

Differentiated Care Pathways for Antiretroviral Therapy Monitoring in Malawi: Expanding Viral Load Testing in Setting of Highly Prevalent Resistance

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We quantified resistance to first-line antiretroviral therapy among previously unmonitored patients in Malawi with viremia (≥ 1000 copies/mL). Ninety-five percent ($n = 57/61$) harbored nucleoside/tide reverse transcriptase inhibitor/non-nucleoside reverse transcriptase inhibitor resistance; resistance was more common comparing >2 (97%) versus ≤ 2 years (87%) on therapy. Immediate switch for persons retained in care may improve monitoring efficiency and maximize clinical outcomes.

Keywords. antiretroviral therapy; human immunodeficiency virus (HIV); resistance; resource-limited settings; viral load monitoring.

Viral load (VL) testing is the preferred strategy for antiretroviral therapy (ART) monitoring in resource-limited settings [1]. Alternatives to plasma-based testing, including use of dried blood spots (DBSs), has facilitated expansion of VL testing to remote populations [2, 3]. As scale-up of VL testing proceeds, appropriate guidelines for regimen switching after viremia is identified require understanding human immunodeficiency virus (HIV) drug-resistance profiles among previously unmonitored patient populations.

Patients with treatment failure have repeatedly revealed high rates of first-line ART resistance. Failing patients in sub-Saharan

Africa demonstrate resistance rates ranging from 57% to 97% with at least 1 major non-nucleoside reverse transcriptase inhibitor (NNRTI) or nucleoside/tide reverse transcriptase inhibitor (NRTI) mutation [4–6]. Current VL monitoring guidelines require confirmation of viremia after 3 months [7, 8]. Designed to preserve first-line options, this algorithm delays treatment switch for patients with first-line drug resistance, compromising outcomes and contributing to ongoing transmissions [9, 10]. Although adherence counseling must remain central to ART programs, adjustment of algorithms to address resistance among previously unmonitored patients in the context of expanding VL testing may maximize ART outcomes.

We sought to quantify the prevalence of resistance to first-line ART among previously unmonitored patients in rural Malawi identified with virological failure [2]. Malawi's ART program includes approximately 500 000 persons alive and retained on ART; many have been retained in care for >4 years [11]. As VL monitoring is rolled out, understanding resistance patterns is essential for devising appropriate, resource-efficient confirmatory testing and treatment switch algorithms.

METHODS

Remnant specimens from a public health evaluation of DBSs for VL monitoring in Malawi were used for resistance testing [2, 12]. The study enrolled HIV-infected adults (≥ 18 years) from district hospital-based ART clinics in Malawi. Patients were eligible for testing if they had been on first-line therapy for 6 months, 24 months, or any 24-month period thereafter ± 3 months (routine monitoring) or if they met clinical failure criteria after 6 months on treatment. When a participant was enrolled under clinical failure criteria, time on ART was abstracted from health records rounded to the nearest 2-year time point for purposes of analysis. Persons with VL >5000 copies/mL received adherence counseling and confirmatory testing after 3 months. Patients were eligible for switch to second-line ART if confirmatory testing demonstrated continued viremia >5000 copies/mL.

Dried blood spot cards were collected using fingerstick or venous blood draws and underwent VL testing using Abbott RealTime HIV-1 Assay (Abbott Laboratories) [12]. Upon completion of the parent study, confirmatory cards with VLs ≥ 1000 copies/mL (or initial cards if no confirmatory specimen available) were shipped on dry ice to the United States for sequencing.

