J Antimicrob Chemother 2015; **70**: 2639–2647
doi:10.1093/jac/dkv143 Advance Access publication 16 June 2015

Journal of Antimicrobial Chemotherapy

Determinants of virological failure and antiretroviral drug resistance in Mozambique

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Received 2 February 2015; returned 16 April 2015; revised 29 April 2015; accepted 1 May 2015

Objectives: The objective of this study was to inform public health actions to limit first-line ART failure and HIV drug resistance in Mozambique.

Methods: This was a cross-sectional study. HIV-1-infected adults on first-line ART for at least 1 year attending routine visits in the Manhiça District Hospital, in a semi-rural area in southern Mozambique with no HIV-1 RNA monitoring available, were evaluated for clinical, socio-demographic, therapeutic, immunological and virological characteristics. Factors associated with HIV-1 RNA ≥1000 copies/mL and HIV drug resistance were determined using multivariate logistic regression.

Results: The study included 334 adults on first-line ART for a median of 3 years, of which 65% (214/332) had suppressed viraemia, 11% (37/332) had low-level viraemia (HIV-1 RNA 150–999 copies/mL) and 24% (81/332) had overt virological failure (HIV-1 RNA ≥1000 copies/mL). HIV drug resistance was detected in 89% of subjects with virological failure, but in none with low-level viraemia. Younger age [OR=0.97 per additional year (95% CI=0.94–1.00), *P*=0.039], ART initiation at WHO stage III/IV [OR=2.10 (95% CI=1.23–3.57), *P*=0.003] and low ART adherence [OR=2.69 (95% CI=1.39–5.19), *P*=0.003] were associated with virological failure. Longer time on ART [OR=1.55 per additional year (95% CI=1.00–2.43), *P*=0.052] and illiteracy [OR=0.24 (95% CI=0.07–0.89), *P*=0.033] were associated with HIV drug resistance. Compared with HIV-1 RNA, clinician's judgement of ART failure, based on clinical and immunological outcomes, only achieved 29% sensitivity and misdiagnosed 1 out of every 4.5 subjects.

Conclusions: Public health programmes in Mozambique should focus on early HIV diagnosis, early ART initiation and adherence support. Virological monitoring drastically improves the diagnosis of ART failure, enabling a better use of resources.

Keywords: HIV-1, antiretroviral treatment, public health, low-income countries

Introduction

Since 2001 the WHO has promoted the provision of ART following a public health approach to enable scale-up of access in low-income countries.[1] The use of standardized, affordable and simplified treatment protocols characterized by a limited number of regimens and decentralized service delivery allowed >9 million people in low- and middle-income countries to receive ART in 2012, which represents a 30-fold increase since 2003.[2]

This increase in access to ART for the most vulnerable populations is accompanied by major challenges. One of the main difficulties has been in the capacity to follow the WHO recommendations of viral load monitoring for guiding treatment changes. Indeed, in settings where advanced laboratory infrastructure is not available, clinical and immunological assessment guides decisions on when to start, stop, substitute and switch ART.[3] Although such an approach has been associated with overall good long-term clinical outcomes, it may delay the diagnosis of true

virological failure (VF), preventing the need for treatment switches to avoid the accumulation of HIV drug resistance mutations (DRMs) and disease progression.[4–7] Continuation on a failing regimen may be associated with more complex mutation patterns, development of cross-resistance to NRTIs and NNRTIs and onward transmission of drug-resistant HIV to ART-naive patients. The prevalence of transmitted HIV resistance has recently increased to ~5% in some areas of South Africa, Kenya and Zambia and has reached >15% in Uganda, representing a major public health challenge in the control of the HIV epidemic in sub-Saharan Africa.[8–10] It is thus critical to develop informed strategies to prevent the development of VF to first-line ART and the emergence of HIV resistance.

Although surveys have been conducted in other countries in the region, data on ART effectiveness and HIV drug resistance in adults in Mozambique, a country with one of the highest HIV prevalences in the world, are still very limited.[11–16] Mozambique has a long-standing tradition of labour migrants to neighbouring countries, especially from the southern part of the country to South Africa. Major transport corridors link inner countries such as Zimbabwe, Zambia and Malawi to important seaports and the regional hub of South Africa. As ART coverage continues to grow, achieving a detailed knowledge of the effectiveness of first-line ART in Mozambique and its determinants is key to optimizing programme planning and management. Ultimately, this would make it possible to protect and extend the benefits of the public health approach to reducing the HIV epidemic in Mozambique and other Southern African countries.

Methods

Study area and population

The study was carried out at the outpatient clinic of Manhiça District Hospital. Manhiça is a semi-rural area of Maputo Province, southern Mozambique. The Centro de Investigação em Saúde de Manhiça (CISM) has been conducting, since 1996, continuous demographic surveillance in the Manhiça District, which covers a population of ~95 000 inhabitants in an area of 500 km^2. A recent community-based study in adults in the Manhiça area reported nearly 40% HIV prevalence, with 30% HIV prevalence among pregnant women attending antenatal clinics.[17,18] At the time of the study, national guidelines recommended zidovudine+lamivudine+nevirapine as the first ART option. Stavudine was administered instead of zidovudine in case of anaemia (haemoglobin <8 g/dL) and efavirenz instead of nevirapine if concomitant TB treatment was needed. Available second-line regimens at the time of the study are listed in Table S1 (available as Supplementary data at *JAC* Online). Although roll out of ART started in Mozambique in 2004, ART coverage among eligible adults is estimated to be <50%.[19] First- and second-line ART is freely provided, following a population-based model of care. Treatment switches are guided by clinical and immunological criteria in the absence of virological monitoring. Standard follow-up of HIV-infected patients on ART consists of outpatient visits every 6 months, which include physical examination and CD4+ T cell count, haematology and biochemistry.[20]

Study design

This was a cross-sectional study conducted among patients attending the outpatient clinic of the Manhiça District Hospital. The study protocol was approved by the institutional review boards and ethics committees of the Hospital Clínic in Barcelona, the Hospital Germans Trias i Pujol in Badalona, Spain, and the National Committee on Health Bioethics, Mozambique. All study participants provided signed informed consent.

