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Chapter 10

Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa

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ABSTRACT

Objectives

This study aimed to investigate the consequences of using clinico-immunological criteria to detect antiretroviral treatment (ART) failure and guide regimen switches in HIV-infected adults in sub-Saharan Africa. Frequencies of unnecessary switches, patterns of HIV drug resistance, and risk factors for the accumulation of nucleoside reverse transcriptase inhibitor (NRTI)–associated mutations were evaluated.

Methods

Cross-sectional analysis of adults switching ART regimens at 13 clinical sites in 6 African countries was performed. Two types of failure identification were compared: diagnosis of clinico-immunological failure without viral load testing (CIF only) or CIF with local targeted viral load testing (targeted VL). After study enrollment, reference HIV RNA and genotype were determined retrospectively. Logistic regression assessed factors associated with multiple thymidine analogue mutations (TAMs) and NRTI cross-resistance (≥2 TAMs or Q151M or K65R/K70E).

Results

Of 250 patients with CIF switching to second-line ART, targeted VL was performed in 186. Unnecessary switch at reference HIV RNA <1000 copies per milliliter occurred in 46.9% of CIF only patients versus 12.4% of patients with targeted VL ($P < 0.001$). NRTI cross-resistance was observed in 48.0% of 183 specimens available for genotypic analysis, comprising ≥2 TAMs (37.7%), K65R (7.1%), K70E (3.3%), or Q151M (3.3%). The presence of NRTI cross-resistance was associated with the duration of ART exposure and zidovudine use.

Conclusions

Clinico-immunological monitoring without viral load testing resulted in frequent unnecessary regimen switches. Prolonged treatment failure was indicated by extensive NRTI cross-resistance. Access to virological monitoring should be expanded to prevent inappropriate switches, enable early failure detection and preserve second-line treatment options in Africa.

INTRODUCTION

Over the past decade, there has been an unparalleled effort to provide access to antiretroviral treatment (ART) for HIV-infected individuals in sub-Saharan Africa, the region with the highest HIV burden [1]. Although short-term outcomes of first-line ART have been favorable [2], extended follow-up data are still limited. A proportion of patients receiving ART will inevitably experience treatment failure, putting them at increased risk of HIV-related morbidity and mortality. Recent guidance from the World Health Organization (WHO) recommends viral load determination, if feasible, to improve the identification of treatment failure [3]. Due to financial and logistical constraints in resource-limited settings, however, access to this expensive and technically demanding test is limited. Therefore, as a substitute, WHO-recommended clinical criteria and CD4 cell counts are commonly used by clinicians to diagnose ART failure and guide switches to second-line regimens.

Several studies in African countries, however, have shown poor association of clinico-immunological criteria with virological failure in patients on first-line ART [4-7]. In absence of viral load testing, incorrectly diagnosed or presumed virological failure may result in inappropriate switches to more expensive and toxic second-line regimens. Additionally, delayed failure detection and continuation of a failing regimen can result in the selection of viruses with extensive resistance to antiretroviral (ARV) drugs [8]. In particular, the accumulation of mutations associated with cross-resistance within the nucleoside reverse transcriptase inhibitor (NRTI) drug class may compromise the effectiveness of standard second-line regimens, which are based on a dual backbone of new or recycled NRTIs and a ritonavir-boosted protease inhibitor (PI).

This multicenter study, conducted in a collaborative network of 13 ART sites in 6 African countries [9], aimed to investigate the consequences of using clinicoimmunological criteria to detect treatment failure and guide regimen switching. To this end, we sought to evaluate frequencies of unnecessary switches to second-line regimens, patterns of HIV drug resistance in patients failing first-line ART, and risk factors for the accumulation of NRTI-associated mutations.

METHODS

Study design and population

The PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) study is a multicenter prospective observational cohort of HIV-1–infected adults who receive

ART in routine circumstances at 13 clinical sites in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe. Collaborating sites were selected to represent a variety of ART programs in terms of site administration (government, nongovernment, and private), ART experience, and geography. Sites were considered eligible to collaborate in PASER-M if they had accruing ART programs and minimum standards of administration and laboratory capacity. Eight sites had access to viral load testing, whereas 5 sites used clinico-immunological monitoring only. Cohort and site characteristics have been profiled previously [9]. The study was approved by local Ethics Committees and the Academic Medical Center Institutional Review Board. Written informed consent was obtained from all participants before the start of study procedures.

