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High turnaround times and low viral resuppression rates after reinforced adherence counselling following a confirmed virological failure diagnostic algorithm in HIV-infected patients on first-line antiretroviral therapy from Tanzania

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Abstract

OBJECTIVE Early identification of confirmed virological failure is paramount to avoid accumulation of drug resistance in patients on antiretroviral therapy (ART). Scale-up of HIV-RNA monitoring in Africa and timely switch to second-line regimens are challenged.

METHODS A WHO adapted confirmed virological treatment screening algorithm (HIV-RNA screening, enhanced adherence counselling, confirmatory HIV-RNA testing) was evaluated in HIV-infected patients on first-line ART from Tanzania. The main endpoints included viral resuppression and virological failure rates, retention and turnaround time of the screening algorithm until second-line ART initiation. Secondary endpoints included risk factors for virological treatment failure and patterns of genotypic drug resistance.

RESULTS HIV-RNA >1000 copies/ml at first screening was detected in 58/356 (16.3%) patients (median time-on-treatment 6.3 years, 25% immunological treatment failure). Adjusted risk factors for virological failure were age <30 years (RR 5.2 [95% CI: 2.5–10.8]), years on ART ≥3 years (RR 3.0 [1.0–8.9]), CD4-counts <200 cells/μl (RR 9.3 [4.0–21.8]) and poor self-reported treatment adherence (RR 2.0 [1.2–3.4]). Resuppression of HIV-RNA <1000 copies/ml was observed in 5/50 (10%) cases after enhanced adherence counselling. Confirmatory testing within 3 months was performed in only 46.6% and switch to second-line ART within 6 months in 60.4% of patients. Major NNRTI-mutation were detected in all of 30 patients, NRTI mutations in 96.7% and ≥3 thymidine-analogue mutations in 40%. No remaining NRTI options were predicted in 57% and limited susceptibility in 23% of patients.

CONCLUSION We observed low levels of viral resuppression following adherence counselling, associated with high levels of accumulated drug resistance. High visit burden and turnaround times for confirmed virological failure diagnosis further delayed switching to second-line treatment which could be improved using novel point-of-care viral load monitoring systems.

keywords HIV, confirmed virological treatment failure, drug resistance, Africa, treatment monitoring

Sustainable Development Goals (SDGs): SDG 3 (good health and well-being), SDG 17 (partnerships for the goals)

Introduction

In recent years, the coverage of antiretroviral treatment (ART) in sub-Saharan Africa has rapidly expanded and led to effective declines in HIV-infected patients' morbidity and mortality. After the launch of the UNAIDS 90-90-90 cascade targets in 2014, in 2017 globally 79% of people with known HIV-infection were on ART, and 81% of people on treatment were virally suppressed; however, cascade progress varies among regions [1]. In Tanzania, the reported national adult HIV prevalence was 5% by 2016, and among the estimated 1.4 million

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people living with HIV, viral suppression is estimated at 52% [2].

Virological treatment success has been associated with several risk factors including the retention of patients in the healthcare system, adherence to therapy and the availability of drugs [3]. Subsequently, the number of patients in sub-Saharan Africa with first-line treatment failure in need of second-line ART is increasing and was recently predicted to reach 0.5–3 million patients by 2030 [4]. In the absence of viral load monitoring, ascertainment of treatment failure has been based on immunological and clinical treatment failure criteria, although both are poor predictors of virological treatment failure [5–7]. There are several reports that in the presence of immunological and clinical treatment failure a switch to second-line ART is often not performed, which might be due to uncertainties about the predictive value of CD4 count-based failure criteria, suspected lack of treatment adherence, negligence or unavailability of previous CD4 values [8,9]. Untreated subsequent virological treatment failure accumulates the amount of acquired drug resistance mutations in the long term [10] and furthermore increases the risk of transmitted drug resistance [11].

Annual virological treatment monitoring has therefore been recommended as the preferred option for ART monitoring by WHO [12] and was adopted in 2015 by the Tanzanian National Guidelines [13]. Following WHO recommendations, virological treatment failure is defined by a persistent detectable HIV-RNA >1000 copies/ml at two consecutive viral load measurements within a 3-month interval, with adherence support between measurements [14].