Study procedures

Adults ≥18 years of age attending routine scheduled outpatient visits for clinical management of HIV/AIDS were consecutively recruited for the study if they had documented HIV infection, documented ART initiation ≥12 months earlier and provided a signed informed consent. Socio-demographic and clinical data were registered using standardized questionnaires. Information on HIV diagnosis, ART initiation, line regimen modifications, treatment withdrawals, TB treatment, suspected treatment failure and clinician-reported low treatment adherence were obtained from the patient's medical chart.

A 10 mL blood sample was drawn into EDTA tubes for CD4+ T cell count, HIV-1 RNA viral load, HIV DRM and nevirapine plasma level determinations. A urine pregnancy test was performed in women of childbearing age.

Laboratory methods

HIV-1 RNA levels were determined from cryopreserved plasma samples with the Abbott m2000 RealTime System (detection limit 150 copies/mL for 0.2 mL sample volume). CD4+ T cell counts were determined by flow cytometry after staining of whole blood for CD3, CD8, CD4 and CD45 using fluorochrome-labelled antibodies and acquisition using FACSCalibur (BD Biosciences) and Trucount tubes (Becton Dickinson, San Jose, CA, USA). Analysis of genotypic drug resistance of plasma HIV was attempted in all subjects with HIV-1 RNA ≥150 copies/mL. HIV-1 RNA was extracted from 140 μL of plasma samples (QIAamp Viral RNA Mini Kit, Qiagen). The *pol* region was reverse transcribed and amplified in a one-step reaction followed by nested PCR. Nested PCR products were inspected by electrophoresis and positive reactions were column-purified (QIAquick PCR Purification Kit, Qiagen) for in-house bulk sequencing. Cycling conditions and primers used for one-step RT–PCR, nested PCR and in-house bulk sequencing are detailed in Table S2. The fully automated sequence analysis pipeline RECall was used to analyse sequence chromatograms.[21,22] Drug resistance-associated mutations in protease and reverse transcriptase gene regions from all quality-assured sequences were interpreted with the HIV database (HIVdb) program implemented in the Stanford HIV drug resistance database web site. To predict susceptibility to NRTIs, NNRTIs and PIs, the Stanford HIVdb scoring system was applied and a resistance score was calculated as susceptible, potentially low level, low level, intermediate level and high level (http://hivdb.stanford.edu). Nevirapine concentrations in plasma were determined by HPLC.

Statistical methods and definitions

Malnutrition was defined as a BMI <18.5 kg/m^2. Suspected treatment failure was defined as reported clinician's judgement of treatment failure based on clinical and immunological failure specified in Mozambican guidelines. Immunological failure was defined as CD4+ T cell counts lower than the pre-ART count, or <50% of the highest value achieved after initiating ART, or <100 cells/mm^3 after being on ART for at least 1 year. Clinical failure was defined as a new or recurrent clinical event indicating WHO clinical stage IV after 6 months of ART.[20] Low ART adherence was defined as the presence of at least one of the following: self-reported low adherence, clinician-reported low adherence or previous voluntary treatment withdrawal. Self-reported low adherence was defined as the patient's confirmation of at least one missed dose of ART in the 7 days before the study survey. Clinician-reported low adherence and treatment withdrawals were defined according to missing on-time ART drug pickups on pharmacy records. ART modification refers to changes in one drug in the regimen within the same first-line ART approach. Treatment

withdrawal was defined as the interruption of ART drug pickups for at least 2 months.[20] Detectable viral load was defined as HIV-1 RNA levels ≥150 copies/mL and VF as HIV-1 RNA ≥1000 copies/mL. Viral load between 150 and 999 copies/mL was defined as low-level viraemia (LLV). Severe immune depression was defined as a CD4+ T cell count ≤200 cells/mm^3. HIV drug resistance was considered as the presence of one or more major resistance mutations as defined by the Stanford HIV DRM database (http://hivdb.stanford.edu).

Baseline characteristics of study patients were described using standard statistics. Associations between socio-demographic and HIV-related variables and each of the primary outcomes (VF and presence of HIV DRMs) were assessed using the χ^2 test for categorical variables and Student's *t*-test or the Mann–Whitney test for continuous variables, as required. To measure the association of variables with primary outcomes, all variables with evidence of association in the univariate analysis (different OR for different categories and a *P* value <0.15) were included in a multiple logistic regression analysis. Pooled *P* values were obtained for categorical variables through Wald tests or likelihood ratio tests depending on the need for adjustment of correlated data. Both multivariate analyses for VF and HIV DRMs were adjusted by CD4+ T cell counts and suspected treatment failure. The HIV resistance model was also adjusted for VF. Statistical analyses were performed using Stata 12™ software (Stata Corp., Houston, TX, USA).

