Response to highly active antiretroviral therapy among severely immuno-compromised HIV-infected patients in Cambodia

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Background: HAART efficacy was evaluated in a real-life setting in Phnom Penh (Médecins Sans Frontières programme) among severely immuno-compromised patients.

Methods: Factors associated with mortality and immune reconstitution were identified using Cox proportional hazards and logistic regression models, respectively.

Results: From July 2001 to April 2005, 1735 patients initiated HAART, with median CD4 cell count of 20 (inter-quartile range, 6–78) cells/μl. Mortality at 2 years increased as the CD4 cell count at HAART initiation decreased, (4.4, 4.5, 7.5 and 24.7% in patients with CD4 cell count >100, 51–100, 21–50 and ≤ 20 cells/μl, respectively; $P < 10^{-4}$). Cotrimoxazole and fluconazole prophylaxis were protective against mortality as long as CD4 cell counts remained ≤ 200 and ≤ 100 cells/μl, respectively. The proportion of patients with successful immune reconstitution (CD4 cell gain >100 cells/μl at 6 months) was 46.3%; it was lower in patients with previous ART exposure [odds ratio (OR), 0.16; 95% confidence interval (CI), 0.05–0.45] and patients developing a new opportunistic infection/immune reconstitution infection syndromes (OR, 0.71; 95% CI, 0.52–0.98). Similar efficacy was found between the stavudine–lamivudine–nevirapine fixed dose combination and the combination stavudine–lamivudine–efavirenz in terms of mortality and successful immune reconstitution. No surrogate markers for CD4 cell change could be identified among total lymphocyte count, haemoglobin, weight and body mass index.

Conclusion: Although CD4 cell count-stratified mortality rates were similar to those observed in industrialized countries for patients with CD4 cell count >50 cells/μl, patients with CD4 cell count ≤ 20 cells/μl posed a real challenge to clinicians. Widespread voluntary HIV testing and counselling should be encouraged to allow HAART initiation before the development of severe immuno-suppression.

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Introduction

In industrialized countries, highly active antiretroviral therapy (HAART) has significantly improved the life expectancy of HIV-infected patients [1,2]. However, the vast majority of HIV-infected patients is located in developing countries, causing the World Health Organization (WHO) propose guidelines for scaling-up antiretroviral therapy (ART) in resource-poor settings and launch a '3*5' plan to provide ART to 3 million patients by 2005 [3]. Together with this initiative, numerous programmes have been started in recent years to offer HAART to HIV-infected patients in developing countries. Recent publications have shown good response to treatment in most resource-poor settings [4–7], with an estimated 1-year mortality of 6.4% in a pooled analysis of 18 programmes [5]. Such good response may not be seen with all HIV-infected patients in the developing world, and particularly among those who discover their HIV infection as a result of hospitalization for an opportunistic infection. In such circumstances, patients often have very advanced immuno-suppression, with CD4+ T-cell (CD4 cell) count less than 50 cells/μl at treatment initiation. This article presents the results of a cohort study among 1735 patients with advanced immuno-suppression (median CD4 cell count = 20 cells/μl at HAART initiation) as part of a hospital-based treatment programme in Cambodia.

Patients and methods

Patients

Since July 2001, HIV-infected patients attending the Preah Bath Norodom Sihanouk Hospital in Phnom Penh (Cambodia) have had access to free HAART through the *Médecins Sans Frontières* (MSF) programme. Following WHO recommendations, eligibility criteria for HAART were WHO stage IV or a CD4 cell count below 200 cells/μl [3]. Several first-line treatments were available, the choice depending on drug availability as well as national policy recommendations. Thus, the combination stavudine (d4T), lamivudine (3TC), efavirenz (EFV) was predominantly administrated until 2004, and the combination d4T, lamivudine (3TC) and nevirapine (NVP), given as a fixed-dose combination (FDC) was largely used thereafter. Physicians were instructed to prescribe cotrimoxazole (480 mg/day) and fluconazole (200 mg/day) prophylaxis to patients with CD4 cell count ≤ 200 and ≤ 50 cells/μl, respectively (in 2003, the decision was made to extend fluconazole indication to those with CD4 cell count ≤ 100 cells/μl). Following HAART initiation, patients were seen monthly for 6 months, and then every 2 months. All patients' data were routinely entered in Fuchia v1.5 (Epicentre – MSF), and included the following information: (1) at enrolment: age, sex, residence, height, weight, WHO clinical stage [8], ART history, first-line therapy and prophylactic therapy; (2) at each follow-up or unscheduled visit: weight, clinical events since last visit, treatment changes, treatment side effects, prophylactic therapy.

