Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia

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Objectives: African and Asian cohort studies have demonstrated the feasibility and efficacy of HAART in resource-poor settings. The long-term virological outcome and clinico-immunological criteria of success remain important questions. We report the outcomes at 24 months of antiretroviral therapy (ART) in patients treated in a Médecins Sans Frontières/Ministry of Health programme in Cambodia.

Methods: Adults who started HAART 24 ± 2 months ago were included. Plasma HIV-RNA levels were assessed by real-time polymerase chain reaction. Factors associated with virological failure were analysed using logistic regression.

Results: Of 416 patients, 59.2% were men; the median age was 33.6 years. At baseline, 95.2% were ART naive, 48.9% were at WHO stage IV, and 41.6% had a body mass index less than 18 kg/m^2. The median CD4 cell count was 11 cells/μl. A stavudine–lamivudine–efavirenz-containing regimen was initiated predominantly (81.0%). At follow-up (median 23.8 months), 350 (84.1%) were still on HAART, 53 (12.7%) had died, six (1.4%) were transferred, and seven (1.7%) were lost to follow-up. Estimates of survival were 85.5% at 24 months. Of 346 tested patients, 259 (74.1%) had CD4 cell counts greater than 200 cells/μl and 306 (88.4%) had viral loads of less than 400 copies/ml. Factors associated with virological failure at 24 months were non-antiretroviral naive, an insufficient CD4 cell gain of less than 350 cells/μl or a low trough plasma ART concentration. In an intention-to-treat analysis, 73.6% of patients were successfully treated.

Conclusion: Positive results after 2 years of advanced HIV further demonstrate the efficacy of HAART in the medium term in resource-limited settings.

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Introduction

African and Asian cohort studies have demonstrated the feasibility and efficacy of HAART in resource-poor settings [1–8]. Adherence to treatment in these settings was found to be as good or even better than that observed in northern countries [1,2]. Even if early adherence to HAART appears to be a crucial factor to ensure

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immunovirological success [9], however, adherence remains a dynamic process [10] and concerns about its long-term maintenance at optimal levels in sub-Saharan Africa have recently been raised [11]. In addition, the occurrence of progressive antiretroviral drug side effects might also impair long-term adherence and virological outcomes of the patients.

Reports on medium or long-term cohorts of patients on HAART in resource-poor settings are still scarce [6,7], and are still performed on only a limited number of patients.

With an estimated HIV prevalence of 1.9% of the adult population (15–49 years) at the end of 2003 and 126 000 individuals living with HIV, Cambodia presents one of the highest HIV prevalences in south-east Asia [12,13]. Access to antiretroviral therapy (ART) is still limited, and up to end of June 2006, almost 16 000 of the 30 000 patients supposed to be in immediate need effectively received ART [14]. The present study aimed to assess patient outcomes 24 months after HAART initiation in a Médecins Sans Frontières (MSF)/Ministry of Health (MoH) observational cohort in Phnom Penh, Cambodia, by analysing survival indicators, CD4 cell count evolution, virological response and pharmacological characteristics.

Methods

Setting

In collaboration with the MoH of Cambodia, MSF began an HIV programme in 1997 supporting the Infectious Disease Department of the Khmero-Sovietic Friendship (KSF) Hospital in Phnom Penh. The project involved MoH staff (eight physicians, 17 nurses) and MSF staff (two physicians, one nurse, four counsellors, two pharmacists), training, patient education, social support, and adherence consultations for information and education about HIV/AIDS disease and treatment. A peer support group was created to support patient advocacy issues and to participate in the activities of the clinic. Since 2001, HAART was offered free of charge to patients either at World Health Organization (WHO) stage IV (AIDS) irrespective of CD4 cell count or at WHO stages I, II or III with CD4 cell counts of 200 cells/μl or less according to WHO recommendations [15]. Preparation for HAART initiation is accomplished by three pre-HAART counselling visits. The initial first-line regimen proposed was the association of stavudine, lamivudine and efavirenz. Patients with intolerance to this combination were offered other alternative first line regimens. Clinical follow-up visits were performed monthly for the first 6 months then bimonthly. The CD4 T-cell count was monitored every 6 months and no individual viral load monitoring was available. Adherence support was provided at each visit by counsellors through individual interviews or 'talk' groups. In the case of treatment failure, a protease inhibitor-based second-line treatment was available as recommended by WHO [15].