Results

Characteristics of the study patients

There were 334 patients enrolled between February and March 2013. Most of them were women (70%), median age was 39 years (IQR 33–48), median time on ART was 3.2 years (IQR 2.0–5.2) and 90% were receiving zidovudine/lamivudine/nevirapine at the time of the survey. Overall only 37% (125/334) had a primary education level, 32% (106/334) had no schooling and 34% (115/334) were illiterate. Forty-three per cent of subjects (144/334) initiated ART in WHO stage III/IV. Malnutrition was observed in 8% (26/334) of the subjects. Eleven per cent (38/334) had had concomitant TB treatment while receiving ART, whereas 23% (53/232) of women became pregnant during ART. Sixteen per cent (52/334) had estimated low adherence at the time of the survey and 13% (44/334) reported previous ART withdrawals. According to the clinician's judgement, 12% (39/334) of subjects had suspected ART failure (Table 1).

Immunological status

Two thirds of subjects (220/332) had CD4+ counts >350 cells/mm^3 and 40% (134/332) had >500 cells/mm^3 at the time of the study (Table 1). Severe immune suppression was observed in 16% (52/332) of the subjects and it was more frequent among male subjects in the univariate analysis [OR=1.83 (95% CI=1.00–3.37), *P*=0.05].

VF

Of the 332 subjects assessed for HIV-1 RNA levels, 118 (36%) had detectable viraemia, 37 (11%) had LLV and 81 (24%) had VF (Table 1). HIV-1 RNA levels were missing for two patients. Undetectable nevirapine concentrations were found in 9/103 (9%) of the patients in whom nevirapine plasma levels were measured, 8 of whom had HIV-1 RNA ≥1000 copies/mL. Severe immune suppression [OR=22.4 (95% CI=10.6–47.1), *P*<0.001] and suspected treatment failure [4.2 (95% CI=2.5–8.7), *P*<0.001] were significantly associated with VF, but, due to co-linearity with the primary endpoint, they were not included in the multivariate model. Factors associated with VF included initiation of ART at WHO stage III/IV [OR 2.10 (95% CI=1.23–3.57), *P*=0.003], younger age [OR 0.97 per additional year (95% CI=0.94–1.00), *P*=0.039] and estimated low adherence [OR 2.69 (95% CI=1.39–5.19), *P*=0.003] (Table 2).

HIV drug resistance

Genotyping resistance data were obtained from 61/81 (75%) and 11/37 (30%) of subjects with VF and LLV, respectively (Table 3). HIV DRMs were only detected in subjects with HIV-1 RNA ≥1000 copies/mL. Of these, 89% had at least one HIV DRM. Out of 61 subjects, 54 (89%) presented mutations conferring resistance to NNRTIs and 49 (80%) presented mutations conferring resistance to NRTIs, always alongside NNRTI resistance mutations. As expected, no PI resistance was detected. The most common mutations were 184V, which was present in 45/72 individuals (63%), followed by 103N, 190A and 181C, found in 20/72 (28%), 19/72 (26%) and 16/72 (22%) subjects, respectively (Table S3). The phenotypic impact of the available genotypes is shown in Figure 1. Of note, no HIV DRMs were found in the 11 subjects with LLV and available genotypic data. Factors associated with HIV DRMs were longer time on ART [OR=1.55 per additional year (95% CI=1.00–2.43), *P*=0.052] and illiteracy [OR=0.24 (95% CI=0.07–0.89), *P*=0.033] (Table 2).

Performance of clinician's judgement for predicting VF

The clinician's judgement of treatment failure, based on clinical and immunological criteria, had a sensitivity of 29% for predicting treatment failure, detecting only 22 out of the 76 cases with confirmed VF (HIV-1 RNA ≥1000 copies/mL), and thus leaving >70% of the patients in need without a required ART change (Table S4). Specificity was 94% (243/260). Positive predictive value was 56% (22/39), and thus 44% of the patients who were classified by the clinician as having treatment failure had HIV-1 RNA <1000 copies/mL and the premature change of ART in them could have been avoided. The number of patients needed to misdiagnose was 4.5, meaning that 1 out of every 4.5 subjects evaluated for the presence of ART failure was misdiagnosed in one direction or another (Table S4).

Discussion

This cross-sectional study among HIV-infected adults attending the outpatient clinic of a district hospital in southern Mozambique showed that two-thirds of the subjects retained undetectable HIV-1 RNA levels after 3 years of first-line ART. Overt VF was observed in a quarter of subjects, whereas LLV was found in 11%. The main determinants of VF were younger age, having a WHO III/IV stage at ART initiation and the presence of estimated low adherence to ART. VF had serious consequences, as almost 90% of subjects with HIV-1 RNA ≥1000 copies/mL had developed resistance to first-line treatment options. Literacy and years on ART were associated with the presence of HIV DRMs. However, in this study HIV resistance was only observed in subjects with HIV-1 RNA ≥1000 copies/mL. This suggests that