Laboratory analysis

At treatment initiation, and every 6 months thereafter, whole blood samples were collected on anticoagulant for total lymphocyte count, CD4 cell count, and haemoglobin determination. Measurements were performed at the Institut Pasteur in Phnom Penh. CD4 cell counts were obtained using FACSCalibur flow cytometry technology (BD Biosciences, Immunocytometry Systems, San Jose, California, USA).

Statistical methods

The analysis included all adult patients (≥ 13 years of age) who initiated HAART between July 2001 and April 2005. The effect of HAART was first investigated in terms of progression to death, by estimating Kaplan–Meier estimates. In this analysis, patients lost to follow-up, i.e. not seen for more than 3 months, were considered dead ($n = 37$). A Cox model was used to identify prognostic factors of death. Factors included in the model were (a) baseline data such as age, sex, WHO clinical stage, biological markers (CD4 cell count, total lymphocyte count, haemoglobin), body mass index (BMI), year of HAART initiation, drug regimen used and previous exposure to ART; and (b) time-dependent variables such as cotrimoxazole and fluconazole prophylaxis. The d4T–3TC–NVP FDC and the combination d4T–3TC–EFV were compared using an intention to treat approach, thus ignoring treatment modifications as well as interruptions. Total lymphocyte count and haemoglobin were categorized according to quartiles, whereas for CD4 cell count the four following categories were defined: ≤ 20, 21–50, 51–100 and > 100 cells/μl. Three categories were defined for the BMI (< 17, 17–18.5 and > 18.5 kg/m^2); the first cut-off corresponds to the first quartile, while the second cut-off correspond to the Food and Agriculture Organization threshold for malnutrition [9]. For CD4 cell counts and other laboratory markers, a category for missing data was introduced when results were not available. In most cases, missing data corresponded to patients with very poor health status who did not need CD4 cell count to decide on HAART indication. Furthermore, the mortality rate ratios associated with the use of cotrimoxazole and of fluconazole were compared across time-varying CD4 cell count categories.

The CD4 distributions at HAART initiation (measured within a maximum of 6 months preceding HAART initiation), and at 6 (± 1) months and 12 (± 2) months after HAART initiation were compared to study immune reconstitution. The time to reach the critical threshold of 200 CD4 cells/μl, at which the risk of opportunistic infections (OI) is reduced, was estimated for each of the baseline CD4 cell count categories. Immune reconstitution was considered as successful if the CD4 cell count

gain, 6 months after HAART initiation, was at least 100 cells/μl [10,11]. Factors associated with a successful immune reconstitution were identified using a logistic regression model.

Due to the limited clinical investigations available on site, it was often difficult to distinguish new OIs from immune reconstitution infection syndromes (IRIS) after HAART initiation, thus the two conditions were not differentiated for the analysis. Post-HAART incidence of OI/IRIS was estimated, and factors associated with the occurrence of OI/IRIS were studied using a Cox model.

Total lymphocyte count, haemoglobin, weight and BMI gains were evaluated as potential surrogate markers for CD4 cell gain 1 year after HAART initiation. For this purpose, the strength of the correlation between CD4 cell gain and each surrogate marker gain was assessed by computing the Spearman correlation coefficient. The sensitivity and specificity of specific marker changes (e.g., lowest quartile of lymphocyte count gain) in predicting CD4 cell count gain ≤ 60 cells/μl (lowest decile) after 1 year were also estimated.

Statistical analyses were performed using Stata 8 software (Stata Corporation, College Station, Texas, USA); all significance tests were two-sided and P-values < 0.05 were considered significant.

Results

Between July 2001 and April 2005, 1735 adults initiated HAART, among which 792 (45.6%) had clinical AIDS (Table 1). The median duration of follow-up under HAART at the time of analysis was 13 months [inter-quartile range (IQR), 5–21 months]. Median CD4 cell count at HAART initiation was 20 cells/μl (IQR, 6–78). Men were at a more advanced stage of infection in comparison with women, as shown by the larger proportion with clinical AIDS (54.5 versus 33.3%; $P < 0.001$), and the lower median CD4 cell count (11 versus 46 cells/μl, $P < 0.001$).