At the end of March 2005, 2048 HIV-infected adult patients were still on HAART in the programme.

Study population

All adults, aged 18 years or more, who had initiated HAART for 24 ± 2 months at the date of analysis (31 March 2005) were eligible for the study. Medical background and follow-up information were routinely collected at each consultation and entered into FUCHIA monitoring software (Follow-Up and Care of HIV Infection and AIDS, Epicentre, Paris).

Cross-sectional survey

Between 13 December 2004 and 31 March 2005, a cross-sectional survey was conducted to assess the clinical, adherence and virological status of all patients who had been receiving HAART for 24 ± 2 months and presented to the HIV clinic during the study period. CD4 cell counts were performed using flow cytometry (Facscount; Beckton Dickinson, Franklin Lakes, New Jersey, USA) at the Institut Pasteur du Cambodge. Plasma viral load measures were performed on -80°C frozen samples at the HIV/Hepatitis laboratory, Necker Enfants Malades Hospital (Paris, France), using real-time polymerase chain reaction technology, which allows the quantification of HIV-1 non-B subtypes including those circulating in Asia [16–18]. Positive samples from Thailand and Cambodia have been studied in parallel with the Cobas Amplicor HIV-1 Monitor v1.5 test (Roche Diagnostic Systems, Pleasanton, California, USA) and gave highly correlated results (data not shown). For viral loads above 400 copies/ml, HIV-1 reverse transcriptase (RT) genotyping was performed at the Institut Pasteur du Cambodge [19]. The resistance profiles to antiretroviral molecules were defined according to Agence Nationale de Recherches sur le Sida (ANRS) algorithms [20].

Trough plasma concentrations of nevirapine and lopinavir or 12 h post-dose concentrations of efavirenz were measured at the Clinical Pharmacology Department, Bicêtre Hospital (Kremlin Bicêtre, France) using validated high performance liquid chromatography assay [21,22]. Patients were classified by to their trough plasma concentrations according to the following therapeutic windows: nevirapine (3000–8000 ng/ml), efavirenz (1000–4000 ng/ml) and lopinavir (3000–8000 ng/ml) [23].

The survey was approved by the National Ethics Committee for Health Research of Cambodia on 3 December 2004. All participants gave their written informed consent.

Statistical analysis

Product-limit estimates (Kaplan–Meier) of survival were determined for all patients on an intention to treat basis. Patients alive and in care on 31 March 2005 were right-censored on the date of their last visit before this date. Patients who had not attended services for 2 months or more beyond their last scheduled appointment were classified as being lost to follow-up, and statistically considered on their last recorded visit to the clinics. CD4 cell gains were calculated every 6 months after HAART initiation. Logistic regression analysis was performed to identify factors associated with viral loads greater than 1000 copies/ml 24 months after HAART initiation. Variables with a $P < 0.05$ were included in the multivariate analysis. Receiver operating characteristic (ROC) curve analysis was performed to define the threshold of percentage CD4 cell gain between month 12 and month 24, which gives optimum sensitivity and specificity for the detection of viral load greater than 1000 copies/ml. Analyses were performed using STATA 8.2 software (Stata Corp., College Station, Texas, USA).

Results

Among the 2048 HIV-infected patients who were on HAART up to 31 March 2005 in the programme, 416 adults had started 24 ± 2 months earlier and were included in the analysis (M24 cohort, Fig. 1). A total of 247 (59.2%) were men and the median age was 33.6 years [interquartile range (IQR) 30.1–38.2; Table 1]. Almost all (396/416, 95.2%) were ART naive. Most were already at an advanced stage of HIV disease when entering the programme: 192 (46.0%) were at WHO stage III and 204 (48.9%) were at WHO stage IV. The body mass index (BMI) was below 18.0 kg/m^2 for 161 out of 387 patients (41.6%) and was below 15.0 kg/m^2 for 40 out of 387 patients (10.3%). Patients were severely immunocompromised, with a median baseline CD4 cell count of 11 cells/μl (IQR 3–60, $n = 416$) and 297 out of 416 patients (71.4%) had a CD4 cell count below 50 cells/μl. A total of 337 patients (81.0%) received a first-line antiretroviral regimen associating stavudine, lamivudine and efavirenz, whereas 64 (15.4%) received stavudine, lamivudine and nevirapine, and 15 (3.6%) received another first-line regimen (Table 1).

