OPTIMIZING HIV THERAPY: OUTCOMES, CHALLENGES, AND OPPORTUNITIES FOR VIROLOGICAL MONITORING IN RESOURCE-LIMITED SETTINGS

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Health Policy and Management in the Gillings School of Global Public Health.

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ABSTRACT

Sarah E. Rutstein: Optimizing HIV Therapy: outcomes, challenges, and opportunities for virological monitoring in resource-limited settings
(Under the direction of Andrea K. Biddle)

Despite extraordinary advances in antiretroviral therapy (ART) coverage, only a fraction of the millions of African ART patients have access to routine viral load (VL) monitoring. The goal of this dissertation was to address how to design and implement effective, efficient, and feasible VL monitoring strategies in resource-limited settings. In Aim 1, we studied programmatic and clinical outcomes of dried blood spots (DBS) for VL monitoring, enrolling 1,498 ART patients from five district hospitals in Malawi. Result delivery was a challenge. Providers frequently failed to deliver available results, and nearly half (665/1498) of participants had a clinic visit without receiving results. Nonetheless, 80% of participants received results within 3 months. We observed a lower-than-expected failure rate; only 88 (5.9%) participants had an elevated VL (>5,000 copies/ml) at baseline. Most (92.6%) eligible patients initiated second-line therapy. In Aim 2, we interviewed 17 providers involved in the DBS study. Providers identified a complex set of interconnected barriers and facilitators to VL monitoring. Echoing Aim 1 results, providers described challenges with result delivery and tracking, exposing gaps in data management systems. For many providers, the study was the first time they used an objective marker of ART response to guide clinical management. Provider empowerment emerged as an unexpected facilitator of VL monitoring. In Aim 3, we used data from a Phase IV open-label trial to develop a risk score identifying persons with resistance among those with elevated VLs. Facilitating

eventual risk score implementation, we only used parameters likely available to providers in resource-limited settings. We developed three model iterations, increasingly more restrictive in terms of assumptions regarding availability of a patient's laboratory information. The sensitivity for the three models ranged from 10.0%-26.0%, and specificity ranged from 97.4%-99.5%. Our studies identified programs that reliably identified virological failure, are feasible and offer unexpected provider benefits in the resource-limited ART clinical setting, and equip providers with point-of-care algorithms facilitating rapid treatment change for patients with ART resistance. Together, these findings bring us closer towards our longer-term goal of optimizing ART use and improving the quality of ART management and HIV care delivered in resource-limited settings.

To my parents, for their love of adventure.

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3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
ACTG	AIDS Clinical Trials Group
ART	Antiretroviral therapy
ATV/r	Atazanavir/ritonavir
AUROC	Area under receiver operating characteristic
AZT	Zidovudine
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CRF	Case report form
d4T	Stavudine
DBS	Dried blood spot
EDTA	Ethylenediaminetetraacetic acid
EFV	Efavirenz
HIV	Human Immunodeficiency virus

HL	Hosemer-Lemeshow
HPTN	HIV Prevention and Trials Network
HAS	Health surveillance assistant
IRB	Institutional Review Board
LR	Likelihood ratio
LPV/r	Lopinavir/ritonavir
MOH	Ministry of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NPV	Negative predictive value
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OR	Odds ratio
PCR	Polymerase chain reaction
PEARLS	Prospective Evaluation of Antiretrovirals in Resource-Limited Settings
POC	Point-of-care
PPV	Positive predictive value
RLS	Resource-limited setting
ROV	Receiver operating characteristic

RR	Risk ratio
SEM	Social ecological model
SMS	Short Message Service
TDF	Tenofovir
TB	Tuberculosis
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNC	University of North Carolina
VL	Viral load
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

When used appropriately, antiretroviral therapy (ART) reduces viral load (VL) and improves quality of life for persons infected with HIV, reducing HIV-associated morbidity and mortality [1-6]. Global treatment access initiatives have resulted in millions receiving life-saving therapy in resource-limited settings, and the potential for reduced HIV transmission through early ART use has reinvigorated efforts to further increase access to therapy [7]. Eligibility for ART also has expanded under the revised 2013 World Health Organization (WHO) guidelines, raising the CD4 count threshold, and extending coverage to all pregnant or breastfeeding HIV-infected women as well as tuberculosis patients [8]. However, maximizing the potential benefits of ART requires appropriate selection of drug regimens and early identification of drug resistance [9]. With more than 9.7 million people receiving ART in low- and middle-income countries, 7.5 million of whom live in sub-Saharan Africa, the issue of how to appropriately monitor patients is now an urgent international issue [10-13]. Effective and acceptable treatment monitoring strategies in these settings remain unclear.

HIV viral suppression is a key indicator of successful treatment for HIV-infected patients on ART. An elevated VL indicates poor adherence or resistance [14-18]. Current VL monitoring algorithms require confirmatory testing in the event of an elevated initial test [8]. For patients harboring resistance, this requirement for a second test unnecessarily delays the treatment switch. Delaying ART switch for patients with resistance increases the risk of sexual transmission of ART-resistant strains [19-21], as well as the likelihood of subsequently failing second-line therapy [14-17, 22-24]. However, for patients with an elevated VL due to inadequate adherence, the confirmatory testing process allows for time

and adherence counseling that may result in improved pill-taking behavior and viral resuppression [25, 26]. Distinguishing between modifiable poor adherence and ART resistance is critical to reduce the spread of resistance and to increase the effectiveness of second-line therapies in the absence of resistance testing.

Only a fraction of the millions of African patients receiving ART have routine VL monitoring [27, 28]. Traditional VL tests used in developed countries are prohibitively expensive and too complex for routine use in sub-Saharan Africa [29]. Point-of-care (POC) VL tests that meet the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) are currently being evaluated [30], and may eventually transform the delivery of HIV care in resource-limited settings [31]. However, effective and affordable POC technologies are not yet available. The use of dried blood spots (DBS) for specimen collection and subsequent transport for testing at a centralized laboratory has emerged as an appealing alternative. By simplifying blood collection and specimen transport, as well as enabling longer-term storage of blood samples, DBS alleviates technological and cold-chain barriers, expanding access to VL monitoring in more remote settings [32-34], improving identification of ART failure [35, 36]. However, effectiveness of VL monitoring using DBS outside of controlled clinical trial settings is unknown.

DBS effectiveness relies on the ART providers appropriately screening and acting on VL results. The acceptability and feasibility of incorporating routine VL monitoring using DBS into existing ART clinics in sub-Saharan Africa has not been assessed. Providers on the front-line of HIV care delivery, including ART clinic coordinators, clinicians, nurses, health surveillance assistants (HSAs), and ART clerks, are all essential to the eventual success of any clinic-based VL monitoring strategy. Numerous potential barriers to implementation exist, some more logistical in nature such as the ability to reliably identify patients who are eligible for VL monitoring at a given ART visit, and others less explicit such as challenges

with patient education and counseling. Unanticipated benefits of VL testing are also important, including provider engagement in care given new monitoring tools, and empowering providers to make informed and timely regimen decisions based on previously-unavailable laboratory-based results.

Successful and sustainable scale-up of VL monitoring requires greater understanding of DBS technology limitations and provider-perceived challenges, and must consider opportunities to strategies to improve efficiency in identifying failing patients. Additional information about rates of virological failure among previously unmonitored ART patients will be essential to guide VL monitoring policy and roll-out. This dissertation assesses the programmatic and clinical outcomes from a real-world evaluation of DBS use for VL monitoring in the sub-Saharan African context, and examines barriers to implementation and optimization of VL monitoring in resource-limited settings. Specific aims include:

Aim 1: Evaluate the feasibility and effectiveness of using dried blood spots for viral load monitoring among ART patients in district hospitals in Malawi

DBS are an enticing alternative to traditional plasma-based VL monitoring strategies, potentially improving access to virological monitoring to ART patients in more remote settings within resource-limited settings. The Centers for Disease Control and Prevention (CDC)-funded public health evaluation (*An evaluation of use of dried blood spot specimens for viral load monitoring of antiretroviral therapy in Malawi* [hereafter "DBS study"] enrolled approximately 1,500 patients from five district hospitals in the central and southern regions of Malawi. The study collected baseline demographic characteristics, ART history and adherence, and relevant clinical history. In this aim, I describe characteristics of enrolled participants, as well as virological and treatment outcomes for this previously unmonitored ART population. Feasibility assessments are based on the proportion of participants

receiving VL results. I evaluated differences in characteristics between participants who are suppressed (<5,000 copies/ml) and those participants with elevated (>5,000 copies/ml) VLs using independent group t-tests (continuous variables) and Pearson's chi-squared tests (categorical variables). I investigated the association between ART history, participant characteristics, and virological failure (baseline VL >5,000 copies/ml) using logistic regression. Finally, I examined rates of virological resuppression among participants with an elevated VL at baseline.

Aim 2: To evaluate antiretroviral therapy provider acceptability and perceived benefits of and barriers to use of dried blood spots for virological monitoring in district hospitals in Malawi

Understanding provider acceptability regarding use of DBS for VL monitoring is essential to eventual successful implementation. Effective VL monitoring using DBS depends not only on accuracy of DBS as compared to the referent standard of plasma, but also on the willingness and ability of providers to identify patients who are eligible for VL testing. Furthermore, providers must be able to successfully collect specimens and complete necessary documentation. The evaluation of use of DBS for VL monitoring therefore encompasses the identification of eligible ART patients, feasibility of DBS specimen preparation, completion of documentation to link DBS cards to the ART patient, timely return of results once available, and making appropriate clinical decisions in terms of switching patients to second-line therapy.

I conducted 17 in-depth interviews with providers and other involved clinic personnel. Interviews explored provider-perceived barriers to and benefits of DBS for VL monitoring including: identifying eligible patients; specimen collection; specimen transport logistics; delivery of results to patients and ability to act on those results (i.e., switching to second-line therapy); perceived patient reactions, understanding, and acceptability; and lastly, approach

to adherence counseling. I also evaluated basic participant descriptors including number of years in their current position and the highest level of certification/education.

Qualitative data was organized and managed in Atlas.ti. Transcribed interviews were read to identify major concepts, potential codes, and additional central themes. Analysis of the coded data included an investigation of frequency of codes across participants, and mapping of codes and themes. I selected quotes that illustrated key findings relevant to the research question of VL from DBS implementation barriers and provider-perceived benefits.

Aim 3: Develop a predictive model to identify patients with resistance from a single elevated VL result.

Adherence counseling will be effective only for patients without resistance mutations. Delaying treatment changes for patients who are resistant to 1st-line ART increases his or her chances of transmitting resistant virus to a sexual partner and may reduce responsiveness to second-line ART once switched. Appropriate identification of patients who would benefit from immediate change in therapy is critical. I conducted a retrospective analysis using data from AIDS Clinical Trials Group (ACTG) 5175 trial conducted at sites around the world, including sub-Saharan Africa. Using these data, I developed a predictive model and associated risk score to distinguish patients with elevated VL secondary to biological resistance from patients for whom improved adherence may result in viral suppression. By developing and validating a risk score to distinguish resistance from inadequate adherence, the results of this study may improve the efficiency of VL monitoring and facilitate provider decision-making regarding regimen switches at the point of initial elevated VL, negating the costs and delays associated with the currently-required confirmatory VL. I anticipate these results may improve ART outcomes by allowing timelier regimen switches for patients with resistant virus.

Dissertation Outline

The dissertation is organized as follows: Chapter 2 outlines the current peer-reviewed literature on VL monitoring as it pertains to roll-out in resource-limited settings, as well as the gaps in this body of literature. This chapter provides background to the relevance and timeliness of the evaluation of VL monitoring strategies, highlighting opportunities and potential barriers to implementation in these settings. Chapter 3 outlines the methods for each proposed aim, including an overview of the study design, data sources, and analytic approaches summarized in an aim-by-aim format. Chapters 4 through 6 are the individual, self-contained manuscripts that correspond to aims 1 through 3, respectively. Chapter 7 summarizes the findings from each aim, describing the strengths and weaknesses of this body of work, the relevance to HIV treatment and treatment monitoring policies, and plans for future research. All cited references are presented in the references section at the end of the dissertation.

CHAPTER 2: BACKGROUND

HIV burden and expanding ART access in sub-Saharan Africa

Sub-Saharan Africa remains the region most heavily burdened by the HIV/AIDS epidemic, accounting for 71% of all persons living with HIV, 71% of all new HIV infections, and 73% of all AIDS-related deaths [13]. Although the rate of new infections in the region has slowed, the total number of persons living with HIV continues to increase as more people access life-extending ART. Nearly 12 million persons receive ART in low- and middle-income countries, nine million of whom live in sub-Saharan Africa (**Figure 2.1**) [37, 38]. Recently revised ART guidelines expand treatment eligibility, potentially leading to more than 20 million HIV infected persons on ART in Africa alone [13]. Identifying appropriate ART monitoring strategies in resource-limited settings is an urgent global health priority [10, 12, 13, 39-41].

Patient and public health benefits of ART

The morbidity and mortality benefits to the individual on ART are well known [42-47]. ART suppresses viral replication, preserving cell-mediated immune response and reducing incidence of opportunistic infections. Recognized benefits of ART spurred widespread implementation in some of the highest HIV-burdened settings, most notably through the joint WHO and UNAIDS “three by five” initiative, setting the goal of having three million persons on ART by 2005 [48].

ART also has important public health benefits. ART use is a promising HIV prevention opportunity, with well-managed therapy resulting in a 96%-reduction in transmission between serodiscordant couples [7]. HIV transmission is strongly correlated

with HIV concentration in body fluids (blood and genital fluids) (**Figure 2.2**) [49-51]. Treatment as prevention has gained traction with policy makers, with recent ART guidelines strongly advocating ART use in serodiscordant couples [8]. Importantly, both the individual and public health benefits of ART use are contingent upon the individual being virologically suppressed.

Virological suppression relies on the person being on the correct regimen, that is, the person's dominant viral strain is not resistant to the class of therapy they are prescribed, and that the person is adequately adherent to that regimen. Emphasis on HIV testing and ART scale-up have successfully increased HIV-awareness and the proportion of HIV-infected persons accessing therapy. However, testing and care-linking efforts are still falling short, with only 45% of all infected persons aware of their status (**Figure 2.3**) [37]. The trend extends to sub-Saharan Africa, where less than half of all HIV-infected persons are aware of their status; however, among HIV-infected persons who are aware of their status, 86% are on ART, and nearly three-quarters of these persons have achieved viral suppression. Although a tremendous public health achievement, these numbers demonstrate the substantial gap in ART management. Specifically, almost one-in-four persons who are on ART in sub-Saharan Africa are not virologically suppressed. However, only 2% of ART patients in sub-Saharan Africa are on second-line regimens [52]. Undiagnosed virological failure represents not only a tremendous waste of resources as governments continue to maintain persons on failing, ineffective therapies, but also threatens the effectiveness of treatment as prevention.

Virological suppression key to maximizing ART benefits

Virological suppression is essential to maximize the individual and public health benefits of ART, and adequate drug adherence is vital. Taking at least 95% of prescribed

doses is generally necessary to achieve virological suppression and to delay progression to AIDS [53, 54], though this threshold may be lower with the addition of non-nucleoside reverse-transcriptase inhibitors (NNRTI) or boosted protease inhibitor regimens [55]. Exposure to therapeutic agents without adequate adherence increases the risk of developing resistance and subsequent treatment failure. Sub-optimal drug levels due to inadequate adherence create selective pressure facilitating viral replication and mutation, potentially propagating the spread of resistant strains [53, 55].

In the event that the elevated VL is due to ART resistance, failure to switch patients to second-line therapy in a timely manner increases morbidity and mortality, the likelihood of second-line treatment failure, and transmission of resistant virus [14, 15, 17-19, 22-24, 56-58]. The objective of ART monitoring is to identify persons who are failing treatment, either due to modifiable adherence behavior or to resistance. Adequate, efficient, and cost-effective ART monitoring will be increasingly critical to sustain and eventually to improve long-term treatment outcomes as ART use expands.

Focus on Malawi

Malawi is among those countries hardest hit by the HIV pandemic with recent adult prevalence estimates >10% [59]. In 2003, in coordination with the WHO's three-by-five Initiative, Malawi initiated a government-sponsored ART program [60, 61]. Under this program, limited first- and second-line ART combination regimens are available free of charge through the public sector, or subsidized through the private sector [62, 63]. As of September 2013, more than 450,000 HIV-infected patients in Malawi were receiving ART, representing nearly three-quarters of all ART-eligible HIV-infected persons [64]. However, <1% of these patients were on second-line therapy. Although VL monitoring has been incorporated into HIV treatment and management policies, widespread implementation, particularly outside of the major metropolitan areas, is yet to be realized [65].

ART monitoring

VL testing is the preferred method for monitoring ART to identify potential adherence problems and treatment failures [8]. With VL monitoring, failing patients are identified sooner, facilitating earlier treatment switches [9, 20, 22, 57, 66-68]. Additionally, the avoidance of premature switching (i.e., switching a patient to second-line therapy when she or he is not actually failing first-line therapy) prevents the loss of potential life-years on first-line therapy and the costs associated with having patients on more expensive, complicated second-line regimens [69]. These concerns are especially relevant in resource-limited settings where third-line options are not widely available.

Unfortunately, numerous barriers prevent widespread implementation of VL testing in resource-limited settings. Traditional VL tests used in developed countries are prohibitively expensive and complex for routine use in resource-limited settings, such as sub-Saharan Africa [29]. VL tests cost approximately $30 per test, four to five times the cost of CD4 testing and much higher than the ~$1 for the widely-used HIV antibody test [70]. The VL testing platform can cost upwards of $250,000, including installation and training. VL testing also requires laboratory infrastructure for plasma processing, phlebotomy-trained providers, highly-trained laboratory personnel, and a continuous cold-chain to keep necessary specimens and reagents refrigerated [29]. Despite VL testing being widely accepted as an effective means of monitoring patients on therapy, its costs will need to decrease to meet acceptable cost-effectiveness thresholds for widespread implementation.

In the absence of well-equipped laboratories with polymerase chain reaction technology to detect virological failure, clinicians use WHO immunological and clinical staging criteria to identify treatment failure [8, 71]. Immunological responses are measured with CD4 cell counts; a lower CD4 count indicates a weakened immune system. However, laboratory investigations such as CD4 counts are not universally available: many clinics have neither laboratory facilities on-site nor phlebotomy staff available for venous draws. In

the absence of these tools and resources, patients are monitored mainly through clinical staging criteria, with immunological monitoring (i.e., CD4 counts) available in only a few clinical centers of excellence. Unfortunately, the sensitivity of both immunological and clinical staging for identifying treatment failure is highly variable and generally low (**Table 2.1**), and thus the utility of these approaches in driving treatment decisions has been mixed [16, 22, 72-84]. Specificity, or our ability to correctly identify persons who are not failing, is also variable with the different monitoring approaches. In general, evaluations of alternative monitoring strategies demonstrate high negative predictive values (i.e., a high probability that a person identified as "not failing" is in fact not failing therapy), but extremely low positive predictive values (i.e., a low probability that a person who is failing will be identified as "failing"). Without access to VL information, the rate of treatment failure misclassification is unacceptably high.

Rates of virological failure in sub-Saharan Africa range from 6% to 53%, depending on failure threshold, clinical setting, and ART exposure time [57, 85-92]. Pooled estimates from low- and middle-income countries suggest that 16% of ART patients fail by 12 months of ART exposure [90]. With only 2% of patients in sub-Saharan Africa on second-line regimens, the rate of treatment misclassification, namely *missing* patients who are failing first-line ART, is substantial [52].

Consequences of treatment failure misclassification

Serious consequences are associated with misclassification (i.e., a false positive or false negative evaluation of ART response). Prolonged exposure to ineffective drugs decreases ART efficacy, reduces effectiveness of second-line ART, and increases transmission of resistant virus [14-17]. Falsely identifying a patient as failing when she or he is still responding to first-line ART may result in prematurely switching him or her to a more complicated, costly second-line therapy. In addition, switching from first-line therapy while

virologically suppressed may be associated with increased risk of subsequent virological failure on second-line therapy [93].

Second-line combination therapy options are expanding in resource-limited settings, now including two different fixed dose drug combination options, but alternatives are still extremely limited (**Table 2.2** and **Table 2.3**). The cost of second-line options are declining with more competitive country-specific contracting, but drugs remain significantly more expensive than first-line options—up to ten times the cost per patient [65, 69]. Once a patient has been switched to second-line therapy, return to first-line ART is not possible. Third-line therapy options are not commonly available in sub-Saharan Africa.

Delaying treatment changes for patients failing first-line ART increases morbidity and mortality [29, 58, 94, 95] and may lead to accumulation of resistance mutations that compromise second-line ART response [14, 15, 17, 23, 24]. Failure to identify treatment failure using either immunological (i.e., CD4 count) or clinical criteria is associated with significant resistance to both nucleoside reverse transcriptase inhibitors (NRTI) and NNRTI [14]. Furthermore, severe immunosuppression at time of second-line treatment initiation, as seen with delayed identification of treatment failure, may be associated with increased mortality [17].