Of the 1735 patients, 968 (55.8%) initiated HAART with the combination d4T–3TC–EFV, 711 (41.0%) with the FDC d4T–3TC–NVP, and 56 (3.2%) with other three-drug combinations. In 30 patients, HAART combination was changed, introducing a new therapeutic class (switch to second line), after a median delay of 11.4 months (IQR, 3.7–17.8). In 766 other patients, HAART combination was changed without introduction of a new therapeutic class; changes were switch from EFV to NVP (42.5%), d4T to zidovudine (40.7%) or NVP to EFV (13.1%), other changes were rare. Switches from EFV to NVP were related to changes in national policy recommendations, other switches were mostly motivated by intolerance. During the follow-up, 209 patients discontinued HAART for a median time of 1.1 months (IQR, 0.5–2.4 months).

Mortality after HAART initiation

Of the 1735 patients who initiated HAART, 186 patients died and 37 were lost to follow-up during 1955 person-years of follow-up. Out of these patients, 103 (46.1%) died within 3 months after HAART initiation. Using Kaplan–Meier estimates, the death rates [95%confidenceinterval(CI)] at 24 months according to the four CD4 cell count categories were: 24.7% (21.4–28.4%), 7.5%

Table 1. Individual characteristics at HAART initiation by gender.

	Men $N = 1010$ (58.2%)	Women $N = 725$ (41.8%)	P-value
Age (years)			
Median (IQR)	35 (31–39)	32 (28–38)	< 0.001
WHO stage			
I	13 (1.3%)	40 (5.5%)	< 0.001
II	37 (3.7%)	64 (8.8%)	
III	409 (40.5%)	380 (52.4%)	
IV	551 (54.5%)	241 (33.3%)	
BMI			
N (%)	857 (84.9%)	600 (82.8%)	
Median (IQR) (kg/m^2)	18.5 (16.6–20.4)	18.7 (16.4–21.0)	0.87
CD4 cell count			
N (%)	923 (91.4%)	693 (95.6%)	
Median (IQR) (cells/μl)	11 (4–41)	46 (11–118)	< 0.001
Lymphocyte count			
N (%)	876 (86.7%)	667 (92.0%)	
Median (IQR) (cells/μl)	1320 (960–1790)	1370 (1000–1800)	0.79
Haemoglobin level			
N (%)	705 (69.8%)	510 (70.3%)	
Median (IQR) (g/dl)	11.9 (10.2–13.0)	10.6 (9.5–12.0)	< 0.001

BMI, body mass index; IQR, inter-quartile range; WHO, World Health Organization.