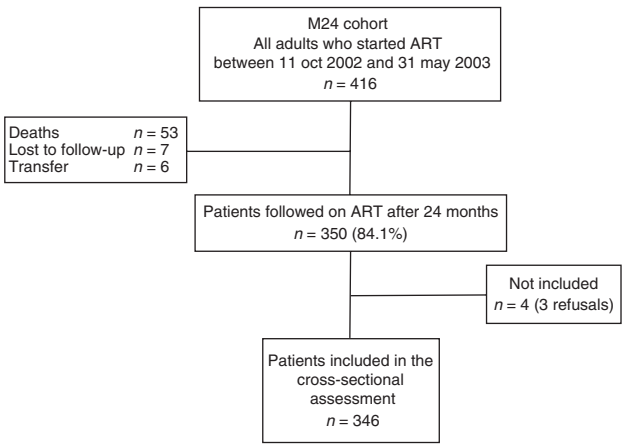

Fig. 1. Details of the M24 cohort. ART, Antiretroviral therapy.

Table 1. Baseline characteristics of the patients of the M24 cohort.

Characteristics	M24 cohort ($N = 416$)
Demography	
Male [in (%)]	247 (59.2)
Median age [years (IQR)]	33.6 (30.1–38.2)
ART naive [*n* (%)]	397 (95.2)
Clinico-immunology	
WHO stage at HAART initiation	
Stage I/II [*n* (%)]	21 (5.0)
Stage III [*n* (%)]	192 (46.0)
Stage IV [*n* (%)]	204 (48.9)
Body mass index (*n*)	387
Median [kg/m^2 (IQR)]	18.6 (16.4–20.4)
< 18 kg/m^2 [*n* (%)]	161 (41.6)
< 15 kg/m^2 [*n* (%)]	40 (10.3)
CD4 T-cell counts at initiation (*n*)	416
Median × cells/μl [*n* (IQR)]	11 (3–60)
Count < 50 cells/μl [*n* (%)]	297 (71.4)
Treatment	
Initial antiretroviral treatment	
d4T/3TC/EFV [*n* (%)]	337 (81.0)
d4T/3TC/NVP [*n* (%)]	64 (15.4)
ZDV/3TC/NVP [*n* (%)]	4 (0.9)
ZDV/3TC/EFV [*n* (%)]	6 (1.4)
ZDV/3TC/LPV [*n* (%)]	3 (0.7)
d4T/3TC/LPV [*n* (%)]	2 (0.5)
Follow-up time on HAART (months)	
Median for all patients (IQR)	23.8 (22.8–24.0)
Outcomes	
Deaths [*n* (%)]	53 (12.7)
Lost to follow-up [*n* (%)]	7 (1.7)
Transfers [*n* (%)]	6 (1.4)
Still on HAART [*n* (%)]	350 (84.1)

ART, Antiretroviral therapy; d4T, stavudine; EFV, efavirenz; IQR, interquartile range; LPV, lopinavir; NVP, nevirapine; 3TC, lamivudine; WHO, World Health Organization; ZDV, zidovudine.

Clinico-immunological outcomes and patient survival

The median time of follow-up was 23.8 months (IQR 22.8–24.0). Among the 416 patients included, 53 patients (12.7%) had died, seven (1.7%) were lost to follow-up, six (1.4%) were transferred and 350 (84.1%) still remained on HAART.

Estimates of survival given by the Kaplan–Meier method were 0.87 at 12 months [95% confidence interval (CI) 0.83–0.90] and 0.85 at 24 months (95% CI 0.81–0.88) when death events were taken into account exclusively (Fig. 2). Two-thirds of the deaths (35/53, 66.0%) occurred within the first 6 months after HAART initiation (median time to death 3.6 months, IQR 1.7–9.8).