VL monitoring and adherence

In addition to identifying failing patients sooner compared to alternative ART monitoring strategies, VL monitoring also may improve rates of virological resuppression by promoting improved adherence—enhancing the person-level and population benefits of ART. WHO ART management guidelines recommend adherence counseling after an elevated VL test (i.e., ≥ 1,000 copies/ml), followed by repeat assessment in 3-6 months (**Figure 2.4**) [8]. The goal of adherence counseling is to improve drug-taking behavior in hopes of suppressing VL and allowing patients to remain on the less toxic, less expensive

first-line ART. VL information, coupled with adherence counseling, may improve virological outcomes [25]. Motivators and facilitators to adherence may be fundamentally different when patients are confronted with a test result that suggests increasing disease severity and treatment failure. For example, the most frequently identified factors associated with non-adherence include symptoms and drug side effects, lack of social support, complexity of the drug regimen, and low patient self-efficacy [96-101]. VL monitoring provides information that may directly or indirectly influence patient self-efficacy.

Virological suppression and the associated improved ART outcomes for the individual are necessary to achieve the full potential public health benefits of ART. Whether it is through resuppression due to reinforced adherence messages or earlier identification of virological failure and faster switch to second-line therapy, VL monitoring is key if treatment as prevention is to be realized outside of the controlled clinical trial setting. Indeed, in settings where routine VL monitoring is not available, ART as a tool for preventing transmission within serodiscordant couples may be less effective [102].

Virological monitoring in resource-limited settings: where do we go from here?

Dried blood spots an appealing alternative to traditional VL technologies

Despite the many advantages, resource-intensive conventional VL monitoring is neither widely available nor well-suited for use in resource-limited settings [28, 29, 40, 69, 103-107]. In advance of the much-anticipated but still unavailable point-of-care (POC) VL testing platforms [108-113], some strategies to streamline or increase the efficiency of VL testing include pooling specimens and targeting VL tests based on clinical or immunological criteria [8, 92, 114-118]. However, alternative specimen collection methodologies may be needed to address the barriers to plasma-based VL testing in resource-limited settings.

The use of DBS for specimen collection and subsequent transport to centralized testing laboratories has emerged as an appealing alternative to traditional, plasma-based VL testing [8, 32, 33, 35, 119-126]. In addition to alleviating the technological and cold-chain barriers for VL testing in more remote clinics, alternative DBS specimen collection options, such as fingerstick rather than venipuncture, could decrease associated costs by permitting task-shifting to lower-level providers and reducing consumable-associated expenses. Fingerstick specimen collection options also may expand monitoring capacity to health centers without phlebotomy capabilities [120]. Indeed, DBS collected from fingerstick by non-laboratory personnel compares well to both venous DBS as well as plasma for identifying virological failure [119].

Notwithstanding the potential advantages of DBS, the feasibility of routine VL monitoring from DBS in ART clinics in sub-Saharan Africa has not been assessed outside of controlled studies. Furthermore, the effectiveness of using DBS for VL monitoring in real-world settings, specifically if eligible patients are appropriately switched to second-line therapy, remains unknown.

Front-line ART providers key to successful virological monitoring implementation

The benefits of VL monitoring for patients and broader HIV prevention objectives are well documented – facilitating resuppression when accompanied by targeted adherence counseling, and referral to more efficacious second-line therapy when indicated. Ultimately, realization of these person- and population-level gains is contingent on ART provider behavior. VL monitoring alone does not increase the rate of switching to second-line ART [127]. The WHO *Technical and Operational Considerations for Implementing HIV Viral Load Testing* report emphasizes human resources, including clinic personnel, as a key component of the Phase II (Scale Up) stage for effective implementation of any VL monitoring programs (**Figure 2.5**) [128].

Understanding the barriers and facilitators of VL monitoring roll-out in resource-limited settings is essential for sustained programmatic success. The social ecological model (SEM) offers an inclusive perspective of factors that influence behaviors, facilitating comprehensive examination of individual and environmental circumstances that affect health [129-131]. A modified SEM framework incorporates patient, provider, facility, system, and policy factors, providing a holistic approach to understanding the barriers and facilitators of incorporating VL monitoring from a provider perspective (**Figure 2.6**).

More efficient monitoring: distinguishing resistance from modifiable adherence behavior

Distinguishing patients with modifiable poor adherence without resistance mutations from patients with resistance, for whom improved adherence will not result in viral resuppression, is critical to reduce the spread of resistance and improve effectiveness of second-line therapies. Current VL monitoring algorithms require confirmatory testing in the event of an elevated initial test – a two-step process that includes adherence counseling to improve pill-taking behavior and possibly to facilitate virological resuppression (**Figure 2.4**) [8]. However, for patients with resistant virus, requiring a second test unnecessarily postpones the treatment switch. The delays introduced with confirmatory testing are especially relevant for ART patients in resource-limited settings: programmatic obstacles and patient-related barriers (e.g., travel distance to clinic) may substantially increase the interval between baseline and confirmatory testing. Among patients with confirmed virological failure in South Africa, switch to second-line therapy took longer than five months from the point of confirmatory VL to treatment switch [85, 91]. Equally complex and considerably more expensive than traditional VL monitoring techniques, ART resistance testing is rarely available in resource-limited settings.

Risk scores can help providers in resource-limited settings appropriately target diagnostic and screening tests, thereby reducing cost [132]. A simple risk score algorithm

may help providers identify patients with probable ART resistance who could be switched to second-line therapy immediately without a confirmatory test. Applying a risk score with high specificity may result in meaningful public health benefits. Assuming virological suppression once on the appropriate second-line regimen, rapidly switching patients with resistance to more efficacious second-line therapy could reduce transmission of resistant viral strains and transmission overall.

Use of a risk score to distinguish patients with elevated viremia *and* resistance mutations from those patients without resistance mutations may not only facilitate faster switch to appropriate second-line regimens, but also may save scarce resources by avoiding unnecessary confirmatory testing. Applying a conservative estimate of treatment failure (16.0% at 12 months) would translate to more than one million ART-taking patients having an elevated VL in sub-Saharan Africa alone [90]. Even a modest reduction in confirmatory test volume as would be facilitated by use of a risk score could substantially reduce expenditures.

Point-of-care technology: horizon and obstacles

POC VL tests may be the key for scale-up of VL testing in resource-limited settings [133]. POC tests that meet the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) are currently being evaluated [30, 134]. Briefly, POC tests are diagnostic tools that can be used at clinics by providers and allow for same-day delivery of results, often within an hour. These diagnostic tools may eventually play an essential role in the management of HIV-positive patients receiving therapy, providing clinicians with vital information about an individual's response to therapy and more accurately identifying patients who are failing first-line ART and therefore require switch to a second-line regimen. However, effective, efficient, and affordable point-of-care technologies are not yet available for widespread implementation.

Scale-up of POC diagnostics has become a priority for many countries, including the Malawian Ministry of Health (MOH), which has convened a task-force to evaluate potential technologies targeting HIV monitoring and diagnostic tools [135]. The guidelines in development by this task force identify numerous potential positive impacts of widespread implementation of POC testing, including: (1) ease-of-use, (2) long shelf-life, (3) potential increases in patient retention on ART (using CD4 POC tests), (4) reduced need for costly and logistically challenging specimen transport, (5) task-shifting away from over-burdened laboratory technicians, and, (6) improvements in patient outcomes by enabling health workers to make treatment decisions in a more timely manner. POC testing helps overcome the poor adaptation of technologies originally created for developed countries, technologies that require complex specimen transportation, progressive laboratory infrastructure, and advanced patient tracking systems.

Important limitations of POC technologies, also recognized by the Malawian task force, include: (1) lower per-device throughput; (2) additional responsibilities for health center staff; (3) challenges in managing supply chain, quality control and training; and, (4) difficulty monitoring testing data in the decentralized setting. Important considerations for platform selection include specimen type (i.e., plasma versus venous whole blood versus fingerstick) and qualitative (i.e., present vs. absent) or quantitative (i.e., copies/ml) VL detection capabilities. Throughput may be one of the principal barriers to POC devices achieving the stated implementation goals. Currently, most platforms under development use a modular system, with each test requiring 60-90 minutes [70]. Even if the maximum daily throughput is sufficient for lower volume clinics (most platforms can accommodate eight samples per day), the reality of returning patient results during the same visit is compromised when only two specimens can be running at any time. Queuing for hours at a time in order to receive same-day results may not be feasible in most ART clinics.

POC testing is unlikely to replace centralized testing and DBS in the near future, but these technologies could eventually facilitate VL testing expansion to more remote settings. Two products are identified in the current technology pipeline as likely to be launched in 2014 [70]. Insufficient funding and technological challenges continue to impede their release. Nonetheless, continued emphasis on VL monitoring, by funding agencies and international ART management guidelines, may expedite the introduction of these devices.

Significance and contribution

Maximizing the morbidity and mortality benefits of ART, as well as the public health potential of treatment as prevention, requires routine assessment of a patient's response to treatment. VL monitoring identifies patients who are failing sooner and is the preferred monitoring approach for both resource-wealthy and resource-limited settings. However, virological monitoring for HIV-infected persons on ART in sub-Saharan Africa has historically been overshadowed by the need for urgent scale-up of ART access. Efficient and feasible VL monitoring strategies will be increasingly critical to improve long-term treatment outcomes as ART use continues to expand.

In this study, we have evaluated implementation of alternative VL monitoring strategies in resource-limited settings. We explored the effectiveness and feasibility of implementation of DBS for VL monitoring in Malawi. Although numerous studies have demonstrated the comparability of DBS to the referent standard plasma-based testing, our study extends previous investigations by evaluating the effectiveness and feasibility of a centralized testing model in clinical settings. We also have examined provider perceived barriers and facilitators of VL monitoring. ART providers are essential to successful implementation of any monitoring strategy. Providers' ability and willingness to act on laboratory outcomes and implement indicated treatment switches are critical to realize the benefits of VL monitoring. Through in-depth interviews with providers at clinics implementing

DBS for VL monitoring, our study uncovers important multilevel motivators and obstacles that should be addressed by policymakers looking to scale-up VL monitoring.

Our study also identified opportunities to improve efficiency of VL monitoring by identifying patients with resistance among those patients with high VLs. Patients with resistance who are not switched to appropriate second-line regimens in a timely manner are at increased risk of poorer outcomes and are more likely to transmit resistant virus. The requirement of confirmatory testing in current VL monitoring algorithms may unnecessarily delay treatment switch, particularly for patients in resource-limited settings where programmatic obstacles and patient-related barriers could further increase the interval between baseline and confirmatory VL testing. By developing a risk score using only variables routinely available in resource-limited settings, our study may help to reduce delays for patients with resistance to first-line ART. The cost of VL testing will remain a barrier to widespread implementation for the foreseeable future – especially as the number of patients on ART increase and, with it the demand for VL testing. Use of the risk score also could have economic advantages, avoiding unnecessary confirmatory testing expenses.

Improved ART monitoring is urgently needed for the nearly 10 million persons on therapy in resource-limited settings. Taken together, our studies can provide new insights to improve the quality of care for ART patients in sub-Saharan Africa, and resource-limited settings more broadly, by maximizing therapeutic outcomes through treatment monitoring. These studies will contribute to the development of effective, acceptable, and efficient evidence-based guidelines for ART monitoring.

Table 2.1: Precision of WHO clinical and immunologic criteria in identifying treatment failure in resource-limited settings.

Report	Setting	Patients (n)	Virological failure definition (copies/ml)	Prev (%)	Sens (%)	Spec (%)	PPV	NPV
ART-LINC [72, 136]	Multi-site	2009	VL >10,000	3.1	17	97.1	9.5	98.5
Chaiworth *et al. [74]*[1]	Thailand	327	VL>50	10.7	10	95.6	25	91.6
Lynen *et al. [137]*	Cambodia	764	VL >1,000	8	29	90	12	96
Mee *et al. [16]*	South Africa	324	VL <10,000 or VL>1,000 or two VL>400	10	33	86	21	92
Meya *et al. [77]*	Uganda	496	VL >1,000	8	31	87	16	94
Moore *et al. [138]*	Uganda	39	VL ≥ 500	NA*	8	98	16	96
Palombi *et al. [139]*[2]	Multi-site	158	VL >10,000	NA	73.6	30.2	NA	NA
Reynolds *et al. [79]*	Uganda	1133	Two VL >400	11	23	90	14	94

[1]Presenting values for calculated values based on assessment with clinical criteria alone; [2]Treatment switches were confirmed with virological testing, helping to explain the significantly increased sensitivity observed in this study.
*Study analyzed only subjects with elevated viral load after 12 months
NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; Prev, prevalence of immunologic failure; Sens, sensitivity; Spec, specificity; VL, viral load; WHO, World Health Organization

Table 2.2: Antiretroviral drug classifications

Drugs	Mode of Action	Dosing interval
d4T, AZT, ABC	Reverse transcriptase inhibitor (NRTI)	12-hourly
3TC	Reverse transcriptase inhibitor (NRTI)	12- or 24-hourly
TDF	Reverse transcriptase inhibitor (NRTI)	24-hourly
NVP	Reverse transcriptase inhibitor (NNRTI)	12-hourly
EFV	Reverse transcriptase inhibitor (NNRTI)	24-hourly
ATV/r	Protease inhibitor	24-hourly
LPV/r	Protease inhibitor	12-hourly

3TC, Lamivudine; ABC, Abacavir; ATV/r, Atazanvir/ritonavir; AZT, Zidovudine; d4T, Stavudine; EFV, Efavirenz; LPV/r, Lopinavir/ritonavir; NRTI, nucleoside reverse transcriptase inhibitor; NPV, Nevirapine; NRTI, non-nucleoside reverse transcriptase inhibitor; TDF, Tenofovir

Table 2.3: Standard ART regimens in Malawi[1]

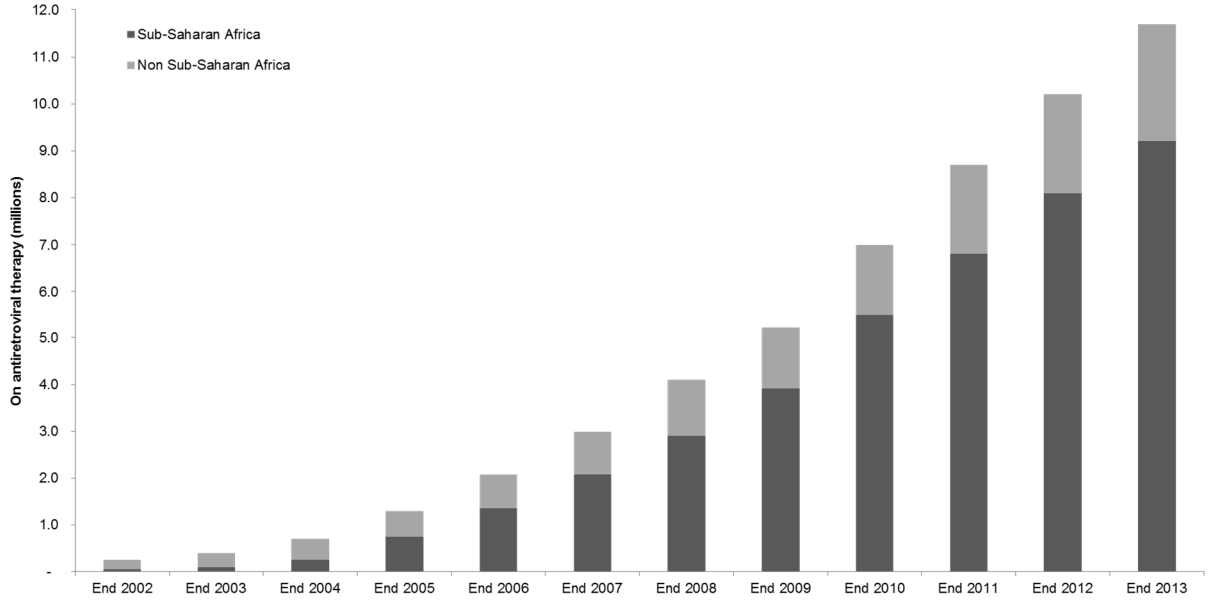
Adult Formulation	Contraindications	Possible Adverse Events
Standard First-line ART (initiation)		
ABC 600mg/ 3TC 300mg & NVP 200mg	• ABC hypersensitivity • Jaundice/hepatitis	• Fever, body pains, vomiting, cough[2] • Hepatitis • Skin rash • Lipodystrophy • Lactic acidosis
d4T 30mg/ 3TC 150mg/ NVP 200mg	• Jaundice/hepatitis	• Neuropathy • Hepatitis • Skin rash • Lipodystrophy • Lactic acidosis
AZT 300mg/ 3TC 150mg/ NVP 200mg	• Anemia < 8g/dL • Jaundice/hepatitis	• Anemia • Hepatitis • Skin rash • Lipodystrophy • Lactic acidosis
TDF 300mg/ 3TC 300mg/ EFV 600mg	• History of psychiatric illness • Renal failure • Child under 12 years	• Renal failure • Hepatitis • Skin rash • Psychiatric disorder
Alternative First-line ART		
d4T 30mg/ 3TC 150mg & EFV 600mg	• History of psychiatric illness	• Neuropathy • Hepatitis • Skin rash • Psychiatric disorder • Lipodystrophy • Lactic acidosis
AZT 300mg/ 3TC 150mg & EFV 600mg	• Anemia (hemoglobin < 8g/dL) • History of psychiatric illness	• Anemia • Hepatitis • Skin rash • Psychiatric disorder • Lipodystrophy • Lactic acidosis
TDF 300mg/ 3TC 300mg & NVP 200mg	• Jaundice/hepatitis • Renal failure • Child under 12 years	• Renal failure • Hepatitis • Skin rash
Second-line ART[3]		
TDF 300mg/ 3TC 300mg & ATV/r 300/100	• Renal failure • Child under 12 years	• Renal failure • Nausea/vomiting • Jaundice
AZT 300mg/	• Anemia < 8g/dL	• Anemia • Nausea/vomiting

Table 2.3: Standard ART regimens in Malawi[1]

Adult Formulation	Contraindications	Possible Adverse Events
3TC 150mg & ATV/r 300/100		• Jaundice
Third-line	*Currently available only through clinical studies.*	

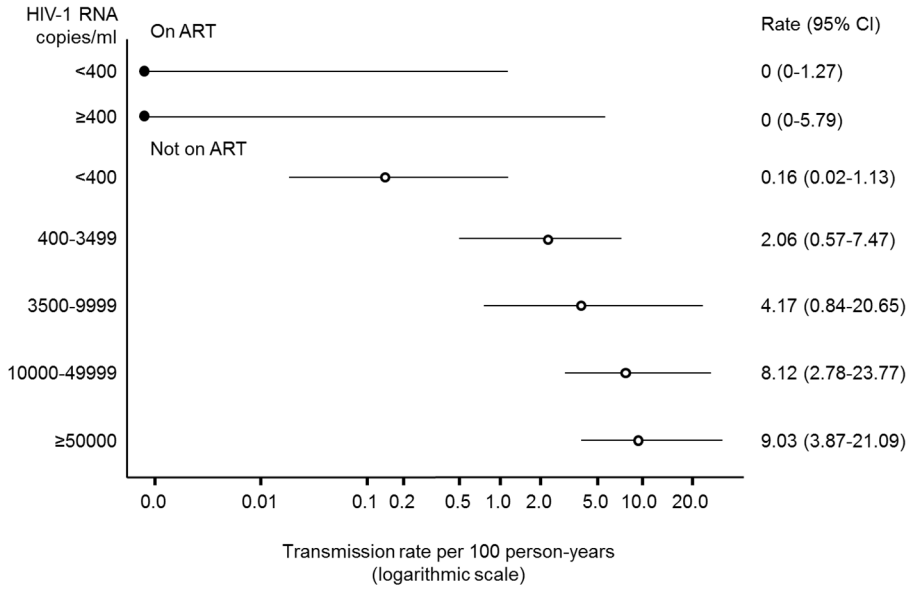
3TC, Lamivudine; ART, antiretroviral therapy; ATV/r, Atazanavir/ritonavir; AZT, Zidovudine; d4T, Stavudine; EFV, Efavirenz; LPV/r, Lopinavir/ritonavir; NPV, Nevirapine; TB, tuberculosis; TDF, Tenofovir
[1]Adapted from Malawi Ministry of Health ART guidelines[140], [2]Symptoms indicative of potential life-threatening ABC hypersensitivity, [3]Patients with pre-existing jaundice or suspected hepatitis should be started on Lopinavir/ritonavir (LPV/r) instead of ATV/r.