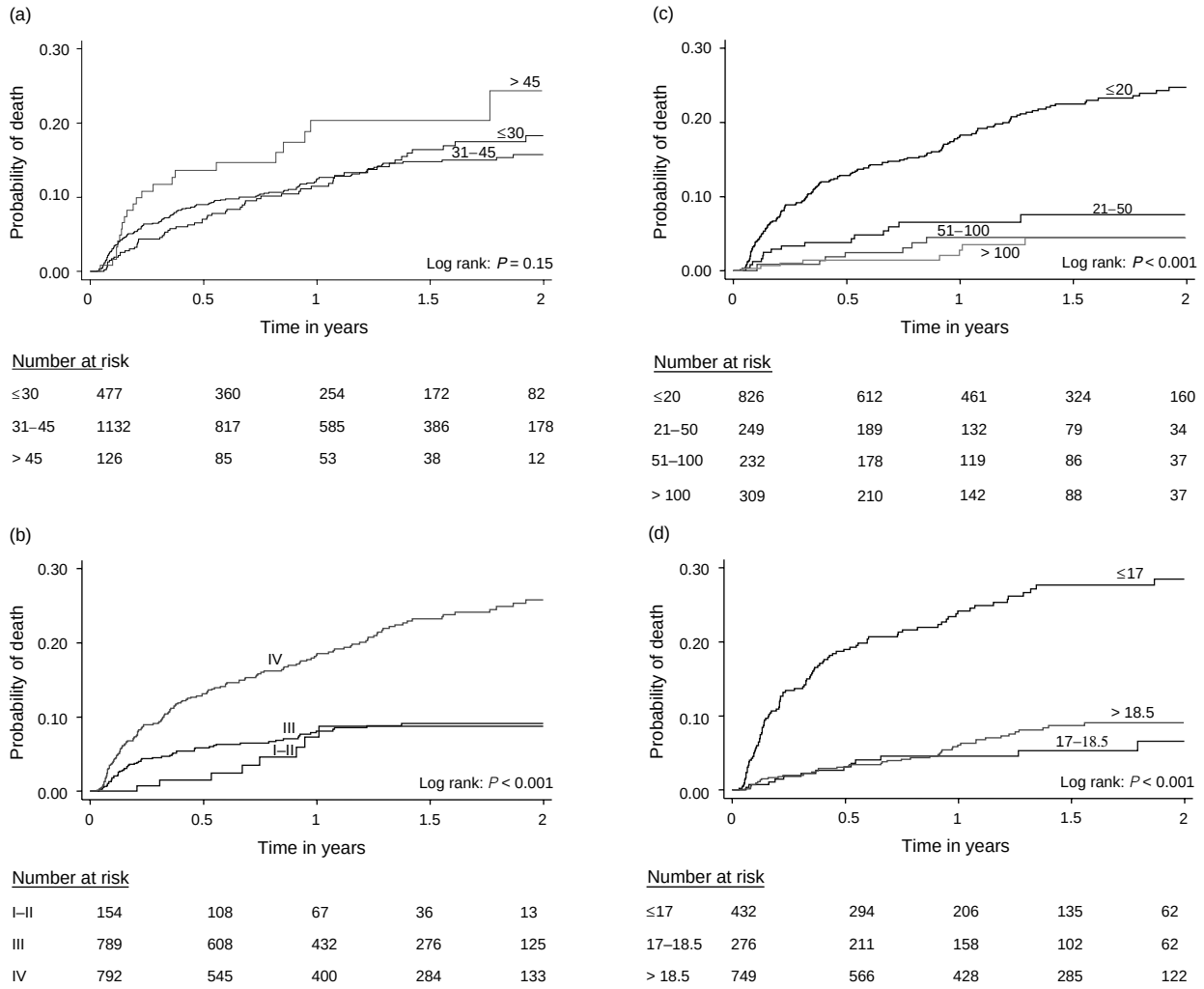

Fig. 1. Kaplan–Meier survival curve according to (a) age (in years); (b) WHO clinical stage; (c) baseline CD4 cell count (in cells/μl); and (d) body mass index (BMI, in kg/m^2).

(4.5–12.5%), 4.5% (2.2–8.9%) and 4.4% (2.1–9.0%), respectively ($P < 0.001$) (Fig. 1). *P*-values comparing the categories 21–50 versus 51–100, 21–50 versus > 100, and 51–100 versus > 100 were all non-significant.

Table 2 shows the factors associated with the death rate in a multivariate Cox model: age above 45 years, low CD4 cell count (≤ 50 cells/μl), WHO clinical stage IV (AIDS), BMI ≤ 17 kg/m^2, and HAART discontinuation > 1 month were all independently associated with a higher risk of death, whereas cotrimoxazole and fluconazole prophylactic treatments were protective. In Table 3, the protective effect of cotrimoxazole and fluconazole prophylaxis is described according to time-dependent CD4 cell count categories. It can be seen that the recommendation to provide cotrimoxazole prophylaxis to patients with CD4 cell count ≤ 200 cells/μl was properly followed (only 37.9 person-years of follow-up without cotrimoxazole), whereas that of providing fluconazole prophylaxis was less properly followed (259.6 person-years without fluconazole). Of interest, the protective effect of cotrimoxazole was limited to patients with CD4 cell count ≤ 200 cells/μl, and that of fluconazole to patients with CD4 cell count ≤ 100 cells/μl.

Immune reconstitution after HAART

The median CD4 cell count at HAART initiation, available in 1616 patients, was 20 cells/μl (IQR, 6–78), and was above 200 cells/μl in only 16 patients. The CD4 cell count increased after HAART initiation (Fig. 2) to reach median levels of 130 cells/μl (IQR, 87–189) at 6 months and 189 cells/μl (IQR, 135–250) at 12 months. Times to reach CD4 cell counts above 200 cells/μl were median 17.7, 16.4, 11.1, and 6.5 months for initial CD4 cell counts at ≤ 20, 21–50, 51–100 and 101–150 cells/μl, respectively.