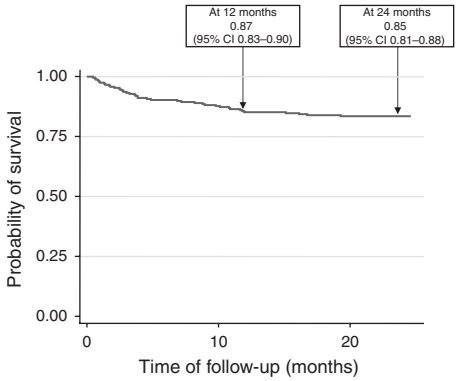

Fig. 2. Kaplan–Meier curve showing the probability of survival of the patients from the M24 cohort. CI, Confidence interval.

Among patients with available CD4 cell counts at baseline ($n = 416$), the median CD4 cell gain was found to be +101 cells/μl (IQR 62–137) at 6 months ($n = 344$), +154 cells/μl (IQR 95–217) at 12 months ($n = 337$) and +233 cells/μl (IQR 156–332) at 24 months ($n = 346$; Table 2). Alhough they presented with a very low baseline CD4 cell count, 259 out of 346 patients (74.9%) had a CD4 cell count greater than 200 cells/μl at 24 months.

Cross-sectional virological survey of patients continuing HAART

Among the 350 patients still on treatment after 24 ± 2 months at the date of analysis, four could not be included (three refusals, one out of time) and 346 were assessed virologicaly (Fig. 1). Among them, 86 (24.8%) were on stavudine–lamivudine–efavirenz, 74 (21.4%) were on zidovudine–lamivudine–efavirenz, 115 (33.2%) were on stavudine–lamivudine–nevirapine, 50 (14.4%) were on zidovudine–lamivudine–nevirapine and 10 (2.9%) were on other alternative first-line regimen. Only 11 patients (3.2%) were on a protease inhibitor-based regimen (lopinavir/ritonavir).

Viral loads were below 40 copies/ml for 276 out of 346 patients (79.8%), were below 400 copies/ml for 306 (88.4%) and below 1000 copies/ml for 315 (91.0%). Among the 40 patients with a viral load above 400 copies/ml (11.6%), only 15 (4.3%) were above 30 000 copies/ml, corresponding to major virological failure (Table 3).

Table 3. Viral load measures of patients from the M24 cohort included in the cross-sectional virological survey ($n = 346$).

Viral load (copies/ml)	*N*	%	95% CI	Cumulative %
< 40	276	79.8	75.1–83.9	79.8
40–400	30	8.7	6.0–12.3	88.4
400–1000	9	2.6	1.3–5.1	91.0
1000–5000	7	2.0	0.9–4.1	93.0
5000–30 000	9	2.6	1.3–5.1	95.7
≥ 30 000	15	4.3	2.5–7.2	100.0
Total	346	100		

CI, Confidence interval.

Overall, in intention-to-treat analysis of the whole M24 cohort ($n = 416$) taking into account both deaths, loss to follow-up and missing data (Fig. 1) as treatment failures, 66.3% (95% CI 61.5–70.8) and 73.6% (95% CI 69.0–77.7) of treatment successes were observed when considering viral loads as failures when above 40 copies/ml or 400 copies/ml, respectively.

HIV reverse transcriptase genotyping was performed for the 40 patients with viral loads above 400 copies/ml. Among interpretable sequences ($n = 39$), 10 (25.6%) did not show any drug-resistance-associated mutations, 23 (59.0%) presented with nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations, and 28 (71.8%) presented with non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations. The most frequent NRTI mutation was the lamivudine-induced M184V mutation (23/39), the stavudine-induced T215Y/F mutation (9/39), the T69D/N (7/39) and the D67N (7/39) mutations. Among NNRTI mutations, K103N (15/39), Y181C/I (8/39) and G190A/S (8/39) were most frequently observed (Fig. 3). According to ANRS algorithms [20], 12 patients with a viral load greater than 400 copies/ml were resistant to both zidovudine, lamivudine, stavudine and nevirapine/efavirenz.