Figure 2.1: Number of people receiving antiretroviral therapy in low- and middle-income countries



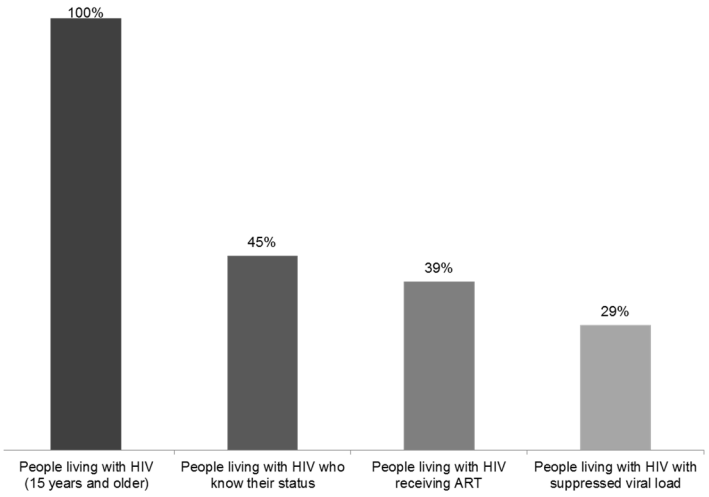
Caption: Figure 2.1: There has been tremendous expansion of access to antiretroviral therapy over the past decade [37, 38]. World Health Organization guidelines, which serve as the basis for determining antiretroviral therapy eligibility in many countries, continue to expand criteria emphasizing increased access for all HIV-infected pregnant women, and stressing the role of treatment as prevention within serodiscordant couples

Figure 2.2: Relationship between VL and transmission



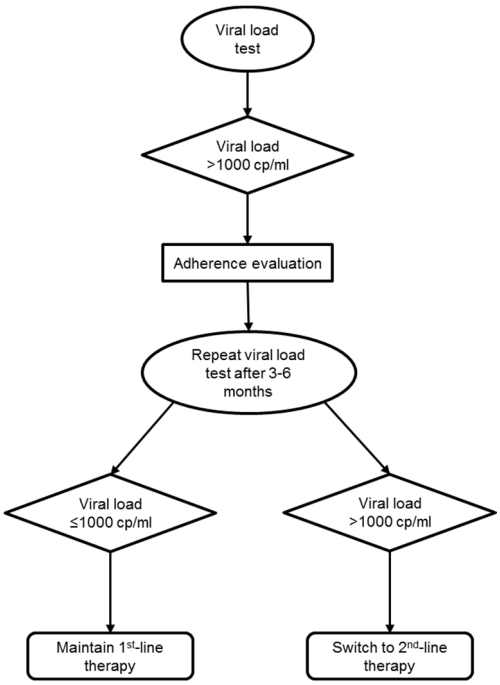
Caption: Figure 2.2: In a systematic review of studies assessing transmission within serodiscordant couples, higher transmission rates are consistently observed with higher VL among persons not on ART (*reproduced with permission)* [51]. The relationship between virological suppression, as facilitated by ART, and reduced risk of HIV transmission has been more definitely demonstrated in the context of a randomized clinical trial, with 96%-reduction in transmission [7]. ART, antiretroviral therapy; VL, viral load

Figure 2.3: Cascade of HIV treatment for adults (≥15 years old) in sub-Saharan Africa



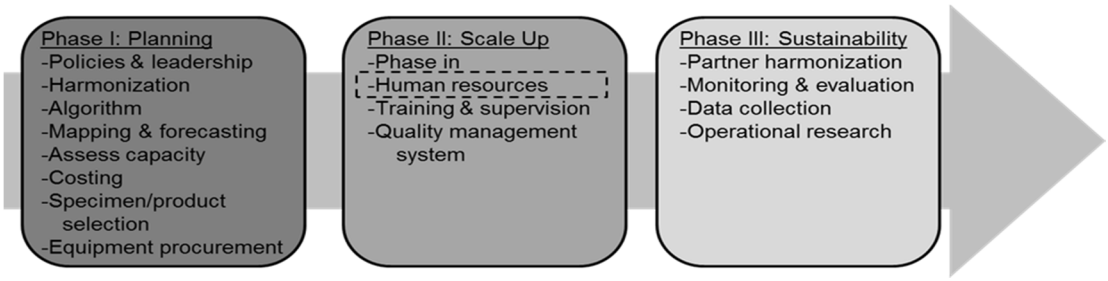
Caption: Figure 2.3: Substantial gaps in linking and retaining HIV-infected adults in care compromise the potential of treatment as prevention [37]. Although diagnosis remains the largest drop-off in this cascade, undetected virological failure is also a significant concern and improved coverage of both HIV testing and adequate treatment monitoring will be essential to achieve the prevention promise of expanded antiretroviral therapy coverage.

Figure 2.4: WHO VL monitoring algorithm



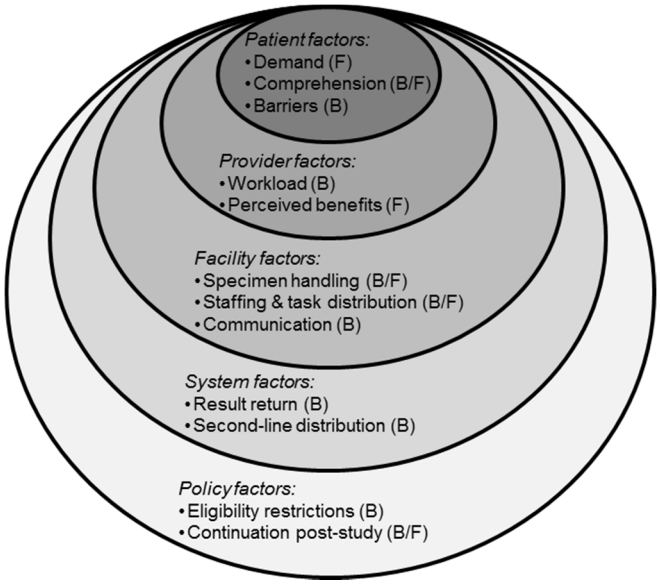
Caption: Figure 2.4: The WHO recommends, where feasible, use of VL monitoring to replace immunological and clinical criteria [8]. Adherence counseling and confirmatory testing are incorporated into the algorithm to minimize inappropriate second-line switches and preserve first-line options. Importantly, the 1,000 copy/ml (cp/ml) threshold is specified for plasma-based evaluations, appreciating that alternative thresholds may be more appropriate for DBS-based assessments. DBS, dried blood spot, VL, viral load; WHO, World Health Organization

Figure 2.5: World Health Organization VL Monitoring Scale-up



Caption: Figure 2.5: Phased implementation of VL monitoring as described in the World Health Organization's Technical and Operational Considerations for Implementing HIV Viral Load Testing identifies human resources, including training ART providers, in Phase II of the scale-up activities[128]. ART, antiretrovireal therapy; VL, viral load.

Figure 2.6: Modified social ecological model framing barriers and facilitators of virological monitoring implementation



Caption: Figure 2.6: The SEM provides a suitable conceptual model, encompassing the multilevel factors that relate to provider acceptability, perceived barriers and facilitators of VL monitoring using DBS. Our modified SEM examines the patient, provider, facility, system and policy factors examined in our assessment of barriers to (B), and facilitators (F) of, incorporating VL monitoring into clinical practice.

CHAPTER 3: METHODS

Overview and study design

This study comprises three distinct studies, each of which utilize different data sets and methodologies but all of which address the same fundamental research question: how can we design and implement effective, efficient, and feasible VL monitoring strategies in resource-limited settings? Methods for each aim are presented below.

Aim 1: Evaluate the feasibility and effectiveness of using dried blood spots for viral load monitoring among ART patients in district hospitals in Malawi

Study population

We enrolled adult (≥18 years) patients from five ART clinics in central and southern Malawi. Inclusion and exclusion criteria mirrored MOH eligibility criteria for routine and targeted VL monitoring [65]. Patients were eligible for VL testing if they were on first-line ART for 6 months, 24 months, or any 24-month period (+/- 3 months) thereafter (milestone-driven monitoring). Alternatively, patients who did not meet milestone criteria were eligible if they were on first-line therapy ≥6 months and showed signs of clinical failure (World Health Organization [WHO] Stage 3 or 4) (targeted monitoring). Patients were excluded if currently hospitalized, imprisoned, or involuntarily incarcerated in a medical facility.

Site selection and enrollment

ART clinics within district hospitals were selected based on the size of their retained ART patient population and willingness to both train providers and enroll participants. We validated DBS vs. plasma VL at the two sites with adequate capacity for plasma-processing.

During this validation period, all participants provided a venous and fingerstick sample from which a plasma sample, venous DBS (vDBS), and fingerstick DBS (fsDBS) were produced. Interim analyses demonstrated acceptable agreement between plasma, vDBS, and fsDBS [119], and upon expansion to the remaining three sites, participants received fsDBS only to simplify specimen collection procedures.

Sample collection and transport

Sample collection and virological testing methods are presented elsewhere [119]. Briefly, sites were provided with pre-packed kits containing: DBS card, capillary tubes, gloves, sterile lancet, alcohol swab, plastic zip bag, and desiccant. All specimens were collected by ART clinic or laboratory staff. Once dried, cards were transferred to individual zip bags with desiccant sachets and stored at room temperature.

DBS specimens were transported at ambient temperature to the central laboratory in Lilongwe (4-6 hours away) approximately weekly using existing hospital-based vehicles or shipped via specimen shipment service.

VL testing and result return

Specimens were tested using the Abbott RealTime HIV-1 Assay (Abbott Laboratories, Chicago, IL) (reportable range of 40 to 10,000,000 copies/ml for plasma and lower limit of detection of 550 copies/ml for DBS). Testing was conducted at an internationally monitored research laboratory.

Results were returned to clinics using e-mail, short message service (SMS), or phone. Hard-copies of results were delivered via hospital vehicles returning to the clinic or by study coordinators during routine (approximately bi-weekly) site visits. Providers delivered results to participants during scheduled clinic visits.

Data collection

All activities were conducted by non-study ART clinic personnel. ART staff members were trained in identifying eligible participants, obtaining consent, specimen collection, study sensitization, adherence counseling, and case report form (CRF) completion. We collected participant demographics, clinical history, and ART adherence data (**Appendix 1**). ART history, including date of diagnosis, ART initiation, and reason for initiation, was abstracted from patient clinic records.

Study visits

Participants were asked to return for VL results one month after enrollment. Participants with elevated VLs (>5,000 copies/ml) were counseled on the importance of adherence and instructed to return after two months for a confirmatory draw. Participants who returned for confirmatory draws were told to return within one month for results. Providers were instructed to refer patients with two elevated VLs for second-line therapy.

Treatment failure definition

Per 2011 MOH guidelines, virological failure was defined as having two sequential VLs >5,000 copies/ml [65]. For patients with plasma results available (validation period), plasma results were used to guide treatment decisions. If vDBS and fsDBS were available, vDBS results were used; fsDBS was used for treatment decisions in all other cases. “Undetectable” results (i.e., results below the platform’s lower limit of detection) were treated as having a value at the midpoint between 0 and the lower detection threshold (20 copies/ml and 275 copies/ml for plasma and DBS, respectively).

Programmatic Outcomes

Primary outcomes were feasibility and effectiveness of DBS for VL monitoring. Feasibility was measured by: proportion of participants receiving VL results within 3 months of enrollment; laboratory testing turnaround time and associated delayed result return (participant seen at clinic but result unavailable); frequency of "provider misses" (participant seen at clinic but did not receive results despite being available at the clinic); proportion of participants with baseline elevated VL receiving confirmatory DBS; time from participant receipt of results to collection of confirmatory specimen; and time from enrollment to second-line treatment initiation among eligible participants. Participants were terminated from the study if results were not delivered ≥6 months of enrollment.

Effectiveness measures of DBS for VL monitoring included: proportion of participants who resuppressed (≤5000 copies/ml on confirmatory specimen) and proportion of eligible participants who initiated second-line therapy within 12 months (365 days) of enrollment.

Statistical methods

We used student's t-tests (continuous variables) and Pearson's chi-squared or Fisher's exact test (categorical variables) to identify demographics and clinical characteristics associated with VL failure and resuppression (≤5000 copies/ml) [141]. We used generalized linear models with a log link and binomial distribution to explore the relationship between time on ART and VL failure (>5000 copies/ml) at enrollment [142]. Factors considered included age, sex, WHO clinical stage at ART initiation, body mass index (BMI), ART regimen, self-reported adherence, and clinical symptoms. We used likelihood ratio (LR) tests to decide which variables to include. We tested interactions between time on ART and symptoms at enrollment to asses if the effect of ART exposure on likelihood of treatment failure was different for participants who showed signs of clinical failure. We evaluated agreement of time on ART (clinic records versus CRFs) using kappa

statistics [143]. We conducted an post-hoc sub-group analysis exploring the relationship between CD4 cell count at ART initiation and treatment failure as this may be an important predictor of virological failure [85, 144, 145].

All analyses were performed using Stata (version 13.0; StataCorp, College Station, TX). P-values <0.05 were considered significant.

Ethical approval

The National Health Sciences Research Committee of Malawi, the Centers for Disease Control and Prevention Ethics Review, and the Biomedical Institutional Review Board at University of North Carolina, Chapel Hill approved this study. All participants provided written informed consent.

Aim 2: To evaluate antiretroviral therapy provider acceptability and perceived benefits of and barriers to use of dried blood spots for virological monitoring in district hospitals in Malawi

Parent study

The parent study was a public health evaluation of DBS for VL monitoring at five ART clinics in central and southern Malawi [119, 146]. ART clinics affiliated with district hospitals were identified based on pre-existing relationships between study leadership and nationwide laboratory mentoring projects. All providers received a 2-day training on study protocols including DBS collection (**Figure 3.1**). The central lab was approximately a 3- to 4-hour drive from clinics in the central region, and 4-6 hours from clinics in the southern region. VL results were returned to the clinic using a combination of e-mail, short message service (SMS), mobile phone, and hand-delivered hardcopy results. To retain confidentiality, SMS and email messages identified patients using only unique patient IDs.

Study Population

Between July 2013 and January 2014, we conducted in-person interviews with all providers who were involved in DBS study activities at each of the five enrolling clinics. Provider responsibilities ranged from retrieval of patient records to patient counseling and second-line ART referral. We identified providers via onsite point-persons–frequently a nurse who assumed additional study-related duties[147]. All providers agreed to participate and gave written informed consent.

Interview guide & conceptual model

We developed the interview guide to explore providers' perceptions of the barriers and facilitators to implementing VL monitoring (**Appendix 2**). The interview guide encouraged discussion and a flexible conversation about specimen handling, return of results to the clinic, and their overall reaction to VL testing activities. We used probing questions to explore emerging themes. All interviews were conducted in English by trained study staff and audio-recorded. Audio-recorded interviews were transcribed [147, 148].

Analysis

All transcripts were coded by the primary researcher (SER) using ATLAS.ti (version 7.0, ATLAS.ti Scientific Software Development GmbH, Berlin, Germany) [149]. A second coder (SH), independently coded 30% of transcripts. The two coders reviewed double-coded transcripts, and any differences in code application were resolved through discussion and negotiated consensus [148, 150].

We based initial structural codes upon interview topics, such as specimen handling and returning VL results to clinic. Thematic content analysis of transcripts guided identification, analysis, and reporting of themes [151]. We reviewed transcripts for broad concepts and used early memos to generate an initial codebook [152]. As more complex

themes emerged, we coded in more depth, revising and accumulating codes [153]. As new themes were added to the coding process, previously coded transcripts were reviewed to ensure coding logic completeness and consistency. The codebook was therefore a living document–adapting to the themes and concepts as they surfaced during analysis. When we completed the coding, we conducted a line-by-line analysis to ensure that all coded transcripts reflected the final codebook [152].

The social ecological model (SEM) emerged as a useful perspective for evaluating the multilevel factors that influenced provider-reported barriers and facilitators of VL monitoring. The SEM facilitated a comprehensive examination of individual and environmental circumstances that effected provider perceptions[129-131]. At its most basic level, the SEM considers two key concepts: 1) that behavior affects and is affected by multiple different levels, and 2) that individual behaviors are shaped by a larger social environment. Because our data had acquired a clear, multilevel nature reminiscent of the SEM, we grouped themes according to the five levels of the modified SEM framework: patient, provider, facility, system, and policy factors (**Figure 3.2**) [154]. These levels represent a contextual adaptation from the original SEM, which included intrapersonal, interpersonal, organizational, community, and public policy levels. We present illustrative representative quotes for each emergent theme. This strategy provides a holistic approach to understanding the barriers and facilitators of incorporating VL monitoring from a provider perspective

Ethical approval

The National Health Sciences Research Committee of Malawi, the Centers for Disease Control and Prevention Ethics Review, and the Biomedical Institutional Review Board at University of North Carolina, Chapel Hill approved this study.

Aim 3: Develop a predictive model to identify patients with resistance from a single elevated VL result

Study setting and population

We studied eligible participants enrolled in the Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) trial (Adult AIDS Clinical Trials Group (ACTG) A5175, NCT00084136). PEARLS was an open-label, Phase IV, randomized clinical trial that investigated efficacy and safety of once- vs. twice-daily regimen dosing. Details of the PEARLS study population and design have been described elsewhere [155]. In brief, A5175 enrolled 1,571 HIV-infected participants ≥18 years old from nine countries, over-sampling participants from resource-limited settings. Participants were excluded if they: had a CD4 cell count >300 cells/mm^3, previous exposure to ART (with an exception for women who received ART for prevention of mother-to-child transmission), were pregnant, or were acutely ill and/or clinically unstable. PEARLS was approved by institutional review boards and ethics committees at participating institutions.

Our study is a secondary analysis of de-identified data among participants initiated on nucleoside reverse transcriptase inhibitor (NRTI)-based regimens and who had a VL ≥1000 copies/ml at any point after week 16 of study enrollment. The 16-week restriction was based on A5175 definitions of virological failure (two successive measurements of plasma HIV-1 RNA ≥1000copies/ml, with the elevated VL on or after week 16). Primary analyses included participants from all study sites, with a sensitivity analysis restricting the study population to participants enrolled from resource-limited settings. This analysis was approved by the University of North Carolina, School of Medicine Institutional Review Board.

Data collection

Per A5175 study protocol, participants received a targeted physical exam, adherence interview, serum chemistries, CD4 lymphocyte count, and plasma HIV RNA (Roche

Amplicor Monitor assay [v1.5]) at least every eight weeks. Any treatment modification (participant, provider, or protocol-mandated) was assessed at each visit. Diagnosis criteria were collected using a standardized case report form.

Resistance tests were done retrospectively (Celera Diagnostics ViroSeq HIV-1 Genotyping Assay) on stored specimens for participants meeting virological failure criteria (defined below) or who had disease progression (new or recurrent AIDS-defining opportunistic infection or malignancy) at least 12 weeks after randomization.

Measures

The outcome variable (resistance) was assessed using stored specimens collected at the time of confirmation of virological failure. Participants with NRTI or non-NRTI (NNRTI) resistance mutations, defined by 2008 International AIDS Society (IAS) guidelines, were classified as resistant [156]. Resistance testing was not done on participants who had a VL ≥1000 copies/ml and resuppressed (<1000 copies/ml) at their subsequent study visit. We classified any participant who resuppressed as not resistant. Participants who had two sequential study visits with VL ≥1000 copies/ml, but who did not have a resistance test, were excluded.

Potential predictors of resistance included demographics, clinical diagnoses prior to treatment initiation, immunological markers (CD4 cell count), self-reported and provider-assessed ART adherence, and therapy duration. Therapy duration is based on the number of days between ART initiation and a participant's first VL ≥1000 copies/ml. Per WHO and other country ART guidelines, the six-month visit is frequently identified as the first point that a participant is eligible for VL monitoring [8, 65, 157] . A six-month visit was defined as any time point at or after the 16-week visit and ≤ 212 days after ART initiation; this time frame includes an acceptable 30-day extension of the six-month window period. The 12-month

visit was similarly classified as any time after the six-month window up to and including 30 days after 12 months on ART (395 days).

Statistical analyses

All analyses were conducted using Stata statistical software (Version 13.0; Stata Corporation, College Station, TX).

We constructed three multivariable models to predict resistance; potential predictors included participant demographics, clinical diagnoses prior to ART initiation, immunological markers, self-reported adherence, and therapy duration prior to having an elevated VL. The three models reflect variations in availability of CD4 and VLs at time of ART initiation. WHO guidelines for ART monitoring suggests VL testing only occurs after a patient has been on ART for six months [8]. Although many countries have scaled up access to CD4 testing to determine ART eligibility, the roll-out of Option B+, in which HIV-infected pregnant women are initiated on lifelong ART regardless of CD4, could mean that many patients will not have a baseline CD4 cell count [8]. In light of these policies and the capacity constraints in resource-limited settings, we constructed models to reflect three scenarios: Model 1 assumed that VL and CD4 at ART initiation were available, so both were included as eligible predictor variables. Model 2 assumed that baseline CD4 was available but that baseline VL was not and thus excluded as an eligible predictor variable. Finally, Model 3 assumed that neither baseline VL nor baseline CD4 were available; thus neither were included as eligible predictor variables. To evaluate the association between predictors and ART resistance, we calculated unadjusted prevalence odds ratios (OR) and 95% confidence intervals (CI) for each potential predictor in each model [158].

The full models contained all variables with bivariate p-values <0.5; this high threshold was chosen to ensure that available important predictors were not excluded [159]. Variables with low frequency, extreme collinearity, or insufficient detail to permit clinical

implementation were excluded from the models, regardless of p-value. We tested four categorizations of time on treatment and selected the category with the lowest Akaike's information criteria (AIC) value for our reference models [160].