For the current cross-sectional analysis, PASER-M study participants were included if they were switched to second-line ART by their treating clinician, regardless of whether clinical, immunological, and/or virological criteria had been used to diagnose treatment failure. Exclusion criteria were a positive pregnancy test at study screening, and, in Nigeria, HIV-2 co-infection. We compared patients based on the type of failure identification used by the treating clinician: diagnosis of CIF in absence of viral load testing ("CIF only" group) or CIF with local targeted viral load (VL) testing ("targeted VL" group). In the CIF only group, the clinician's decision to switch was based on a new WHO clinical stage 3 or 4 condition or immunological deterioration, as defined by a CD4 cell count fall to pretreatment value, CD4 cell count decrease of 50% or persistent CD4 levels <100 cells per cubic millimeter. In the targeted VL group, treating clinicians had access to a local real-time HIV RNA test result to confirm suspected treatment failure based on clinical and immunological information. Drug changes because of side effects or toxicity were not considered regimen switches and were excluded from analysis. Demographic and clinical information were collected using standard case report forms.

Laboratory procedures

Routine laboratory results including CD4 cell count and HIV RNA were obtained from local laboratory records. Before switch to second-line ART, an additional phlebotomy was performed and EDTA anti-coagulated plasma specimens were stored at −80°C and batch-shipped to 2 reference laboratories in South Africa (Genotyping Unit, Department of Molecular Medicine and Hematology, University of the Witwatersrand) and Uganda (Genotyping Laboratory, Joint Clinical Research Centre) for retrospective determination of reference HIV RNA and genotypic resistance testing on all specimens with HIV RNA >1000 copies per milliliter. The Ugandan laboratory performed testing for the Ugandan sites and the South African laboratory performed testing for all other sites. Both laboratories participated in external quality assessment schemes for HIV RNA and genotypic testing. Tests performed at the reference laboratory are denoted "reference HIV-RNA"

throughout this report, and results obtained from the clinic are referred to as "local HIV RNA". For patients in the targeted VL group, both local and reference HIV RNA results were available. The reference HIV RNA was considered the gold standard.

The South African laboratory used the NucliSens EasyQ real-time assay version 2.0 (bioMérieux, Lyon, France) for reference HIV RNA determination and an in-house sequencing method encompassing the whole of protease and codons 1–300 of reverse transcriptase with an ABI Prism 3730 Genetic Analyzer Genetic Analyzer (Applied Biosystems, Foster City, CA) [10]. Sequences were assembled and manually edited using Sequencher v4.8 (Genecodes, Ann Arbor, MI). The Ugandan laboratory used the COBAS Ampliprep/ COBAS Taqman HIV-1 test (Roche, Branchburg, NJ) for HIV RNA determination and an in-house sequencing method encompassing the whole of protease and codons 1–300 of reverse transcriptase with a Beckman Coulter CEQ 8000 analyzer (Beckman Coulter Inc, Fullerton, CA) [11]. Sequences were assembled and manually edited using BioEdit version 7.0.9.0. All final sequences were submitted to the ViroScore database (Advanced Biological Laboratories SA, France) for quality control and data storage. Drug resistance mutations were scored according to the 2009 International AIDS Society–USA list of December 2009 [12]. HIV with genetic mixtures of wild-type and mutant sequences at amino acid sites that code for drug resistance mutations were considered resistant. Subtypes were determined using the REGA HIV-1 subtyping algorithm version 2.0 [13] and additional STAR genotype analysis if required [14].

Statistical analysis

An unnecessary switch to second-line ART was defined using 3 reference HIV RNA cut-offs: <400 copies per milliliter, <1000 copies per milliliter, and the WHO-recommended threshold of <5000 copies per milliliter [3]. The sensitivity, specificity, positive predictive ratio (PPV) and negative predictive values of the type of failure identification used (CIF only vs. targeted VL) were calculated with 95% confidence intervals using 2 × 2 contingency tables, compared with the reference HIV RNA load. The positive and negative likelihood ratios were calculated from the sensitivity and specificity. Patient characteristics were compared using the χ^2 test for categorical data and the Wilcoxon rank-sum test for continuous data. NRTI cross-resistance was defined as the presence of ≥2 thymidine analogue mutations (TAMs), the tenofovir (TDF)-associated mutations K65R or K70E, or the Q151M complex. Univariate and multivariate logistic regression was performed to identify factors associated with the following outcomes: ≥2 TAMs, NRTI cross-resistance, or selected single mutations. Explanatory variables included in the analysis were sex, age, type of failure identification (CIF only vs. targeted VL), WHO clinical stage, CD4 cell count, and HIV-RNA load at time of treatment failure, HIV-1 subtype, total duration of previous ARV exposure and type of prior non-nucleoside reverse transcriptase inhibitors

(NNRTIs) or NRTIs. Explanatory variables that were associated with the outcome variables ($P < 0.10$) in univariate analysis were forwarded to a multivariate prediction model, using a step forward procedure. Results were expressed as odds ratios (ORs) with 95% confidence intervals and P values with $P < 0.05$ regarded statistically significant. Analyses were performed using the statistical software package Stata version 10 (StataCorp LP, TX).