We therefore developed an HIV cohort study with the aim to investigate the scale-out and operational feasibility for viral monitoring and confirmed virological failure diagnosis in Tanzania as recommended by WHO. In the ALISA Study, we focused on virological treatment failure in HIV-infected patients on first-line antiretroviral regimens who had evidence of immunological treatment failure and/or had been on ART for longer than 2 years. The study objectives were to assess the virological treatment failure rates, evaluate the operational feasibility of the WHO confirmed virological treatment failure algorithm as well as factors affiliated with treatment failure and to assess the genotypic resistance profiles at the time of virological failure diagnosis.

Methods

Study design and ethics

This was a prospective, longitudinal cohort study in HIV-infected patients on first-line ART with suspected virological treatment failure defined as a viral load >1000 copies/ml during first screening. The study was approved by the institutional review boards of the Mbeya Medical Research Ethics Committee and the National Institute for Medical Research (NIMR) Ethics Committee in Tanzania, and at the Ethics Committee at the University of Munich (LMU) in Germany. This study was conducted according to the ethical principles of the Helsinki Declaration.

Study setting and population

The study was carried out at the HIV Care and Treatment Clinic (CTC) of the Mbeya Zonal Referral Hospital (MZRH) in Mbeya, Tanzania, which serves as a referral point for the Tanzanian Southern Highlands regions covering more than 5 million inhabitants. During the time of our recruitment period between November 2013 and October 2015, overall 5329 adult patients were registered at the MZRH CTC who received ARTs as extracted from the local database of the HIV National Control Programme (NACP). Of those, 5076 patients were on first-line ART. Routine viral load treatment monitoring was not yet implemented, HIV-RNA assessments were done sporadically (mainly if clinical and/or immunological treatment failure criteria were noticed), and routine monitoring procedures were based on 6-monthly consultations by a CTC doctor and CD4-count assessments. No standardised treatment adherence tools were used.

For study inclusion, we targeted patients aged 18 years or older on first-line ART whom we prioritised to receive virological treatment monitoring. This included patients with evidence for clinical and/or immunological treatment failure as defined by national guidelines (new WHO III/IV disease, 50% drop in CD4 count from peak value, or return to pre-ART baseline CD4 count or lower, or CD4 counts <100 cells/μl) [12,13], and those who were on first-line ART for a longer duration (at least 2 years). Patients were excluded if they were imprisoned, mentally disturbed or critically sick. All patients received written and oral information before study inclusion. Patients who received a protease inhibitor (PI) containing first-line regimen, for example because of previous Kaposi's sarcoma or intolerance of non-nucleoside reverse transcriptase inhibitors (NNRTI) were included in our analysis. For the study recruitment, doctors and nurses at the MZRH CTC were sensitised towards the screening algorithm and our inclusion criteria; furthermore, potential study participants were flagged from the local NACP database and contacted by the study personnel.

Study procedures

The confirmed virological failure algorithm was projected based on regular monthly ART pick-up visits

and a turnaround time of viral load results within 1 month. Thus, after the first HIV-RNA screening, intensified adherence counselling in patients with HIV-RNA >1000 copies/ml was performed after 1 month, and confirmatory viral load testing 1 month after intensified adherence counselling. For patients with confirmed virological failure after the second viral load testing, the optimal expected time from first viral screening to switch of ART because of confirmed virological failure was 3 months. All patients underwent treatment adherence assessment at the first viral load screening. We used a composite approach including an adapted 6-items self-reported adherence questionnaire [15–17] and a visual analogue scale (VAS) [18]. The self-reported adherence included a 4-day recall asking for numbers of skipped doses, a 1-month recall of voluntary treatment interruptions, questions on skipped, shifted or double doses over the last 2 weeks and a question about following the prescription over the past month. The VAS ranged from 0 (no medication taken) to 10 (all medication taken).

Enhanced adherence counselling was performed during a single session at the same visit when the first elevated viral load results were discussed with the patient. Trained HIV CTC counsellor with an HIV counselling degree according to national requirements performed counselling as stipulated in the NACP guidelines [13], thus enhanced counselling procedures were not further specified by study procedures.