Antiretroviral concentrations

The median concentration of efavirenz for 169 patients was 2946 ng/ml (range < 50–31 559 ng/ml), with seven

Table 2. Immunological restoration of patients from the M24 cohort.

	Baseline $n = 416$	6 months $n = 344$	12 months $n = 337$	18 months $n = 312$	24 months $n = 346$
CD4 cell count (median cells/μl)	11	134	193	240	274
(IQR)	(3–60)	(90–198)	(139–263)	(160–324)	(199–371)
% With CD4 cell count					
< 50 cells/μl	71.4	6.7	0.9	1.3	0.9
< 200 cells/μl	98.8	75.6	51.9	35.6	25.1
CD4 cell count gain (median cells/μl)	–	+101	+154	+194	+233
(IQR)	–	(62–137)	(95–217)	(131–278)	(156–332)

IQR, Interquartile range.

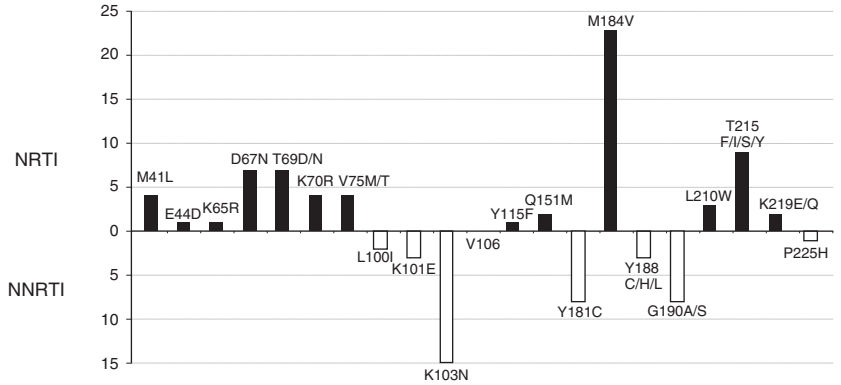

Fig. 3. Most frequently found mutations. NNRTI, Non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

patients (4.1%) having efavirenz levels either undetectable ($n=4$) or less than 1000 ng/ml ($n=3$) and 47 (27.8%) having levels greater than 4000 ng/ml. Median trough nevirapine concentrations in 165 patients were 5643 ng/ml (range <25–16 971 ng/ml), with two patients undetectable (1.2%), six patients (3.6%) with nevirapine levels less than 3000 ng/ml and 21 (12.7%) with levels greater than 8000 ng/ml. For the 11 patients on lopinavir/ritonavir at the time of the study, the median trough plasma concentration of lopinavir was 4962 ng/ml (range 2215–8797 ng/ml) with only one patient less than 3000 ng/ml.

Among the 15 patients with low NNRTI trough or 12-h concentrations at month 24, seven had a viral load greater than 1000 copies/ml (47%). Among them, three displayed a wild-type HIV strain with neither NRTI nor NNRTI-induced RT mutations and had a viral load greater than 30 000 copies/ml. On the other hand, when considering patients with high trough plasma antiretroviral concentrations, none among the 21 patients who had high nevirapine concentrations and three out of 47 patients (6.4%) with high efavirenz levels were found to have a virological failure.

Side effects of patients from the M24 cohort

As a result of drug intolerance, nine patients from the M24 cohort (2.2%) stopped zidovudine (eight for anaemia, and one for anaemia and neutropenia), 131 (31.5%) stopped stavudine (50 for neuropathy, 71 for lipodystrophy, nine for both neuropathy and lipodystrophy, and one for mitochondrial toxicity), and four stopped HAART because of mitochondrial toxicity. Considering NNRTI, three patients (0.7%) stopped efavirenz (one for neuropsychiatric disorders and two for gynaecomasty), and 13 (3.1%) stopped nevirapine (one for hepatitis, eight for hypersensibility and three for severe cutaneous rash). The median duration before stopping the drug was 93 days (IQR 63–140) for zidovudine, 13.9 months (IQR 11.8–16.7) for stavudine, 22 days (IQR 12–28) for nevirapine and between 10 and 674 days for efavirenz ($n=4$).

