μl. A total of 568 (2.4%) deaths were recorded during the study period. In the CD4 response categories 500 cells/μl or more, 350–499, 200–349, 50–199 cells/μl and less than 50 cells/μl, unadjusted mortality rates were 0.36; 0.58; 0.88; 1.91 and 7.43 per 100 person-years, respectively. In multivariate analysis, higher mortality was observed in patients with CD4 response levels 350–499 cells/μl [adjusted hazard ratio (aHR) 1.70, 95% confidence interval (CI) 1.26–2.30] and for those between 200–349 (aHR 2.56; 95% CI 1.93–3.38), compared to those with 500 cells/μl or more.

Interpretation: The observed higher survival of patients with a CD4 response to ART higher than 500 cells/μl supports the need of further research to evaluate the individual benefit of initiating ART at higher CD4 levels in sub-Saharan Africa.

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Introduction

By the end of 2009, almost 4 million patients were receiving antiretroviral therapy (ART) in sub-Saharan Africa [1]. Many national ART scaling-up programs in sub-Saharan African began in 2004 or 2005 and there are increasing numbers of patients that have medium or long-term experience with ART. Reports from evaluations of long-term mortality are relatively scarce and few studies have investigated risk factors associated with longer term mortality. In Senegal, mortality decreased from 12.5 per 100 person-years during the first year to 2.2 per 100 person-years during the fifth year on ART [2]. In Botswana, survival estimates at 1 and 5 years of ART reported little difference, 0.82 and 0.79, respectively [3]. Additionally, a recent South African study reported a corrected mortality estimate of 20.9% after 5 years of treatment [4].

In resource-limited settings, the optimal CD4 cell count level to initiate ART is still debated. Current WHO guidelines recommend starting ART when CD4 cell count levels reach 350 cells/μl, whereas US national guidelines have raised this threshold to 500 [5,6]. As clinical trials studying the potential individual benefits of starting ART earlier are still years from completion, observational studies exploring the relation between CD4 response to ART and mortality could help by providing indirect evidence.

This analysis aimed at describing mortality among HIV-infected patients receiving ART for 9 months or more and studying its association with CD4 response and other risk factors in four large sub-Saharan African programs supported by Médecins Sans Frontières.

Methods

Data collection

We used data from four HIV programs (Malawi, Uganda and two programs in Kenya) where ART became available between 2001 and 2002. All care and treatment, including antiretroviral drugs, were provided free of charge.

Routine demographic, clinical, biological and treatment data (including ART regimens) were recorded using a standardized monitoring system (FUCHIA software, Epicentre, Paris, France).

Deaths were recorded by the medical teams, reported by a close relative or friend, or from 2006, by a member of the tracing team after house visits.

CD4 cell counts were initially quantified using manual methods (Dynabeads; Dynal Biotech SA, Compiègne, France) and, since 2003, with semiautomated techniques (Cyflow counter; Partec, Munster, Germany or FACS-count; Becton-Dickinson Immunocytometry Systems, San Jose, California, USA). During treatment, CD4 cell counts were routinely monitored every 6–12 months.

The study protocol was approved by the 'Comité de protection des personnes d'Ile de France', Paris, France.

Inclusion criteria

All HIV-infected patients who, at ART start, were aged 15 years or older, had no history of ART use, had been receiving therapy for more than 9 months and had at least one CD4 measure after 9 months on ART, were included in the analysis.

Statistical analysis

Study follow-up started 9 months after ART initiation. Censoring occurred at death, loss to follow-up or at the last consultation, whatever came first. The database was locked on 30 November 2010.

Loss to follow-up was defined as the absence of recorded clinical visit for at least 9 months at the time of the analysis among patients who were not dead or transferred out of the program.

Two types of CD4 measures were included in the analysis: initial (closest measurement between three months before and one month after ART start) and updated CD4 cell counts (all tests performed after 9 months of ART). Initial CD4 cell counts were less than 50, 50–199, 200 cells/μl or more and missing. Updated CD4 cell counts were grouped into five categories: less than 50, 50–199, 200–349, 350–499 and 500 cells/μl or more. BMI and WHO clinical stage at ART initiation were also considered for the analysis as categorical variables.

We estimated the mortality rates per 100 person-years with 95% confidence interval (CI) from 9 months after ART initiation and up to 5 years of therapy use.