Table 1. Study population characteristics

Socio-demographic characteristics		
Age (years), median (IQR)		39 (33–48)
Women, *n* (%)		232 (69.5)
Civil status	single, *n* (%)	38 (11.6)
	widow, *n* (%)	63 (19.2)
	divorced, *n* (%)	25 (7.6)
	married/union, *n* (%)	202 (60.5)
Education[a]	no schooling, *n* (%)	106 (31.8)
	primary education, *n* (%)	125 (37.5)
	secondary education, *n* (%)	99 (29.7)
	higher education, *n* (%)	3 (0.9)
Literacy (able to read and write)	yes, *n* (%)	219 (65.6)
	no, *n* (%)	115 (34.4)
HIV and ART-related characteristics		
At the time of first-line ART initiation	age (years), median (IQR)	36.2 (29.7–44.6)
	time since HIV diagnosis (months), median (IQR)	4.8 (1.9–16.6)
	WHO clinical stage of HIV/AIDS, *n* (%)	
	I/II	188 (56.6)
	III/IV	144 (43.4)
	starting ART, *n* (%)	
	d4T+3TC+NVP	157 (47.0)
	ZDV+3TC+NVP	153 (45.8)
	d4T+3TC+EFV	14 (4.2)
	ZDV+3TC+EFV	10 (3.0)
At the time of the survey	malnutrition (BMI[b] <18.5 kg/m^2), *n* (%)	26 (7.8)
	any type of symptoms, *n* (%)	125 (37.4)
	time on ART (years), median (IQR)	3.2 (2.0–5.2)
	time since HIV diagnosis (years), median (IQR)	4.8 (2.9–6.4)
	at least one previous ART modification[c], *n* (%)	184 (55.1)
	current ART, *n* (%)	
	ZDV+3TC+NVP	299 (89.5)
	d4T+3TC+NVP	28 (8.4)
	ZDV+3TC+EFV	6 (1.8)
	d4T+3TC+EFV	1 (0.3)
	women pregnant while on ART, *n* (%)	53 (22.8)
	TB treatment while on ART, *n* (%)	38 (11.4)
	suspected ART failure, *n* (%)	39 (11.7)
	previous ART withdrawals, *n* (%)	44 (13.2)
	self-reported low adherence, *n* (%)	11 (3.3)
	recorded low adherence, *n* (%)	11 (3.3)
	estimated low adherence[d], *n* (%)	52 (15.6)
	therapeutic NVP plasma levels[e], *n* (%)	94 (91.3)
	HIV-1 RNA (copies/mL), *n* (%)	
	$\leq$150	214 (64.5)
	150–999	37 (11.1)
	$\geq$1000	81 (24.4)
	CD4+T cell count (cells/mm^3), *n* (%)	
	$\leq$50	12 (3.6)
	51–200	40 (12.0)
	201–350	60 (18.1)
	351–500	86 (25.9)
	$>$500	134 (40.4)

ZDV, zidovudine; 3TC, lamivudine; NVP, nevirapine; d4T, stavudine; EFV, efavirenz.
[a]Primary education comprises seven grades of elementary education at primary school. Secondary education comprises four grades of pre-university education. Higher education refers to acquisition of a university degree.
[b]Weight (kg)/height (m)^2.
[c]Refers to changes in one drug in the regimen within the same first-line ART approach.
[d]Estimated low adherence if positive answer in at least one of the three preceding fields on adherence (ART withdrawals, self-reported low adherence and recorded low adherence).
[e]Sub-sample of 103 subjects.

Table 2. Factors associated with VF and with the presence of at least one DRM at VF

	VF						HIV drug resistance					
	univariate analysis			multivariate analysis[a]			univariate analysis			multivariate analysis[a]		
Variable	OR	95% CI	*P*	AOR	95% CI	*P*	OR	95% CI	*P*	AOR	95% CI	*P*
Age at time of study evaluation, per each additional year	0.98	0.95–1.00	0.063	0.97	0.94–1.00	0.039	0.97	0.92–1.02	0.251	—	—	—
Male	1.09	0.64–1.87	0.758	—	—	—	2.23	0.56–8.77	0.253	—	—	—
No schooling	0.95	0.55–1.63	0.849	—	—	—	0.47	0.15–1.47	0.195	—	—	—
Illiteracy (not able to read or write)	1.01	0.70–1.72	0.960	—	—	—	0.31	0.10–0.98	0.045	0.24	0.07–0.89	0.033
Years on ART, per additional year	1.07	0.94–1.22	0.314	—	—	—	1.46	1.00–2.14	0.051	1.55	1.00–2.43	0.052
Years since HIV diagnosis, per additional year	1.01	0.89–1.14	0.937	—	—	—	1.07	0.78–1.47	0.664	—	—	—
Age at ART initiation, per additional year	0.97	0.95–1.00	0.040	—	—	—	0.96	0.91–1.01	0.145	—	—	—
Months between HIV diagnosis and ART, per additional month	0.99	0.97–1.00	0.182	—	—	—	0.95	0.92–1.00	0.029	—	—	—
WHO stage III/IV at ART initiation	1.83	1.10–3.05	0.019	2.10	1.23–3.57	0.003	1.73	0.54–5.51	0.354	—	—	—
Malnutrition (BMI[b] <18.5 kg/m^2)	2.21	0.95–5.16	0.064	—	—	—	2.14	0.24–18.84	0.492	—	—	—
Any symptoms at the time of the survey	1.38	0.83–2.29	0.220				0.56	0.18–1.75	0.317	—	—	—
At least one previous ART modification	1.30	0.78–2.19	0.308	—	—		1.89	0.61–5.85	0.266	—	—	—
Women pregnant while on ART	1.40	0.70–2.83	0.344	—	—	—	3.75	0.42–33.63	0.238	—	—	—
Concomitant TB treatment	1.81	0.87–3.74	0.110	—	—	—	0.84	0.15–4.63	0.841	—	—	—
Estimated low adherence	2.73	1.47–5.09	0.001	2.69	1.39–5.19	0.003	1.20	0.34–4.28	0.779	—	—	—
Starting with ZDV/3TC/NVP versus d4T/3TC/NVP	0.71	0.42–1.20	0.206				0.54	0.18–1.82	0.346	—	—	—
Current ART with ZDV/3TC/NVP versus d4T/3TC/NVP	0.50	0.22–1.10	0.086				0.39	0.05–3.39	0.395	—	—	—

AOR, adjusted OR; ZDV, zidovudine; 3TC, lamivudine; NVP, nevirapine; d4T, stavudine.
AOR and corresponding 95% CI and *P* value are only shown for those variables showing a significant association with VF in the multivariate analysis.
[a]Variables included in the multivariate analysis had *P* values <0.15 in the univariate analysis.
[b]Weight (kg)/height (m)^2.