Baseline demographic information (gender, age), HIV status (WHO stage, previous CD4 values) and ART history (start date of first-line regimen, current and past antiretroviral drugs) were collected on standardised study questionnaires or extracted from hospital charts as applicable. Blood was drawn for plasma HIV-RNA (Roche COBAS® TaqMan®2.0) and CD4 counts (Becton Dickinson FACSCount™ system), analyses were performed at the College of American Pathologists-accredited research laboratory at the NIMR-MMRC.

Genotypic resistance testing (GRT) was performed from stored plasma samples at the time of time of the first diagnosis of confirmed virological failure at the Institute for Immunology and Genetics in Kaiserslautern using the using the 'deepTypeHIV' assay as previously described [19]. Genotypic resistance mutations were interpreted using the HIV-Grade System (Version 12/2015; http://www.hiv-grade.de/cms/grade/homepage/).

Outcome measures and statistical analysis

The primary outcome of this study was to assess the proportion of patients with first and confirmed virological failure defined as HIV-RNA of >1000 copies/ml including (i) the proportion of patients who suppressed viral loads <1000 copies/ml following enhanced adherence counselling, and (ii) turnaround time of procedures involved in the confirmed virological treatment failure screening algorithm until second-line ART initiation. Secondary outcomes included risk factors affiliated with evidence of virological failure, and the assessment of genotypic resistance patterns in patients with confirmed virological failure.

All data were recorded in study-specific case report forms, double-entered into a study-tailored SQL database, compared and corrected for errors and inconsistencies before they were extracted for analysis. Descriptive categorical data were summarised as proportions and percentages of outcome characteristics, continuous variables such as CD4 count, age, years on ART or intervals within the screening algorithm were summarised as medians and ranges. Treatment adherence was graded as good if all adherence questionnaires indicated optimal compliances (no interruption, never shifted, skipped or doubled doses, totally followed prescription); moderate if doses were once or rarely skipped, shifted or doubled, or prescriptions were generally followed; poor if doses were skipped or shifted more than once or often, or if prescriptions were modified often or worse. Treatment adherence by VAS was graded as good if values indicated ≥9.5, medium between 8 and 9, and poor if <8 on the scale. The composite adherence measure for both assessments was adapted from Steel *et al.*, [20] discrepant adherence grades were combined based on the worst case for adjacent grades (poor + moderate = poor) or using the middle value (poor + good = medium).

Demographic, HIV, treatment regimens and adherence-related variables were associated with evidence of virological failure at first screening using binary regression analysis, which reported risk ratios (RR), their accompanying confidence interval and Fisher's exact *P*-value in univariate analysis. Risk factors with a *P*-value ≤0.1 were included in the adjusted model. All reported *P*-values were two-sided, and for all statistical tests an alpha level of <0.05 was used to define significance. All statistical analyses were performed using Stata statistics software (Version 14, StataCorp, College Station, TX, USA), graphs were drawn in MS Excel.

Results

Between November 2013 and October 2015, 356 HIV-infected patients (59% males, median age 46 years) on first-line ART were screened for virological treatment failure, baseline characteristics are shown in Table 1.

In brief, 63.2% were in WHO stage III/IV disease, the median CD4 count was 331/μl, 24.7% met the criterion for immunological treatment failure, the majority were on a zidovudine, lamivudine plus nevirapine or efavirenz-containing regimen with a median duration on first-line ART of 6.3 years. Poor self-reported composite treatment adherence was indicated by 15.4% of patients. Our study population represented about 7% of patients in care at the MZRH CTC who received first-line ART during our recruitment period. The proportion of patients still on a zidovudine-containing NRTI backbone was overrepresented in our cohort, which was explained by the long-term first-line duration for most of our patients, and the predominant NRTI backbone recommended at the time when ART was initiated.

Virological treatment failure and screening algorithm feasibility

Following first virological screening 269 (75.6%) patients had an HIV-RNA <50 copies/ml. Low viral replication between 50 and <400 copies/ml was detected in seven (2.0%) patients, and between 400 and <1000 copies/ml in 22 (6.2%) patients. High viral loads >1000 copies/ml at first screening were detected in 58 (16.3%) patients who were subsequently subject to enhanced adherence counselling (Figure 1a). In 50 (86.2%) of those patients confirmatory viral load testing was performed. Two patients were immediately switched to second-line ART without confirmatory testing due to low CD4 counts, and for six patients confirmatory testing or switch to second-line ART was either not performed or information could not be obtained. Confirmed virological treatment failure was observed in 45 (90%) cases, while in five patients (10%) HIV-RNA was re-suppressed following enhanced adherence counselling (Figure 1b).