Factors associated with a viral load greater than 1000 copies/ml at 24 months

In order to identify patients at risk of having a detectable viral load, we analysed the association of distinct factors present either at baseline, at 12 or at 24 months with a viral load greater than 1000 copies/ml at 24 months. The aim here was more to identify the group of patients potentially needing a decision to switch their treatment than describing factors associated with virological failures. For this reason, and because of the well-known existence of virological blips, we chose 1000 copies/ml as a reasonable threshold. Higher viral load thresholds could not be tested because of too small sample sizes.

Using univariate logistic regression analysis, the following factors were found to be associated with viral loads greater than 1000 copies/ml at 24 months: age greater than 35 years ($P=0.008$), previous antiretroviral exposure ($P=0.004$), CD4 cell count less than 200 cells/μl ($P=0.001$) or between 200 and 350 cells/μl ($P=0.041$), an opportunistic infection between months 9 and 24 ($P=0.041$), low plasma antiretroviral concentrations at month 24 ($P<0.001$) and an insufficient percentage CD4 cell gain at month 24 ($P<0.001$). On the other hand, hospitalization between months 9 and 24, antiretroviral drug stops, weight loss between months 12 and 24, BMI at month 24 or being on HAART second line at month 24 were not statistically associated with a viral load greater than 1000 copies/ml (Table 4). Other factors such as sex ($P=0.7$) or being a smoker ($P=0.57$) were also not associated. Finally, no criteria present at month 12 among the following were found to be predictive of a viral load greater than 1000 copies/ml at month 24: CD4 cell count ($P=0.68$), CD4 cell gain ($P=0.7$), percentage CD4 cell gain ($P=0.14$), weight gain between months 0 and 12 ($P=0.16$) or between months 6 and 12 ($P=0.1$).

Table 4. Logistic regression analysis showing odds ratios for factors associated with virological failure (> 1000 copies/ml) of patients from the M24 cohort, Phnom Penh, Cambodia ($n = 346$).

	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)
Sex		
Male	1	
Female	1.2 (0.5–2.4)	
Age (years)		
< 35	1	
≥ 35	2.9 (1.3–6.5)	2.5 (0.9–6.6)
Previous ART exposure		
No	1	
Yes	5.3 (1.7–16.5)	12.6 (2.6–62.8)
Body mass index[a] (kg/m^2)		
≥ 18.5	1	
< 18.5	0.8 (0.3–2.3)	
CD4 cell count[a] (cells/μl)		
≥ 350	1	
200–350	4.8 (1.1–21.9)	
< 200	11.7 (2.6–52.5)	
M12–M24% CD4 cell gain (%)		
> +23	1	
≤ +23	5.4 (2.3–12.6)	
CD4 cell count at M24 & M12–M24% CD4 cell gain		
CD4 > 350	1	
CD4 < 350 & % CD4 cell gain > +23	2.6 (0.5–12.7)	2.2 (0.4–12.0)
CD4 < 350 & % CD4 cell gain ≤ +23	13.1 (2.9–58.3)	10.6 (2.2–51.7)
Plasma ART concentrations		
Within the therapeutic window	1	
Low	11.3 (3.7–33.8)	11.1 (2.6–48.4)
On HAART second line[a]		
No	1	
Yes	1.5 (0.2–12.3)	
No. of drug stops[b]		
0	1	
1	1.1 (0.5–2.3)	
≥ 2	0.7 (0.1–5.4)	
Hospitalization[b]		
No	1	
Yes	1.9 (0.7–4.8)	
Opportunistic infection[b]		
No	1	
Yes	3.4 (1.1–11.3)	3.6 (0.7–17.5)
M12–M24% weight loss (%)		
< 5	1	
≥ 5	1.0 (0.4–2.6)	
Smoker		
No	1	
Yes	0.8 (0.35–1.8)	

ART, Antiretroviral therapy; CI, confidence interval; OR, odds ratio.
[a]At 24 months.
[b]Between 9 and 24 months.