We then explored factors associated with mortality after the first 9 months of ART. A mixed Poisson survival model was used to explore the association between mortality and CD4 response measured after 9 months of ART. Time at risk started from the date of blood sample collection of the first CD4 measure taken after 9 months on ART until death, end of follow-up or the next CD4 cell count measure, whatever came first. The model was adjusted for program, sex and characteristics measured at ART start (age, BMI, CD4 cell count level and clinical WHO stage), all included as categorical variables. Missing initial values of BMI, clinical stage, and CD4 cell counts were included as separate categories in the analysis.

Data were analysed using Stata 11 (Stata Corp, College Station, Texas, USA).

Results

Study population characteristics

A total of 24 037 patients met the inclusion criteria and contributed 69 516.2 person-years of follow-up (Fig. 1). Women represented 68% of the study population. At ART initiation, 5718 patients (23.8%) were in WHO clinical stage 4 and 1587 (7%) had a BMI below 16 kg/m^2. The median CD4 cell count was 152 cells/μl [interquartile range (IQR) 84–211] and 2568 patients (11%) had a CD4 cell count below 50 cells/μl (Table 1). During the study follow-up, 568 (2.4%) patients died and 2796 (10.3%) were lost to follow-up. Median time of follow-up in the programs after ART start was 3.33 years (IQR 2.10–5.00).

Mortality rates

Overall mortality rate was 0.82 deaths per 100 person-years (95% CI 0.75–0.88). Mortality rates were 1.03 (95% CI 0.91–1.18), 0.93 (95% CI 0.79–1.09) and 0.64 (95% CI 0.48–0.86) deaths/100 person-years, during the second, third and fourth years of ART, respectively.

Associations between mortality and CD4 response

Overall, 77 970 CD4 measures were available for the analysis. The median number of measures per patient was 3 (IQR 2–5).

Unadjusted mortality rates were 0.36 (95% CI 0.29–0.44), 0.58 (95% CI 0.48–0.71), 0.88 (95% CI 0.75–1.01), 1.91 (95% CI 1.65–2.22) and 7.43 (95% CI 5.43–10.2) in the CD4 response strata at least 500, 350–499, 200–349, 50–199 cells/μl and less than 50 cells/μl.

In the multivariate analysis, mortality decreased with increasing CD4 cell count levels during treatment (likelihood ratio test for trend $P < 0.001$; Table 1). Patients with CD4 cell counts greater than 500 cells/μl had better survival than those with levels between 350 and 500 cells/μl [adjusted hazard ratio (aHR) 1.70; 95% CI 1.26–2.30] and with CD4 cell counts between 200 and 349 cells/μl (aHR 2.56; 95% CI 1.93–3.38).

Initial BMI and clinical stage were associated with mortality. Mortality among patients in clinical stage 4 was 1.99 times higher than among those in stage 1 (95% CI 1.22–3.23). Patients with initial BMI below 16 kg/m^2 had higher mortality than those with BMI over than 18 kg/m^2 (aHR 1.61; 95% CI 1.17–2.21). Overall women experienced better survival than men (aHR 1.33; 95% CI 1.10–1.61).

Discussion

We studied the association between mortality and CD4 response following the first 9 months of ART use