To assess the operational feasibility of the screening algorithm, we analysed intervals between screening procedures. In 47% of patients with HIV-RNA >1000 copies/ml at first screening, confirmatory virological testing was performed within 3 months, and in 36% after 3–6 months. In 16% confirmatory testing was delayed beyond 6 months or patients were lost to follow-up (Figure 2a). A switch to second-line ART in 45 patients with confirmed virological failure occurred in 60.4% within 6 months of the first viral load screening, in 15.1% this was delayed beyond 6 months and in 20.8% we lost contact to the patients (Figure 2b). Based on not systematically obtained CTC nurse and counsellor information, reasons for delayed or interrupted procedures included 'no time', 'having another appointment', 'fear of stigma' or 'disappointment about ART drug intake without receiving good results'.

Table 1 Baseline characteristics, HIV status and antiretroviral treatment information of HIV-infected patients on 1st line ART undergoing virological treatment monitoring

Variables	*N* = 356
Gender	
Female	147 (41.3)
Male	209 (58.7)
Age (years)	46 (20–71)
Age categories	
<30 years	12 (3.4)
30–39 years	78 (21.9)
40–49 years	145 (40.7)
≥50 years	121 (34.0)
WHO stage	
Stage 1/2	131 (36.8)
Stage 3/4	223 (63.2)
CD4 count (cells/μl)	331 (5–1289)
CD4 count categories	
<200 cells/μl	78 (21.9)
200 to <350 cells/μl	103 (28.9)
350 to <500 cells/μl	76 (21.4)
≥500 cells/μl	75 (21.1)
Missing data	24 (6.7)
Immunological failure*	
No	242 (68.5)
Yes	88 (24.7)
Missing data	24 (6.7)
Years on 1st line ART	6.3 (0.3–10.8)
Years on 1st line ART categories	
<3 years	45 (12.6)
3 to <5 years	71 (19.9)
5 to <7 years	97 (27.3)
≥7 years	143 (40.2)
1st line ART regimen	
ZDV/3TC/NVP	197 (55.3)
ZDV/3TC/EFV	88 (24.7)
3TC or FTC/TDF/EFV	64 (18.0)
FTC/TDF/NVP	1 (0.3)
ZDV/3TC/LPV/r	4 (1.1)
ABC/3TC/LPV/r	1 (0.3)
FTC/TDF/LPV/r	1 (0.3)
Composite adherence (questionnaire + VAS)	
Good	168 (47.2)
Medium	133 (37.4)
Poor	55 (15.4)

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; EFV, efavirenz; FTC, emtricitabine; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; TDF, tenofovir; VAS, Visual Analogue Scale; ZDV, zidovudine.
Data are presented as numbers (%) or median (range).
*Defined as >50% drop of CD4 count from peak-level and/or persistent CD4 count <100 copies/ml and/or drop below baseline level prior to ART initiation.

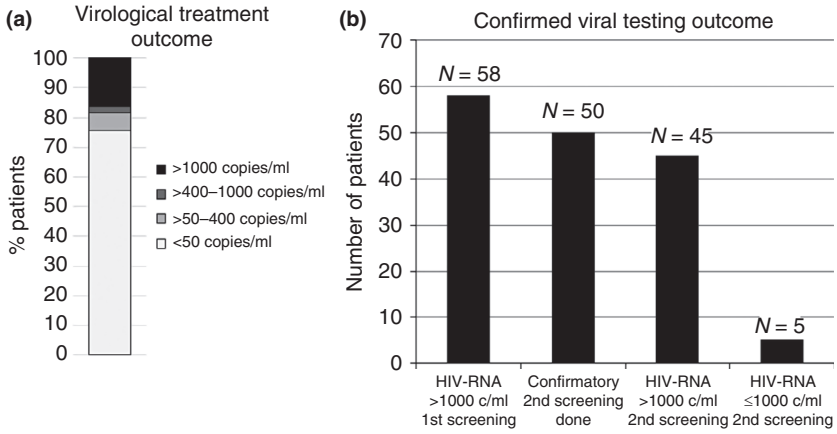

Figure 1 (a) Virological outcome in $N = 356$ patients screened on first-line ART; and (b) numbers of patients with HIV-RNA >1000 copies/ml at first HIV-RNA screening, patients who received confirmatory HIV testing, and patients with confirmed HIV-RNA > or ≤1000 copies/ml at confirmation.