RESULTS

Patient characteristics

Between March 2007 and September 2009, 250 patients with CIF were switched to a second-line regimen (figure 1). The treating clinician had diagnosed CIF only in 64 (25.6%) patients and used targeted VL testing in 186 (74.4%) patients. Patients originated from

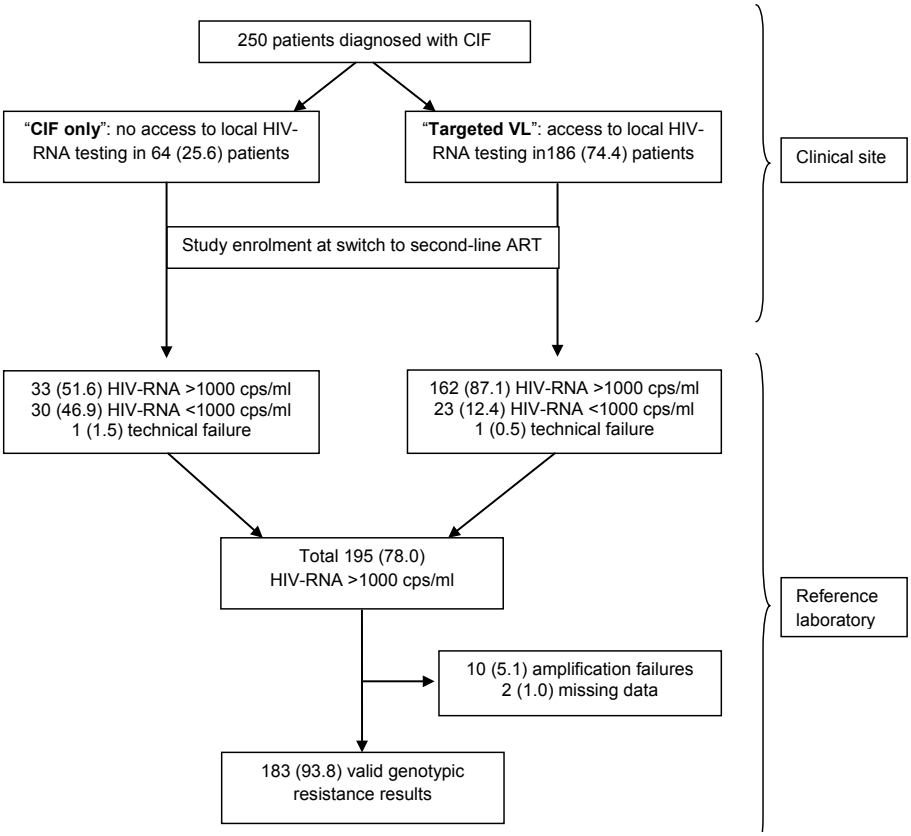

Figure 1. Flow chart of type of failure identification, study enrollment, and laboratory testing.
Data are n (%) of patients. CIF, clinico-immunological failure; VL, viral load; ART, antiretroviral treatment.

Uganda (n = 78, 31.2%), South Africa (n = 66, 26.4%), Kenya (n = 37, 14.8%), Nigeria (n = 32, 12.8%), Zambia (n = 27, 10.8%), and Zimbabwe (n = 10, 4%). Patient characteristics at switch to second-line ART are summarized in table 1. Advanced HIV disease (WHO clinical stage 3 or 4) and severe immunodeficiency (CD4 count <100 cells/mm^3) was more frequently observed in patients with CIF only (P = 0.007 and P = 0.021, respectively). The median reference HIV RNA $\log_{10}$ level was 3.3 [interquartile range (IQR): 1.4–4.4] in patients with CIF only and 4.4 (IQR: 3.7–5.0) in patients with targeted VL (P < 0.001).

The median duration of the first-line ART was 28.3 months in patients with CIF only and 25.3 months in patients with targeted VL (P = 0.310). Most patients (n = 236, 95.5%)

Table 1. Patient Characteristics at Switch to Second-Line Treatment, by Type of Identification of Failure