We developed the predictive models using multiple logistic regression with backward elimination [158]. Beginning with the variable with the largest p-value, we removed variables one at a time until five or fewer variables remained (regardless of p-value). The five-variable limit was selected to facilitate eventual implementation of risk scores in resource-limited clinical settings [161, 162]. We assessed the equality of the area under the receiver operating characteristic curves (AUROC) between each model (chi-squared test) [163]. AUROC is a measure of the risk score's discriminatory power –where 1.0 indicates a perfect test (i.e., 100% sensitivity and 100% specificity) [164]. Likelihood ratio (LR) comparing successive models were evaluated to confirm that variable removal did not adversely affect the model's predictive capacity. We also compared LR-test statistics from each reduced model to the full model.

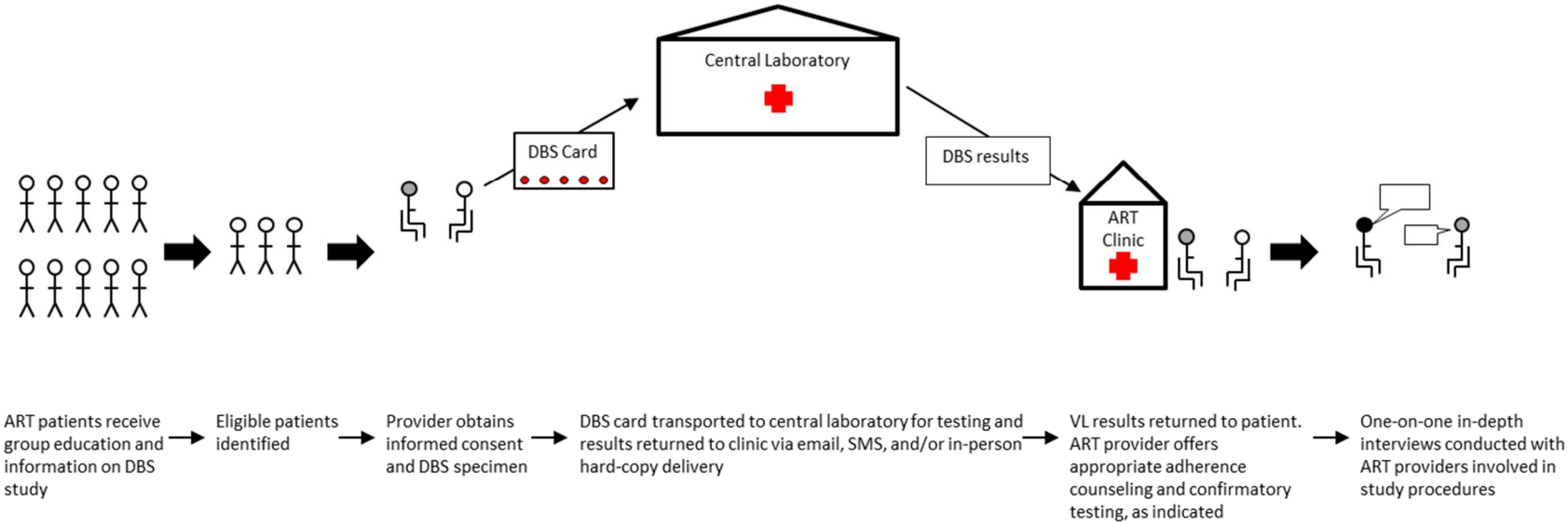
We used the three predictive models to develop the associated risk scores by assigning each variable in the final models a predictor score equal to two times the beta coefficient rounded to the nearest integer. We doubled the coefficient to retain inherent discrimination between betas. Patients with a high VL (≥1000 copies/ml) and a risk score equal to or greater than a pre-specified cutoff are classified as likely resistant to first-line ART and should be switched to second-line ART without a confirmatory VL test. For each model, we assessed sensitivity, specificity, and associated risk scores at cutoffs selected based on clinically-acceptable model-performance criteria [165, 166]. Given the undesirable consequences of prematurely switching patients to second-line therapy, we maintained a high specificity threshold (>95.0%) for all models to minimize false positives. We also calculated the number of patients in a hypothetical cohort of 10,000 ART patients who would be switched without confirmatory testing at each cutoff. We internally validated the model

and risk score performance using 1,000 bootstrap samples with replacement [158, 167]. Bootstrapping is preferred over data splitting and cross validation for the purposes of internal validation [168-172]. Model calibration was assessed using Hosmer-Lemeshow (HL) goodness-of-fit tests [173].

Sensitivity Analyses

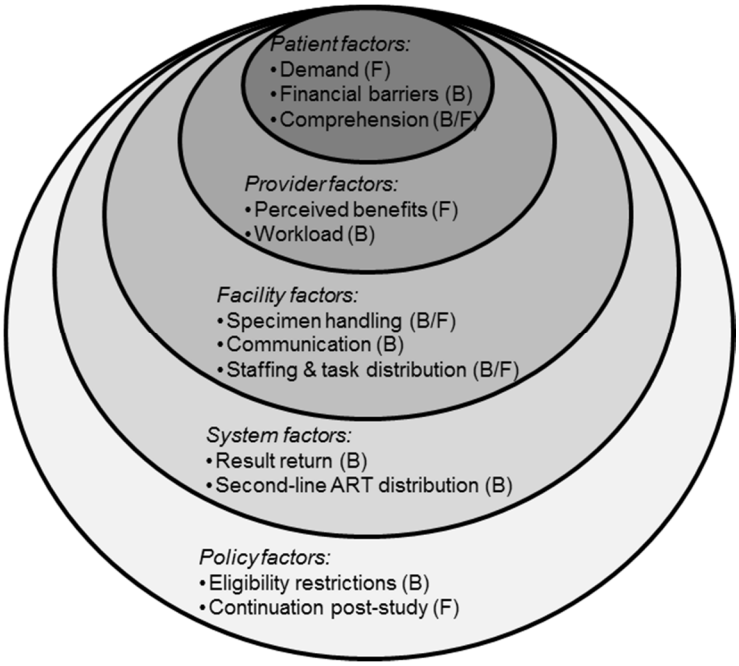
We conducted a sensitivity analysis to evaluate model performance using only study participants from resource-limited settings only. Given the implementation and policy implications and hypothesized biological association of ART duration and resistance, we tested multiple forms of the treatment time variable (**Appendix 3**). Models 4-6 evaluate therapy duration categorized as <7, 7-24, and >24 months; models 7-9 dichotomized duration (<7 vs ≥7 months). We compared these alternatives to the primary models using AIC.

Figure 3.1: Dried blood spot (DBS) study flow



Caption: Figure 3.1: ART patients receiving care at enrolling clinics were briefed as to study purpose and eligibility during the morning education section. After identifying eligible patients, providers completed informed consent forms and study-specific case report forms for patient demographics, clinical history, and adherence. DBS specimens were collected and, after appropriate drying time, transported to the central laboratory in Lilongwe where specimens were tested. Results were returned to clinics using email, SMS and/or in-person hard-copy printouts. Patients were supposed to receive the results at their next visit. Each site was encouraged to designate tasks and responsibilities to clinic personnel in a manner that suited existing clinic flow, patient volume, and staffing constraints. The provider interviews, the topic of this paper, occurred once the study procedures had begun at a given clinic.

Figure 3.2: Modified social ecological model conceptual framework



Caption: Figure 3.2: The social ecological model (SEM) provides a suitable conceptual model, encompassing the multilevel factors that relate to provider acceptability, perceived barriers and facilitators of viral load (VL) monitoring using DBS. Our modified SEM examines the patient, provider, facility, system and policy factors examined in our assessment of barriers to and facilitators of incorporating VL monitoring into clinical practice. ART, antiretroviral therapy; B, barrier; DBS, dried blood spot; F, facilitator

CHAPTER 4: AIM 1

Dried blood spots for viral load monitoring in Malawi: feasible and effective

Introduction

Viral load (VL) testing is the preferred method for monitoring antiretroviral therapy (ART) to identify potential adherence problems and treatment failures [8]. Compared to immunological (CD4 cell counts) or clinical staging, VL testing is more sensitive and specific for accurately diagnosing treatment failure, reducing premature or inappropriate switching to second line therapy [16, 68, 74, 79, 81, 138, 174, 175]. Delaying treatment changes for patients failing first-line ART increases morbidity and mortality [29, 58, 94, 95] and may lead to accumulation of resistance mutations that compromise second-line ART response [14, 15, 17, 23, 24]. With VL monitoring, failing patients are identified sooner, facilitating earlier treatment switches [9, 20, 22, 57, 66-68]. Additionally, the avoidance of premature switching prevents the loss of potential life-years on first-line therapy and costs associated with having patients on more expensive and complicated second-line regimens [69]. These concerns are especially relevant in resource-limited settings where third-line options are not widely available.

As recently revised ART guidelines expand treatment eligibility, potentially leading to >20 million HIV infected patients on ART in Africa alone, access to VL monitoring remains poor and identifying appropriate monitoring strategies in resource-limited settings is an urgent global health priority [10, 12, 13, 39]. The benefits of ART, specifically reducing transmission [7] and disease progression [53], are realized only if viral replication is suppressed [176]. Rates of virological failure in sub-Saharan Africa range from 6% to 53%, depending on failure threshold, clinical setting, and ART exposure time [57, 85-92]. Pooled

estimates from low- and middle-income countries at 12 months of ART exposure suggest 16% failure [90].

Despite the benefits of VL monitoring, numerous barriers impede widespread implementation in resource-limited settings. Traditional VL tests used in developed countries are prohibitively expensive and complex for routine use in resource-limited settings because they require laboratory infrastructure for plasma processing, continuous cold-chain, and phlebotomy-trained providers [29]. Point-of-care technologies are under evaluation but are not yet available [31, 70].

The use of dried blood spot (DBS) for specimen collection and subsequent transport to centralized testing laboratories is an appealing alternative to plasma-based VL testing [8, 32, 35, 119-126]. Malawi is one of many countries attempting to incorporate VL monitoring from DBS into ART care [59, 64]. After 10 years of operation, <1% of Malawian ART patients are on second-line regimens [64], which may reflect providers' relying primarily on clinical staging criteria to diagnose treatment failure and subsequent under-diagnosis of virological failure.

The feasibility of routine VL monitoring from DBS in ART clinics in sub-Saharan Africa has not been assessed outside of controlled studies. Furthermore, the effectiveness of using DBS for VL monitoring in real-world settings, specifically if eligible patients are appropriately switched to second-line therapy, remains unknown. In coordination with the Malawi MOH, we conducted a prospective, non-randomized evaluation of DBS for VL monitoring among ART patients managed at districts hospitals in Malawi.

Methods

Study population

We enrolled adult (≥18 years) patients from five ART clinics in central and southern Malawi. Inclusion and exclusion criteria mirrored MOH eligibility criteria for routine and

targeted VL monitoring [65]. Patients were eligible for VL testing if they were on first-line ART for 6 months, 24 months, or any 24-month period (+/- 3 months) thereafter (milestone-driven monitoring). Alternatively, patients who did not meet milestone criteria were eligible if they were on first-line therapy ≥6 months and showed signs of clinical failure (World Health Organization [WHO] Stage 3 or 4) (targeted monitoring). Patients were excluded if currently hospitalized, imprisoned, or involuntarily incarcerated in a medical facility.

Site selection and enrollment

ART clinics within district hospitals were selected based on the size of their retained ART patient population and willingness to both train providers and enroll participants. We validated DBS vs. plasma VL at the two sites with adequate capacity for plasma-processing. During this validation period, all participants provided a venous and fingerstick sample from which a plasma sample, venous DBS (vDBS), and fingerstick DBS (fsDBS) were produced. Interim analyses demonstrated acceptable agreement between plasma, vDBS, and fsDBS [119], and upon expansion to the remaining three sites, participants received fsDBS only to simplify specimen collection procedures.

Sample collection and transport

Sample collection and virological testing methods are presented elsewhere [119]. Briefly, sites were provided with pre-packed kits containing: DBS card, capillary tubes, gloves, sterile lancet, alcohol swab, plastic zip bag, and desiccant. All specimens were collected by ART clinic or laboratory staff. Once dried, cards were transferred to individual zip bags with desiccant sachets and stored at room temperature.

DBS specimens were transported at ambient temperature to the central laboratory in Lilongwe (4-6 hours away) approximately weekly using existing hospital-based vehicles or shipped via specimen shipment service.

VL testing and result return

Specimens were tested using the Abbott RealTime HIV-1 Assay (Abbott Laboratories, Chicago, IL) (reportable range of 40 to 10,000,000 copies/ml for plasma and lower limit of detection of 550 copies/ml for DBS). Testing was conducted at an internationally monitored research laboratory.

Results were returned to clinics using e-mail, short message service (SMS), or phone. Hard-copies of results were delivered via hospital vehicles returning to the clinic or by study coordinators during routine (approximately bi-weekly) site visits. Providers delivered results to participants during scheduled clinic visits.

Data collection

All activities were conducted by non-study ART clinic personnel. ART staff members were trained in identifying eligible participants, obtaining consent, specimen collection, study sensitization, adherence counseling, and case report form (CRF) completion. We collected participant demographics, clinical history, and ART adherence data. ART history, including date of diagnosis, ART initiation, and reason for initiation, was abstracted from patient clinic records.

Study visits

Participants were asked to return for VL results one month after enrollment. Participants with elevated VLs (>5,000 copies/ml) were counseled on the importance of adherence and instructed to return after two months for a confirmatory draw. Participants who returned for confirmatory draws were told to return within one month for results. Providers were instructed to refer patients with two elevated VLs for second-line therapy.

Treatment failure definition

Per 2011 MOH guidelines, virological failure was defined as having two sequential VLs >5,000 copies/ml [65]. For patients with plasma results available (validation period), plasma results were used to guide treatment decisions. If vDBS and fsDBS were available, vDBS results were used; fsDBS was used for treatment decisions in all other cases. "Undetectable" results (i.e., results below the platform's lower limit of detection) were treated as having a value at the midpoint between 0 and the lower detection threshold (20 copies/ml and 275 copies/ml for plasma and DBS, respectively).

Programmatic Outcomes

Primary outcomes were feasibility and effectiveness of DBS for VL monitoring. Feasibility was measured by: proportion of participants receiving VL results within 3 months of enrollment; laboratory testing turnaround time and associated delayed result return (participant seen at clinic but result unavailable); frequency of "provider misses" (participant seen at clinic but did not receive results despite being available at the clinic); proportion of participants with baseline elevated VL receiving confirmatory DBS; time from participant receipt of results to collection of confirmatory specimen; and time from enrollment to second-line treatment initiation among eligible participants. Participants were terminated from the study if results were not delivered ≥6 months of enrollment.

Effectiveness measures of DBS for VL monitoring included: proportion of participants who resuppressed (≤5000 copies/ml on confirmatory specimen) and proportion of eligible participants who initiated second-line therapy within 12 months (365 days) of enrollment.

Statistical methods

We used student's t-tests (continuous variables) and Pearson's chi-squared or Fisher's exact test (categorical variables) to identify demographics and clinical

characteristics associated with VL failure and resuppression (≤5000 copies/ml) [141]. We used generalized linear models with a log link and binomial distribution to explore the relationship between time on ART and VL failure (>5000 copies/ml) at enrollment [142]. Factors considered included age, sex, WHO clinical stage at ART initiation, body mass index (BMI), ART regimen, self-reported adherence, and clinical symptoms. We used likelihood ratio (LR) tests to decide which variables to include. We tested interactions between time on ART and symptoms at enrollment to asses if the effect of ART exposure on likelihood of treatment failure was different for participants who showed signs of clinical failure. We evaluated agreement of time on ART (clinic records versus CRFs) using kappa statistics [143]. We conducted an post-hoc sub-group analysis exploring the relationship between CD4 cell count at ART initiation and treatment failure as this may be an important predictor of virological failure [85, 144, 145].

All analyses were performed using Stata (version 13.0; StataCorp, College Station, TX). P-values <0.05 were considered significant.

Ethical approval

The National Health Sciences Research Committee of Malawi, the Centers for Disease Control and Prevention Ethics Review, and the Biomedical Institutional Review Board at University of North Carolina, Chapel Hill approved this study. All participants provided written informed consent.

Results

Study population

Of 1,498 ART patients enrolled, 1494 (99.7%) had VL results available (**Figure 4.1**). The average age was 42.1 years, 444 (29.7%) were male, and most participants had been on ART for ≥2 years (**Table 4.1**). Eighty-three (5.5%) were enrolled under “targeted

monitoring" criteria. Approximately one quarter (338, 22.8%) had at least one clinical symptom. Only 524 (35.0%) had a quantitative CD4 recorded when initiating ART (mean 187 cells/mm^3). Nearly three-quarters (1,067, 71.3%) of participants reported 100% adherence over the last 30 days and 1,261 (84.5%) reported 100% adherence over the last week. Pill count was only available for 229 (15.3%) participants, according to which ART adherence was 99.2%.

Baseline virological failure

Nearly all participants (1,406, 94.1%) were virologically suppressed at baseline (≤5000 copies/ml) (**Table 4.1**). Compared to participants with suppressed VLs, participants with elevated VLs were younger (37.3 vs 42.4, p<0.01). Sex, BMI, self-reported perfect adherence within the past 30-days or week, and proportion with clinical symptoms were similar across suppression status. Median VL among participants failing at baseline was 30,329 copies/ml [IQR: 16,483-102,029].

Among 83 persons enrolled based on targeted VL monitoring eligibility, 10 (12.1%) had elevated baseline VLs, compared to 78 (5.5%) of persons enrolled based on milestone eligibility (p=0.01) (**Table 4.1**). Targeted participants were older (45.1 vs 41.9, p=0.01), and more likely to be male (41.0% vs 29.0%, p=0.02), have advanced WHO stage at ART initiation (p<0.01), and report no missed doses in the last 30 days (81.7% vs 70.7%, p=0.03). Nearly half of participants enrolled for targeted monitoring had multiple symptoms (48.2% vs 3.7% milestone, p<0.01). Median VL for targeted and milestone monitoring groups was 275 copies/ml, but the distributions differed significantly: 10^{th} to 90^{th} percentile: milestone: 275-1247 versus targeted: 275-20,150 (Mann-Whitney z=-2.98, p<0.01 two-tailed).

Resuppression

Among 78 persons with a confirmatory VL, 24 (30.8%) resuppressed (**Figure 4.1, Table 4.2**). Compared to participants who did not resuppress, participants who resuppressed had longer periods between receipt of baseline results and confirmatory testing (median 81.5 vs 60 days, $p<0.01$). Participants who resuppressed has slightly lower baseline VL than those who did not resuppress (23,167 copies/ml vs 32,562 copies/ml, $p=0.06$). Rates of resuppression varied by enrolling clinic – ranging from 16.7% to 56.3% - although the differences were not significant according to Fisher's exact test ($p=0.13$).

Programmatic Outcomes

Feasibility: About 80% (1189/1498) of participants received results within 3 months. Median period from enrollment to receipt of results was 42 days (**Figure 4.2**). Nearly 45% (665/1498) of participants had a clinic visit during which VL results were not delivered. The median period between enrollment and a specimen being tested at the central lab was 23 days. Results were generally communicated back to clinics within 3 days of testing. Lab-based delays, in which a participant came to the clinic but results were not available, accounted for most delays. However, 47.4% of participants (315/665) had at least one visit at which results were available, but not delivered. Among participants with elevated VLs at baseline, 93.2% (82/88) received results and 88.6% (78/88) of all eligible participants had a confirmatory VL test. The median days between receipt of elevated VL results and collection of confirmatory specimen was 62. Among participants with confirmed elevated VLs, median time from enrollment to second-line treatment initiation was 164 days (range: 125-381). Over half (54%; 27/50) of participants who were eventually switched, initiated second-line therapy on the same day they received confirmation of high VL.

Over 100 (6.8%) of participants never received results during the study follow-up period: 6 died, 2 moved, 1 was referred immediately for second-line therapy, 4 defaulted

from care, and 89 were terminated from the study prior to receiving results, if they had been enrolled ≥6 months without being given results. Four participants did not have VL results because of failed redraw attempts (n=3) or ineligibility at enrollment (n=1). Patients that were terminated from the study without receiving results were enrolled an average of 195 days (range: 179-322).

Effectiveness: Nearly one-third (30.8%, 24/78) of participants with an elevated VL at baseline resuppressed. Among participants with a confirmed elevated VL, 92.6% (50/54) initiated second-line therapy. Over 90% (49/54) of participants who were confirmed as eligible for second-line therapy were switched within 365 days of their first elevated VL; over half (31/54) were switched within 180 days. If we assume that the 4 participants who were switched before confirmatory VLs would not have resuppressed, 91.4% (53/58) of participants reached the primary effectiveness endpoint—initiating second-line therapy within 12 months of enrollment.

