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Should viral load thresholds be lowered?
Revisiting the WHO definition for virologic failure in patients on antiretroviral therapy in resource-limited settings

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Abstract

The World Health Organization (WHO) guidelines on antiretroviral therapy (ART) define treatment failure as 2 consecutive viral loads (VLs) ≥1000 copies/mL. There is, however, little evidence supporting 1000 copies as an optimal threshold to define treatment failure. Objective of this study was to assess the correlation of the WHO definition with the presence of drug-resistance mutations in patients who present with 2 consecutive unsuppressed VL in a resource-limited setting.

In 10 nurse-led clinics in rural Lesotho children and adults on first-line ART for ≥6 months received a first routine VL. Those with plasma VL ≥80 copies/mL were enrolled in a prospective study, receiving enhanced adherence counseling (EAC) and a follow-up VL after 3 months. After a second unsuppressed VL genotypic resistance testing was performed. Viruses with major mutations against ≥2 drugs of the current regimen were classified as "resistant".

A total of 1563 adults and 191 children received a first routine VL. Of the 138 adults and 53 children with unsuppressed VL (≥80 copies/mL), 165 (116 adults; 49 children) had a follow-up VL after EAC; 108 (74 adults; 34 children) remained unsuppressed and resistance testing was successful. Ninety of them fulfilled the WHO definition of treatment failure (both VL ≥1000 copies/mL); for another 18 both VL were unsuppressed but with <1000 copies/mL. The positive predictive value (PPV) for the WHO failure definition was 81.1% (73/90) for the presence of resistant virus. Among the 18 with VL levels between 80 and 1000 copies/mL, thereby classified as "non-failures", 17 (94.4%) harbored resistant viruses. Lowering the VL threshold from 1000 copies/mL to 80 copies/mL at both determinations had no negative influence on the PPV (83.3%; 90/108).

The current WHO-definition misclassifies patients who harbor resistant virus at VL below 1000 c/mL as "nonfailing." Lowering the threshold to VL ≥80 copies/mL identifies a significantly higher number of patients with treatment-resistant virus and should be considered.

Abbreviations: ART = antiretroviral therapy, c/mL = copies/mL, DBS = dried blood spot, EAC = enhanced adherence counseling, HIV = human immunodeficiency virus, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, VL = viral load, WHO = World Health Organization.

Keywords: Africa, antiretroviral therapy, drug resistance, genotyping, Lesotho, treatment failure, WHO guidelines

1. Introduction

The 2013 Consolidated Guidelines of the World Health Organization (WHO) "On the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection" introduced routine viral load (VL) monitoring of patients on antiretroviral therapy (ART) in resource-limited settings.[1] For VLs ≥1000 copies/mL (c/mL) the WHO recommends adherence support for the following 3 to 6 months and then a confirmatory VL. If the follow-up VL remains ≥1000 c/mL despite good adherence, the patient is to be empirically switched to a second-line regimen. In contrast, patients with VLs below <1000 c/mL should be continued with unchanged first-line ART.[1]

Only in 2013, the WHO had adjusted the level from previously 5000 to 1000 c/mL, although an "optimal threshold" for defining treatment failure had never been scientifically defined; the WHO rationale for the 1000 copies reads: "clinical and epidemiological studies show that the risk of human immunodeficiency virus (HIV) transmission and disease progression is very low when the VL is lower than 1000 c/mL."[1] Another important rationale for a threshold at 1000 c/mL is that many programs in remote rural areas rely on dry blood spot (DBS) specimens. DBS does not reliably detect viremias below 1000 c/mL. Thus the recommendation to use 1000 c/mL for defining treatment failure is maintained in the revised 2015 version of the WHO guidelines.[2]

In contrast, various recent studies from high-income countries revealed that a high proportion of patients on ART with unsuppressed VL far below 1000 c/mL already harbor mutations known to confer resistance to the current ART regimen that will ultimately lead to treatment failure.[3–5] Consequently US- and European guidelines today recommend thresholds below 1000 c/mL for second line switching.[6,7] To our knowledge, there are currently no studies from resource-limited settings assessing if the WHO-proposed threshold for failure discriminating "true failure," defined as presence of relevant drug-resistance mutations, from "non-failure" without relevant virus mutations.