Dried blood spots are an alternative source to plasma for drug-resistance genotyping [13–15]. The mSample Preparation System was used for RNA extraction on the Abbott m2000sp (Abbott Laboratories). Depending on availability, 1–3 spots were

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eluted in 1.7 mL of RNA Lysis Buffer (Promega) and extracted using the Abbott 0.6-mL protocol. Previously described primers [16] did not produce amplicons; new primers for the reverse transcriptase (RT) gene were used. RNA was concentrated using RNA Clean and Concentrator columns (Zymo Research), eluting into 10 μ L of water. Concentrated RNA was used in a single cDNA synthesis reaction with 2 downstream primers (RT1Adn [5'-TAGGTATGGTGAATGCAGTATACTTCCT] and RT2Adn [5'-TGTACTGTCCATTTGTCAGGATG]) and SuperScript III reverse transcriptase (Invitrogen). One-quarter of the reaction was used in nested polymerase chain reaction using the Expand High Fidelity PCR System (Roche Life Science). First-round (labeled A) upstream primers were RT1Aup (5'-GGCCATTGACAGAAGAAAAATAAAAGC) and RT2Aup (5'-GAAGACTTCAGGAAGTATACTGC); second-round (labeled B) primers were RT1Bup (5'-TAAAAGCATTAACAGAAATTTGTACAGA), RT1Bdn (5'-TCCTGAAGTCTTCATCTAAAGGAAGTGA), RT2Bup (5'-TATACTGCATTCACCATACCTAG), and RT2Bdn (5'-TTGTCAGGATGGAGTTCATATCC). Two regions were amplified and sequenced, encompassing RT codons 41–116 (region RT1) and 135–230 (region RT2). Sequences were analyzed using Sequencher 5.3 software and submitted to Stanford University Drug Resistance Database (<http://hivdb.stanford.edu>) to determine mutations. Resistance was only scored for the region(s) that amplified.

Statistical analyses were performed using Stata (version 13.0; StataCorp). The National Health Sciences Research Committee of Malawi and the Biomedical Institutional Review Board at the University of North Carolina, Chapel Hill approved this study. All participants provided written informed consent for the parent study.

RESULTS

Among 1498 patients enrolled in the parent study, 88 (5.8%) met protocol-defined failure (initial VL \geq 5000 copies/mL); 90% ($n = 78/88$) had confirmatory testing. After adherence counseling, 24 of 78 (30.8%) participants had repeat VL <5000 copies/mL and 16.7% ($n = 13$) had VL <1000 copies/mL. Persons who resuppressed were more likely to have lower initial VL, but time on ART was similar (data not shown) [2]. We performed resistance testing on 75 specimens; 62 (83%) were confirmatory specimens. Both regions (RT codons 41–116 and 135–230) amplified for 43 of 75 (58%); 18 (24%) had partial amplification (41–115 [3] and 135–230 [14]). Neither region amplified for 14 of 75 (19%), consistent with historical sequencing success rates of fingerstick DBSs [13]. Most amplified specimens were obtained as confirmatory tests ($n = 49/61$); among initial specimens ($n = 12$) that amplified, 11 of 12 harbored resistance.

Among participants with full or partial amplification ($n = 61$), most (70.5%) were female; average age was 36, and average time

on ART was 4.1 years (SD = 2.3). Median VL among amplified specimens was 46 313 versus 5440 copies/mL among specimens without amplification (Mann-Whitney $P < .05$; 2-tailed).

Nearly 95% ($n = 57/61$) of amplified sequences demonstrated resistance to NRTI ($n = 51$) and/or NNRTI ($n = 55$) classes. Primary mutations within the NRTI class included M184M/V ($n = 48/57$), A62V ($n = 7/47$), D67N ($n = 7/47$), K65K/R ($n = 9/47$), and T215F/I/Y ($n = 16/57$). The most common NNRTI mutations included Y181C/Y/I/T ($n = 27/57$), G190A/G/R/S ($n = 18/57$), and K103K/N/S ($n = 17/47$). Most participants harbored high levels of resistance to lamivudine (3TC) (85.7%; $n = 48/56$); only a quarter (26.8%; $n = 11/41$) demonstrated high resistance to tenofovir. Among partially amplified specimens, 89% ($n = 16/18$) demonstrated resistance. Resistance tended to increase with duration of therapy: 87% ($n = 20/23$) of persons on therapy ≤ 2 years harbored resistance compared with 97% ($n = 37/38$) among those on therapy > 2 years ($P = .10$). This trend continued comparing persons on therapy ≤ 4 years to those on ART > 4 years (92% vs 96%; $P = .60$).