Risk factors for virological failure

In univariate and multivariate analyses, HIV-RNA >1000 copies/ml at screening was significantly associated with younger age or age groups, declining CD4 counts or lower CD4 count categories, evidence for immunological treatment failure and poor self-reported adherence (Table 2). No association was observed for gender, WHO stage, the choice of ZDV *vs.* TDF based NRTI backbone or EFV *vs.* NVP NNRTI based regimens. Immunological treatment failure as a criterion for targeted viral load screening was present in 36/58 (40.9%) of patients with HIV-RNA >1000 copies/ml and in 52/298 (17.5%) with suppressed viral load, resulting in a sensitivity and specificity to predict HIV-RNA >1000 copies/ml of 66.7% (95% CI: 52.5; 78.9) and 81.3% (95% CI: 76.2; 85.7), respectively, with a positive predictive value of 40.9% (95% CI: 30.5; 51.9). Annual increase of ART duration was not associated with virological failure in univariate analysis, however, using time on ART categories >3 years *vs.* treatment duration <3 years was significantly associated with HIV-RNA >1000 copies/ml in the adjusted multivariate analysis (Table 2). The vast majority of patients with immunological failure had been treated for >3 years (92.1%), resulting in collinearity in the regression analysis.

Genotypic resistance testing

Samples for GRT were available and amplifiable for 30 of 45 patients with confirmed virological failure who had a median time on first-line ART of 6.4 years (range 2.7–9.8). Of those, 23 (77%) were on a zidovudine and 7 (23%) on a tenofovir-containing NRTI backbone, 13 (43%) were on an efavirenz and 17 (57%) on a nevirapine-containing NNRTI regimen. The majority represented subtype C viruses (63%), followed by subtype A1 (30%) and others (7%, subtype D, CRF31_BC). In all samples, major drug resistance mutations according to Stanford definitions were detected and dual-class resistance against NRTI and NNRTI was present in almost all participants (97%). Frequencies of thymidine-analogue NRTI mutations (TAM), non-TAM NRTI mutations and NNRTI major drug resistance mutations are listed in Table 3. At least one TAM was detected in 70% of cases, accumulation of two TAMs and three or more TAM's were detected in 57% and 40%, respectively. The M184V mutation was detected in 87%, the L74I and K65R was each detected in 7% of patients. The latter was selected only in patients on a tenofovir-containing regimen. The most frequently detected major NNRTI mutations were the K103N (43%), G190A (27%), Y181C (23%) and GK101E (20%). In general, minority variants (<20% of the viral population) were almost not present, possibly indicating already long-lasting viral replication and drug resistance pressure. We did not observe a clear pattern in respect to greater numbers of TAM's or major NNRTI resistance mutations with longer time on ART.

Full or intermediate predicted drug resistance was reported for efavirenz and nevirapine in all patients, and cross-resistance was predicted against etravirine in 67% and rilpivirine in 77%, in most cases some drug susceptibility could be assumed. Full drug resistance against lamivudine or emtricitabine was almost complete in all patients, and against tenofovir, zidovudine, stavudine,