Logistic Regression

We used time on ART as documented in patient clinic records (Kappa statistic comparing time on ART on CRFs vs. clinic records =0.89). After adjusting for time on therapy, clinical symptoms, sex, WHO stage at initiation, and self-reported adherence, increasing age was associated with decreased risk of failure (RR 0.95, 95% confidence interval (CI) 0.92-0.98) (**Table 4.3**). Participants on ART >4 years were 1.7 times more likely to fail compared to participants on therapy 1-4 years (RR 1.70, 95% CI 1.01-2.84); participants on ART ≤1 year were less likely to be failing (RR 0.57, 95% CI 0.18-1.83), although the association was not statistically significant.

The effect of time on ART on likelihood of treatment failure did not differ meaningfully among patients with and without documented symptoms of clinical failure (p>0.05). Removing this interaction term did not change model fit (LR test p=0.26).

Sub-group analysis

Limiting analyses to participants with CD4 count, compared to participants with a CD4 count >100 cells/mm^3 at ART initiation, participants with CD4 ≤100 cells/mm^3 were 2.2 times more likely to have an elevated VL after adjusting for time on therapy, age, sex, symptoms, adherence, and BMI (RR 2.22, 95% CI 1.02-4.84). Increasing age remained associated with decreased risk of failure (RR 0.93, 95%CI 0.89-0.98). However, being on ART >4 years was no longer associated with increased risk of failure (RR 1.18, 95% CI 0.50-2.82).

Discussion

DBS for VL monitoring was feasible and effective when implemented by ART providers in a resource-limited setting at district hospitals in Malawi. Greater than 99% of the VL results were available on site, and nearly 80% of participants received their VL results within 3 months of testing. Among the participants with confirmed elevated VL, 92.6% initiated second-line therapy, and 91% were switched within one year of their first high VL. Participants were rapidly transitioned to second-line therapy: >50% of participants initiated second-line therapy the same day that they received confirmatory VL results.

Our results are considerably better than VL monitoring outcomes elsewhere in sub-Saharan Africa, where only 62% of patients meeting guideline-dictated failure definition were switched and the median delay between confirmation of failure and treatment switch approached 5 months [85, 91]. There are many potential explanations for the observed differences. Providers involved in our study were aware of study endpoints, which may have motivated them to initiate indicated timely treatment switches, whereas retrospective programmatic evaluations in comparator studies are less likely to be subject to that bias.

Similar to rates observed in the region, 31% of participants with elevated VLs resuppressed [26, 177, 178]. Per national guidelines, providers were instructed to

emphasize the importance of adherence for patients with elevated VL [65]. However, we observed substantial inter-clinic variation in rates of resuppression – potentially indicating variation in adequacy of adherence counseling. Alternatively, inter-clinic resuppression variation may be explained by the differences across clinics in time between enrollment and confirmatory testing among participants with elevated VLs.

We observed surprisingly low virological failure (5.9%) among this previously unmonitored mature ART-patient cohort [57, 85, 87, 88, 90-92]. The lower-than-expected failure rate may be at least partially explained by cohort variability in failure definitions [8, 91, 119]. Our results represent virological failure rates among persons retained in care and thus may underestimate the true rate of failure: 2% of Malawian ART patients default from care each quarter and >18% of patients initiated on ART have been lost to follow-up since 2004, with an additional 10% known to have died [64].

Both age and time on therapy were associated with treatment failure in multivariable models. Younger participants were at increased risk of failure, highlighting the importance of targeting adherence interventions to youth, regardless of how long they are retained on therapy [85, 144, 145, 179-181]. The increased risk of failure is not limited to adolescents. We only enrolled participants ≥18 years, the majority (>95%) of whom initiated ART in their early or mid-20s and yet were still at increased risk of failure. Expanding the definition of youth to include young adults and tailoring interventions to this group may be an efficient strategy for reducing virological failure. Participants who had been on therapy longer were at increased risk of failing, even after adjusting for clinical signs of failure. Our sub-group analysis findings confirm the relationship between lower CD4 (≤100 cells/mm^3) at initiation and increased risk of treatment failure [91, 182], an observation that may be relevant for identifying patients at highest risk of failure [183].

Clinical symptoms were not associated with increased risk of failure, emphasizing shortcomings of relying on clinical staging for predicting virological failure [8, 16, 81].

However, participants recruited under targeted monitoring criteria were significantly more likely to fail than milestone-based eligible participants. This demonstrates an apparent contradiction, in which participants eligible according to milestone criteria who also had clinical symptoms were not at increased risk of failure but patients targeted for VL monitoring based only on clinical symptoms were at increased risk. The difference may be explained by the extent of symptoms: patients who were enrolled under targeted monitoring criteria frequently had multiple symptoms concurrently. Targeted monitoring has been advocated as an important alternative in settings where routine monitoring is cost prohibitive [8, 184]. Our findings reaffirm the efficiency of targeted monitoring, but demonstrate that nearly 75% of failing patients would be missed.

We attempted to have study conditions mimic real-world circumstances, but elements of our evaluation may not be replicated beyond the study setting. Providers were aware of the data collection procedures, leading to an unavoidable observer effect. We also emphasized result delivery and confirmatory specimen collection, especially for participants with elevated VLs. Due to staffing constraints, we were not able to assess the proportion of eligible ART patients visiting the clinics who were enrolled. When hospital vehicles were not available for specimen transfer, study coordinators retrieved specimens during weekly or bi-weekly site visits. Even with the 30-day downtime, the laboratory turn-around time likely represents an ideal scenario given staffing and experience at the research laboratory. Having a “point person” in each hospital or district to facilitate specimen transfer and result follow-up may expedite monitoring activities, including ensuring all VL eligible clients are reached.

Numerous barriers remain to widespread implementation of DBS for VL monitoring. More than three-fourths of participants who went >90 days without receiving results had at least one interim clinic visit. Laboratory-driven delays (result not available at the visit)

accounted for some of the “missed” result delivery opportunities, but many were due to providers failing to retrieve results and deliver to participants.

We have demonstrated that DBS for VL monitoring is both feasible and effective in real-world resource-limited settings. The centralized laboratory testing was efficient and results were successfully distributed back to clinics. Our results help validate the potential for central laboratory testing of DBS to improve access to VL monitoring in more remote settings. Delays in returning results to participants were largely due to inadequate result tracking and provider notification in the existing paper-based and electronic ART management systems. Modifications to these systems will be essential in advance of widespread implementation. We observed remarkable performance in terms of proportion of eligible participants switched to second-line therapy in a timely manner. We also observed a lower-than-expected virological failure rate. At this failure rate, pooling specimens may be a cost-effective testing alternative [92]. Our findings demonstrate the importance of virological monitoring among patients on ART for extended periods, regardless of clinical symptoms: targeted monitoring alone would miss nearly 75% of patients with treatment failure. Important next steps include assessment of resistance among patients who do not resuppress to distinguish between modifiable inadequate adherence and biological failure.

Table 4.1: Participant baseline demographics, ART history, & clinical characteristics

	All (n=1,494)[♦] N (%)	*Suppressed* ≤5,000 copies/ml (n=1,406)* N (%)	*Elevated* >5,000 copies/ml (n=88)* N (%)	*p-value*	*Monitoring*		*p-value*
					Milestone (n=1,415) N (%)	*Targeted (n=83)* N (%)	
Participant demographics							
Age (years)				<0.01			0.01
18-24	38 (2.5)	31 (2.2)	7 (8.0)		36 (2.6)	2 (2.4)	
25-34	323 (21.6)	290 (20.6)	33 (37.5)		318 (22.5)	5 (6.0)	
35-44	576 (38.6)	548 (39.0)	28 (31.8)		543 (38.5)	33 (39.8)	
45-54	363 (24.3)	350 (24.9)	13 (14.8)		337 (23.9)	26 (31.3)	
55-64	158 (10.6)	152 (10.8)	6 (6.8)		143 (10.1)	15 (18.1)	
≥65	36 (2.4)	35 (2.5)	1 (1.1)		34 (2.4)	2 (2.4)	
Sex				0.61			0.02
Male	444 (29.7)	420 (29.9)	24 (27.3)		410 (29.1)	34 (41.0)	
Female	1050 (70.3)	986 (70.1)	64 (72.7)		1001 (70.9)	49 (59.0)	
ART history							
Time on ART (CRF)[†]				0.12			
6 months	140 (9.7)	135 (10.0)	5 (5.9)		- -	- -	
2 years	481 (33.3)	453 (33.4)	28 (32.9)		- -	- -	
4 years	340 (23.6)	321 (23.7)	16 (18.8)		- -	- -	
> 4 years**	402 (27.8)	374 (27.6)	27 (31.8)		- -	- -	
Time on ART (clinic records)[†]							<0.01
≤1 years	144 (9.7)	- -	- -		139 (9.9)	6 (7.3)	
1-2 years	467 (31.4)	- -	- -		460 (32.6)	7 (8.5)	
2-4 years	351 (23.6)	- -	- -		340 (24.1)	12 (14.6)	
4-6 years	288 (19.3)	- -	- -		263 (18.6)	26 (31.7)	
>6 years	239 (16.1)	- -	- -		209 (14.8)	31 (37.8)	
Clinical stage at initiation				0.33			<0.01
Stage 1	213 (16.6)	204 (16.9)	9 (12.5)		210 (17.3)	3 (4.0)	
Stage 2	193 (15.0)	177 (14.6)	16 (22.2)		189 (15.6)	4 (5.3)	
Stage 3	775 (60.3)	730 (60.3)	41 (56.9)		715 (59.0)	61 (80.3)	
Stage 4	105 (8.2)	99 (8.2)	6 (8.3)		97 (8.0)	8 (10.5)	
ART regimen				0.36			<0.01
d4T/3TC/NVP (1A)	835 (55.9)	786 (56.0)	46 (52.9)		806 (57.0)	29 (35.4)	
AZT/3TC/NVP (2A)	79 (5.3)	76 (5.4)	3 (3.5)		75 (5.3)	4 (4.9)	
TDF/3TC/EFV (5A)	541 (36.2)	505 (36.0)	35 (40.2)		494 (35.0)	47 (57.3)	
Other	38 (2.5)	36 (2.6)	2 (2.3)		37 (2.6)	1 (1.2)	
Adherence							

No missed doses last 30 days (self-report)	1,067 (71.3)	1003 (71.3)	60 (69.0)	0.64	1,000 (70.7)	67 (81.7)	0.03
No missed doses last week (self-report)	1,261 (84.5)	1,189 (84.8)	68 (78.2)	0.10	1,187 (84.1)	74 (90.2)	0.14
Clinical Characteristics							
Any symptoms of clinical failure º	338 (22.8)	315 (22.6)	22 (25.3)	0.56	258 (18.4)	80 (97.6)	<0.01
>1 symptom	92 (6.2)	- -	- -		52 (3.7)	40 (48.2)	<0.01
>2 symptoms	31 (2.1)	- -	- -		19 (1.4)	12 (14.5)	<0.01
Targeted VL monitoring eligibility	83 (5.5)	73 (5.2)	10 (11.4)	0.01	- -	- -	
Virological failure (baseline)	88 (5.9)	-	-		78 (5.5)	10 (12.1)	0.01
Viral load copies/ml‡							<0.01
≤5,000	1406 (94.1)	1406 (100.0)	0 (0.0)		1333 (94.5)	73 (88.0)	
5,000-10,000	11 (0.7)	-	11 (12.5)		10 (0.7)	1 (1.2)	
10,001-100,000	54 (3.6)	-	54 (61.4)		52 (3.7)	2 (2.4)	
100,001-1,000,000	21 (1.4)	-	21 (23.9)		15 (1.1)	6 (7.2)	
≥1,000,000	2 (0.1)	-	2 (2.3)		1 (0.1)	1 (1.2)	

ᵠ 1,498 participants enrolled, 1,494 with VL results available

* "suppressed" and "elevated" refers to baseline viral load measurement as below or above the failure threshold of 5,000 copies/ml;

† Time on therapy collected on study CRFs but only available for patients enrolled under milestone eligibility. ART time was abstracted from clinic records for all enrolled participants.

**267 (59%) on ART for 6 years, 5 (1.1%) for 7 years, 174 (38.6%) for 8 years, and 5 (1.1%) for 10 years

º Symptoms included: Herpes Zoster, popular pruritic eruption, unexplained chronic diarrhea (>1 month), unexplained persistent fever, moderate unexplained weight loss, oral candidiasis, esophageal candidiasis, pulmonary TB, extra-pulmonary TB, pneumonia, Crytpococcal meningitis, Kaposi's Sarcoma, and Other.

‡ Viral load values assigned as midpoint between 0 and lower limit of detection (40 copies/ml for plasma, 550 copies/ml for DBS). Reported values based on per protocol assessment (so plasma or vDBS if available). Median and IQR unchanged among suppressed group if using fsDBS only. Median [IQR] based on fsDBS among patients with elevated VL per fsDBS results was 30,870 [17,156-121,306]

3TC – Lamivudine; ART – antiretroviral therapy; AZT – Zidovudine; CRF – case report form; d4T – Stavudine; DBS – dried blood spot; EFV – Efavirenz; IQR – interquartile range; NVP – Nevirapine; SD – standard deviation; TB – tuberculosis; TDF – Tenofovir; VL – viral load

Table 4.2: Demographic, ART, and clinical outcomes among patients with baseline viral loads >5000 copies/ml

	Resuppress (n= 24)[†] N (%)	*No resuppression (n=54)*[†] N (%)	*p-value*
Baseline Characteristics			
Age (years)			0.69
18-24	2 (8.3)	5 (9.3)	
25-34	8 (33.3)	21 (38.9)	
35-44	7 (29.2)	16 (29.6)	
45-54	5 (20.8)	8 (14.8)	
55-64	1 (4.2)	4 (7.4)	
≥65	1 (4.2)	0 (0.0)	
Sex			0.55
Male	7 (29.2)	15 (27.8)	
Female	17 (70.8)	39 (72.2)	
Time on treatment			0.43
6 months	2 (8.7)	2 (3.8)	
2 years	10 (43.5)	18 (34.0)	
4 years	5 (21.7)	11 (20.8)	
> 4 years	4 (17.4)	20 (37.7)	
Enrollment Site			0.13
Site 1	9 (56.3)	7 (43.7)	
Site 2	3 (27.3)	8 (72.7)	
Site 3	4 (33.3)	8 (66.7)	
Site 4	4 (16.7)	20 (83.3)	
Site 5	4 (26.7)	11 (73.3)	
Correct understanding of VL and adherence[‡]	21 (87.5)	50 (92.6)	0.47
Time between baseline and confirmatory VL (days)			0.27
≤90	2 (8.3)	14 (25.9)	
91-180	19 (79.2)	34 (63.0)	
181-270	3 (12.5)	5 (9.3)	
>270	0 (0.0)	1 (1.9)	
Viral load (baseline) copies/ml*			0.35
≤5,000	0 (0.0)	0 (0.0)	
5,000-10,000	4 (16.7)	7 (13.0)	
10,001-100,000	18 (75.0)	33 (61.1)	
100,001-1,000,000	2 (8.3)	13 (24.1)	
≥1,000,000	0 (0.0)	1 (1.9)	

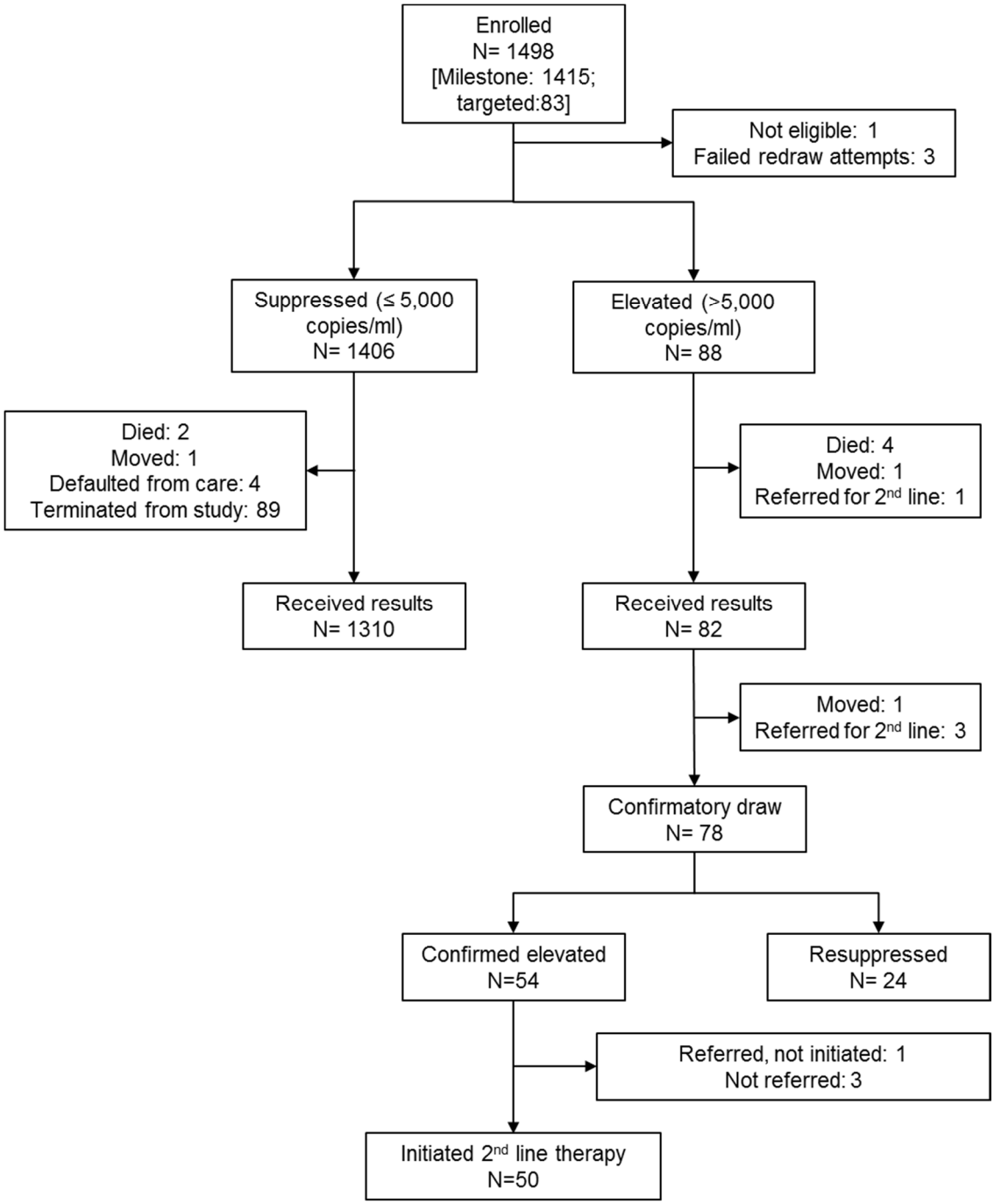
† Among patients with elevated VL, 10 were terminated prior to confirmatory testing; 4 died, 2 moved from the enrolling clinic, and 4 were referred immediately for second-line therapy. Among these 10 terminated patients, 4 were from the milestone group and 6 from the targeted VL group.
*viral load (VL) values assigned as midpoint between 0 and lower limit of detection (40 copies/ml for plasma, 550 copies/ml for DBS)
‡ Question: For most people, if you take all of your medications your viral load will: go up/be higher (*correct*) or go down/be lower (*incorrect*)
3TC – Lamivudine; ART – antiretroviral therapy; AZT – Zidovudine; d4T – Stavudine; DBS – dried blood spot; EFV – Efavirenz; IQR – interquartile range; LPV/r – lopinavir/ritonavir; NVP – Nevirapine; SD – standard deviation; TDF – Tenofovir; VL – viral load

Table 4.3: Factors associated with baseline virological failure (>5,000 copies/ml)

Variable	Unadjusted RR (95% CI)		Adjusted RR (95% CI)	
Time on ART				
≤1 year	0.81	(0.38-1.71)	0.57	(0.18-1.83)
1-4 years	0.90	(0.60-1.35)	1.0	
>4 years	1.21	(0.80-1.82)	1.70	(1.01-2.84)
Age (per year increase)	0.95	(0.93-0.97)	0.95	(0.92-0.98)
Sex				
Male	0.89	(0.56-1.40)	1.42	(0.85-2.36)
Female	1.13	(0.71-1.78)	1.0	
Any clinical symptoms at enrollment (yes)	1.15	(0.72-1.83)	1.17	(0.65-2.11)
WHO stage 3 or 4 at ART initiation (yes)	0.87	(0.54-1.40)	0.77	(0.46-1.29)
Self-reported 100% adherence in last 30 days	0.90	(0.58-1.40)	1.13	(0.68-1.89)
Eligible based on targeted monitoring criteria	2.20	(1.17-4.05)	1.54	(0.63-3.77)
BMI (kg/m^2)	0.99	(0.93-1.06)	N/a	

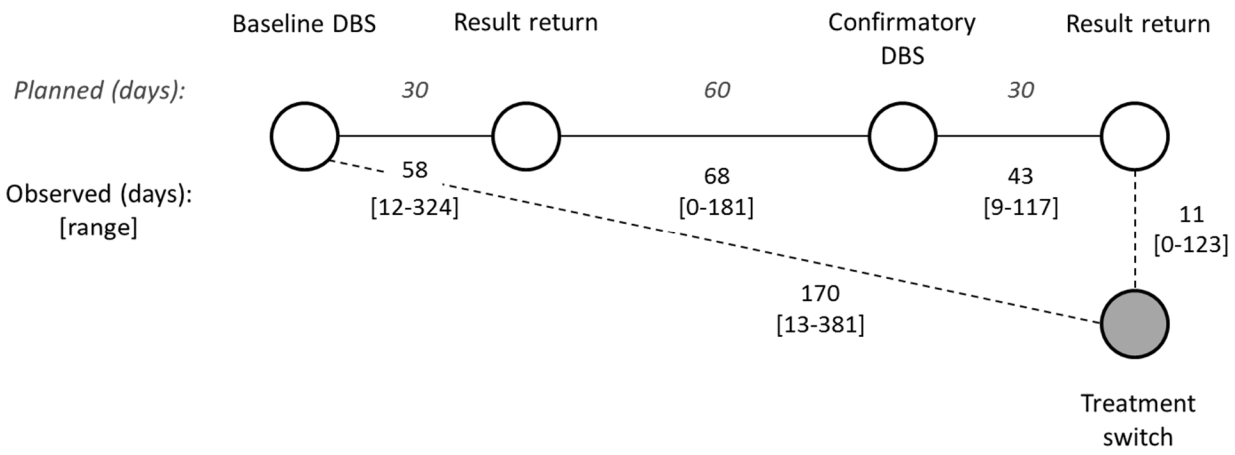
ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; RR, risk ratio; WHO, world health organization

Figure 4.1: Flow diagram of study enrollment and follow-up



Caption: Figure 4.1: Patients enrolled and eligible for study participation had viral load tests run at a central laboratory. Results were communicated back to enrolling ART clinics where providers were instructed to deliver results to participants. Providers proceeded with clinical care according to if the result was suppressed (≤5,000 copies/ml) or elevated (>5,000 copies/ml). Patients with elevated viral loads received confirmatory testing. Per national guidelines, patients with confirmed elevated viral loads were eligible for second-line ART.