The CD4 cell count gain, estimated in 1040 patients, was in median +94 cells/μl (IQR, 59–131 cells/μl); within the four baseline CD4 cell count categories (≤ 20, 21–50, 51–100 and > 100) the median gains were

Table 2. Factors associated with progression to death after HAART initiation.

	N (%)	Crude HR (95% CI)	Adjusted HR (95% CI)
Sex			
Men	1010 (58.2)	1	
Women	725 (41.8)	0.60 (0.50–0.87)	
Age at HAART initiation (years)			
<30	477 (27.5)	1.03 (0.77–1.39)	0.88 (0.65–1.19)
30–45	1132 (65.2)	1	1
≥ 45	126 (7.3)	1.55 (0.99–2.44)	1.83 (1.16–2.90)
WHO stage at HAART initiation			
I–II	154 (8.9)	0.74 (0.36–1.56)	0.73 (0.33–1.63)
III	789 (45.5)	1	1
IV	792 (45.6)	2.66 (1.98–3.58)	1.86 (1.37–2.53)
CD4 cell count at HAART initiation (cells/μl)			
≤ 20	826 (47.6)	7.09 (3.49–14.42)	8.93 (4.17–19.14)
20–50	249 (14.3)	2.20 (0.93–5.20)	2.78 (1.14–6.81)
50–100	232 (13.4)	1.22 (0.46–3.24)	1.65 (0.61–4.43)
>100	309 (17.8)	1	1
Missing	119 (6.9)	10.03 (4.57–22.01)	10.58 (4.69–23.89)
Lymphocyte count at HAART initiation (cells/μl)			
≤ 970	390 (22.5)	4.22 (2.66–6.71)	
970–1340	382 (22.0)	1.97 (1.19–3.26)	
1340–1800	393 (22.7)	1.37 (0.81–2.33)	
>1800	378 (21.7)	1	
Missing	192 (11.1)	4.16 (2.50–6.94)	
Haemoglobin level at HAART initiation (g/dl)			
≤ 10	384 (22.1)	2.04 (1.14–3.67)	
10–11	242 (14.0)	1.74 (0.92–3.27)	
11–13	405 (23.3)	1.31 (0.71–2.42)	
>13	184 (10.6)	1	
Missing	520 (30.0)	2.89 (1.64–5.10)	
BMI at HAART initiation (kg/m^2)			
≥ 18.5	749 (43.2)	1	1
17–18.5	276 (15.9)	1.14 (0.71–1.82)	0.88 (0.54–1.41)
<17	432 (24.9)	3.80 (2.78–5.22)	2.47 (1.77–3.43)
Missing	278 (16.0)	1.37 (0.86–2.17)	1.11 (0.69–1.78)
Baseline HAART regimen			
d4T–3TC–EFV	968 (55.8)	1	
d4T–3TC–NVP	711 (41.0)	0.74 (0.54–1.01)	
Other	56 (3.2)	1.06 (0.52–2.16)	
HAART discontinuation >1 month[a]			
No	–	1	1
Yes	–	4.18 (2.88–6.06)	3.23 (2.19–4.77)
Switch to second line[a]			
No	–	1	
Yes	–	2.61 (0.96–7.06)	
Year of HAART initiation			
2001–2002	202 (11.6)	1.45 (1.00–2.09)	1.22 (0.84–1.78)
2003	669 (38.6)	1	1
2004–2005	864 (49.8)	1.45 (1.07–1.98)	1.64 (1.19–2.27)
Previous exposure to ART			
No	1652 (95.2)	1	
Yes	83 (4.8)	1.27 (0.71–2.27)	
Cotrimoxazole during HAART[a]			
No	–	1	1
Yes	–	0.16 (0.11–0.21)	0.15 (0.11–0.21)
Fluconazole during HAART[a]			
No	–	1	1
Yes	–	1.13 (0.84–1.52)	0.50 (0.35–0.72)

ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; d4T, stavudine; EFV, efavirenz; HR, hazard ratio; NVP, nevirapine; WHO, World Health Organization' 3TC, lamivudine.
[a]Time-dependent covariate in the Cox model.

Table 3. Effect of cotrimoxazole and fluconazole prophylaxis on the mortality rate.