	Overall (n = 250)	**CIF Only (n = 64)**	**Targeted VL (n = 186)**	***P****
Women (%)	125 (50)	36 (56.3)	89 (47.8)	0.246
Median age (IQR) (yrs)	38.4 (34.2–45.0)	39.5 (34.9–45.9)	38.0 (33.8–44.9)	0.305
Median duration of prior ART regimen (IQR) (months)	26.1 (14.3–43.7)	28.3 (17.0–47.4)	25.25 (13.9–43.2)	0.310
ART regimen at failure (%)				0.016
ZDV + 3TC + NVP	67 (26.8)	15 (23.4)	52 (28.0)	
ZDV + 3TC + EFV	40 (16.0)	9 (14.1)	31 (16.7)	
D4T + 3TC + NVP	52 (20.8)	22 (34.4)	30 (16.1)	
D4T + 3TC + EFV	47 (18.8)	10 (15.6)	37 (19.9)	
TDF + FTC + NVP	12 (4.8)	1 (1.6)	11 (5.9)	
TDF + FTC + EFV	12 (4.8)	0	12 (6.5)	
NNRTI-based, other	6 (2.4)	1 (1.6)	5 (2.7)	
Triple NRTI	4 (1.6)	3 (4.8)	1 (0.5)	
PI-based	7 (2.8)	1 (1.6)	6 (3.2)	
WHO clinical stage 3 (%)	78 (31.2)	27 (42.9)	51 (27.4)	0.031
WHO clinical stage 4 (%)	40 (16.0)	12 (19.1)	28 (15.1)	
Median CD4 cell count (IQR) (cells/mm^3)	124 (59.5–200)	119 (58–177)	125.5 (61.5–215)	0.195
<100 (n, %)	103 (41.5)	30 (46.8)	73 (39.7)	0.021
100–200 (n, %)	82 (33.1)	26 (40.6)	56 (30.4)	
>200 (n, %)	63 (25.4)	8 (12.5)	55 (29.9)	
Median HIV RNA (IQR) ($\log_{10}$ c/mL)	4.2 (3.3–5.0)	3.3 (1.4–4.4)	4.4 (3.7–5.0)	<0.001
<1000 (n, %)	53 (21.2)	30 (46.9)	23 (12.4)	<0.001
1000–5000 (n, %)	25 (10.0)	2 (3.1)	23 (12.4)	
5000–10000 (n, %)	19 (7.6)	3 (4.7)	16 (8.6)	
>10000 copies/mL (%)	151 (60.4)	28 (43.8)	123 (66.1)	

Percentages are shown in parentheses and IQRs in square brackets. *Categorical data were compared using the χ^2 test; continuous were compared using the Wilcoxon rank-sum test. 3TC, lamivudine; FTC, emtricitabine.

used a NNRTI triple regimen at time of treatment failure. Others had received PI-based regimens (n = 7, 2.8%) or triple nucleoside regimens (n = 4, 1.6%). The most frequently used NRTI was zidovudine (ZDV, n = 107, 43.3%), followed by stavudine (D4T, n = 99, 40.1% and TDF (n = 24, 9.7%). The prior use of D4T was more common in patients with CIF only ($P = 0.049$), and TDF was used more frequently in patients with targeted VL ($P = 0.011$). In addition to first-line ART, 9 (4.8%) patients with targeted VL had a history of non-suppressive ARV use, either as mono-therapy (n = 2) or dual therapy (n = 3) or for the prevention of mother to child transmission (n = 4).

Diagnostic performance of treatment failure criteria

The median difference between the local and reference HIV RNA was 0.032 $\log_{10}$ copies (IQR: −0.23 to 0.52, $P = 0.0101$). Table 2 summarizes the performance of CIF only and targeted VL criteria to identify virological failure. Using the HIV RNA cut-off of <5000 copies per milliliter (table 2), unnecessary switches occurred in 78 (31.2%) patients: 32 (50.0%) in the CIF only group versus 46 (24.7%) in the targeted VL group ($P < 0.001$). CIF only criteria had a sensitivity of 18.2%, a specificity of 59%, and a PPV of 49.2%. Using the HIV RNA cut-off of <1000 copies per milliliter (table 2), unnecessary switches occurred

Table 2. Performance of CIF only* and CIF with targeted VL† criteria to identify virological failure at switch to second-line treatment

	Sensitivity (%)‡§	Specificity (%)‡§	PPV (%)‡§	NPV (%)‡§	PLR	NLR
Compared with who definition for regimen switch (HIV RNA > 5000 copies/mL)						
CIF only	18.2 (12.7 to 24.9) [31/170]	59.0 (47.3 to 70.0) [46/78]	49.2 (36.4 to 62.1) [31/63]	24.9 (18.8 to 31.7) [46/185]	0.4	1.4
Targeted VL	81.8 (75.1 to 87.3) [139/170]	41.0 (30.0 to 52.7) [32/78]	75.1 (68.3 to 81.2) [139/185]	50.8 (37.9 to 63.6) [32/63]	1.4	0.4
Compared with reference standard (HIV RNA > 1000 copies/mL)						
CIF only	16.9 (11.9 to 22.9) [33/195]	43.4 (29.8 to 57.7) [23/53]	52.4 (39.4 to 65.1) [33/63]	12.4 (8.0 to 18.1) [23/185]	0.3	1.9
Targeted VL	83.1 (77.1 to 88.1) [162/195]	56.6 (42.3 to 70.2) [30/53]	87.6 (81.9 to 92.0) [162/185]	47.6 (34.9 to 60.6) [30/63]	1.9	0.3
Compared with stricter definition for virological failure (HIV RNA >400 copies/mL)						
CIF only	16.7 (11.9 to 22.6) [34/203]	35.6 (21.9 to 51.2) [16/45]	54.0 (40.9 to 66.7) [34/63]	8.6 (5.0 to 13.7) [16/185]	0.3	2.3
Targeted VL	83.3 (77.4 to 88.1) [169/203]	64.4 (48.8 to 78.1) [29/45]	91.4 (86.3 to 95.0) [169/185]	46.0 (33.4 to 59.1) [29/63]	2.3	0.3