Figure 4.2: Planned (*italics)* and observed participant progression through study activities and follow-up



Caption: Figure 4.2: According to the study protocol, participants were supposed to return for results 30 days after dried blood spot (DBS) collection (baseline DBS). For participants with an elevated viral load (>5,000 copies/ml), confirmatory test specimens were to be collected an additional 60 days later (90 days after enrollment). Again, participants were to return 30 days after DBS collection for receipt of results. This diagram describes the observed periods (mean number of days and range) between each participant study encounter.

CHAPTER 5: AIM 2

On the front line of HIV virological monitoring: barriers and facilitators from a provider perspective in resource-limited settings

Introduction

Virological testing is recommended by the World Health Organization (WHO) as the preferred method for monitoring response to antiretroviral therapy (ART) and identifying treatment failure[8]. Alternative methods for identifying treatment failure in resource-limited settings, such as immunological (CD4 cell counts) and clinical staging, are considerably less sensitive and specific than viral load (VL) monitoring[16, 68, 74, 79, 81, 138, 174, 175]. By identifying failure, VL monitoring reduces morbidity and mortality, improves second-line ART outcomes by avoiding accumulation of ART resistance mutations, and guides providers as they counsel patients and reinforce adherence behavior [14, 15, 17, 23, 24, 29, 58, 94, 95]. Despite proven health benefits, VL monitoring is rarely available in the highest HIV-burden settings, including sub-Saharan Africa. Traditional VL tests require expensive laboratory equipment, complex specimen collection procedures, and highly-trained personnel[12, 13, 29]. Dried blood spots (DBS) have emerged as an alternative to traditional plasma-based VL testing for remote, resource-limited settings by simplifying specimen collection and storage; centralizing laboratory testing; and reducing the need for extensive clinic-level laboratory infrastructure. DBS testing using ART providers at outlying clinics and a centralized laboratory for specimen testing may increase access to VL monitoring in remote clinics, and is a promising alternative VL monitoring model [33, 185].

ART providers are critical to achieving the potential individual and public health benefits of VL monitoring. Currently, providers are responsible for clinical staging,

adherence counseling, and drug distribution. With expansion of VL monitoring, ART providers may be tasked with VL specimen collection and asked to incorporate other monitoring activities into daily practice, including second-line ART referral and provision of more focused adherence counseling. More than 70% of patients with high VLs resuppress following VL testing and adherence support [25, 186]. Appropriate counseling and referral for second-line ART, if indicated, requires provider commitment and cooperation, as well as adequate comprehension of how to deliver guideline-concordant care using VL monitoring results[127]. Clinic personnel are a key component of the WHO phased approach to VL scale-up preparations (**Figure 5.1**) [128]. Sustained VL monitoring programmatic success is contingent upon ART provider behavior and buy-in.

Recognizing the important role that ART providers will play in implementation of successful VL monitoring programs, we explored provider-perceived barriers to, and facilitators of, incorporating VL monitoring into daily clinical practice. We interviewed providers working at clinics that were participating in a public health evaluation of a VL monitoring program using DBS in Malawi. Providers' input is critical for successful implementation of monitoring activities.

Methods

Parent study

The parent study was a public health evaluation of DBS for VL monitoring at five ART clinics in central and southern Malawi [119, 146]. ART clinics affiliated with district hospitals were identified based on pre-existing relationships between study leadership and nationwide laboratory mentoring projects. All providers received a 2-day training on study protocols including DBS collection (**Figure 5.2**). The central lab was approximately a 3- to 4-hour drive from clinics in the central region, and 4-6 hours from clinics in the southern region. VL results were returned to the clinic using a combination of e-mail, short message

service (SMS), mobile phone, and hand-delivered hardcopy results. To retain confidentiality, SMS and email messages identified patients using only unique patient IDs.

Study Population

Between July 2013 and January 2014, we conducted in-person interviews with all providers who were involved in DBS study activities at each of the five enrolling clinics. Provider responsibilities ranged from retrieval of patient records to patient counseling and second-line ART referral. We identified providers via onsite point-persons–frequently a nurse who assumed additional study-related duties[147]. All providers agreed to participate and gave written informed consent.

Interview guide & conceptual model

We developed the interview guide to explore providers' perceptions of the barriers and facilitators to implementing VL monitoring. The interview guide encouraged discussion and a flexible conversation about specimen handling, return of results to the clinic, and their overall reaction to VL testing activities. We used probing questions to explore emerging themes. All interviews were conducted in English by trained study staff and audio-recorded. Audio-recorded interviews were transcribed [147, 148].

Analysis

All transcripts were coded by the primary researcher (SER) using ATLAS.ti (version 7.0, ATLAS.ti Scientific Software Development GmbH, Berlin, Germany) [149]. A second coder (SH), independently coded 30% of transcripts. The two coders reviewed double-coded transcripts, and any differences in code application were resolved through discussion and negotiated consensus [148, 150].

We based initial structural codes upon interview topics, such as specimen handling and returning VL results to clinic. Thematic content analysis of transcripts guided identification, analysis, and reporting of themes [151]. We reviewed transcripts for broad concepts and used early memos to generate an initial codebook [152]. As more complex themes emerged, we coded in more depth, revising and accumulating codes [153]. As new themes were added to the coding process, previously coded transcripts were reviewed to ensure coding logic completeness and consistency. The codebook was therefore a living document–adapting to the themes and concepts as they surfaced during analysis. When we completed the coding, we conducted a line-by-line analysis to ensure that all coded transcripts reflected the final codebook.[152]

The social ecological model (SEM) emerged as a useful perspective for evaluating the multilevel factors that influenced provider-reported barriers and facilitators of VL monitoring. The SEM facilitated a comprehensive examination of individual and environmental circumstances that effected provider perceptions[129-131]. At its most basic level, the SEM considers two key concepts: 1) that behavior affects and is affected by multiple different levels, and 2) that individual behaviors are shaped by a larger social environment. Because our data had acquired a clear, multilevel nature reminiscent of the SEM, we grouped themes according to the five levels of the modified SEM framework: patient, provider, facility, system, and policy factors (**Figure 5.3**) [154]. These levels represent a contextual adaptation from the original SEM, which included intrapersonal, interpersonal, organizational, community, and public policy levels. We present illustrative representative quotes for each emergent theme. This strategy provides a holistic approach to understanding the barriers and facilitators of incorporating VL monitoring from a provider perspective.

Ethical approval

The National Health Sciences Research Committee of Malawi, the Centers for Disease Control and Prevention Ethics Review, and the Biomedical Institutional Review Board at University of North Carolina, Chapel Hill approved this study.

Results

Participant characteristics

We interviewed 17 ART providers: 3 ART coordinators, 8 non-coordinator nurses, and 6 other clinic personnel (**Table 5.1**). ART coordinators were either nurses or clinical officers (non-physician clinical provider). ART coordinators had been in their positions a median of five years, whereas nurses had been in their positions a median of 7.5 years. Other providers included a hospital attendant, laboratory technicians (n=2), HIV testing and counseling counselors (n=2), and an ART clerk.

Qualitative findings overview

We identified12 emergent themes of provider-reported barriers to, and facilitators of, VL monitoring activities: patient demand for VL monitoring, patient financial barriers to VL monitoring uptake, patient comprehension of VL, provider-reported benefits of VL monitoring, provider workload, specimen handling, communication, staffing and task distribution, delayed result return, second-line ART distribution, eligibility restrictions, and continuation of VL monitoring post-study completion (**Table 5.2** and **Figure 5.3**).

Patient-level factors: 'in my body'

Providers reflected on patient responses to VL monitoring eligibility and identified common patient-initiated questions. We identified three themes at the patient level: demand for VL testing, financial barriers to VL monitoring uptake, and comprehension of VL.

Demand for VL monitoring: Patients' reactions and questions indicated a desire for expanded access to VL monitoring beyond that which the Ministry of Health (MOH) criteria allowed. Nearly all providers described an eager patient population, craving information about VL and their body's response to ART.

> *'We need VL! We need VL! We need VL!' so, we hope. You know these people are very interested to know what is going on in the body, while they are taking these ARV drugs.* – Female nurse

Financial barriers to VL monitoring uptake: Despite the eagerness to enroll in the VL monitoring study, providers identified common patient-reported barriers, including transportation costs and travel time to the clinic. In general, barriers emphasized challenges with attending follow-up visits to both receive results and be referred for second-line therapy, when indicated. Providers emphasized the reluctance of patients to return between the regularly scheduled 3-month visits for receipt of VL results.

> *Of course [we encounter] some [pushback from clients], because some are coming from far. Yes. But still, this doesn't mean that they will not come. They will still come, because they want to know the result.* – Female nurse

Patients' comprehension of VL: The overwhelming majority of providers reported that patients generally understood the concept of VL. But when probed, providers reported high variability. For example, patients commonly confused VL with CD4 cell counts and held potentially dangerous misunderstandings regarding interpretation of an "undetectable" VL.

> *People knew CD4 more than HIV itself... And now, that we are talking about VL, I think we still need to emphasize [what it means] and educate the people, especially with the low literacy levels that we have amongst our clients...However, we have a good number of cases who are understanding what is VL.*– Male ART coordinator

> *But again…to some people it was like a confusion a little bit because undetectable to them it was like maybe the virus[es] are dead so they can stop taking ARVs…*– Female nurse

Providers frequently used patient misconceptions as an opportunity to counsel on the importance of ART adherence, as described further below.

Provider-level factors: 'pressure of work'

We identified two key themes at the provider level–perceived benefits of VL monitoring, and workload as a substantial barrier to VL monitoring.

Perceived benefits of VL monitoring: All providers supported incorporating VL into clinical practice, citing numerous benefits to their clinical practice. Patient education, including reinforcing messages of adherence behavior as facilitating desired clinical outcomes, were frequently identified as benefits of delivering the VL result.

> *So when we are giving out the results, like in my case, I say to them: "before we started treatment, we said the function of how ARVs work, we said they reduce or … they block the lifecycle of the HIV so much so that it does not replicate anymore. And this is what we have been expecting since you started ART. So, now that we have tested your blood, it really shows that the HIV is not replicating, but this does not make you HIV free." So, if they understand, they appreciate. –* Male ART coordinator

Another reported benefit of VL monitoring was helping providers to better identify treatment failure. Clinical symptoms poorly predict virological failure[16, 74, 79, 81, 106, 138, 174]. However, in the absence of VL monitoring, providers relied on the less sensitive and less specific clinical staging to identify ART failure. Providers were surprised at how infrequently the patients with high VLs showed symptoms of failure.

> *The good things we have seen with this study is that it has given us a clear mind, so that patients can switch, switch on the second line, earlier than waiting for them to get sick and present [with] treatment failure.*– Female nurse

Provider empowerment emerged as an unexpected theme related to the benefit of VL monitoring. Most providers had not used VL to guide ART management previously. Although familiar with ART's mechanism of action, many providers observed the impact of

ART on viral replication for the first time. The evidence of ART efficacy increased provider's confidence in ART adherence counseling and contributed to their overall clinical confidence.

> *It has also helped us to know what is going on with the drugs they are taking. Because we didn't know. Like in the past they would ask us 'I want to know! I want to know how the drugs are going on in my body!' but we were not able to answer them...But with this VL it has helped us, the nurses, to know that 'I think this guy is doing well' or 'this drug is not going on, I think we need to do something.'*– Female nurse

> *You are [learning] how to do counseling, how to do adherence, how to monitor them, and you know, suppose somebody has this high VL, what about the next step, you know? So we are learning. I am one of those people!* – Male ART coordinator

> *…You may just be giving the drugs not knowing that maybe the client or patient their body is resisting to that type of drug. With the viral load we see [a] really new aspect of their well-being.* – Female nurse

Workload: Despite the perceived benefits of VL monitoring, providers emphasized that the associated duties overwhelmed already limited personnel. Every provider who was involved in patient management described a burden of work that was unsustainable in the long-term.

> *It has been a headache because it was like an added job for what we have been doing. Like we have always been in the ART clinic, we are always busy. So, when it comes [time] for this VL study, yea it was like, we've added another job. We are even not going for lunch, working very late just to help the clients…Of course it has helped us and the client. But according to the workload, it was too much.* –Female nurse

<u>*Facility-level factors: 'disconnected'*</u>

Providers acknowledged numerous facility-level barriers and facilitators associated with VL monitoring. We identified three themes at the facility level: specimen handling, communication, and staffing and task distribution.

Specimen handling: Inconsistent specimen transport mechanisms complicated DBS specimen movement between outlying clinics and the central laboratory. Specimen

transportation for the parent DBS study relied largely on hospital vehicles. Site personnel were instructed to work with the hospital administrators and other departments to arrange for DBS cards to ride along with any vehicle going from the hospital to the capital, located approximately 3-6 hours from enrolling clinics. Although generally described as successful, providers noted challenges with inconsistent availability of vehicles to facilitate specimen transfer.

> *The reasons might be due to lack of transport to send samples to the testing site in good time (e.g. we still have samples collected earlier last week and no sign of transport to date). So issues of transport may have contributed to the delay of testing and then later to result delivery.* – Female nurse

The hospital that used an established district-wide motorcycle specimen transport system was more satisfied with their specimen transfer arrangements.

In general, providers were pleased with the ease of sample collection and storage with DBS cards[33, 185]. Among the providers who described specimen collection as part of their study duties, most acknowledged the simple and rapid (~3-5 minutes) fingerstick specimen collection procedures. However, when challenges with specimen collection were noted, they were frequently due to cool weather or thickened skin.

> *…it was cold, so you need two pricks to have enough blood. But now, because of the weather, it has changed. Now it's hot. We are no longer experiencing such kind of things…People here, they work in the field each and every time, so their fingers become hard. So you need to prick deeply.* – Male laboratory technician

Communication: Scarce internet connectivity complicated communication between the central lab and clinics. Communication of results, need for additional supplies, or any other VL monitoring-related issue, frequently relied on personal mobile phones.

> *No, there is no consistent email. We had the email, the internet was connected [and] the hospital was paying, but due to funding constraints you know, they disconnected. So currently, you can use your personal phone*. – Male ART coordinator

Staffing and Task Distribution: Perhaps the greatest facility-specific barrier to VL monitoring was the shortage of staff. Echoing the sentiment of work burden described in provider-factors, staff shortage was frequently identified as an impediment to completing VL monitoring activities.

> *I'm just alone here, so I'm doing each and every patient, those who are enrolled and those who are not enrolled. Because we have just this ART room, only this one, so [I] entertain everybody.* – Female nurse

> *We already have a shortage of staff…that's the most challenging.* – Female nurse

Rotating staff also complicated VL enrollment and follow-up activities, which frequently coincided with work-burden, provider reluctance, and discussions of missed result delivery (i.e., failing to return a VL result to a patient during scheduled clinic visits).

> *We usually have nurses who are rotating and we have nurses who are on study. So sometimes it may happen that with the workload, maybe somebody can easily miss somebody.* – Female nurse

Some sites were able to accommodate the extra responsibilities better than others, largely due to more equitable distribution of tasks. Interestingly, providers who identified teamwork as key to their success were exclusively at high-volume sites – none of the low-volume site providers discussed teamwork during interviews.

> *We work as a team and so far I haven't see any kind of resistance from our friends. We have been doing well.* – Male Laboratory technician

Task-shifting to lower-cadre providers, such as health surveillance assistants (HSAs), has emerged as an attractive opportunity to distribute the burden of VL monitoring activities, particularly specimen collection[187-189]. Task-shifting also facilitates expanded access to VL monitoring in more remote clinics and health centers where nurses and clinicians may not rotate regularly. We observed task-shifting at most of the participating clinics.

HSAs they were oriented...And these two [HSAs] they are staying with us, they are not shifting. They are only those ones. – Female nurse

System-level factors: 'at first, it was difficult'

Delayed return of VL results and centralized second-line ART distribution inhibited patient flow and referral efficiency. Neither electronic nor paper-based data management systems were capable of integrating VL results and thus did not alert providers when a result was received from the laboratory. We identified two system-level themes, both of which were classified as provider-reported barriers: delayed result return and second-line ART distribution.

Delayed Result Return: Delays in return of results were due to laboratory-based delays or provider "misses." A month-long machine outage at the central lab created a backlog in returning results to clinics and required rescheduling numerous patient follow-up visits. Unfortunately, without an established notification system, patients still came to the clinic despite results not being available. Provider misses occurred when patients had a clinic visit and the VL result was available but was not returned to the patient. When asked about result delivery delays, providers generally focused on laboratory-driven delays, citing discrepancies between projected and observed result turnaround time.

I think the real turnaround time was like 28 days. But as of yesterday and today…we did not receive the results and the clients were expected to get their results on these particular two days. They did not get their results because the results were not with us at our site. – Male ART coordinator

Though not widely available, and not available at any of the enrolling clinics in this study, most providers supported use of an SMS printer to facilitate more rapid, real-time return of results. SMS printers are distinct from the mobile-phone based SMS method used in this study. The SMS printers are located onsite, and receive data directly from the central laboratory, generating a receipt-like output containing patient ID and VL results.

I think [SMS printers] would be better … because it would be faster getting the results immediately after testing has been done and we may not have experienced the hiccups already shared on result delays encountered here. – Female nurse

That [SMS printer] would work to our advantage …when it's printed, you can clearly see. … When it's on the phone… you can miscode them and then you can give the results to the wrong person. – Male ART coordinator

Second-line ART distribution: Second-line ART distribution is tightly controlled in Malawi and frequently only available at larger district or central hospitals. Providers described the travel time and costs required to collect second-line ART as obstructing referral.

We don't have second-line, so we are supposed to refer to Lilongwe, [to] Lighthouse. So you know [our] district, with some villagers, they can't afford to travel… It's a challenge. – Female nurse

Just to tell [the patients] that they have to go there, it was easy. But for them it was difficult…they were always complaining about the transport monies. …For second-line drugs…it's very difficult…When they go there also, they are just given one bottle. So for them to go back again next month, it becomes a problem. – Female nurse

Policy-level factors: 'why not us?'

Reported policy factors highlight the challenges of practicing in extremely resource-constrained settings where care rationing complicates the desire to provide comprehensive services to patients. We identified two policy-level themes: eligibility restrictions and the desire to continue monitoring post-study completion.

Eligibility restrictions: Strict eligibility restrictions were seen as thwarting providers' efforts to deliver high-quality HIV care. Malawi policy dictates that persons are eligible for VL monitoring at 6-months, 24-months of ART exposure, and then biannually thereafter, or if they are showing clinical signs of treatment failure [140]. Providers were frustrated with VL monitoring eligibility criteria, and the challenge of turning away willing patients, many of whom had been on ART for many years. They were forced to ration a service that, per their

own feedback, was tremendously useful for guiding clinical practice and counseling patients. Perceived rationing of this ART management tool challenged their empowerment, making pleas on behalf of their patients for expanded eligibility criteria. Relaying the response of patients who failed to meet eligibility criteria, providers almost universally described a patient sentiment of '*why not us?'*.