This registered prospective study assessed the correlation of the WHO definition of virologic failure with the presence or absence of critical drug-resistance mutations. The study was conducted in 10 rural nurse-led clinics in rural Lesotho, Southern Africa.

2. Materials and methods

2.1. Study design

The study entitled "Comorbidities and Virologic Outcome Among Patients on Antiretroviral Therapy in Rural Lesotho" (CART-1 study) is a registered prospective observational study assessing comorbidities and virologic outcomes among patients on first-line ART in 10 rural facilities in Lesotho (www.clinicaltrials.gov; ID: NCT02126696). This study has 2 parts: first, a cross-sectional assessment of routine VL among patients on first-line ART and, thereafter, a cohort study involving those patients with unsuppressed VL during part 1. One of the 2 predefined primary outcomes of the CART-1 study was to assess the WHO VL algorithm in a typical resource-limited setting with sensitive plasma VL. Using genotypic resistance testing, we probed the suitability of the WHO failure definition for predicting the presence of major drug-resistance mutations against the current first-line regimen.

2.2. Study setting

The study was conducted during the year 2014 in 2 districts of Lesotho, Thaba-Tseka and Butha-Buthe, each including 1 hospital and 4 health centers, where HIV care is exclusively provided by trained nurses. All sites receive support through SolidarMed, a Swiss not-for-profit organization that is assisting the Ministry of Health in the rollout of ART in Lesotho since 2005. The setting has been described previously.[8]

In 2013, Lesotho had revised its own national ART guidelines, adopting the WHO recommendation of routine VL monitoring, which suggests for VL ≥1000 c/mL to strengthen and monitor adherence over a period of 3 to 6 months, followed by a second VL. A VL ≥1000 c/mL at follow-up triggers the switch to second-line ART. With VL below that level patients stay on first-line ART.[1,2,9] During the conduct of the study, VL monitoring and genotypic resistance testing were unavailable for patients in routine HIV care.

2.3. Participants and study procedure

Participants were recruited between May and June 2014 for the cross-sectional study of part 1, which assessed virologic outcomes and comorbidities among patients attending routine ART care at the 10 study facilities. Eligibility criterion was prior continuous first-line ART for ≥6 months. Exclusion criteria were shorter periods on ART, documented treatment interruption of ≥7 consecutive days during the last 3 months, or being on a second-line regimen. Outcomes of part 1 in adults and children have been reported elsewhere.[10,11] Based on the specifications of our validated test system, pediatric and adult patients with VL above 80 c/mL during part 1 were defined as "unsuppressed."

Part 2 of the study was limited to patients with unsuppressed VL in part 1: Following the WHO guidelines, these patients received enhanced adherence counseling (EAC) and follow-up VL after 3 months. But, deviating from the WHO guidelines, all patients with any detectable VL, even below 1000 copies, received EAC and follow-up VL.

A health professional informed the patients about an unsuppressed VL and organized a follow-up for EAC. Adherence interventions included at least 1 of the following: focus-group discussion among patients with unsuppressed VL, one-to-one adherence counseling with nurse or lay-counselor, or directly observed therapy in the community. A follow-up VL was obtained 3 months after first EAC (October to November 2014), and viral genotyping was performed on the follow-up VL sample if VL remained unsuppressed with at least 80 c/mL.

2.4. Outcome measures

Primary outcome of this analysis was the predictiveness of the WHO definition of virologic treatment failure with repeated VL elevation to ≥1000 c/mL for detecting therapy-relevant viral resistance mutations. Following the current WHO recommendation only patients with first and follow-up VL ≥1000 c/mL were labeled "failures," whereas patients with any unsuppressed level below 1000 c/mL continued to be considered "non-failures". The prevalence of critical viral resistance mutations was assessed in both groups for all samples with successful HIV amplification. Resistance against the first-line regimen was defined by the presence of at least "low-level resistance" against at least 2 of the 3 drugs in a patient's current regimen according to *Stanford Drug Resistance Database version 7.0*.