*CIF only: CIF diagnosis based on clinical (WHO clinical staging) and/or immunological (CD4 cell count) parameters. †Targeted VL: targeted viral load testing used by treating clinician in decision to switch to second-line treatment. ‡Numbers in parentheses refer to the 95% confidence interval. §Numbers in square brackets refer to the numerator and denominator from which the relevant percentages are calculated. PLR, positive likelihood ratio; NPV, negative predictive value; NLR, negative likelihood ratio; PPV, positive predictive value.

in 53 (21.2%) patients: 30 (46.9%) in the CIF only group versus 23 (12.4%) in the targeted VL group ($P < 0.001$). The CIF only criteria had a sensitivity of 16.9%, specificity 43.3%, and PPV of 52.4%. Applying a more stringent HIV RNA cut-off of <400 copies per milliliter (table 2), unnecessary switches occurred in 45 (18.1%) patients: 29 (46.0%) in the CIF only group versus 16 (8.6%) the targeted VL group ($P < 0.001$).

Genotypic analysis

Of the 195 specimens with HIV-1 RNA >1000 copies per milliliter, 183 (93.8%) valid genotypic test results were available (Fig. 1). The HIV-1 subtype distribution was C (n = 82, 44.8%), A (n = 45, 24.6%), D (n = 28, 15.3%), G (n = 14, 7.7%), CRF02_AG (n = 12, 6.6%), and other (n = 2, 1.2%). Resistance profiles are listed in table 3. At least 1 drug resistance

Table 3. Resistance patterns, by type of identification of failure

Resistance pattern	Overall (n=183)	CIF only (n=32)	Targeted VL (n=151)	*P*
Any resistance mutation	161 (88.0)	25 (78.1)	136 (90.1)	0.059
NRTI and NNRTI mutations	149 (81.4)	23 (71.8)	126 (83.4)	0.126
NRTI, NNRTI, and PI mutations	3 (1.6)	1 (3.1)	2 (1.3)	0.440
Any NRTI mutation	155 (84.7)	24 (75.0)	131 (86.8)	0.093
M184V/I	150 (82.0)	23 (71.9)	127 (84.1)	0.102
M184V/I only	0			n/a
M184V/I + TAM	97 (53.0)	18 (56.3)	79 (52.3)	0.686
TAM-containing virus*	100 (54.6)	19 (59.4)	81 (53.6)	0.554
≥2 TAMs	69 (37.7)	14 (43.8)	55 (36.4)	0.437
≥3 TAMs	44 (24.0)	12 (37.5)	32 (21.2)	0.050
K65R	13 (7.1)	3 (9.4)	10 (6.6)	0.404
K65R + TAM	3 (1.5)	1 (3.0)	2 (1.2)	0.428
K70E	6 (3.3)	0	6 (4.0)	n/a
Q151M	6 (3.3)	2 (6.3)	4 (2.7)	0.282
Q151M + TAM	3 (1.5)	0	3 (1.9)	n/a
Q151M + K65R	3 (1.5)	2 (6.0)	1 (0.6)	0.075
NRTI cross-resistance‡	87 (48)	17 (53.1)	70 (46.4)	0.486
Any NNRTI mutation	155 (84.7)	24 (75.0)	131 (86.8)	0.093
K103N	73 (39.9)	6 (18.8)	67 (44.4)	0.007
V106A/M	17 (9.3)	0	17 (11.3)	n/a
Y181C/V	57 (31.1)	13 (40.6)	44 (29.1)	0.202
G190A/S	50 (27.3)	11 (34.4)	39 (25.8)	0.324
Any PI mutation	6 (3.3)	2 (6.3)	4 (2.7)	0.282

Data are n (%) of patients. ‡NRTI cross-resistance, defined as ≥2 TAMs or Q151M or K65R/K70E. *Frequencies of individual TAMs: M41L, 40 (21.9); D67N, 46 (25.1); K70R, 39 (20.6); L210W, 17 (9.3); T215Y/F, 64 (34.9); K219Q/E, 37 (20.2). n/a, not applicable.