Participants with clinical failure had elevated VL compared with persons enrolled based on routine monitoring (11.4% vs 5.2%; $P = .01$) [2]. Resistance did not differ based on presence of clinical failure: 100% ($n = 14/14$) of clinical failure enrollees versus 91.5% ($n = 43/47$) among routine monitoring enrollees ($P = .26$) (Figure 1).

Among participants who resuppressed (<5000 copies/mL), 54.2% ($n = 13/24$) had detectable viremia ≥ 1000 copies/mL. Of these, 85% ($n = 11/13$) were available for resistance testing, 5 of 11 (45.5%) were sequenced successfully, and all 5 showed intermediate or high resistance to NRTIs or NNRTIs.

DISCUSSION

We observed a high rate of ART resistance among persons with elevated VLs in a cohort of retained, previously unmonitored ART patients in Malawi. The 95% resistance rate is comparable with elsewhere in sub-Saharan Africa [5, 17] but is considerably higher than reports from other Malawian cohorts, likely because of the proportion of persons on therapy > 2 years [18]. Among Malawian pregnant women initiated on ART and retained in care at 6 months, resistance was only 35% [19]. Our findings highlight the challenges that Malawi and other sub-Saharan African countries face in managing persons with newly identified virological failure in the context of extended, unmonitored ART.

Current guidelines require confirmatory testing for all patients, regardless of time on therapy [7]—a process that incurs additional costs and delays treatment switch to second-line therapy for persons with resistance. Among patients eligible for second-line therapy with confirmed elevated VL, average time from first elevated VL to initiation of second-line therapy was 181 days; this delay is even longer in other sub-Saharan African programs [2, 20, 21].

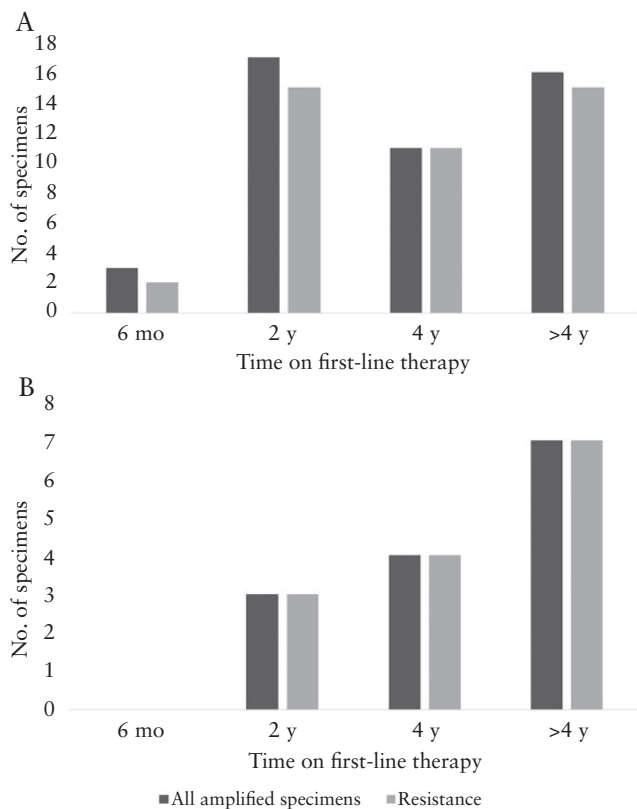


Figure 1. Frequency of resistance among amplified specimens according to time on therapy by participants enrolled under routine monitoring eligibility criteria (A) and participants enrolled under targeted eligibility criteria (ie, those with clinical signs of failure at time of enrollment) (B). Persons enrolled using routine criteria were eligible for 6 (+/-1), 24, or 48 months or any 24-month increment (+/-3). Persons enrolled under clinical criteria who were not also eligible based on antiretroviral therapy exposure time points were categorized as follows: 0–11 months “6 months”; 12–35 months “2 years”; 35–51 months “4 years”; and >51 months “>4 years.”