	With cotrimoxazole			Without cotrimoxazole			
CD4 count (cells/μl)	Deaths	Person-years	Mortality rate[a]	Deaths	Person-years	Mortality rate[a]	Mortality rate ratio (95% CI)
≤100	100	702.4	14.2	34	18.0	183.8	0.08 (0.05–0.11)
101–200	19	582.5	3.2	4	19.4	20.6	0.16 (0.05–0.46)
>200	6	314.2	1.9	6	168.0	3.6	0.53 (0.17–1.65)
	With fluconazole			Without fluconazole			
CD4 count (cells/μl)	Deaths	Person-years	Mortality rate[a]	Deaths	Person-years	Mortality rate[a]	Mortality rate ratio
≤50	81	419.2	19.3	34	26.0	130.8	0.15 (0.10–0.22)
51–100	10	198.5	5.0	9	76.7	11.7	0.43 (0.17–1.06)
101–200	2	84.8	2.4	21	517.1	4.1	0.58 (0.14–2.48)
>200	0	14.7	0.0	12	467.6	2.6	0.0 (0.0–15.8)

[a]Per 100 person-years.

90 cells/μl (IQR, 62–123), 107 cells/μl (IQR, 67–145), 99 cells/μl (IQR, 67–140) and 74 cells/μl (IQR, 34–133), respectively. At 12 months, the CD4 cell count gain was in median +149 cells/μl (IQR, 99–207) (estimated in 795 patients).

A CD4 cell count gain of at least 100 cells/μl at 6 months was considered as a successful immune reconstitution, and was achieved in 481 (46.3%) patients. In the multivariate logistic regression model, female gender [odds ratio (OR), 1.67; 95% CI, 1.28–2.19], age lower than 30 years (OR, 1.43; 95% CI, 1.07–1.90, when compared with age 30–45 years as the reference category), CD4 cell count between 21 and 50 cells/μl at HAART initiation (OR, 2.15; 95% CI, 1.38–3.37, when compared with CD4 cell count >100 cells/μl as the reference category) were all positively associated with a successful immune reconstitution, while previous exposure to ART (OR, 0.16; 95% CI, 0.05–0.45) and occurrence of a new OI/IRIS in the 6 months after HAART initiation (OR, 0.71; 95% CI, 0.52–0.98) were negatively associated with a successful immune reconstitution.

Six and 12 months after HAART initiation, the median total lymphocyte gains were +525 cells/μl (IQR, 50–1040) and +790 cells/μl (IQR, 260–1340), respectively; the median haemoglobin gains were +1.3 g/dl (IQR, 0.4–2.6) and +2.0 g/dl (IQR, 0.8–3.1), respectively; the median weight gains were +4 kg (IQR, 1–7) and +4 kg (IQR, 1–8), respectively; and the median BMI gains were +1.4 kg/m^2 (IQR, 0.4–2.8) and +1.6 kg/m^2 (IQR, 0.3–3.2), respectively. The Spearman correlation coefficients between CD4 cell count gain and total lymphocyte gain at 6 and 12 months were 0.45 and 0.51 ($P<0.001$), respectively; although significant, correlations with haemoglobin, weight and BMI gains were weaker (data not shown). When trying to identify surrogate markers changes that could predict CD4 cell count gains ≤60 cells/μl at 12 months (lowest decile), none came out with satisfactory sensitivity and specificity (data not shown).