mutation was present in 161 of 183 (88.0%) specimens; 149 (81.4%) harbored dual-class and 3 (1.6%) triple-class resistance. Wild-type virus was detected in 7 (21.9%) patients in the CIF only group and 15 (9.9%) patients in the targeted VL group ($P = 0.059$). The most frequently observed mutation was the M184V/I (n = 150, 82.0%), followed by TAMs (n = 100, 54.6%). At least 2 TAMs were present in 69 (37.7%) specimens and 3 or more in 44 (24%). Both TAM pathways 1 and 2 were observed; the M41L was present in 40 (21.9%) specimens and the D67N in 46 (25.1%) specimens. The M184V/I mutation was combined with TAMs in 97 (53.0%) sequences. The K65R and K70E mutations were observed in 13 (7.1%) and 6 (3.3%) specimens, respectively. Three (1.5%) specimens harbored both the K65R and TAM(s). Six (3.3%) specimens harbored the Q151M complex, of which 3 with TAM(s) and 3 with the K65R. Overall, NRTI cross-resistance mutations were observed in 87 (48.0%) specimens. NRTI-associated mutational patterns did not differ by type of identification of failure (CIF only vs. targeted VL). The most frequent mutation conferring resistance to NNRTIs was the K103N (n = 73, 39.9%), followed by the Y181C/V (n = 57, 31.1%) and the G190A/S (n = 50, 27.3%). Major PI mutations occurred in 6 (3.3%) patients.

Factors associated with accumulation of drug resistance mutations

Table 4 summarizes factors associated with the presence of ≥2 TAMs and NRTI cross-resistance. In univariate analysis, the presence of ≥2 TAMs was associated with HIV-1 subtype, the total duration of previous ARV exposure, a history of ZDV use in failing and/or previous regimens and ≥2 different NRTIs. In multivariate analysis, the association persisted for ZDV use (OR: 3.49, 95% CI: 1.46 to 8.32, $P = 0.005$) and the duration of previous ARV exposure (OR for >24 months 2.90, 95% CI: 1.05 to 8.00, $P = 0.040$; OR for >36 months 4.47, 95% CI: 1.89 to 10.59, $P = 0.001$). The presence of multiple TAMs was not associated with sex, age, type of failure identification, WHO clinical stage, CD4 count, HIV RNA load, or TDF use.

The presence of NRTI cross-resistance was univariately associated with HIV RNA load, duration of ARV exposure, ≥2 different NRTIs, ZDV and TDF use, and CD4 count. Multivariate analysis showed that the risk of NRTI cross-resistance was significantly increased for longer duration of previous ARV exposure (OR: for >36 months 3.95, 95% CI: 1.58 to 9.85, $P = 0.003$), ZDV use (OR: 2.66, 95% CI: 1.12 to 6.28, $P = 0.026$) and TDF use (OR: 5.00, 95% CI: 1.67 to 14.94, $P = 0.004$). The association with HIV RNA load was close to significance (OR: 1.57, 95% CI: 1.00 to 2.47, $P = 0.052$). NRTI cross-resistance was not associated with sex, age, type of failure identification, WHO clinical stage, or HIV-1 subtype. In multivariate analysis, the K65R mutation was associated with TDF use (OR: 14.33, 95% CI: 2.92 to 70.31, $P = 0.001$) and HIV RNA load (OR: 2.27, 95% CI: 1.02 to 5.08, $P = 0.045$), but not with D4T or HIV-1 subtype.

Table 4. Factors associated with the accumulation of drug resistance mutations among patients failing first-line ART

	≥2 TAMs		NRTI cross-resistance	
	Univariate	**Multivariate**	**Univariate**	**Multivariate**
Sex				
Female	1.0	—	1.0	—
Male	1.17 (0.64 to 2.13)	—	1.16 (0.65 to 2.08)	—
Age (yrs)	1.02 (0.99 to 1.05)	—	1.01 (0.97 to 1.04)	—
Type of failure identification				
Targeted VL	1.0	—	1.0	—
CIF only	1.36 (0.63 to 2.94)	—	1.31 (0.61 to 2.82)	—
WHO clinical stage				
Stage I/II	1.0	—	1.0	—
Stage III/IV	0.99 (0.54 to 1.80)	—	1.23 (0.69 to 2.21)	—
CD4 count (cells/mm3)*	0.80 (0.61 to 1.06)	—	0.69 (0.53 to 0.92)	—
HIV RNA (log_{10})	1.09 (0.73 to 1.60)	—	1.60 (1.08 to 2.38)	1.57 (1.00 to 2.47)
HIV-1 subtype				
C	1.0	—	1.0	—
A	1.72 (0.81 to 3.65)	—	1.86 (0.89 to 3.87)	—
D	2.49 (1.03 to 5.97)	—	2.29 (0.95 to 5.52)	—
Other†	0.86 (0.34 to 2.21)	—	1.11 (0.47 to 2.66)	—
Duration of ARV use				
<24 months	1.0	1.0	1.0	1.0
24–36 months	2.97 (1.11 to 7.94)	2.90 (1.05 to 8.00)	1.6 (0.66 to 3.85)	1.99 (0.71 to 5.52)
>36 months	4.47 (1.95 to 10.23)	4.47 (1.89 to 10.59)	2.6 (1.27 to 5.31)	3.95 (1.58 to 9.85)
Thymidine analogue				
D4T	1.0	1.0	1.0	1.0
ZDV	3.02 (1.34 to 6.80)	3.49 (1.46 to 8.32)	2.43 (1.12 to 5.24)	2.66 (1.12 to 6.28)
TDF use				
No	1.0	—	1.0	1.0
Yes	0.75 (0.30 to 1.84)	—	4.19 (1.59 to 11.06)	5.00 (1.67 to 14.94)
Number of NRTIs used‡				
1	1.0	—	1.0	1.0
2 or more	2.71 (1.46 to 5.01)	—	2.83 (1.55 to 5.18)	1.09 (0.45 to 2.69)