A secondary outcome described the frequencies of resistance mutations against nucleosidic (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI) among patients with sustained VL above 80 c/mL.

2.5. Data collection

Patients' baseline characteristics were recorded when blood for the first VL measurement was drawn. Trained, supervised lay-counselors interviewed participants using a structured questionnaire on social and demographic characteristics and adherence. Adherence was assessed using pill-count and self-reported adherence on a visual analog scale. Following the interview, participants had their regular ART visits with clinical assessment and review of medical and therapeutic history. Questionnaires were digitalized in Lesotho and subsequently processed with Data-Scan 5.7.7 (Neoptec, Montpellier, France) for electronic data capture. Prior to analysis, all data were manually cross-checked against the original records.

Routine laboratory exams (full blood count, CD4-cell count, transaminases, serum creatinine) were performed at the certified national laboratories of the 2 study hospitals. Blood for virologic analysis was collected in plasma preparation tubes (PPTs), centrifuged within 8 hours and stored frozen at −80°C. Within 3 weeks of storage, PPTs were then shipped on dry ice to a reference laboratory in Switzerland. Samples were thawed without agitation, and viral RNA extraction was performed from the top 1 mL plasma fraction using NucliSENS easyMag (bioMérieux, Geneva, CH). VL determination was performed with a sensitive in-house protocol with a quantification limit at below 80 c/mL, targeting the viral LTR[12] and validated against the commercial diagnostic protocol COBAS TaqMan HIV-1 test, v2.0. For genotypic resistance testing we used material retained after NucliSENS easyMag extraction of the clinical specimen for the second VL measurement. Wherever sequencing was not successful, the sample of the first VL was used. (Five of the 108 determinations had to be repeated on the first VL sample.)

2.6. Statistical analysis

The study sample size was chosen to estimate the prevalence of unsuppressed VL during the cross-sectional part 1 of this study: we assumed that among the adults about 15% would have an
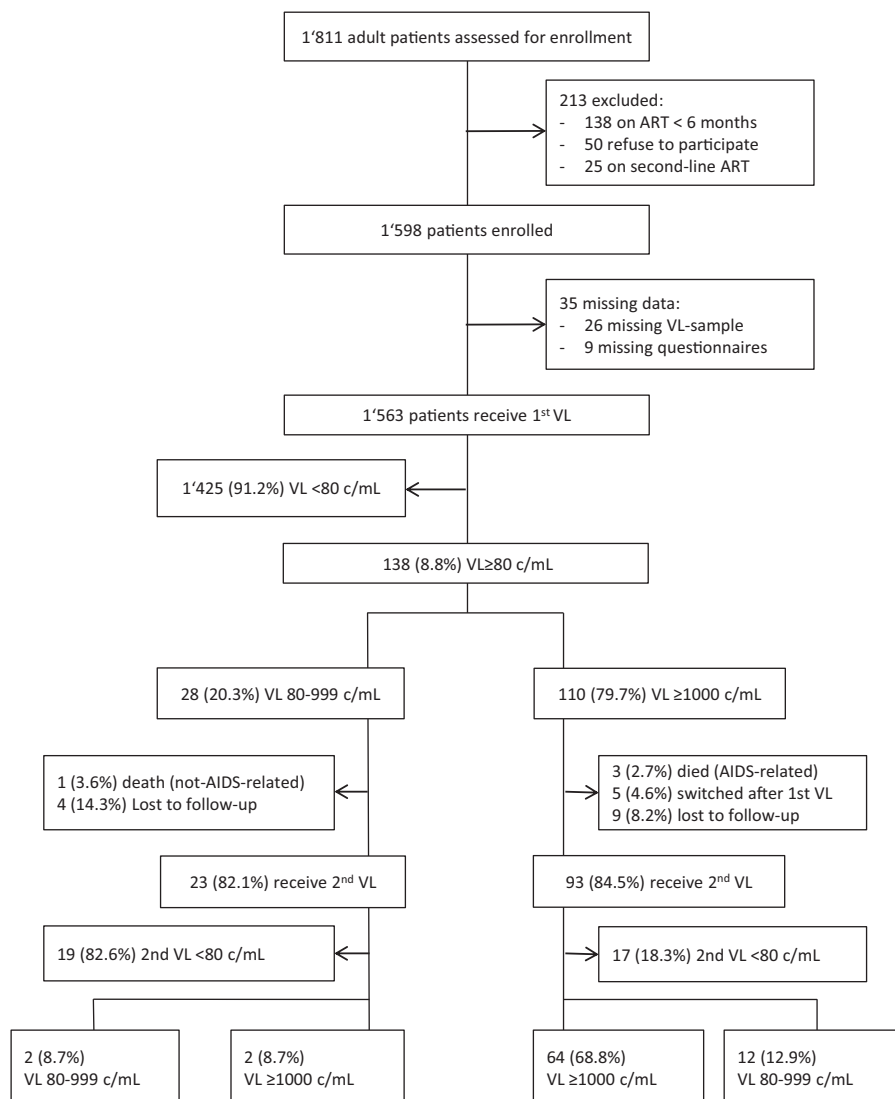