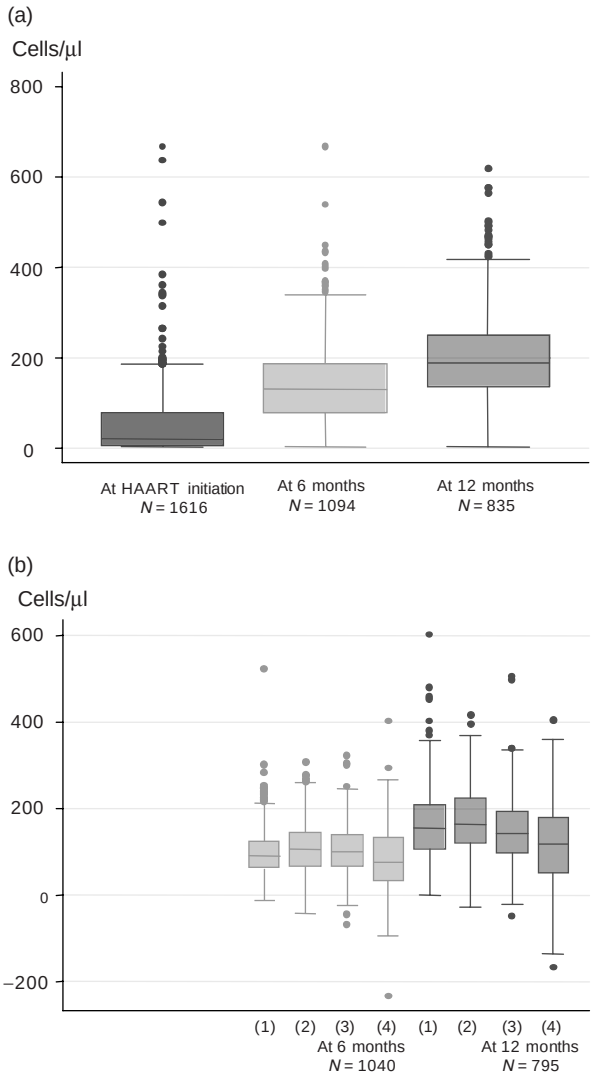

Fig. 2. Immune reconstitution after HAART initiation. (a) CD4 cell count distribution; (b) CD4 cell count gain from HAART initiation, according to the CD4 level at HAART initiation [(1) ≤20 cells/μl; (2) 21–50 cells/μl; (3) 51–100 cells/μl; (4) >100 cells/μl].

Occurrence of opportunistic infection/immune reconstitution infection syndromes after HAART initiation

Following HAART initiation, 496 (28.6%) patients experienced either incident pulmonary tuberculosis, or a WHO stage IV condition (OI/IRIS). Extra-pulmonary tuberculosis was reported in 145 patients, pulmonary tuberculosis in 108, wasting syndrome in 63, atypical disseminated mycobacteriosis in 25, extra-pulmonary Cryptococcus in 18, and other WHO stage IV conditions in 50 (some patients presented more than one clinical manifestation). The delay from HAART initiation to the diagnostic of OI/IRIS was in median 4 week (IQR, 2–12 weeks).

Using a multivariate Cox model, WHO stage IV (AIDS) at HAART initiation [hazard ratio (HR), 1.35; 95% CI, 1.11–1.64, when compared with WHO stage III as the reference category], CD4 level ≤ 100 cells/μl (HR, 3.20; 95% CI, 2.05–5.00), and BMI $\leq 17\,kg/m^2$ (HR, 1.73; 95% CI, 1.42–2.11, when compared with BMI > 18.5 as the reference category) were all independently associated with an increased rate of OI/IRIS following HAART initiation. Similar results were obtained when observations were censored 1 year after HAART initiation.

Discussion

In this MSF programme, 1735 patients initiated HAART with a median CD4 cell count of only 20 cells/μl. The very severe immuno-suppression at HAART initiation found in this study is not unusual in developing countries, where many patients discover their infection while hospitalized for their first opportunistic infection [12,13]. Indeed, of over 8000 patients treated worldwide by MSF, 40.4% had CD4 cell count < 50 cells/μl at HAART initiation [14]. Having detailed information about treatment response and prognosis for patients with very low CD4 cell counts is therefore of great relevance to physicians working in developing countries. The strength of the present study is to be based in a single centre with well established routines for patient management and data collection, to reflect the outcome of a 'real-life' treatment programme (i.e., not a clinical trial with restrictive inclusion criteria and intensive monitoring), to have limited attrition (2.1% lost to follow-up), and to be sufficiently large ($n = 1735$) for precise estimations of CD4-stratified mortality rates.

The first important finding of this study is that mortality in the first 2 years after HAART initiation in this resource-poor setting was not markedly different from that observed in the industrialized world. The CD4-stratified survival curves displayed in Fig. 1 are very similar to those shown in the ART Cohort Collaboration initiative, a grouped analysis of over 12 000 adult patients treated with HAART in the Western world [15]. The only difference was in the severely immuno-compromised group. Patients with CD4 cell count ≤ 20 cells/μl represented half of the study population, and had a much worse prognosis compared with the rest of the group. Such highly immuno-compromised patients are no longer commonly seen in industrialized countries, and pose a real challenge to treating physicians in resource-poor settings. How to initiate HAART while treating opportunistic infections, among which tuberculosis, in these patients remains to be further studied to minimize early mortality [14,16].