Table 3. Prevalence of DRMs in subjects with detectable viraemia (HIV-1 RNA ≥150 copies/mL)

	Subjects with genotypic resistance data available		At least one DRM		At least one NNRTI DRM		At least one NRTI DRM		At least one NNRTI DRM plus one NRTI DRM		At least one PI DRM	
Viral load (copies/mL)	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%
Overall (≥150)	72/118	61.0	54/72	75.0	54/72	75.0	49/72	68.1	49/72	68.1	0	0.0
150–999	11/37	29.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
≥1000	61/81	75.3	54/61	88.5	54/61	88.5	49/61	80.3	49/61	80.3	0	0.0

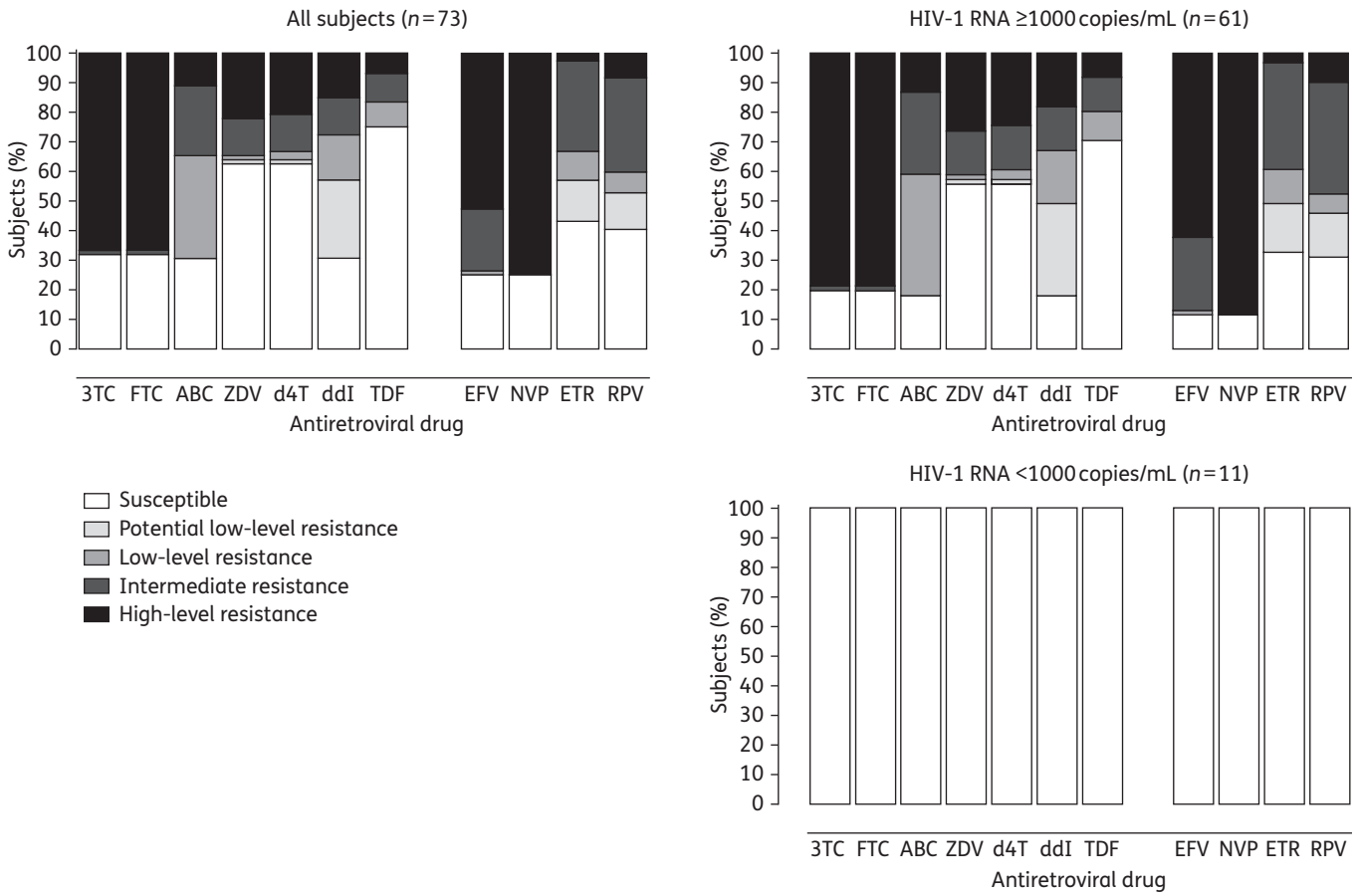

Figure 1. Predicted NRTI and NNRTI susceptibility according to the Stanford HIVdb. 3TC, lamivudine; FTC, emtricitabine; ABC, abacavir; ZDV, zidovudine; d4T, stavudine; ddI, didanosine; TDF, tenofovir disoproxil fumarate; EFV, efavirenz; NVP, nevirapine; ETR, etravirine; RPV, rilpivirine.

subjects with LLV may have a window of opportunity to re-suppress viral replication through adherence interventions, whereas those with overt VF should be switched to second-line ART without further delay. Of note, the clinician's judgement of ART failure, based on immunological and clinical criteria currently in place for patient management, was extraordinarily inaccurate in predicting VF, highlighting the urgent need for fostering point-of-care virological monitoring tools for low-income countries.

To our knowledge, this is the first comprehensive, well-powered study addressing the clinical and virological determinants of VF in Mozambique. Previous studies in the country were conducted before or at the beginning of the ART rollout, providing little information on the effectiveness of ART, or did not evaluate the determinants of virological outcomes of first-line ART.[11–16] Both are essential for guiding healthcare policies to improve the effectiveness of therapy and to prevent resistance emergence, most particularly if virological monitoring is not available.