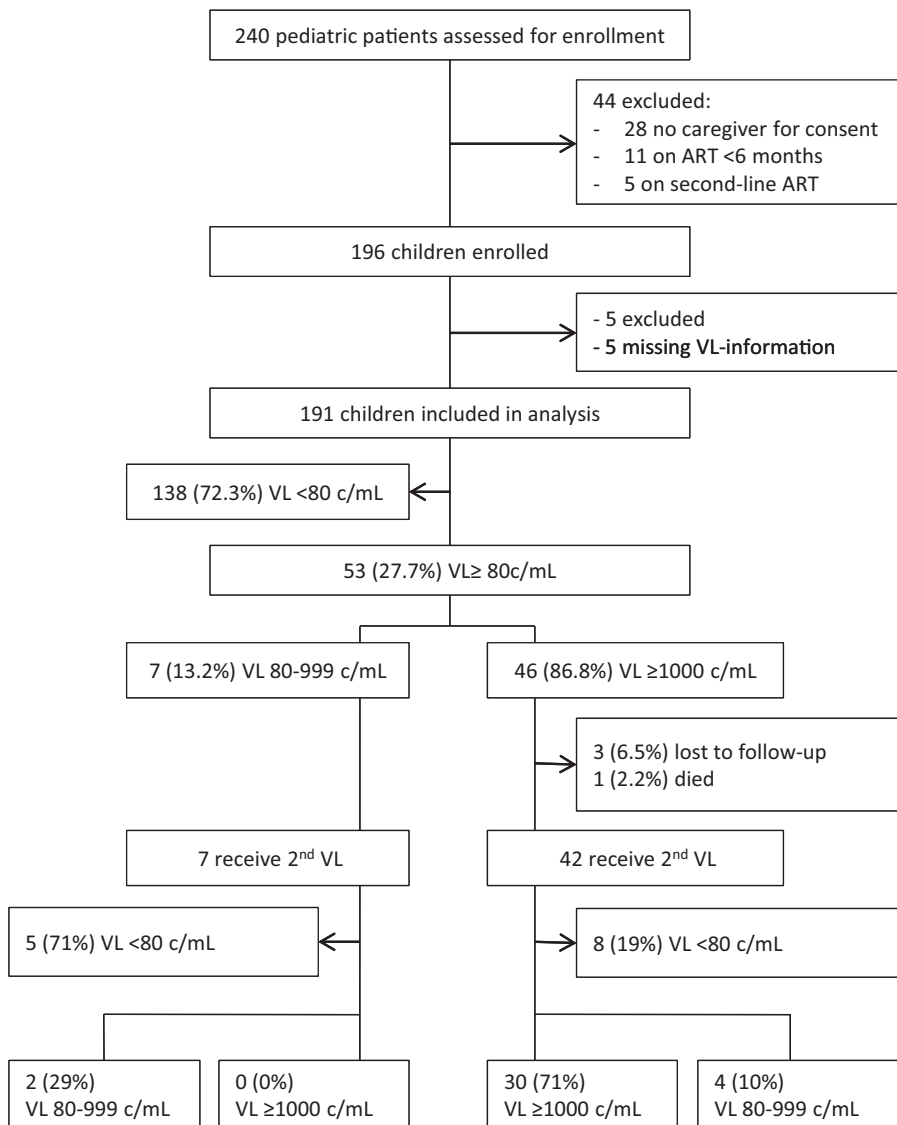**Figure 1A.** Enrolment and follow-up of adult study participants.