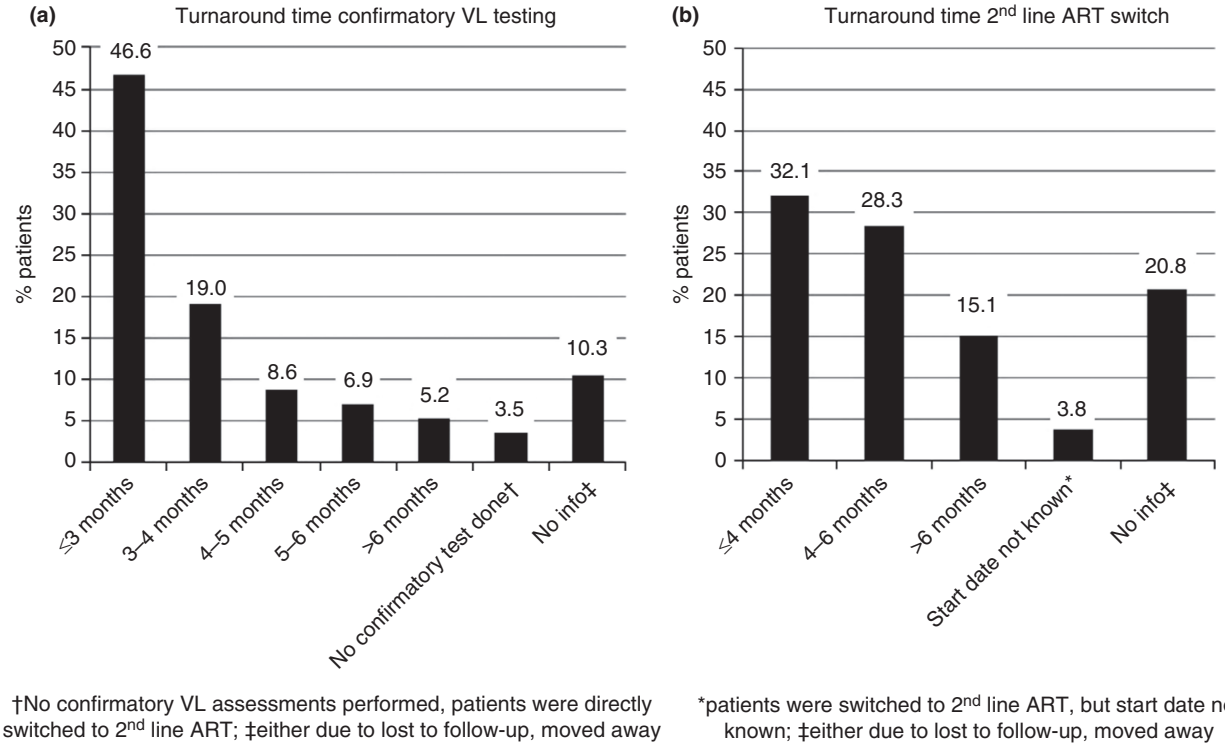

Figure 2 Turnaround time of virological failure screening procedures between first HIV-RNA screening and (a) confirmatory second HIV-RNA testing (N = 58) and (b) switch to second-line ART regimen (N = 53).

didanosine or abacavir in 50–57% of patients, with additional 20–27% of cases limited drug susceptibility. Overall, no susceptible NRTI treatment options were present in 17 (57%), limited susceptible options in 7 (23%) and still good options in 6 (20%) of patients as the backbone for PI-based second-line regimens.

Discussion

In our cohort study, we targeted virological treatment screening in HIV-infected patients on first-line ART for at least 2 years on ART, or if there was evidence for immunological treatment failure. High viral loads >1000 copies/ml were detected in 16.3% of patients at first screening, and after reinforced adherence support we observed HIV-RNA resuppression <1000 copies/ml in 10% of those who received confirmatory testing following an WHO adapted recommended confirmed virological failure algorithm. This was lower than rates reported in earlier studies with 27% resuppression without treatment switch in patients from Uganda [21] and 41% from South Africa [22]. As reviewed by Bonner *et al.* [23], the pooled estimate of resuppression from five studies including African countries was even 70.5% and 86% in a study from Haiti [24]. Resuppression rates in these studies were, however, evaluated between larger intervals (mostly 12 months) and patient populations had shorter treatment durations than in our study. The main reason for this large difference in resuppression rates between studies is likely the predominance of viral load assessments as a surrogate of poor treatment adherence, especially in settings where routine viral monitoring is implemented and high viral loads can still be reverted through adherence support. In our settings, high viral load was rather a surrogate for accumulated drug resistance in the absence of regular viral load monitoring.