In the multivariate logistic regression model, evident interactions between CD4 cell level at month 24 and percentage CD4 cell gain between months 12 and 24 (M12–M24%CD4 gain) were taken into account and a new variable combining both CD4 cell count and percentage CD4 cell gain was introduced in the model (Table 4). Finally, three independent factors at month 24 remain positively associated with a viral load greater than 1000 copies/ml (Table 4): previous antiretroviral exposure [odds ratio (OR) 12.6, 95% CI 2.6–62.8, $P = 0.002$], low plasma antiretroviral concentrations at month 24 (OR 11.1, 95% CI 2.6–48.4, $P = 0.001$), and having both a CD4 cell count less than 350 cells/μl and a M12–M24%CD4 gain of +23% or less (OR 10.6, 95% CI 2.2–51.7, $P = 0.003$).

ROC curve analysis was used to define this cut-off of M12–M24% CD4 gain giving the highest sensitivity and specificity for the detection of viral loads greater than 1000 copies/ml. The percentage CD4 cell gain cut-off at 23% gave optimal sensitivity (66.7%, 95% CI 46.0–83.5) and specificity (73.4%, 95% CI 68.0–78.3).

When combining such criteria to improve the detection of patients with viral loads greater than 1000 copies/ml at 24 months, we found that the following association was

optimal: previous exposure to ART, or CD4 cell count at month 24 less than 350 cells/μl and a M12–M24%CD4 gain of +23% or less. Using such combined criteria greatly improved the sensitivity (74%, 95% CI 53.7–88.9) with a similar specificity (75%, 95% CI 69.3–79.4). The positive and negative predictive values were 20.6% (95% CI 13.1–30.0) and 97% (95% CI 93.9–98.8), respectively.

Discussion

The present analysis of 416 patients performed 24 months after HAART initiation in Cambodia found 75% of treatment successes (patients still followed with a viral load < 400 copies/ml) in an intention-to-treat analysis and a 0.85 probability of survival at 24 months. Such favourable outcomes are similar or even better than those reported so far in western cohorts [24,25], and are remarkable given the extreme severity of the disease at HAART initiation (see Table 1) in such a resource-limited setting.

Intensive therapeutic education, counselling and psychosocial support might explain the very low rate of lost-to-follow-up patients observed. Such a result was obtained given an important investment in the recruitment and training of counsellors. Human resources remain a key factor in resource-limited settings. As already reported by other studies in similar settings [6,26–31], most of the deaths occurred during the first 6 months after HAART initiation. Advanced clinical stage and low CD4 cell counts might explain such an increased early mortality. Difficulties in managing opportunistic infections as a result of late presentation or limited diagnostic and therapeutic tools as well as malnutrition or immune reconstitution syndrome might also have played a role in worsening the prognosis of such patients [31–34]. Noteworthy is the fact that 20.4% of the patients analysed had either nevirapine or efavirenz concentrations above the therapeutic range without apparent impairment of their tolerance to the antiretroviral regimen. The reasons for such high concentrations are still unclear and do not seem to be related to the low weight of Asian patients compared with western patients [35,36].

Progressive immune restoration was observed over time (Table 2) similar to that reported in similar settings [6,26–28,37] or even in high-income countries for similar patients [26,38]. It is, however, noteworthy that after 2 years a quarter of the patients remained below 200 CD4 cells/μl probably partly because of late HAART initiation with very low baseline CD4 cell counts. It has been reported that patients with poor immune reconstitution at 6 months (CD4 cell count < 100 cells/μl) have a high risk of an AIDS-defining event or progression to death at 5 years [39]. As in our case, the long-term outcomes of patients still below 200 CD4 cells/μl at 24 months remain largely unknown. Further studies are clearly warranted in resource-limited settings in order to determine the long-term outcomes of these patients and understand the factors governing such poor immune restoration.