Figure 1B. Enrolment and follow-up of pediatric study participants.

unsuppressed VL. To achieve a precision of 2%, a minimum sample size of 1225 individuals was needed. Due to much lower numbers of children on ART in the study facilities, convenience sampling was applied for children. Unless specified otherwise, all quantitative variables are given in median and interquartile ranges (IQR). Exact binomial quantiles were used to compute the confidence intervals of proportions. Data from children (<16 years) and adults were analyzed separately. Calculations of sensitivity and specificity at VL ≥1000 and VL ≥80 c/mL were based on the assumption that all study participants with suppressed VL (<80 c/mL) would not harbor resistant HIV and could thus be categorized as true negatives in the four-by-four table. All analyses were run on R 2.15.3 (the R Foundation for Statistical Computing), and TIBCO Spotfire S+ 8.1 for Windows (TIBCO Software Inc., Munich, GER).

2.7. Ethical considerations

Ethics approval was received by the National Health Research and Ethics Committee of Lesotho (ID 01-2014) and the

"Ethikkomission Nordwest- und Zentralschweiz" (EKNZ) in Switzerland (ID 2014-029). Prior to enrolment patients provided individual oral and written informed consent. For children aged <16 years, a main caregiver had to provide oral and written informed consent.

2.8. Role of the funding source

The Swiss Foundation for Talent and Excellence on Biomedical Research, funder of this study, was not involved in design, implementation, and publication of the study.

3. Results

3.1. Enrolment of adult participants

The enrolment scheme of adult patients is displayed in Fig. 1A: A total of 1811 adult patients attended ART consultations during the recruitment period, 213 (11.8%) were excluded based on the prespecified exclusion criteria and 35 (1.9%) due to missing data.

Table 1

Baseline characteristics of the 138 adult patients with unsuppressed VL (≥80 c/mL) who were enrolled in the cohort study.

Parameters	
Clinical characteristics	
Median age (IQR)	41.1 (32.4–49.9)
Female gender (%)	65.9
Median CD4-cell count (cells/mL) (IQR)	351 (182–520)
Median hemoglobin (g/dL) (IQR)	13.4 (12.2–14.3)
Median BMI (kg/m^2)	22.2 (20.2–25.8)
Clinical failure (new WHO 3 or 4 condition)	10 (7.4%)
Type of facility	
Health center	77 (55.8%)
District hospital	61 (44.2%)
Median time on ART (y) (IQR)	4.1 (2.4–5.7)
First-line regimen's NRTI backbone	
Zidovudine/lamivudine	70 (50.7%)
Tenofovir/lamivudine	67 (48.6%)
Abacavir/lamivudine	1 (0.7%)
First-line regimen's NNRTI	
Efavirenz	90 (65.2%)
Nevirapine	48 (34.8%)
History of NRTI substitution	34 (24.6%)
Sociodemographic characteristics	
Median travel time to clinic (min) (IQR)	70 (60–150)
Mode of travel	
Walking	96 (72.2%)
Taxi	28 (21.1%)
Donkey/horse	4 (3.0%)
Other	5 (3.8%)
Educational level	
No completed primary education	74 (54.0%)
Completed primary education	36 (26.3%)
Secondary education and higher	27 (19.7%)
Civil status	
Single	14 (10.3%)
Concubinary	4 (2.9%)
Widowed	38 (27.9%)
Married	80 (58.88%)
Employment status	
Employed	17 (12.6%)
Self-employed	31 (23.0%)
Unemployed	87 (64.4%)

BMI = body-mass index, IQR = interquartile range, NNRTI = nonnucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, WHO = World Health Organization.