> *Just a plea for the other [patients] … because there are many who want to know their VL while they are on the drugs. If ever, [the government should] expand to everybody who is willing to.* – Female nurse

> *So for those who have taken ARVs for so long, was it not possible at least to check everybody? … Because some people again, they … will not meet the eligibility if the government starts today, they will…they will miss it. And it will be painful.* – Female nurse

Continue monitoring post-study completion: At the end of each interview, providers were given the opportunity to ask any questions and provide feedback regarding study procedures (i.e., what worked? What could be improved?). Requests for expansion of eligibility criteria were common, as were queries into plans for continuation of VL monitoring activities after meeting study enrollment targets.

> *I would say, because it's a study, I would wish that you would roll this out country-wide. It should be routine to each and every client, maybe, [every] client who is HIV-positive and he is on ART.* – Male laboratory technician

Discussion

Successful implementation of VL monitoring in resource-limited settings requires coordination of, and buy-in from, numerous stakeholders–chief among them, the ART providers. We interviewed all providers engaged in a public health evaluation of DBS for VL monitoring in Malawi. The providers identified numerous barriers to effective VL monitoring implementation. These provider-reported barriers and facilitators to VL monitoring underscore the multidimensional nature of the challenges and opportunities associated with expanding monitoring activities. Fitting within the framework of a modified SEM, we

identified a complex set of interconnected provider-identified barriers and facilitators to VL monitoring.

Integrating patient, provider, facility, system, and policy levels, the modified SEM contextualizes the diverse factors that contribute to provider perceptions of VL monitoring [129, 131]. The SEM has been used to explore HIV service utilization [190] and individual risk behaviors [191, 192]. Inter-level interactions reinforce the intricacies of provider perceptions. Themes cut across individual and environmental factors, emphasizing the importance of addressing multi-level barriers and facilitators when planning for VL implementation.

The patients' demand for VL testing reinforced the provider-perceived benefits of monitoring. Provider empowerment emerged as an unexpected facilitator. For many providers, the DBS study was the first time they used an objective marker of ART response to guide clinical management. Providers' knowledge of a patient's virological status increased confidence in adherence counseling and clinical decision making. Emphasizing provider empowerment during VL scale-up activities may improve providers' willingness to adopt additional clinical duties and counseling responsibilities. Based on our results, VL monitoring can modify provider behavior, and should be presented as a tool to help providers improve the quality of HIV care they deliver to patients.

Despite provider-reported benefits of VL monitoring, new clinician responsibilities are often met with uncertainty or resistance [193-195]. Resistance to adopting additional duties may be intensified in inadequately staffed clinics. Human resource capacity among clinical health care workers is a key consideration in VL monitoring implementation in resource-limited settings[128]. In our study, nurses frequently pointed to staff shortages as contributing to the work burden associated with VL monitoring. Task-shifting to lower-cadre health workers could redistribute current responsibilities, especially with non-phlebotomy-based specimen collection (i.e., fingerstick DBS cards) [119, 187, 188]. Based on our

results, task-shifting only for specimen collection will be insufficient; rather than specimen collection, providers' frustration with workload frequently focused on data management, patient counseling, and patient referral. Given the time constraints reported by providers, expanding provider-to-patient ratios at ART clinics, or broadening the scope of practice, as well as training, for lower-cadre health workers, may facilitate program sustainability.

The WHO proposed phased implementation for VL monitoring scale-up emphasizes harmonization between facility-, system-, and policy-levels (**Figure 5.1**) [128]. We observed shortcomings in data management systems, confirming a critical weak integration at the facility- and system-levels. Data management issues highlight challenges in a central laboratory model. Lack of integration with centralized laboratory systems complicated the process for alerting providers when results were available; these communication gaps were exacerbated by poor facility connectivity. The delays in result availability frustrated providers and patients. These obstacles could be addressed by point-of-care VL technologies, but devices are still years away from meeting standards necessary for widespread use [30, 112, 133, 135]. Despite the challenges, the centralized VL testing remains the best alternative for expanding access to VL monitoring, but data management systems must be enhanced to ensure successful implementation. Furthermore, improving coverage of mobile networks and increasing internet connectivity to outlying clinics will be critical to maintain reliable clinic-laboratory communication, and crucial for the success of the centralized VL monitoring model.

In contrast to centralized laboratories, decentralized drug distribution will be imperative for effective patient referral and efficient initiation of second-line ART. Providers frequently identified patients' irritation with the time and money required for transportation to centralized drug distribution sites. For some patients, the cost of travelling to a central distribution point considerably delayed initiation of second-line therapy. Patients were forced to make these long journeys monthly, as initiation on second-line drugs requires clinic visits

every four weeks for at least the first six months[140]. Although a six month drug supply can be sent to the referring clinic, based on the provider interviews, drugs were not transferred. Providing enough medications to cover longer periods would reduce the travel burden, but could compromise clinician's assessment of patients' drug response and adherence to the new ART regimen. Many providers indicated that they had been told their clinic would be receiving second-line ART, but stocks had not arrived or had been exhausted without re-supply. Currently, less than 2% of ART patients in Malawi are on second-line therapy, a trend mirrored elsewhere in sub-Saharan Africa[64, 196]. VL monitoring will likely increase the proportion of patients initiating second-line therapy[197]. Decentralized second-line ART distribution should be considered with any scale-up of VL monitoring, along with supply chain procedures to minimize stock-outs.

Another system-wide policy that discouraged patients and providers was the strict monitoring eligibility criteria set forth by MOH[140]. Patients craved information regarding their VL, and providers were frustrated as they were forced to ration monitoring based on restrictive policies. Many providers closed out interviews with a plea for expansion of VL monitoring eligibility to include all persons on ART. Although more restrictive than WHO VL monitoring standards, the Malawi MOH criteria were designed to maximize extremely limited resources while still providing VL monitoring opportunities for ART patients at highest risk of treatment failure[8, 140]. Anticipating these frustrations, policymakers should design provider trainings and patient education materials explaining the biannual eligibility. Another option is to implement "catch up" testing, in which every patient on therapy for greater than two years receives a single test, and then returns to the biannual eligibility. Extended exposure to ART is associated with increased risk of virological failure, even in the absence of clinical symptoms [197]. A catch up approach might satisfy providers and patients and improve detection of virological failure.

Provider-reported barriers and facilitators to VL monitoring may be generalizable to other mid- or high-volume ART clinics in resource-limited settings. However, generalizability of the provider experience must be evaluated against the back-drop of the DBS study. Providers were aware that VL monitoring activities, including specimen collection and form completion, were components of the ongoing DBS public health evaluation. Perceptions of activities may be different when they are within the context of a study, compared to standard clinical procedures. This limitation is particularly salient in terms of interpreting provider-perceived work burden. In an attempt to mimic real-world implementation, we restricted data collection responsibilities to basic demographics and assessment of adherence and clinical symptoms. However, we cannot rule out that completion of these forms contributed to the provider-reported frustration with workload.

Study site selection and the small sample size are potential sources of bias. However, site selection criteria were not based on clinic functionality or performance and sites were diverse in terms of ART clinic volume, clinic staffing, distance to central labs, and data management systems. Despite a small sample size, we interviewed every provider directly involved in DBS study activities and achieved saturation in emergence of themes. We asked providers to participate in a training session before study initiation, but individual provider willingness to participate in DBS study activities was not assessed nor required prior to study initiation. The broad site selection criteria and universal sampling of providers improve generalizability of our study.

In this study, we offer insight into the multi-level barriers to, and facilitators of, VL monitoring from providers who serve on the frontline of ART management. We observed latent demand from both patients and providers for additional information regarding ART response. The most salient provider-reported barrier to VL monitoring implementation was the workload associated with monitoring activities, taxing an overextended provider workforce Provider empowerment was a striking provider-reported facilitator of VL

monitoring – enabling providers to use laboratory results to focus adherence counseling and guide clinical management. We believe ours is the first study to investigate provider perceptions of implementing VL monitoring in resource-limited settings. Our results may help decision-makers design programs that are responsive to provider-reported barriers and facilitators, helping to anticipate obstacles and take advantage of identified opportunities to improve feasibility and sustainability of VL scale-up.

Table 5.1: Demographic details of clinic staff participating in interviews

Characteristic	ART clinic coordinator (n=3)	Nurse (n=8)	Other[1] (n=6)
Sex			
Male	3	0	6
Female	0	8	0
Years in position			
Median (range)	5 (1.5-8)	7.5 (4-30)	6.5 (3-10)
Time since study initiation at clinic (days)			
Median (range)	57 (44-71)	140 (44-292)	134.5 (57-190)
Study enrollment status at time of interview			
Enrolling	3	5	3
Closed to enrollment	0	3	3

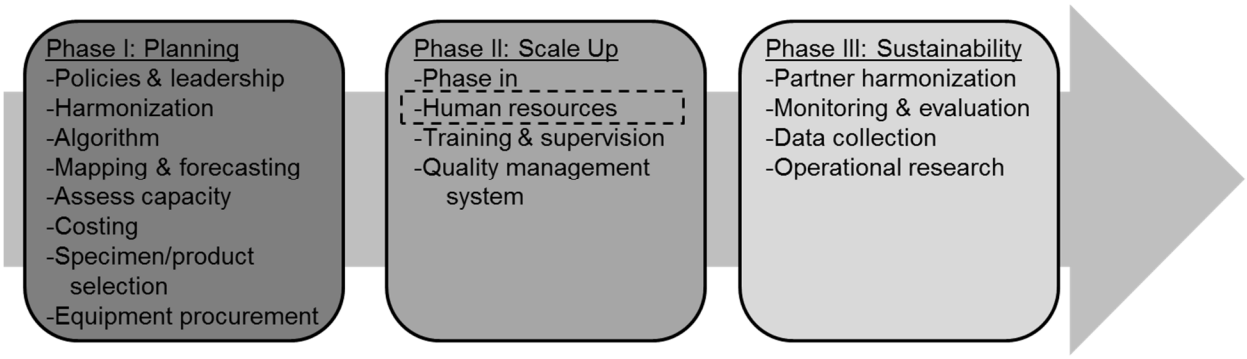
[1]Includes hospital attendants (1), lab technicians (2), HIV testing and counseling counselors (2), and ART clerks (1)
ART: antiretroviral therapy

Table 5.2: Theme frequency

Theme	Frequency		
	Interviews (n=17)	Barrier	Facilitator
Patient-level			
Demand for VL monitoring	14	n/a	37
Financial barriers to VL monitoring uptake	10	22	n/a
Patients' comprehension of VL	9	12	13
Provider-level			
Perceived benefits of VL monitoring	12	n/a	56
Workload	14	36	n/a
Facility-level			
Specimen handling	13	24	32
Communication	3	7	n/a
Staffing & task distribution	13	20	18
System-level			
Delayed Result Return	11	54	n/a
Second-line ART distribution	7	15	n/a
Policy-level			
Eligibility restrictions	13	22	n/a
Continue monitoring post-study completion	11	n/a	20

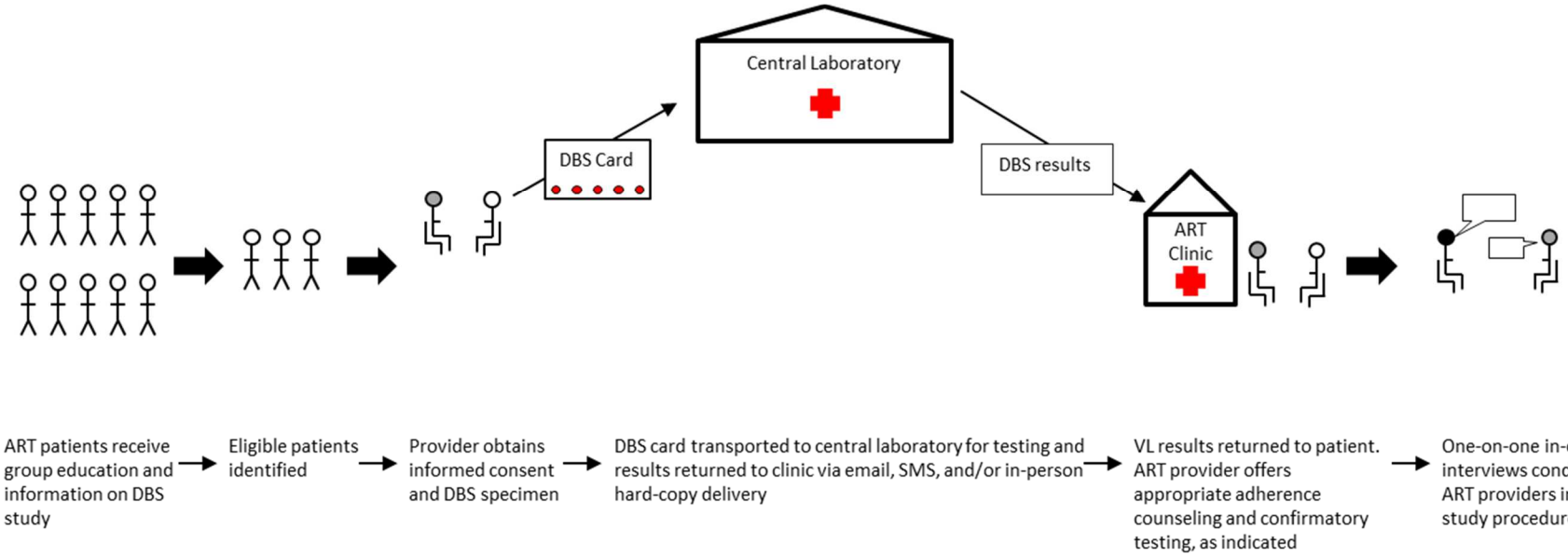
ART, antiretroviral therapy; n/a, not applicable

Figure 5.1: WHO viral load monitoring scale-up



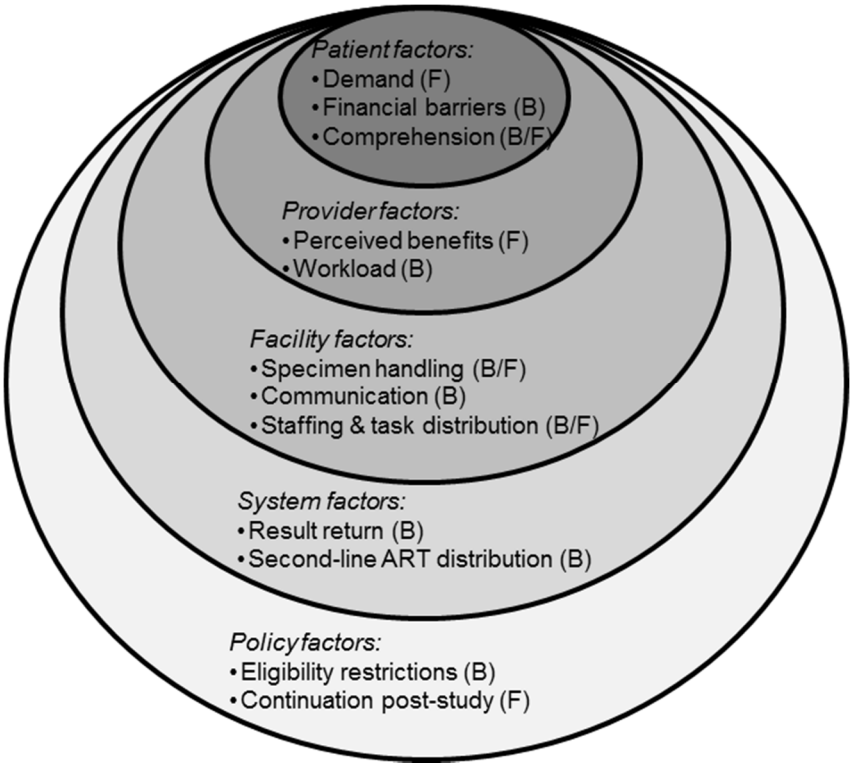
Caption: Figure 5.1: Phased implementation of viral load monitoring as described in the World Health Organization's *Technical and Operational Considerations for Implementing HIV Viral Load Testing* identifies human resources, including training ART providers, in Phase II of the scale-up activities [128].

Figure 5.2: Dried blood spot (DBS) study flow



Caption: Figure 5.2: ART patients receiving care at enrolling clinics were briefed as to study purpose and eligibility during the morning education section. After identifying eligible patients, providers completed informed consent forms and study-specific case report forms for patient demographics, clinical history, and adherence. DBS specimens were collected and, after appropriate drying time, transported to the central laboratory in Lilongwe where specimens were tested. Results were returned to clinics using email, SMS and/or in-person hard-copy printouts. Patients were supposed to receive the results at their next visit. Each site was encouraged to designate tasks and responsibilities to clinic personnel in a manner that suited existing clinic flow, patient volume, and staffing constraints. The provider interviews, the topic of this paper, occurred once the study procedures had begun at a given clinic.

Figure 5.3: Modified social ecological model conceptual framework



Caption: Figure 5.3: The social ecological model (SEM) provides a suitable conceptual model, encompassing the multilevel factors that relate to provider acceptability, perceived barriers and facilitators of viral load (VL) monitoring using DBS. Our modified SEM examines the patient, provider, facility, system and policy factors examined in our assessment of barriers to and facilitators of incorporating VL monitoring into clinical practice. ART, antiretroviral therapy; B, barrier; DBS, dried blood spot; F, facilitator

CHAPTER 6: AIM 3

Predicting first-line antiretroviral therapy resistance among patients with elevated viral loads: development of a risk score algorithm

Introduction

The World Health Organization (WHO) recommends viral load (VL) as the preferred method of monitoring antiretroviral therapy (ART) and for diagnosing treatment failure in HIV-infected patients [8]. An elevated VL is an important gauge of treatment effectiveness, indicating poor adherence and/or resistance [14, 16, 20, 21]. Failing to switch patients with resistance to second-line therapy in a timely manner increases morbidity and mortality, likelihood of second-line treatment failure, and transmission of resistant virus [14, 15, 17-19, 22-24, 56-58]. ART resistance testing is rarely available in resource-limited settings, where the majority of ART-patients reside [13]. Distinguishing patients with modifiable poor adherence without resistance mutations from patients with resistance (for whom improved adherence will not result in viral resuppression) is critical to reduce the spread of resistance and improve effectiveness of second-line therapies.

Current VL monitoring algorithms require confirmatory testing in the event of an elevated initial test (**Figure 6.1**) [8]. This two-step process presents an opportunity for adherence counseling that may improve pill-taking leading to virological resuppression [25]. However, for patients with resistant viruses, requiring a second test unnecessarily postpones the treatment switch. The delays introduced with confirmatory testing are especially relevant for ART patients in resource-limited settings: programmatic obstacles and patient-related barriers (e.g., travel distance to clinic) may substantially increase the interval between baseline and confirmatory testing. Among patients with confirmed

virological failure in South Africa, switch to second-line therapy took greater than five months after confirmatory VL [85, 91].

In sub-Saharan Africa, >25% of ART patients may not achieve viral suppression by 12 months[90], and rates of virological failure may be as high as 14% at five years [85]. With nearly 10 million persons receiving ART in low- and middle-income countries [10], eliminating confirmatory testing for even a fraction of ART-resistant persons will produce substantial cost savings .

Distinguishing patients with elevated viremia *and* resistance mutations from patients without resistance mutations is challenging in resource-limited settings where resistance testing is currently unavailable. A simple risk score algorithm may help providers identify patients with probable ART resistance who could be switched to second-line therapy immediately without a confirmatory test. Using patient demographics, clinical, and laboratory-based predictors that would be readily available in most clinical settings, we developed a risk score algorithm to predict resistance among patients with elevated VL. Patients exceeding a pre-specified risk score threshold could be switched immediately; patients below this threshold would have confirmatory VL testing prior to treatment switch decisions.

Methods

Study setting and population

We studied eligible participants enrolled in the Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) trial (Adult AIDS Clinical Trials Group (ACTG) A5175, NCT00084136). PEARLS was an open-label, Phase IV, randomized clinical trial that investigated efficacy and safety of once- vs. twice-daily regimen dosing. Details of the PEARLS study population and design have been described elsewhere [155]. In brief, A5175 enrolled 1,571 HIV-infected participants ≥18 years old from nine countries, over-

sampling participants from resource-limited settings. Participants were excluded if they: had a CD4 cell count >300 cells/mm^3, previous exposure to ART (with an exception for women who received ART for prevention of mother-to-child transmission), were pregnant, or were acutely ill and/or clinically unstable. PEARLS was approved by institutional review boards and ethics committees at participating institutions.

Our study is a secondary analysis of de-identified data among participants initiated on nucleoside reverse transcriptase inhibitor (NRTI)-based regimens and who had a VL ≥1000 copies/ml at any point after week 16 of study enrollment. The 16-week restriction was based on A5175 definitions of virological failure (two successive measurements of plasma HIV-1 RNA ≥1000copies/ml, with the elevated VL on or after week 16). Primary analyses included participants from all study sites, with a sensitivity analysis restricting the study population to participants enrolled from resource-limited settings. This analysis was approved by the University of North Carolina, School of Medicine Institutional Review Board.