The participants in this study were HIV-infected subjects attending scheduled visits at the outpatient clinic in Manhiça District Hospital. The selection criteria might have led to the exclusion of those patients who had poorer ART adherence, individuals skipping the scheduled control visits due to severe illness or hospital admittance, or those deceased during early ART. On the other hand, the availability of only one HIV-1 RNA measurement might have inflated the number of cases of detectable viraemia, particularly those with HIV-1 RNA <1000 copies/mL. However, detection rates of HIV DRMs would have increased if ultrasensitive HIV-1 drug resistance testing had been used.[23] The limited number of genotypes available for subjects with LLV limits our ability to generalize our results in this subset of patients. Though the clinician's judgement on ART treatment failure was presumably based on the patient's clinical and immunological outcomes as recommended in national guidelines, information on the CD4+ T cell counts and clinical staging that supported this judgement is not provided. This poses a limitation when interpreting the performance of clinical and/or immunological criteria to predict treatment failure.

The rates of VF observed in this study met the WHO recommendations for the prevention of HIV DRMs (>70% of subjects with HIV-1 RNA <1000 copies/mL after at least 1 year of first-line ART)[24] and were consistent with those from neighbouring countries.[8,25–27] However, more VFs were observed in this study than in the only other similar report from Mozambique, a cross-sectional study conducted in 2009 on 149 patients on first-line ART for a median of 23 months, attending the Maputo Central Hospital, which showed a prevalence of VF of 10%. In comparison with this study, our cohort included a larger sample size and subjects had been on first-line ART for a longer time.[14]

Of note, this is the first known study evaluating the rates of LLV in Southern Africa. The findings from this study showed that ~10% of subjects under first-line ART for 3 years have LLV, concordant with data from low- and middle-income countries in Asia and the Pacific region.[28] The clinical implications and management consequences of LLV remain unclear. First, the risk of HIV transmission and disease progression is very low (<1000 copies/mL).[29,30] Second, viral blips and intermittent low viraemia may not be necessarily associated with an increased risk of treatment failure. On this basis, WHO's recent consolidated guidelines recommend reducing the viral load threshold defining treatment failure from 5000 to 1000 copies/mL.[3] This may not be low enough in view of studies in industrialized settings that have shown that LLV is associated with increased risk of overt VF and, in some cases, also with antiretroviral drug resistance.[31–37] In this study, all resistance mutations were found exclusively in subjects with HIV-1 RNA levels ≥1000 copies/mL. This is likely related to the high levels of estimated adherence in the population evaluated, which were further supported by the presence of therapeutic nevirapine plasma levels in >90% of the participants tested. Considering the small sample size of subjects with LLV with an available genotype, we cannot conclude that resistance cannot occur below 1000 copies/mL. Thus, LLV might be able to regain HIV-1 RNA suppression with the same first-line ART through intensified adherence interventions. Evidence addressing the clinical and virological implications of LLV in Africa is urgently needed.

The main factors predicting VF were having a WHO III/IV stage of HIV disease at ART initiation, being younger and having a low estimated adherence, consistent with previous studies.[26,27,34,38–41] Initiation of ART at a late stage of disease is common in sub-Saharan Africa, where high rates of attrition due to delays or failure to complete ART eligibility assessments have been described.[42] In this study, subjects who initiated ART at WHO III/IV stages were twice as likely to have CD4+ T cell counts ≤200 cells/mm^3 at the time of the survey, which is consistent with previous reports.[43,44] It has also been shown that initiating ART at WHO III/IV stages leads to mortality of up to 80% in the first 6 months of therapy;[44] these early deaths would not be reflected in our survey. In agreement with previous studies, both in industrialized and low-income countries , younger age predicted VF, likely because older age protected against low adherence [OR=0.96 (95% CI=0.93–0.99), P=0.011].[34,45]

VF has severe consequences. As seen in other studies in the sub-Saharan region,[26,27,46,47] almost 80% of subjects with detectable viraemia (90% of those with HIV-1 RNA ≥1000 copies/mL) had lost most first-line drug options available. Resistance to second-generation NNRTIs was present in 50% of subjects with detectable viraemia, limiting their use as second-line ART in the absence of previous genotypic resistance testing. The NRTI backbone was involved in most patients, except for tenofovir disoproxil fumarate, to which susceptibility was retained in ~70% of subjects. Based on our results, second-line ART in Mozambique must thus rely on ritonavir-boosted PIs plus tenofovir disoproxil fumarate and usually lamivudine. Despite the overall high effectiveness of second-line ART with boosted PIs in the field, recent evidence that PI monotherapy performs less well than triple-drug, PI-including ART suggests that the residual activity of the NRTI backbone is important for the long-term efficacy of second-line ART.[48] New drugs with complementary resistance profiles and formulations with enhanced pharmacokinetic properties are urgently needed to construct effective second-line ART in low-income settings.

The 2013 WHO consolidated antiretroviral guidelines recommended, for the first time, viral load monitoring as the preferred monitoring approach to the diagnosis and confirmation of ART failure. Although efforts are being made to adopt these guidelines in sub-Saharan Africa, this option still remains largely out of reach, forcing programmes to rely on clinical outcomes and CD4+ T cell counts for monitoring treatment efficacy. This study suggested that clinical and immunological criteria have extremely poor performance in the diagnosis of treatment failure. The clinician's judgement, based on such criteria, missed almost 75% of VF and erroneously classified 50% of cases as treatment failure. One out of every four subjects evaluated in the clinic was misclassified in terms of their actual virological status. Point-of-care virological monitoring is therefore essential for early detection of treatment failure to guide the clinical management of HIV-infected individuals, limit the emergence of resistance, preserve the effectiveness of second-line regimens and avoid unnecessary costly treatment switches.