Table 2

Baseline characteristics of the 53 pediatric patients with unsuppressed VL who were enrolled in the cohort study.

Parameter	
Clinical characteristics	
Median age at study visit (IQR)	9.7 (5.6 to 13.0)
Median age at ART start (IQR)	5.1 (1.6 to 8.5)
Female gender (%)	47.2
Median CD4 count if age ≥5 years (IQR), n=41	690 (432 to 1015)
Median CD4 percentage if age <5 years, n=12	22% (17% to 32%)
Median hemoglobin (g/dL) (IQR)	12.8 (12.0 to 15.3)
Clinical failure at study visit (new WHO 3 or 4 condition)	2 (3.8%)
Median height for age Z-score (HAZ) at study visit	−2.5 (−3.5 to −1.3)
HAZ ≥−2 at study visit	21 (40.4%)
HAZ <−2 at study visit	31 (59.6%)
Missing information	1
Treatment history	
Type of facility	
Health center	21 (39.6%)
District hospital	32 (60.4%)
Median time on ART (IQR)	3.7 (2.1–4.8)
First-line regimen's NRTI backbone	
Zidovudine/lamivudine	47 (88.7%)
Tenofovir/lamivudine	2 (3.8%)
Abacavir/lamivudine	4 (7.5%)
First-line regimen's NNRTI/PI	
EFV	14 (26.4%)
NVP	36 (67.9%)
Lopinavir-based first line	3 (5.7%)
History of NRTI substitution	13 (24.5%)
Sociodemographic characteristics	
Median travel time to clinic (min)	60 (30 to 105)
Mode of travel	
Walking	32 (60.4%)
Taxi	21 (39.6%)
Donkey/horse	0
Other	0
Orphanhood	
Both parents alive	27 (50.9%)
One parent alive	14 (26.4%)
No parent alive	11 (20.8%)

BMI = body-mass index, IQR = interquartile range, NNRTI = nonnucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, VL = viral load, WHO = World Health Organization.

Out of 1563 patients analyzed, 1425 (91.2%) had VL below the detection limit of 80 c/mL, 138 had unsuppressed VL (28 (1.8%) VL 80–999 c/mL; 110 (7.0%) VL ≥1000 c/mL). Table 1 summarizes the characteristics of the 138 adult patients with unsuppressed VL. They all received information about their ongoing viremia within a median of 28 days (IQR: 22–31 days) after blood draw. Based on the clinical assessment by the study physician, 5 were clinically unstable and were switched without awaiting a follow-up VL.

Of the 133 patients with unsuppressed VL, 116 (87.2%) had a documented follow-up VL after EAC, and 22 failed to have a follow-up VL for the reasons summarized in Fig. 1A. The median time between first and follow-up VL was 107 days (IQR 97–121). Of the 116 adults with available follow-up VL-result, 36 (31.0%) achieved resuppression to <80 c/mL, 14 (12.1%) had a follow-up VL 80 to 999 c/mL, and 66 (56.9%) a VL ≥1000 c/mL (Fig. 1A). There was no significant association between participants' clinical and sociodemographic characteristics (Table 1) and viral resuppression at follow-up VL.