Data are given as odds ratio (95% confidence interval). Outcomes include the emergence of ≥2 TAMs or NRTI cross-resistance, defined as: ≥ 2 TAMs or the Q151M complex or TDF-associated mutations (K65R/K70E). *OR for 100-cell increase of CD4 count. †Includes subtype G and circulating recombinant forms. ‡Excluding lamivudine or emtricitabine.

The NNRTI mutational profiles differed for patients failing efavirenz (EFV) versus nevirapine (NVP)-containing regimens. In univariate analysis, the use of EFV was associated with the K103N and V106A/M mutations, and NVP was associated with the Y181C/V. Multivariate analysis showed that EFV remained associated with the V106A/M (OR: 11.05, 95% CI: 2.39 to 50.98, $P = 0.002$) and NVP with the Y181C/V (OR: 28.5, 95% CI: 6.5 to 123.8, $P < 0.001$).

DISCUSSION

This multicenter observational study in 6 African countries investigated consequences of the common practice of using clinico-immunological criteria to detect ART failure and subsequently guide regimen switches. Without access to viral load testing, 47% of patients was switched to second-line ART inappropriately, at an HIV RNA below 1000 copies per milliliter. Targeted viral load testing to confirm treatment failure reduced unnecessary switches nearly 4-fold. A high frequency (88%) of clinically significant mutations was observed after first-line failure, suggesting late failure detection. Mutations associated with cross-resistance to NRTIs were observed in 48% of patients, comprising multiple TAMs (37.7%), K65R (7.1%), K70E (3.3%), or Q151M (3.3%). Accumulation of TAMs and NRTI cross-resistance were both associated with the duration of previous ARV exposure and ZDV use, and NRTI cross-resistance was additionally associated with TDF use.

Our data provide evidence that the use of clinico-immunological criteria to guide regimen switching has far-reaching public health implications. Treatment switches in patients who do not experience virological failure will result in mounting treatment costs and exhaust drug options. Additional concerns are the potential drug toxicity associated with second-line drugs and the costs of monitoring for adverse effects. This has particularly serious consequences in African countries where access to second-line regimens is limited. Moreover, continuation of treatment regimens in patients with virological failure will compromise their immunological and clinical status and, because of ongoing viral replication, result in the selection of viruses with accumulated resistance mutations. The effectiveness of future regimens, especially of those including NRTI backbones, is likely to be impaired.

An important strength of the study was the inclusion of a large international sample of patients diagnosed with treatment failure at different types of clinics, representative of the current clinical practice in many African ART programs. A limitation related to the cross-sectional design is that the prevalent cases identified in this study may not be rep-

resentative of all incident cases with virological and/or CIF. For example, individuals who died before the study or in whom failure was not yet diagnosed could not be included. Therefore, care should be taken when extrapolating the study results. Unfortunately, information about the duration of treatment failure or possible previous periods of clinical immunological of virological failure was not available. Additionally, historical information about prior ARV use was collected retrospectively and might have been incomplete due to limitations in recall.

Detection of treatment failure

The poor diagnostic performance of clinical and immunological criteria for the identification of virological failure observed in this study confirms previous reports 4-7]. The PPV of CIF only criteria was somewhat higher in this study, which is explained by the fact that the PPV is dependent on the prevalence of treatment failure in the sampled population [15], which was considerably higher in our study sample that consisted of patients who were switched to second-line ART, compared with the general population of patients receiving first-line ART. Our study underscores the importance of targeted viral load testing to maximize the clinical benefits of first-line regimens and prevent unnecessary switches to expensive second-line ART. This approach is in line with new WHO guidelines [3] and also corroborates findings of a recent study in India in which targeted viral load testing prevented unnecessary switch, at an HIV RNA <400 copies per milliliter, in nearly 25% of patients [16]. Taking into account that the cost of second-line regimens is currently up to 10 times higher than the cost of first-line ART, and that in our study approximately half of individuals with CIF only were truly failing, confirmation of failure by a viral load test before switching is likely to be cost saving. As simplified and more affordable methods of HIV RNA determination are being developed, the cost-effectiveness of this strategy is expected to increase further.