The virological results of the M24 cohort revealed that among 346 patients, 40 (11.6%) presented with a detectable viral load (> 400 copies/ml), and only 15 (4.3%) were above 30 000 copies/ml. Overall, among patients with detectable viral loads, 25.6% did not have any ART-induced RT HIV mutations and three had low NNRTI trough plasma concentrations corresponding to non-adherent patients. The mutations observed for the other patients were those expected under the first-line regimen used, and the great majority of patients were already resistant to nevirapine/efavirenz (93%) or lamivudine/emtricitabine (79%) (Fig. 3). Overall, these observations are similar to other first-line cohort studies in resource-limited settings describing HIV-1 RT mutation patterns [7,27,40]. This stresses the importance of keeping available several distinct second-line molecules in such settings, and calls upon the international community to make them really and rapidly affordable. In this study, genotyping analysis allowed the detection of non-adherent patients and the selection of appropriate second-line regimens when necessary. Empiric second-line regimens given according to the type of first-line treatments will have to be evaluated through HIV genotyping pilot studies.

In any case, the decision of when to switch remains critical in order to avoid the accumulation of HIV mutations and preserve the efficacy of the limited number of second-line molecules available. In resource-poor settings where access to viral load is largely limited, better identifying groups of patients at risk of treatment failure might help design accurate viral load monitoring strategies. For operational reasons and because of the existence of potential virological blips, we have chosen 1000 copies/ml as a reasonable threshold in order to identify the group of patients potentially needing to switch to a second-line regimen. Logistic regression analysis revealed that previous ART exposure or CD4 cell counts still less than 350 cells/μl with a CD4% gain of less than +23% between months 12 and 24 were two factors strongly associated with viral loads greater than 1000 copies/ml at 24 months. It is noteworthy that no other characteristics of the patients either at baseline, and specifically the CD4 cell count, or at 12 months of follow-up were found to be associated with viral loads greater than 1000 copies/ml at 24 months. A test using both criteria to identify patients with viral loads greater than 1000 copies/ml gave a 74% sensitivity, a 74% specificity and a 21% positive-predictive value. Using more stringent criteria closer to those recommended by WHO (a decrease in CD4 cell gain > 30% between months 12 and 24) gave a lower sensitivity (26%, 95% CI 11–46) with a higher specificity (98%, 95% CI 96–99)

and only a 54% (95% CI 25–81) positive-predictive value. Therefore, if a decision to switch was made only according to such stringent criteria, half of the patients would have been switched inappropriately, and most patients with viral loads greater than 1000 copies/ml would have been missed. These observations clearly emphasize that deciding to switch solely on the basis of immunological criteria is not an acceptable option and might even be costly to the programme. Using less stringent criteria allows the identification of a larger group of patients including most patients with a viral load greater than 1000 copies/ml and for whom viral load tests could be proposed in order to help appropriate decision making. Such a strategy clearly strengthens the need to improve access to affordable viral load testing in resource-limited countries [41].

Data from all patients on HAART for more than 24 months ($N = 1036$) in this MSF/MoH HIV programme indicate that almost 25.9% ($n = 218$) would have fulfilled the criteria defined above and would have been proposed a viral load. Among them, approximately 50 viral loads greater than 1000 copies/ml would have been detected, whereas other patients would have had undetectable viral loads. In settings where viral load access is limited, such a strategy identifying patients at risk of failure could be useful to restrict the number of viral loads proposed. Our analysis is still preliminary, however, and needs to be strengthened by further studies on larger numbers of patients. In addition, it appears that the criteria for suspecting failure might vary greatly according to the duration of follow-up. Studies analysing success/failure criteria at distinct timepoints after HAART initiation are on the way in other MSF programmes.

A model to monitor the virological efficacy of HAART in resource-limited settings has recently been proposed by Colebunders *et al*. [42]. This interesting proposal, mainly based on patients' clinical history, remains to be validated and will have to take into account that a large number of patients still have CD4 cell count less than 200 cells/μl with WHO stage III or IV manifestations even after 2 years of follow-up. Cost-effectiveness analyses would also be important to compare the 'selective viral load' approach with a 'systematic viral load' approach (proposing a viral load once a year for all patients, for example) in settings where viral load access and second-line regimen availability are limited. As a result of the still high cost of second-line regimens and the complete lack of a third line in resource-poor settings, further studies are clearly warranted to determine the appropriate timepoint for switching to second line, not too early (being aware of poor adherence) but not too late (precluding the accumulation of HIV mutations, which impairs the choice among the limited number of second-line molecules). The efficacy of second-line regimens chosen without genotyping information is currently being assessed in Cambodia.

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