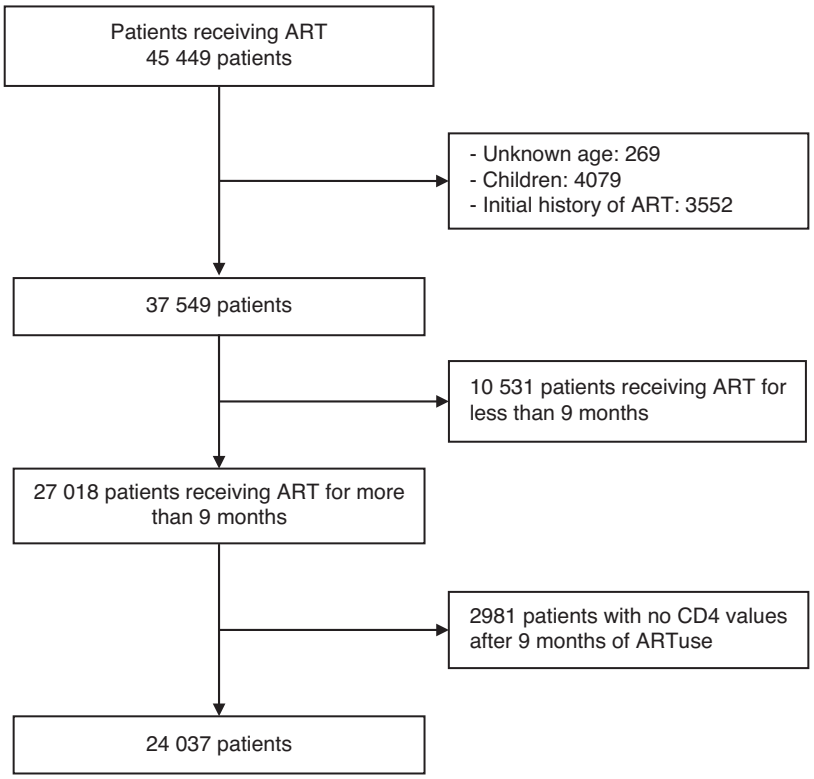

Fig. 1. Patient study flow chart, Kenya, Uganda and Malawi, 2001–2010. ART, antiretroviral therapy.

Table 1. Association between patient characteristics at antiretroviral therapy start and updated CD4 cell count levels, and death after 9 months of antiretroviral therapy initiation.

	Number of patients (%) $N = 24\,037$	Number of deaths $N = 568$	Number of person, years $N = 69\,516.2$	Adjusted HR (95% CI)
HIV program				
Kenya 1	3883 (16.2)	107	14 508.0	1
Kenya 2	2136 (8.9)	67	6132.4	1.74 (1.23–2.47)
Malawi	13 046 (54.3)	298	34 124.3	1.61 (1.25–2.10)
Uganda	4972 (20.7)	96	14 751.8	1.62 (1.25–2.10)
Sex				
Female	16 355 (68.0)	322	48 155.5	1
Male	7682 (32.0)	246	21 360.9	1.33 (1.10–1.61)
Age[a] (years)				
15–30	6254 (26.0)	113	17 609.2	1
30–50	15 356 (63.9)	354	45 407.8	1.11 (0.88–1.41)
>50	2427 (10.1)	101	6499.5	2.42 (1.77–3.31)
Clinical stage[a]				
1	3571 (14.9)	23	6140.5	1
2	4471 (18.6)	72	11 279.1	1.39 (0.85–2.29)
3	9200 (38.3)	205	29 960.2	1.24 (0.77–1.98)
4	5718 (23.8)	243	19 313.2	1.99 (1.22–3.23)
Unknown	1077 (4.5)	25	2823.5	1.75 (0.95–3.22)
BMI[a] (kg/m^2)				
≥18	18 113 (75.4)	349	51 883.0	1
≥17 and <18	2546 (10.6)	78	7514.3	1.36 (1.03–1.80)
≥16 and <17	1628 (6.8)	64	4894.3	1.60 (1.17–2.18)
<16	1587 (6.6)	64	4881.1	1.61 (1.17–2.21)
Unknown	163 (0.7)	13	343.8	5.56 (2.77–11.17)
Initial CD4 cell count[a] (cells/μl)				
≥200	5543 (23.1)	66	12 026.0	1
50–199	10 710 (44.6)	217	31 411.9	0.79 (0.58–1.08)
<50	2568 (10.7)	76	8060.7	0.63 (0.43–0.92)
Unknown	5216 (21.7)	209	18 017.8	1.07 (0.77–1.48)
Updated CD4 cell count (cells/μl)				
>500	26 891 (34.5)	81	22 719.9	1
350–499	19 285 (24.7)	103	17 632.2	1.70 (1.26–2.30)
200–349	20 752 (26.6)	171	19 527.4	2.56 (1.93–3.38)
50–199	10 250 (13.2)	174	9112.6	5.74 (4.30–7.68)
<50	792 (1.0)	39	524.3	25.76 (16.0–41.3)
Period of ART start				
2008–2010	7371 (30.7)	27	8104.7	1
2006–2007	7916 (32.9)	160	21 110.7	2.39 (1.56–3.67)
2004–2005	6300 (26.2)	241	26 902.3	2.93 (1.89–4.54)
2001–2003	2450 (10.2)	140	13 398.5	3.77 (2.37–5.96)

Mixed Poisson model, Uganda, Malawi and Kenya, 2001–2010; 77 970 CD4 values. ART, antiretroviral therapy.
[a]Information measured at ART initiation.

in four large HIV programs in sub-Saharan Africa. We found a significant negative dose-response relationship between on-treatment CD4 and mortality. The lowest mortality estimates were observed among patients who had reached on-treatment CD4 levels higher than 500 cells/μl.