Using the WHO-recommended confirmed virological failure algorithm we assessed the turnaround times, and demonstrated that in patients with initial high viral loads enhanced adherence counselling and confirmatory viral load testing within the recommended 3 months of first viral load screening only occurred in 47%, and a switch to second-line ART within 6 months in 60% of patients. We want to point out that in our setting the awareness of biological failure criteria among HIV care provider was optimal and tracing of patients biased by study

Table 2 Risk factors associated with virological treatment failure (>1000 copies/ml at first screening) among 1st line ART experienced individuals (*N* = 356)

		Non-adjusted*		Adjusted for gender, age, years on ART, CD4, composite adherence*	
Variable	*n*/*N* (%)	RR (95% CI)	*P*	RR (95% CI)	*P*
Gender					
Female	33/209 (15.8)	1		1	
Male	25/147 (17.0)	1.1 (0.7–1.7)	0.760	0.9 (0.6–1.4)	0.708
Age per 5 years increase	58/356 (16.7)	0.9 (0.8–1.0)	**0.030**		
Age categories					
≥50 years	10/121 (8.3)	1		1	
40–49 years	27/145 (18.6)	2.3 (1.1–4.5)	**0.020**	2.1 (1.1–4.1)	**0.022**
30–39 years	17/78 (21.8)	2.6 (1.3–5.5)	**0.009**	2.3 (1.1–4.8)	**0.030**
<30 years	4/12 (33.3)	4.0 (1.5–10.9)	**0.006**	5.2 (2.5–10.8)	**<0.001**
Years on 1st line	58/356 (16.7)	1.0 (0.9–1.1)	0.886		
Years on 1st line categories					
<3 years	3/45 (6.7)	1		1	
3 to <5 years	17/71 (23.9)	3.6 (1.1–11.6)	**0.032**	3.0 (1.0–8.9)	**0.043**
5 to <7 years	17/97 (17.5)	2.6 (0.8–8.5)	0.108	3.4 (1.1–10.4)	**0.034**
≥7 years	21/143 (14.7)	2.2 (0.7–7.1)	0.184	3.2 (1.1–9.7)	**0.036**
CD4 count† per 50 cells/μl increase	54/332 (16.3)	0.7 (0.6–0.8)	**<0.001**		
CD4 count categories					
≥350 cells/μl	6/151 (4.0)	1		1	
≥ 200 to <350 cells/μl	14/103 (13.6)	3.4 (1.4–8.6)	**0.009**	3.5 (1.4–9.0)	**0.008**
<200 cells/μl	34/78 (43.6)	11.0 (4.8–25.0)	**<0.001**	9.3 (4.0–21.8)	**<0.001**
Immunological failure†					
No	18/244 (7.4)	1			
Yes	36/88 (40.9)	5.5 (3.3–9.2)	**<0.001**	–	–
Composite adherence (questionnaire + VAS)					
Good (≥95%)	22/168 (13.1)	1		1	
Medium (80–94%)	20/133 (15.0)	1.1 (0.7–2.0)	0.630	0.9 (0.6–1.5)	0.781
Poor (<80%)	16/55 (29.1)	2.2 (1.3–3.9)	**0.006**	2.0 (1.2–3.4)	**0.009**

Bold values highlight statistical significant *P*-values.
ART, antiretroviral therapy; RR, risk-ratio.
*Poisson regression.
†Twenty-four CD4 samples were missing.

procedures, which exceeds involvements that can be expected in routine HIV management. Still, patient retention and turnaround times for confirmatory viral load testing and treatment switches were far from optimal, which demonstrates the challenges associated with a confirmed virological treatment failure algorithm that requires high multiple visit adherence within a short period. In our view, these findings support the implementation of novel point-of-care viral load monitoring systems at the HIV clinic which provide results within 2 h, hence same-day dissemination to the patient [25,26]. Point-of-care monitoring could greatly reduce visit burden, the duration of a confirmed virological failure algorithm, and thus likely increasing the effectiveness in switching ART regimen.

Treatment duration in our study was associated with higher virological failure rates, and in almost all patients with virological failure in which GRT was performed we detected extensive drug resistance patterns which is likely explained with accumulating drug resistance and long-termed treatment failure in the absence of viral monitoring. Several reports have indicated deficiencies in switching ART despite the presence of clinical or immunological treatment failure marker [8,9,27] which were present in 25% of patients in our population and significantly associated with virological treatment failure. Routine viral monitoring has been shown to reduce the accumulation of drug resistance [23,28] but does not avoid delayed switching to second-line treatments as reported from South Africa [29]. Further risk factors significantly

Table 3 Genotypic resistance mutations, interpretation and HIV subtypes in patients with confirmed virological failing on 1st line ART