Ending the HIV/AIDS pandemic will require further efforts to strengthen healthcare programmes and health systems. The focus must be placed on ensuring universal and prompt HIV testing and linkage with care, avoiding delays in ART initiation, reinforcing adherence interventions, particularly in younger subjects, and in developing and widely implementing affordable HIV-1 RNA monitoring.

Acknowledgements

This work was presented in part at the International Workshop on Antiviral Drug Resistance, Berlin, Germany, 2014 (Abstract 60).

We are grateful to all the study participants and to all the staff of the Manhiça District Hospital and the CISM who contributed to the study. We are particularly grateful to Arsenio Nhacolo for his helpful advice in data management and statistics, and Elisa López Varela for her help, on site, in the data cleaning process.

Funding

The study was supported through an investigator-initiated grant from Gilead Sciences. M. R. is supported by the grant Rio Hortega from the Spanish Ministry of Health (CM11/00278) and partially by the European Developing Countries Clinical Trials Partnership (EDCTP; IP.2007.31080.002), the Malaria in Pregnancy Consortium and the Instituto de Salud Carlos III (PI08/0564), Spain. The CISM receives core funding from the Spanish Agency for International Cooperation (AECI). The Manhiça District Hospital HIV programme receives funding from the Agencia Catalana de Cooperació al Desenvolupament (ACCD).

Transparency declarations

None to declare.

Supplementary data

Tables S1 to S4 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Gilks CF, Crowley S, Ekpini R *et al*. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. *Lancet* 2006; **368**: 505–10.

2 WHO. *Antiretroviral Medicines in Low- and Middle-income Countries: Forecasts of Global and Regional Demand for 2012–2015*. http://www.who.int/hiv/pub/amds/2013forecast_report/en/.

3 WHO. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for a Public Health Approach*. http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf.

4 Keiser O, Orrell C, Egger M *et al*. Public-health and individual approaches to antiretroviral therapy: township South Africa and Switzerland compared. *PLoS Med* 2008; **5**: e148.

5 Rawizza HE, Chaplin B, Meloni ST *et al*. Immunologic criteria are poor predictors of virologic outcome: implications for HIV treatment monitoring in resource-limited settings. *Clin Infect Dis* 2011; **53**: 1283–90.

6 Sigaloff KC, Hamers RL, Wallis CL *et al*. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr* 2011; **58**: 23–31.

7 Team DT, Mugyenyi P, Walker AS *et al*. Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. *Lancet* 2010; **375**: 123–31.

8 Hamers RL, Sigaloff KC, Wensing AM *et al*. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clin Infect Dis* 2012; **54**: 1660–9.

9 Hunt GM, Ledwaba J, Basson AE *et al*. Surveillance of transmitted HIV-1 drug resistance in Gauteng and KwaZulu-Natal Provinces, South Africa, 2005–2009. *Clin Infect Dis* 2012; **54** Suppl 4: S334–8.

10 Wood E, Montaner JS. Time to get serious about HIV antiretroviral resistance. *Lancet Infect Dis* 2011; **11**: 723–4.

11 Bartolo I, Casanovas J, Bastos R *et al*. HIV-1 genetic diversity and transmitted drug resistance in health care settings in Maputo, Mozambique. *J Acquir Immune Defic Syndr* 2009; **51**: 323–31.

12 Bellocchi MC, Forbici F, Palombi L *et al*. Subtype analysis and mutations to antiviral drugs in HIV-1-infected patients from Mozambique before initiation of antiretroviral therapy: results from the DREAM programme. *J Med Virol* 2005; **76**: 452–8.

13 Bila DC, Young P, Merks H *et al*. Evolution of primary HIV drug resistance in a subtype C dominated epidemic in Mozambique. *PLoS One* 2013; **8**: e68213.

14 Maldonado F, Biot M, Roman F *et al*. Viraemia and HIV-1 drug resistance mutations among patients receiving antiretroviral treatment in Mozambique. *Trans R Soc Trop Med Hyg* 2009; **103**: 607–12.

15 Parreira R, Piedade J, Domingues A *et al*. Genetic characterization of human immunodeficiency virus type 1 from Beira, Mozambique. *Microbes Infect* 2006; **8**: 2442–51.

16 Vaz P, Augusto O, Bila D *et al*. Surveillance of HIV drug resistance in children receiving antiretroviral therapy: a pilot study of the World Health Organization's generic protocol in Maputo, Mozambique. *Clin Infect Dis* 2012; **54** Suppl 4: S369–74.

17 Gonzalez R, Desai M, Macete E *et al*. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-infected women receiving cotrimoxazole prophylaxis: a multicenter randomized placebo-controlled trial. *PLoS Med* 2014; **11**: e1001735.

18 Gonzalez R, Munguambe K, Aponte J *et al*. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med* 2012; **13**: 581–8.

19 WHO. *Global Update on HIV Treatment 2013: Results, Impact and Opportunities*. Geneva: WHO, 2013.

20 República de Moçambique. Ministério de Saúde. Direcção Nacional de Assistência Médica. *Guia de Tratamento Antiretroviral e Infecções Oportunistas no Adulto, Adolescente e Grávida 2009/2010*. http://www.who.int/hiv/pub/guidelines/mozambique_art.pdf.

21 Pou C, Bellido R, Casadella M *et al*. RECall for automated genotypic tropism testing. *J Clin Microbiol* 2013; **51**: 2754–7.