Despite access to local HIV RNA testing, 12% of patients were switched to second-line ART at a reference HIV RNA below 1000 copies per milliliter. The local HIV RNA had higher average values than the reference HIV RNA. This could be explained by differences between HIV RNA assays [17, 18] and factors-related specimen storage and shipment which may account for the variation in results. Another reason for the disagreement might be due to reinforcement of drug adherence although awaiting the requested test results, resulting in a suppression of HIV RNA by the continued first-line ART regimen. A previous study has shown that resuppression can occur in up to 41% of patients with viremia on NNRTI-based regimens [19]. Unfortunately, information on adherence or the time-lag between local HIV RNA testing and switch to second-line ART was not available in our study.

Drug resistance patterns and predictors

The most frequently observed mutation in our study, the M184V, causes resistance to lamivudine and emtricitabine, but delays the emergence of mutations associated with ZDV and D4T [20, 21]. Despite this increased susceptibility, we found high frequencies of multiple TAMs and 53% of patients harbored TAMs in combination with the M184V/I mutation. The rate of TAMs found in our study was higher than has previously been reported in similar population [22]. Furthermore, the considerable frequencies of K65R, K70E, and Q151M mutations observed are also likely to reflect a longer duration of treatment failure. Particularly, the Q151M complex usually requires a lengthy period of time to develop and confers broad NRTI cross-resistance [23]. K65R selection has been associated with higher levels of viral replication and lower CD4 cell counts, related to longer duration of virological failure [24]. TDF, and less frequently D4T, select for K65R [25-27], and in vitro studies have demonstrated its preferential acquisition in subtype C [28, 29]. Our analyses indeed showed that K65R was significantly associated with HIV RNA load and TDF use but did not demonstrate an association with D4T use or HIV-1 subtype.

The presence of ≥2 TAMs was associated with ZDV but not with D4T use. This is in accordance with findings of a previous study from South Africa [26], but differs from previous reports in subtype B [30]. Apart from genetic diversity, a possible explanation might be that short-term drug intolerance or toxicity is more frequently reported in relation to ZDV use, possibly leading to suboptimal adherence and prompting drug substitutions more often than in patients starting a D4T-based regimen [31]. Both TAM pathways 1 and 2 were observed, with the latter being slightly more common, which is congruent with previous reports from South Africa [26, 32].

Patterns of NRTI-associated mutations did not differ significantly according to the type of failure identification used. This is due to the fact that patients in the targeted VL group were also diagnosed with CIF and, consequently, failure was detected at a late stage. Indirect evidence for the accumulation of mutations due to late failure detection is provided by the significant associations of ≥2 TAMs and NRTI cross-resistance with the duration of previous ARV exposure. Also, HIV RNA load was marginally associated with NRTI cross-resistance.

NNRTI mutational patterns were in agreement with previous investigations [26, 33] and varied depending on prior EFV or NVP use. EFV was associated with the V106A/M mutation, whereas NVP selected for the Y181C/V mutation. Because only the Y181C/V reduces susceptibility to etravirine [34], this finding is relevant if etravirine would be considered for future salvage therapy and would encourage the use of EFV over NVP as part of first-line regimens.

Implications for clinical management

The high frequency of mutations conferring NRTI cross-resistance detected in our study may have consequences for the effectiveness of the currently available second-line regimens in African countries. Although favorable initial response to second-line ART have been described in resource-limited settings despite resistance at time of regimen switch [35-38], the development of NRTI cross-resistance will result in limited or no additional effect of the NRTI backbone, and standard second-line regimens will therefore primarily offer the benefit of the boosted-PI. Patients will thus receive functional monotherapy as second-line, which lowers the barrier for selection of PI resistance [39]. As a conditional recommendation, the WHO now advocates to perform routine viral load testing to detect virological failure early [3]. This strategy can trigger adherence interventions or changes in therapy that will limit ongoing viral replications, reducing the risk of accumulation of resistance mutations, and protect susceptibility to second-line and subsequent therapies.

In conclusion, this study demonstrated that, in the absence of viral load monitoring, unnecessary regimen switches are common, resulting in increased treatment costs and loss of drug options. Additionally, late detection of treatment failure resulted in high frequencies of accumulated mutations conferring broad cross-resistance to NRTIs, which may impair the effectiveness of second-line regimens. Future studies should determine the optimal frequency of routine virological monitoring and examine the clinical benefits of early failure detection and timely switching. The development of more affordable point-of-care viral load assays is a public health priority for resource-limited settings.

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