Categories	*N* = 30
HIV subtype	
Subtype C	19 (63.3)
Subtype A1	9 (30.0)
Others (subtype D, CRF31_BC)	2 (6.7)
Resistance associated mutations	
NRTI associated	29 (96.7)
NNRTI associated	30 (100.0)
Accumulated thymidine-analogue mutations	
≥1 TAM's	21 (70.0)
≥2 TAM's	19 (56.7)
≥3 TAM's	12 (40.0)
Major NRTI resistance mutations (TAM's)	
K70R	12 (40.0)
D67N	12 (40.0)
M41L	11 (36.7)
T215Y	8 (26.7)
T215F	8 (26.7)
L210W	5 (16.7)
T215I	1 (3.3)
T215V	1 (3.3)
Major NRTI resistance mutations (non-TAM's)	
M184V	26 (86.7)
L74I	2 (6.7)
K65R	2 (6.7)
Major NNRTI resistance mutations	
K103N	13 (43.3)
G190A	8 (26.7)
Y181C	7 (23.3)
K101E	6 (20.0)
V106M	3 (10.0)
K238T	3 (10.0)
Y188L	3 (10.0)
G190S	3 (10.0)
V106A	2 (6.7)
K101P	1 (3.3)

Predicted drug resistance*

ARV	Full resistance	Intermediate resistance
Efavirenz (EFV)	26 (86.7)	4 (13.3)
Etravirine (ETR)	7 (23.3)	13 (43.3)
Nevirapine (NVP)	30 (100.0)	–
Rilpivirine (RPV)	10 (33.3)	13 (43.3)
Lamivudine (3TC)	29 (96.7)	–
Emtricitabine (FTC)	29 (96.7)	–
Tenofovir (TDF)	16 (53.3)	8 (26.7)
Zidovudine (ZDV)	15 (50.0)	7 (23.3)
Stavudine (D4T)	17 (56.7)	7 (23.3)
Didanosine (DDI)	17 (56.7)	7 (23.3)
Abacavir (ABC)	17 (56.7)	6 (20.0)

ART, antiretroviral therapy.
Data are provided for *N* (numbers of cases) and percentages.
*Classified by the HIV-GRADE (v12/2015) drug resistance classification system.

associated with virological failure in our study were younger age and poor self-reported treatment adherence, which is consistent with findings from other African studies [8,17,27].

More recent studies from Tanzania have also described high proportion of drug resistance in patients with virological failure [30,31], and in line with these and other reports the most frequent drug resistance mutations observed in our study were the M184V induced by lamivudine or emtricitabine and the K103N induced by NNRTI's [32,33]. In our predominately zidovudine-treated population, we found high proportions of patients with accumulated thymidine-analogue mutations (TAMs) with two or more TAMs detected in 57%, and three or more TAMs in 40% of patients. This was associated with high levels of cross-resistance including tenofovir resistance, and no susceptible NRTI treatment options were predicted in 57% and limited susceptible options in 23% of patients. A high proportion of patients therefore were switched on a functional PI monotherapy following the available second-line regimen. High jeopardised second-line treatment options were reported from few other studies [34,35], however, other studies did not describe a severe impact on second-line regimens [33,36–39], especially when tenofovir was part of the first-line regimen [40,41]. Full susceptibility against etravirine or rilpivirine was seen in 33% and 23% of our patients, respectively, which is in the range of possible second-line NNRTI susceptibility seen after first-line failure in Africa by others [40,42].

Limitations of our study are related to the sample size that did not allow more in-depth analysis of, for example drug resistance development with time on ART. Furthermore, most patients still received a zidovudine-containing regimen that is no longer the preferred choice for first-line therapies. Reported drug resistance patterns might

therefore not reflect the current reality for most patients that started on recommended tenofovir-based first-line regimens. In addition, the scale-up of viral load monitoring has been ongoing at many African settings since the conduct of our study, but we would argue that our data remain relevant as the availability of regular viral load monitoring is still scarce, especially in rural environments.

In conclusion, in settings where routine viral monitoring has not been implemented viral resuppression rates after reinforced adherence counselling can be relatively low due to high prevalence of accumulating drug resistance. In support of a confirmed virological failure diagnostic algorithm, challenges related to high turnaround times that are associated with further delayed switch to second-line regimens recommend the implementation of routine point-of-care viral load monitoring systems.

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