The second important finding was the similar efficacy, as judged by the mortality rate and the quality of the immune reconstitution, of the FDC d4T–3TC–NVP compared with the combination d4T–3TC–EFV. These results should be considered with caution, as assignment to treatment regimens was not randomized, but reflected changes with time of national policy recommendations. Nevertheless, results remained unchanged after controlling for potential confounders in multivariate analysis. Although previous cohort studies favoured EFV compared to NVP [17–19], and the only large clinical trial gave equivocal results [20,21], the NVP-containing FDC represents today the most practical choice for developing countries, considering its low cost, its ease of use (one pill twice a day), and its lack of teratogenicity. In this regard, the results of this study, showing its comparable effectiveness with another common regimen, are important.

Because of cost and technology limitations, viral load measurements were not available in this study. In such case, WHO recommends that immune reconstitution, together with clinical evaluation, are used to assess treatment efficacy [3]. In this study, the median CD4 cell count gain at 6 months was +94 cells/μl (IQR, 59–131), and at 12 months +149 cells/μl (IQR, 99–207). Similar CD4 cell count gains were observed in developing countries [4,7,22], and slightly higher gains in industrialized countries [10,11,23]. Some factors were found associated with lower CD4 cell count gains in this study, such as male gender [24] and previous ART exposure [24,25]. One should be cautious, however, on the magnitude of these associations in the absence of control for viral load in this study. It is recommended that chemoprophylaxis is used to prevent OIs at HAART initiation if CD4 cell count is still low, but the CD4 cell count threshold at which chemoprophylaxis can be stopped has not been assessed in developing countries. In this study, based on mortality data, cotrimoxazole prophylaxis was protective for CD4 cell counts ≤ 200 cells/μl, and fluconazole prophylaxis was protective for CD4 cell counts ≤ 100 cells/μl. Moreover, chemoprophylaxis was found to strongly reduce the risk of death in a multivariate Cox model. The time to reach the 200 CD4 cells/μl threshold was estimated in this study at 18 and 6 months in patients initiating HAART with baseline CD4 cell count ≤ 20 and 101–150 cells/μl, respectively.

Identification of a surrogate marker of immune response to HAART would be of great help as CD4 cell count may not be available in many places for monitoring patients' response to treatment. Total lymphocyte count, haemoglobin, weight and BMI were considered as potential surrogate markers, as these are easy to obtain. Although improvements in these markers were significantly correlated with the CD4 cell gains, with best correlations for total lymphocyte count changes, the relationships were weak (correlation coefficients < 0.5). Contrary to other publications [26,27], the sensitivity and specificity of various cut-offs of total lymphocyte count changes in predicting poor CD4 immune response never reached satisfactory levels (both $> 70\%$) to be of use in clinical practice. Efforts should be continued to simplify CD4 cell count testing in the developing world, in the absence of proper surrogate marker, to monitor patients under treatment [28].

Following HAART initiation, 496 (28.6%) of the 1735 patients enrolled in this study were diagnosed with an incident pulmonary tuberculosis or a WHO stage IV condition. Although we could not differentiate OIs from IRIS, the delay from HAART initiation to diagnosis (4 weeks in median) and the nature of these OIs (half were extra-pulmonary and pulmonary tuberculoses) suggest that the majority of these patients suffered from IRIS [29,30]. The higher incidence of IRIS in this study, compared to industrialized countries [31,32], could be related to the severe immuno-suppression of the patients (shown as a risk factor of IRIS in this study), and the higher prevalence of past mycobacterial infections in the study population. Management of IRIS is difficult in resource-poor settings, where the use of steroids is hampered by the absence of in-depth microbiological investigations to rule out active OIs. The elevated early mortality seen in this study and elsewhere [5] could be partly related to IRIS, and further studies are required to help sorting out IRIS from OIs for better management of these patients.

In conclusion, HAART proved to be effective in patients with CD4 cell count > 20 cells/μl. Further research is required to know how best to initiate and monitor HAART in patients with very low CD4 cell count (≤ 20 cells/μl), concomitant OIs and high risk of IRIS. Meanwhile, widespread voluntary HIV testing and counselling should be encouraged to allow HAART initiation before the development of severe immuno-suppression. Free access to HAART, as in this programme, is also important as programmes in which patients had to pay for their treatment have shown higher rates of mortality [5,33].

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