22 Woods CK, Brumme CJ, Liu TF *et al*. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. *J Clin Microbiol* 2012; **50**: 1936–42.

23 Pou C, Noguera-Julian M, Perez-Alvarez S *et al*. Improved prediction of salvage antiretroviral therapy outcomes using ultrasensitive HIV-1 drug resistance testing. *Clin Infect Dis* 2014; **59**: 578–88.

24 WHO. *HIV Drug Resistance Report*. WHO, 2012.

25 Aghokeng AF, Monleau M, Eymard-Duvernay S *et al*. Extraordinary heterogeneity of virological outcomes in patients receiving highly antiretroviral therapy and monitored with the World Health Organization public health approach in sub-Saharan Africa and southeast Asia. *Clin Infect Dis* 2014; **58**: 99–109.

26 El-Khatib Z, Ekstrom AM, Ledwaba J *et al*. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS* 2010; **24**: 1679–87.

27 Ramadhani HO, Thielman NM, Landman KZ *et al*. Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania. *Clin Infect Dis* 2007; **45**: 1492–8.

28 Kanapathipillai R, McManus H, Cuong DD *et al*. The significance of low-level viraemia in diverse settings: analysis of the Treat Asia HIV Observational Database (TAHOD) and the Australian HIV Observational Database (AHOD). *HIV Med* 2014; **15**: 406–16.

29 Ledergerber B, Lundgren JD, Walker AS *et al*. Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes. *Lancet* 2004; **364**: 51–62.

30 Tomasoni LR, Patroni A, Torti C *et al*. Predictors of long-term immunological outcome in rebounding patients on protease inhibitor-based HAART after initial successful virologic suppression: implications for timing to switch. *HIV Clin Trials* 2003; **4**: 311–23.

31 Gonzalez-Serna A, Min JE, Woods C *et al*. Performance of HIV-1 drug resistance testing at low-level viremia and its ability to predict future virologic outcomes and viral evolution in treatment-naive individuals. *Clin Infect Dis* 2014; **58**: 1165–73.

32 Laprise C, de Pokomandy A, Baril JG *et al*. Virologic failure following persistent low-level viremia in a cohort of HIV-positive patients: results from 12 years of observation. *Clin Infect Dis* 2013; **57**: 1489–96.

33 Mackie NE, Phillips AN, Kaye S *et al*. Antiretroviral drug resistance in HIV-1-infected patients with low-level viremia. *J Infect Dis* 2010; **201**: 1303–7.

34 Paredes R, Mocroft A, Kirk O *et al*. Predictors of virological success and ensuing failure in HIV-positive patients starting highly active antiretroviral therapy in Europe: results from the EuroSIDA study. *Arch Intern Med* 2000; **160**: 1123–32.

35 Santoro MM, Fabeni L, Armenia D *et al*. Reliability and clinical relevance of the HIV-1 drug resistance test in patients with low viremia levels. *Clin Infect Dis* 2014; **58**: 1156–64.

36 Swenson LC, Min JE, Woods CK *et al*. HIV drug resistance detected during low-level viraemia is associated with subsequent virologic failure. *AIDS* 2014; **28**: 1125–34.

37 Taiwo B, Gallien S, Aga E *et al*. Antiretroviral drug resistance in HIV-1-infected patients experiencing persistent low-level viremia during first-line therapy. *J Infect Dis* 2011; **204**: 515–20.

38 Ahoua L, Guenther G, Pinoges L *et al*. Risk factors for virological failure and subtherapeutic antiretroviral drug concentrations in HIV-positive adults treated in rural northwestern Uganda. *BMC Infect Dis* 2009; **9**: 81.

39 Amoroso A, Etienne-Mesubi M, Edozien A *et al*. Treatment outcomes of recommended first-line antiretroviral regimens in resource-limited clinics. *J Acquir Immune Defic Syndr* 2012; **60**: 314–20.

40 Lahuerta M, Wu Y, Hoffman S *et al*. Advanced HIV disease at entry into HIV care and initiation of antiretroviral therapy during 2006–2011: findings from four sub-Saharan African countries. *Clin Infect Dis* 2014; **58**: 432–41.

41 Rosenblum M, Deeks SG, van der Laan M *et al*. The risk of virologic failure decreases with duration of HIV suppression, at greater than 50% adherence to antiretroviral therapy. *PLoS One* 2009; **4**: e7196.

42 Micek MA, Gimbel-Sherr K, Baptista AJ *et al*. Loss to follow-up of adults in public HIV care systems in central Mozambique: identifying obstacles to treatment. *J Acquir Immune Defic Syndr* 2009; **52**: 397–405.

43 Brinkhof MW, Boulle A, Weigel R *et al*. Mortality of HIV-infected patients starting antiretroviral therapy in sub-Saharan Africa: comparison with HIV-unrelated mortality. *PLoS Med* 2009; **6**: e1000066.

44 Gupta A, Nadkarni G, Yang WT *et al*. Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. *PLoS One* 2011; **6**: e28691.

45 Fatti G, Mothibi E, Meintjes G *et al*. Antiretroviral treatment outcomes amongst older adults in a large multicentre cohort in South Africa. *PLoS One* 2014; **9**: e100273.

46 Barth RE, van der Loeff MF, Schuurman R *et al*. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. *Lancet Infect Dis* 2010; **10**: 155–66.

47 Marconi VC, Sunpath H, Lu Z *et al*. Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. *Clin Infect Dis* 2008; **46**: 1589–97.

48 Antinori A, Arribas J, Fehr J *et al*. The PROTEA trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV-1 RNA below 50 copies/mL. *J Int AIDS Soc* 2014; **17** Suppl 3: 19525.