This cohort study demonstrates on-treatment mortality decreases when patients reach 500 CD4 cells per microlitre compared to 350–500 cells/μl. This analysis reinforces the findings of two recent cohort studies that have showed a higher incidence of tuberculosis and Kaposi sarcoma, two frequent opportunistic infections, among patients with on-treatment CD4 cell counts ranging from 350 to 500 cells/μl compared to those reaching higher CD4 cell count [7,8]. Another recent study reported similar association between levels of CD4 cell counts and mortality among pre-ART patients treated in Europe and north America [9]. Despite the long time frame and the difference in patient characteristics (pre-ART patients and the majority of deaths happened to intravenous drug users), this study provided the first evidence that pre-ART patients with a CD4 cell count falling below 500 cells/μl experienced higher mortality than patients maintaining their CD4 cell count above this level. So far, two observational studies from developed countries found a higher incidence of AIDS-related events for patients initiating ART between 350 and 500 cell/μl compared to those initiating over 500 cell/μl, but found no effect on mortality [10,11]. However, we could hypothesize outcomes to be different in sub-Saharan Africa where capacities to accurately diagnose and treat opportunistic infections are more limited. Therefore, further studies to evaluate the impact of starting patients at higher CD4 cell counts are required in sub-Saharan Africa.

The main strength of our analysis is the long duration of follow-up and the large number of patients included and coming from some of the first HIV programs that provided ART at large scale in sub-Saharan Africa. A further strength was that programs included use of a similar standardized routine monitoring system. Concerning statistical analysis, residual individual heterogeneity due to repeated CD4 measures in same individuals was accounted for using mixed survival models. Furthermore, the multivariate analyses allowed adjusting for the effect of covariates including sex, patient characteristics at ART start, program and adherence.

We possibly underestimated mortality as 10.3% of patients were lost to follow-up during the study period. Results of the sensitivity analyses in which all the patients lost to follow-up were considered as dead weakened all the incidence rate ratios. However, it did not affect the conclusions of the primary analysis; patients with a CD4 response of at least 500 cells/μl still had better survival than those with 350/500 (e-content).

Including missing initial CD4 cell counts in the analysis as a category, and to a lesser extent missing clinical stage and BMI may have contributed to bias our results. We chose this strategy because missing information was not at random and represented patients with very advanced clinical disease. Our first model showed that patients with missing initial CD4 cell count had similar mortality than those severely immunosuppressed. In the sensitivity analysis restricted to patients with complete data (e-content), the conclusions of the main analyses did not change.

We assumed that CD4 cell counts remained constant between two consecutive measures or until the end of follow-up for the last measurement. Given that CD4 cell counts taken were those measured after 9 months of therapy when the slope of immune reconstitution is flatter, potential-related lead-time bias would have been minimized. CD4 cell count is a measure of immunity, which has a strong physiological variability [12]. This variability seems to lead to nondifferential misclassification resulting in underestimation of the magnitude of the CD4 response effect on survival [13].

The generalizability of our findings is reinforced by the inclusion of program data from four different sub-Saharan African cohorts, one in an urban area and three located in rural ones. All the sites are located in countries with high HIV prevalence. However, as our analysis used data from programs supported by Médecins Sans Frontières wherein additional human and diagnostic resources are generally available, we could hypothesize that in sub-Saharan African programs, not receiving this extra support, the survival benefit achieved by reaching 500 CD4 cell counts would be even higher.

Our study highlights the need to further evaluate the impact of starting patients at higher CD4 cell counts in sub-Saharan Africa for their own individual benefits. A consequent decrease in mortality and morbidity would contribute to the simplification of monitoring procedures.

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D.M., M.M.G., S.N. and M.P.R. participated in data collection and cleaning. D.M., R.E. and J.F.E. performed statistical analysis. D.M., M.P.R., R.E., and J.F.E. and interpreted the data. D.M. and J.F.E. drafted the article. All authors reviewed, revised, and approved the final report.

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Conflicts of interest

The authors declare that they have no conflict of interest with respect to this article.

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