3.2. Enrolment of pediatric participants

Figure 1B details the pediatric cohort: out of a total of 240 children 44 (18.8%) were excluded due to prespecified exclusion criteria or missing data (5; 1.7%). Among the analyzed 191 pediatric patients for 138 (72.3%), the VL was below the detection limit, 7 (3.6%) had a VL of 80 to 999 c/mL, and 46 (24.1%) ≥1000 c/mL. Table 2 summarizes the characteristics of the 53 children with a detectable first VL. Caregivers of the 53 children received information about the unsuppressed VL within a median of 25 days (IQR 23–32 days) after blood draw, and 49 (92.5%) received EAC, and their children had a documented follow-up VL (Fig. 1B). Median time between first and second VL was 99 days (IQR 93–109 days). Of the 49 children with an unsuppressed first and a follow-up VL determination, 13 (26.5%) achieved resuppression to <80 c/mL, 6 (12.2%) had 80 to 999 c/mL, and the remaining 30 children a VL ≥1000 c/mL (Fig. 1B). There was no significant association between the participants' clinical and sociodemographic characteristics (Table 2) and viral resuppression at follow-up VL.

Virologic failure according to WHO definition and prevalence of at least "low-level drug resistance" against at least 2 drugs of the current first-line regimen in children and adults.

	N	DRM against ≥2 drugs of first line	DRM against <2 drugs of first line
Adult patients	74	66	8
Failures (first and follow-up VL ≥1000 c/mL)	61	53 (87%)	8 (13%)
Non-failures (first and/or follow-up VL 80–999 c/mL)	13	13 (100%)	0 (0%)
Pediatric patients	34	24	10
Failures (first and follow-up VL ≥1000 c/mL)	29	20 (69%)	9 (31%)
Non-failures (first and/or follow-up VL 80–999 c/mL)	5	4 (80%)	1 (20%)

DRM = drug-resistance mutation, VL = viral load.

3.3. WHO-defined failure and viral resistance

Genotyping was successful in 108 (93.1%) of the 116 patients with unsuppressed follow-up VL (74/80 adults; 34/36 children). For the remaining 8 samples no RT-PCR product could be obtained due to a low VL or variant HIV subtype.

For 90 patients (61 adults, 29 children) the WHO failure definition was fulfilled, and in 18 others first and/or follow-up VL were detectable but below 1000 c/mL. Of those 90 failing according to WHO definition 73 (81%) were found to harbor a virus resistant against ≥2 drugs of their current first-line regimen (at least "low-level resistance" according to Stanford db version 7.0). Assuming that all patients with VL<80 c/mL had a non-resistant virus, the WHO definition of failure had a sensitivity and specificity for the presence of a resistant virus of 81% and 99%, respectively. Of the 18 participants with elevated VL, who did not fulfill the WHO failure definition, 17 (94.4%) harbored resistant viruses (Table 3). Lowering the cut-off to 80 c/mL increased sensitivity to 100% while the specificity remained at 99%. The positive predictive value (PPV) for the presence of a resistant virus was 81.1% (95% CI: 72.2–88.9) using the WHO definition, and 83.3% (95% CI: 75.6–91.1) for a cut-off ≥80 c/mL.

The strict application of the WHO recommended cut-off of 1000 c/mL at first and follow-up VL for definition of failure would thus have missed 13/74 (17.6%) adults and 4/34 (11.8%) children with therapy-threatening viral resistances. Having a VL 80 to 999 c/mL at first and/or follow-up VL had a negative predictive value for a resistant virus of 5.6% (95% CI: 0%–16.7%). The 18 patients with VL 80 to 999 c/mL presented with a median VL of 360 c/mL (IQR: 266–738).

3.4. Detection of RT inhibitor mutations

Among adults, no major mutations for NNRTI and NRTI were present in 12.2% (9/74) and 13.5% (10/74) of all patients, respectively. Similarly, 7 (20.6%) and 8 (23.5%) children were found to harbor no major NNRTI- and NRTI-specific mutations, respectively. All 3 children on a lopinavir-based first-line therapy carried viruses that had no major PI mutations. Figures 1 and 2 of the Supplemental Digital Content, http://links.lww.com/MD/B62 (SDG) of this article display the frequencies of major NRTI- and NNRTI-resistance mutations among the 74 adult and 34 pediatric patients with successful HIV genotyping, who had presented with first- and second-VL above the plasma threshold of 80 c/mL. The SDG Table 1, http://links.lww.com/MD/B62 displays NRTI resistances stratified by the first-line NRTI backbone.