Differentiated care pathways may be 1 opportunity to improve efficiency and effectiveness of VL monitoring scale-up while maximizing ART outcomes. These pathways customize packages of services for patient subgroups. For example, VL-differentiated care is a cost-effective strategy where patients with suppressed viremia require fewer clinic visits [22]. Our results highlight another opportunity to differentiate HIV care pathways: the high rate of resistance among long-term patients with elevated VL represents a cadre of failing patients who could be switched immediately to second-line therapy, avoiding costs and delays of confirmatory testing. Indeed, many patients likely failed earlier, accumulating resistance mutations despite adequate adherence, with some estimates suggesting that median duration of ART prior to failure may be closer to 12 months [23]. For patients on ART for <24 months, adherence support should remain a mainstay of ART programs. But expedited switch pathways for long-term ART patients with high VL are needed to manage patients with viremia and resistance, which is fundamentally different from patients who may resuppress with

improved adherence. Individualized pathways are critical in the context of VL monitoring scale-up.

Our results suggest that providers consider immediate switch for patients retained in care >2 years with VLs >5000 copies/mL, the failure threshold in Malawi guidelines at the time of the study [24]. Importantly, we considered persons to be retained if they engaged in care based on ART clinic visits with or without documented adherence. Persons may have had preceding periods of missed visits and gaps in care, but available data do not permit further characterization of care retention. Low-level viremia (<1000 copies/mL) may also harbor clinically relevant resistance [25–27]. Resistance among persons presenting with VL <5000 copies/mL is particularly important given the updated WHO guidelines that define treatment failure thresholds at 1000 copies/mL [7], and although all amplified specimens between 1000–5000 copies/mL demonstrated resistance, only 50% of specimens amplified in this range. Unsuccessful DBS amplification was a limitation of this study and could represent an over- or underestimation of resistance rates. We hypothesize that substandard long-term storage conditions and fingerstick specimen source compromised extraction efforts [13, 14, 28]. Further advancements in DBS-based amplification techniques will help elucidate implications for those with lower-level viremia and will be critical to expand on the small sample size presented here. We did not assess any pretreatment resistance because study eligibility criteria required a minimum of 6 months on ART, but pretreatment resistance is likely uncommon [29]. The parent study identified a low failure rate (5.9%), and loss to follow-up was rare [2]. The low failure rate is likely due to eligibility-driven selection of persons retained in care, but our resistance results should be verified in settings with higher failure rates. Retention within the study could result in a subpopulation for whom viremia is more likely secondary to resistance rather than inadequate adherence, using retention in care as a proxy for adherence. However, given that this real-world evaluation used existing Ministry of Health infrastructure without patient incentives, study-related retention bias is unlikely. Finally, considering the all-comer nature of our cohort and the widespread use of DBSs for VL monitoring in many resource-limited settings, our results are generalizable.

Identifying efficient and effective means of scaling up VL testing in resource-limited settings requires innovative strategies. Point-of-care VL technologies will improve monitoring efficiency, but even inexpensive point of care assays incur treatment switch delays via mandatory 3-month confirmatory periods. We have identified a public health approach of differentiated care for previously unmonitored persons with extended ART exposure and newly identified virological failure. Although aggressive adherence counseling and confirmatory testing may be important early with ART, the resistance rate among persons on therapy >2 years demonstrates an opportunity to expedite second-line therapy transitions. Delaying second-line switch

for persons harboring resistance mutations has both financial and public health implications. Next steps should include an evaluation of the cost-effectiveness of the proposed differentiated care pathway as well as further evaluation of resistance profiles among persons retained in care with low-level viremia.

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