4. Discussion and conclusions

This prospective study conducted in 10 rural health facilities in Lesotho assessed the WHO definition of virologic ART failure by investigating the VL threshold of 1000 c/mL for the presence or absence of HIV drug resistance. One out of 5 patients fulfilling the WHO failure definition carried a virus with no relevant resistance against the current first-line regimen, indicating a persisting adherence issue. On the other hand, applying the strict WHO threshold of 1000 copies in 2 consecutive VLs resulted in a very significant misclassification of patients indeed harboring resistant viruses with VLs between 80 and 999 c/mL. In 17 of the 18 patients with sustained VL of 80 to 999 c/mL resistant virus was detected. More and more HIV programs in Sub Saharan Africa nowadays use modern VL platforms that have detection limits at below 1000 copies/mL. This fact implies that also in resource-limited settings the threshold for failure should be lowered to, for example, "100 c/mL at 2 consecutive VLs". This would enable an earlier detection of therapy failure and may thus help to conserve, particularly in resource-limited settings, the effectiveness of individual precious drugs in failing regimens.

The discrepancy between the current WHO failure threshold and the detection of relevant genotypic resistance in this study creates the situation that in resource-limited settings the switching of patients strictly according to WHO guidelines may prevent a considerable number of patients from receiving the needed second-line therapy, whereas others, who would not require second-line therapy at this point in time would be switched unnecessarily. Both, delayed switch as well as nonindicated switch to second-line, are of great clinical and public health relevance. Patients, retained on a first-line regimen without full viral suppression, are at risk of acquiring further resistance mutations[3,4] and may spread resistant HIV. Moreover, delayed switching is associated with poorer response to second-line therapy.[13,14]

In contrast, switching those patients to second-line, who present with no mutations against their first-line NRTI backbone has been linked to poor outcome in observational studies[15] and trials in resource-limited settings indicating unsolved adherence problems after switch to second-line.[16,17] In addition, the switch to a second-line regimen typically comes with a higher pill-burden and tends to cause more side effects. Moreover, as currently second-line regimens are still about 3 times more expensive than the first-line, every avoidable switch has also considerable economic implications for resource-limited settings.[18]

Despite intensified adherence support after a first unsuppressed VL 13% of the adults and 31% of all children fulfilling the WHO failure definition did not present any evidence for major viral resistance against the current first-line regimen (Table 3). This is consistent with related studies from similar settings, which showed that probably 10% to 20% of patients with sustained virologic failure are switched to second-line therapy without sufficient virological evidence for resistance.[19–22] These studies support the probably unique value of genotypic resistance testing

for resource-limited settings to discriminate true resistance-based failures and VL elevations due to nonadherence.[23] Modeling studies have already suggested that genotype-guided switching to second-line (i.e., switch only undertaken when resistance is detected), would be cost-effective.[24,25] To our knowledge, however, there are currently no trials assessing the cost-effectiveness of resistance testing in resource-limited settings.

A limitation of this study is the relatively low number of patients with unsuppressed VL at first measurement yielding only a relatively small sample size of the cohort followed-up for second VL. Of note, other reports from similar settings have reported significantly higher rates of unsuppressed VL.[26] Another limitation is that patients included in the study had a median time on first-line ART of more than 4 years. Because patients did not receive any VL monitoring prior to the study, the actual duration of the period of failing therapy before first measurement is unknown. This could have favored a steady accumulation of resistance mutations and might, in part, explain the rather low resuppression rates in this study as compared to other studies.[27]

In conclusion, our study provides evidence that the WHO-recommended VL threshold of 1000 c/mL is likely to miss a substantial part of patients on first-line ART with persisting virus replication below 1000 c/mL who carry drug-resistance mutations. Lowering the VL cut-off to as low as 80 c/mL did not lower the PPV for the detection of therapy-relevant resistance mutations in our study population. For programs using VL platforms with detection limits below 1000 c/mL, and if confirmed by additional studies, a revision of the current WHO threshold definition for virologic failure in resource-limited settings should be considered.

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