Data collection

Per A5175 study protocol, participants received a targeted physical exam, adherence interview, serum chemistries, CD4 lymphocyte count, and plasma HIV RNA (Roche Amplicor Monitor assay [v1.5]) at least every eight weeks. Any treatment modification (participant, provider, or protocol-mandated) was assessed at each visit. Diagnosis criteria were collected using a standardized case report form (ACTG Appendix 60).

Resistance tests were done retrospectively (Celera Diagnostics ViroSeq HIV-1 Genotyping Assay) on stored specimens for participants meeting virological failure criteria (defined below) or who had disease progression (new or recurrent AIDS-defining opportunistic infection or malignancy) at least 12 weeks after randomization.

Measures

The outcome variable (resistance) was assessed using stored specimens collected at the time of confirmation of virological failure. Participants with NRTI or non-NRTI (NNRTI) resistance mutations, defined by 2008 International AIDS Society (IAS) guidelines, were classified as resistant [156]. Resistance testing was not done on participants who had a VL ≥1000 copies/ml and resuppressed (<1000 copies/ml) at their subsequent study visit. We classified any participant who resuppressed as not resistant. Participants who had two sequential study visits with VL ≥1000 copies/ml, but who did not have a resistance test, were excluded.

Potential predictors of resistance included demographics, clinical diagnoses prior to treatment initiation, immunological markers (CD4 cell count), self-reported and provider-assessed ART adherence, and therapy duration. Therapy duration is based on the number of days between ART initiation and a participant's first VL ≥1000 copies/ml. Per WHO and other country ART guidelines, the six-month visit is frequently identified as the first point that a participant is eligible for VL monitoring [8, 65, 157] . A six-month visit was defined as any time point at or after the 16-week visit and ≤ 212 days after ART initiation; this time frame includes an acceptable 30-day extension of the six-month window period. The 12-month visit was similarly classified as any time after the six-month window up to and including 30 days after 12 months on ART (395 days).

Statistical analyses

All analyses were conducted using Stata statistical software (Version 13.0; Stata Corporation, College Station, TX).

We constructed three multivariable models to predict resistance; potential predictors included participant demographics, clinical diagnoses prior to ART initiation, immunological markers, self-reported adherence, and therapy duration prior to having an elevated VL. The

three models reflect variations in availability of CD4 and VLs at time of ART initiation. WHO guidelines for ART monitoring suggests VL testing only occurs after a patient has been on ART for six months [8]. Although many countries have scaled up access to CD4 testing to determine ART eligibility, the roll-out of Option B+, in which HIV-infected pregnant women are initiated on lifelong ART regardless of CD4, could mean that many patients will not have a baseline CD4 cell count [8]. In light of these policies and the capacity constraints in resource-limited settings, we constructed models to reflect three scenarios: Model 1 assumed that VL and CD4 at ART initiation were available, so both were included as eligible predictor variables. Model 2 assumed that baseline CD4 was available but that baseline VL was not and thus excluded as an eligible predictor variable. Finally, Model 3 assumed that neither baseline VL nor baseline CD4 were available; thus neither were included as eligible predictor variables. To evaluate the association between predictors and ART resistance, we calculated unadjusted prevalence odds ratios (OR) and 95% confidence intervals (CI) for each potential predictor in each model [158].

The full models contained all variables with bivariate p-values <0.5; this high threshold was chosen to ensure that available important predictors were not excluded [159]. Variables with low frequency, extreme collinearity, or insufficient detail to permit clinical implementation were excluded from the models, regardless of p-value. We tested four categorizations of time on treatment and selected the category with the lowest Akaike's information criteria (AIC) value for our reference models [160].

We developed the predictive models using multiple logistic regression with backward elimination [158]. Beginning with the variable with the largest p-value, we removed variables one at a time until five or fewer variables remained (regardless of p-value). The five-variable limit was selected to facilitate eventual implementation of risk scores in resource-limited clinical settings [161, 162]. We assessed the equality of the area under the receiver operating characteristic curves (AUROC) between each model (chi-squared test) [163].

AUROC is a measure of the risk score's discriminatory power –where 1.0 indicates a perfect test (i.e., 100% sensitivity and 100% specificity) [164]. Likelihood ratio (LR) comparing successive models were evaluated to confirm that variable removal did not adversely affect the model's predictive capacity. We also compared LR-test statistics from each reduced model to the full model.

We used the three predictive models to develop the associated risk scores by assigning each variable in the final models a predictor score equal to two times the beta coefficient rounded to the nearest integer. We doubled the coefficient to retain inherent discrimination between betas. Patients with a high VL (≥1000 copies/ml) and a risk score equal to or greater than a pre-specified cutoff are classified as likely resistant to first-line ART and should be switched to second-line ART without a confirmatory VL test. For each model, we assessed sensitivity, specificity, and associated risk scores at cutoffs selected based on clinically-acceptable model-performance criteria [165, 166]. Given the undesirable consequences of prematurely switching patients to second-line therapy, we maintained a high specificity threshold (>95.0%) for all models to minimize false positives. We also calculated the number of patients in a hypothetical cohort of 10,000 ART patients who would be switched without confirmatory testing at each cutoff. We internally validated the model and risk score performance using 1,000 bootstrap samples with replacement [158, 167]. Bootstrapping is preferred over data splitting and cross validation for the purposes of internal validation [168-172]. Model calibration was assessed using Hosmer-Lemeshow (HL) goodness-of-fit tests [173].

Sensitivity Analyses

We conducted a sensitivity analysis to evaluate model performance using only study participants from resource-limited settings only. Given the implementation and policy implications and hypothesized biological association of ART duration and resistance, we

tested multiple forms of the treatment time variable (**Appendix 3**). Models 4-6 evaluate therapy duration categorized as <7, 7-24, and >24 months; models 7-9 dichotomized duration (<7 vs ≥7 months). We compared these alternatives to the primary models using AIC.

Results

Study population

Among 1,045 participants, 305 had at least one VL ≥1000 copies/ml after week 16; 15 participants were excluded despite having two sequential VL ≥1000 copies/ml because resistance results were unavailable at the time of failure, leaving a final sample of 290.

Age at time of failure or first elevated VL ranged from 19 to 65 years, and 53% of patients with at least one VL ≥1000 copies/ml were male (**Table 6.1**). Mean CD4 at enrollment was 156 cells/mm^3 . Median VL at enrollment was 115,383 copies/ml (interquartile range [IQR], 36,925-308,000). Twenty participants had a history of an AIDS-defining diagnosis prior to enrollment, and 60 (21%) had either incident or prevalent tuberculosis at enrollment.

Bivariable analyses

The overall prevalence of NRTI or NNRTI resistance at time of failure was 25.9% (95% CI 20.8%, 30.9%). Participants with a higher VL at ART initiation (>100,000 copies/ml) (OR=2.5, 95% CI 1.4, 4.3) were more likely to be resistant compared to participants with a lower VL at ART initiation (≤100,000copies/ml) (**Table 6.1**). At time of failure, VL >100,000copies/ml (OR=3.3, 95% CI 1.6, 6.9) or 10,000-100,000 copies/ml (OR=5.7, 95% CI 3.0, 10.7) also were associated with increased likelihood of resistance, compared to participants with VL <10,000 copies/ml. Participants who were on therapy less than seven months (OR=5.1, 95% CI 2.6, 9.8), or seven to 12 months (OR=3.2, 95% CI 1.5, 6.8) were

more likely to be resistant compared to participants on therapy >12 months. Participants whose BMI>25.0 kg/m^2 at ART initiation were more likely to be resistant at time of first VL ≥1000 copies/ml than participants with BMI ≤25.0 kg/m^2 (OR=2.5, 95% CI 1.4, 4.5).

Multivariable analyses

Model 1 - Including baseline VL and CD4: The full model included 10 predictor variables (AUROC=0.842). The HL calibration test failed to reject the null hypothesis that there was a statistically significant difference between observed and predicted estimates (p=0.70). Our final model contained five predictor variables: age <30, BMI > 25.0, baseline VL ≤100,000 copies/ml, time on treatment, and VL at time of first VL ≥1000 (**Table 6.2**). The AUROC was 0.820 for the reduced model, which showed acceptable calibration, (HL p=0.12)

Model 2 - Excluding baseline VL: The full model included nine predictor variables (AUROC=0.819) and showed acceptable calibration (HL failed to reject null, p=0.84). After backward elimination, the model contained six predictor variables (AUROC=0.807). To meet the predefined criterion of a five-variable model, we eliminated the variable with the lowest OR (self-reported adherence). Our final model contained: age <30, screening CD4 <100 cells/mm^3, BMI >25.0, time on treatment, and VL at time of first VL ≥1000 (AUROC=0.800) (**Table 6.2**). The reduced model showed acceptable calibration (HL p=0.84).

Model 3 - Excluding baseline VL and CD4: The full model included eight predictor variables (AUROC=0.801) and showed acceptable calibration (HL p=0.37). The final model contained: age <30, self-reported missed medications, BMI > 25.0, time on treatment, and VL at time of first VL ≥1000 (AUROC=0.794) (**Table 6.2**). The reduced model showed acceptable calibration (HL, p=0.10).

Reduced Model 1 performed slightly better than reduced Model 2, but the difference was not significant (p=0.23). Reduced Model 3 performed slightly worse again, but

compared to reduced Model 1, the difference was not statistically significant (p=0.22) (**Figure 6.2**). Bootstrapping demonstrated consistent performance for all models over 1,000 replications.

Risk scores

The weighted risk scores ranged from zero to 12 for Models 1 and 2, and zero to 11 for Model 3 (**Table 6.2**). The maximum attained score by any individual in the tested population was 11 for each model. The predictive power of the model was retained when predicted probabilities were transformed to risk scores (AUROC for Model 1=0.813 (p=0.69), Model 2=0.797 (p=0.91), and Model 3=0.802 (p=0.57)). A risk score cutoff of ≥9 met predefined specificity threshold (>95.0%) (**Table 6.3**).

We estimated the number of patients who would be immediately switched to second-line therapy in a hypothetical population of 10,000 ART patients receiving VL monitoring. Given the resistance prevalence observed (~25%), Model 1 risk score would accurately identify 700 persons with resistance (true positives) and would incorrectly classify 248 persons as resistant when they were not (false positives) (**Figure 6.3**). At this same resistance prevalence, Model 2 risk score would correctly switch 400 persons with resistance and would have 105 false positives. Model 3 would correctly switch 368 persons with resistance, with 143 false positives. However, as the resistance prevalence increases, so too does the number of true positives as well as the ratio of true positive:false positive. For example, with a resistance prevalence of 55% in a population of 10,000 ART patients with a VL ≥1000copies/ml, use of the Model 1 risk score would correctly identify 1,540 patients as resistant with only 149 false positives.

Sensitivity analyses

Model performance was comparable when the study population was restricted to persons from resource-limited settings: AUROC =0.823 (Model 1), 0.812 (Model 2), and 0.804 (Model 3). Using the same risk score cutoff as in the unrestricted model (≥9), the sensitivity for the three models ranged from 10.0%-26.0%, and specificity ranged from 97.4%-99.5% (**Table 6.3**).

Discussion

Current WHO guidelines recommend confirmatory testing for ART patients with high VL (≥1000 copies/ml). A subset of patients will be resistant at the time of initial elevated VL; for these persons, requiring confirmatory testing unnecessarily delays switch to second-line therapy. We developed a risk score using only parameters that are likely to be available to providers in resource-limited settings that successfully distinguishes persons with and without resistance among those with elevated VLs. The risk score performed well, >98% specific in most model iterations. Increased specificity comes at the cost of reduced sensitivity; however, the low sensitivity (~15-30%) is less concerning as these "misses" represent someone with resistance proceeding with the current confirmatory test standard-of-care for virological failure [8]. Applying the risk score with high specificity may result in meaningful public health benefits. Rapidly switching patients with resistance to more efficacious second-line therapy could reduce transmission of resistant viral strains and transmission overall.

Utilization of the risk score may also reduce costs and patient burden (e.g., travel) by avoiding unnecessary confirmatory VL tests. Alternative cost-saving strategies for virological monitoring include pooling specimens and targeting VL tests based on clinical or immunological criteria [8, 92, 114, 116-118]. Despite potential cost-savings, pooling requires additional laboratory support for linkage and deconstruction of positive pools. Applying a

conservative estimate of treatment failure (16.0% at 12 months) would translate to more than one million ART patients having an elevated VL in sub-Saharan Africa alone [90]. Even a modest reduction in confirmatory test volume as facilitated by implementation of our algorithm could substantially reduce expenditures.

Our risk score balances predictive ability and practicality. Including additional variables, and allowing variables to remain continuous rather than categorical, would have improved test performance slightly. However, given our goal of point-of-care application by end-users, we limited all analyses to predictors that were likely available in the context of ART clinics in resource-limited settings. We sacrificed some precision for ease-of-use by collapsing continuous variables into discrete categories and limiting the number of included variables.

Maximizing specificity was essential to decrease false positives. We selected 95.0% as the lower threshold for specificity, though the selected risk score thresholds had specificities above this lower limit (96.7%-98.6%). Even at specificities >98%, prematurely switching a patient to second-line therapy (false positive) still occur and have significant person- and system-wide consequences. For the patient, the false positive misclassification results in lost potential life years that that person could have remained on first-line therapy. For the healthcare system, the premature second-line switch results in increased drug costs– as much as six-to-10-times the cost of first-line therapy [65, 69]. Modeling the consequences of delayed second-line initiation versus premature treatment switches may help elucidate the trade-offs inherent to these thresholds. Importantly, trade-off may vary by population: for example, providers may be more willing to “risk” false positive results in HIV-infected pregnant women given the importance of viral suppression at time of delivery to prevent vertical transmission. Acceptable true positive-to-false positive ratios may also differ depending on anticipated time-to-referral, as the patient and public health benefits of

immediate switching may be greater in settings where there are extensive delays in second-line initiation [85, 91].

These data came from a controlled clinical trial, and enrolled patients may not be representative of larger ART populations. Viral suppression was similar to other cohorts with nearly 30% of patients having a VL ≥1000 copies/ml after ≥16 weeks on ART [90]. Participants received frequent virological monitoring in the study –unlikely in the intended settings for this risk score. In that our risk score has >12 months as the reference time category, patients who first receive virological monitoring later in the course of treatment would not receive any "points," meaning that providers would be more likely to conduct confirmatory testing. Sensitivity analyses with alternative categorization of therapy duration did not change model performance (**Appendix 3**). Despite inherent differences within the clinical trial setting, participants were recruited largely from resource-limited settings and the risk score performed well in this subgroup. Furthermore, PEARL's broad inclusion criteria improves generalizability. Study-driven CD4 cell count eligibility were consistent with WHO guidelines (<300 cells/mm^3), but these guidelines have since changed, expanding ART eligibility to HIV-infected patients earlier in the course of disease (<500 cells/mm^3)[8]. For the risk score generated from Model 2, having a greater proportion of patients with high CD4 at baseline could mean that fewer patients exceed the switch threshold, potentially dampening the efficiency gains of the algorithm.

Resistance rates in the trial (25.9%) were lower than observed in sub-Saharan African based cohorts where rates of resistance are as high as 70% [14, 23, 198-200]. A higher prevalence of resistance would favor use of the risk score, increasing the score's positive predictive value. Assuming 55% resistance among patients with an elevated VL, we demonstrated that in a hypothetical cohort of 10,000 ART patients, >1,500 would be appropriately classified as resistant and switched immediately, with only 150 false positives.

To our knowledge, this risk score is the first to predict resistance among persons with an elevated VL. We successfully identified predictors that reliably distinguished between patients with and without resistance at the time of their first VL ≥1000 copies/ml. Our risk score is sensitive to realities in resource-limited settings: we used a limited number of readily-available categorical variables and minimized false positive results. This model performed well and is a promising opportunity to quickly transition patients with resistance to more effective regimens – improving ART morbidity and mortality outcomes. Using this risk score may reduce transmission of resistant viral strains and save healthcare systems scarce resources by avoiding personnel and equipment costs incurred with unnecessary confirmatory VL testing. These potential benefits should be assessed prospectively by evaluating the effect of the risk score on health outcomes and resource utilization, taking into account the trade-offs associated with misclassifying even a small subset of patients as resistant when they are not [162]. External validation is important to confirm model performance among ART patients managed in resource-limited settings.

Table 6.1: Bivariable association of NRTI/NNRTI resistance and potential predictor characteristics

Predictor	Overall (n=290) N (%)	Resistant (n=75)* N (%)	Not resistant (n=215) N (%)	Unadjusted Prevalence OR (95% CI)	p-value
Age, years					0.09
≤30	82 (28.3)	27 (36.0)	55 (25.6)	1.64 (0.93-2.87)	
>30	208 (71.7)	48 (64.0)	160 (74.4)	1.0	
Sex					0.3
Male	154 (53.1)	36 (48.0)	118 (54.9)	0.76 (0.45, 1.28)	
Female	136 (46.9)	39 (28.7)	97 (71.3)	1.0	
BMI, kg/m^2					0.002
Normal/low (<24.9)	229 (79.0)	50 (21.8)	179 (78.2)	1.0	
High (>25.0)	31 (21.0)	25 (41.0)	36 (59.0)	2.48 (1.37-4.52)	
CD4 at screening, cells/mm^3					0.12
≤100	84 (71.0)	27 (36.0)	57 (26.5)	1.56 (0.89, 2.73)	
>100	206 (29.0)	48 (23.3)	158 (76.7)	1.0	
Baseline VL, copies/ml					0.001
≤100,000	135 (46.6)	23 (17.0)	112 (83.0)	1.0	
>100,000	155 (53.4)	52 (33.5)	103 (66.5)	2.46 (1.41, 4.30)	
AIDS history					0.55
Yes	26 (9.0)	8 (30.8)	18 (69.2)	1.31 (0.54-3.14)	
No	264 (91.0)	67 (25.4)	197 (74.6)	1.0	
History of ART exposure					0.02
Yes	4 (1.4)	3 (75.0)	1 (25.0)	8.92 (0.91-87.1)	
No	286 (98.6)	72 (25.2)	214 (74.8)	1.0	
History of TB					0.14
Yes	60 (20.7)	11 (18.3)	49 (81.7)	1.0	
No	230 (79.3)	64 (27.8)	166 (72.2)	1.72 (0.84-3.51)	
Reported symptoms					0.22
Yes	37 (71.2)	11 (29.7)	26 (70.3)	2.75 (0.53-14.3)	
No	15 (28.9)	2 (13.3)	13 (86.7)	1.0	
Imperfect adherence					0.11
Yes	67 (25.6)	22 (32.8)	45 (67.2)	1.63 (0.89, 3.00)	
No	195 (74.4)	45 (23.1)	150 (76.9)	1.0	
Pill count, % taken					0.29
<80%	11 (22.4)	6 (54.5)	5 (45.5)	2.06 (0.53, 8.00)	
≥80%	38 (77.6)	14 (36.8)	24 (63.2)	1.0	
Regimen frequency					0.84
Once daily (FTC/TDF/EFV QHS)	144 (49.7)	38 (26.4)	106 (73.6)	1.06 (0.62, 1.79)	
Twice daily (3TC/ZDV BID+EFV QHS)	146 (50.3)	37 (25.3)	109 (74.7)	1.0	
Time on therapy, months**					<0.001
< 7	102 (35.2)	42 (41.2)	60 (58.8)	5.1 (2.6-9.8)	
7-12	56 (19.3)	17 (30.4)	39 (69.6)	3.2 (1.5-6.8)	
>12	132 (45.5)	16 (12.1)	116 (87.9)	1.0	
VL at time of failure, copies/ml					<0.001

≤10,000	175	(60.4)	25	(14.3)	150	(85.7)	1.0		
10,001-100,000	70	(24.1)	34	(48.6)	36	(51.4)	5.7	(3.0-10.7)	
>100,000	45	(15.5)	16	(35.6)	29	(64.4)	3.3	(1.6-6.9)	
CD4 at failure, cells/mm^3									0.18
≤200	77	(27.6)	24	(31.2)	53	(68.8)	1.49	(0.83-2.7)	
>200	202	(72.4)	47	(23.3)	155	(76.7)	1.0		
Any change in therapy during study									0.28
Yes	42	(14.5)	8	(19.1)	34	(80.1)	0.64	(0.38-1.4)	
No	248	(85.5)	67	(27.0)	181	(73.0)	1.0		

*Resistance indicates identified NRTI or NNRTI resistance mutations detected on stored specimens at time of first elevated (>1000 copies/ml) viral load

**Therapy duration defined by days, <7 months is <213; 7-12 months is 212-395, >12 months is >395 days.

3TC, lamivudine; ART, antiretroviral therapy; BID, twice daily; BMI, body-mass index; CI, confidence interval; EFV, efavirenz; FTC, emtricitabine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; QHS, nightly; TB, tuberculosis; TDF, tenofovir; VL, viral load; ZDV, zidovudine

Table 6.2: Adjusted odds ratios and risk scores of NRTI/NNRTI resistance

Predictor	Model 1 (with baseline VL) (n=290), AUROC=0.820			
	Full model OR (95% CI)	Reduced OR (95% CI)	β^2	Predictor score[1]
Age, years				
≤30	2.2 (1.0-4.6)	2.1 (1.0-4.1)	0.72	1
>30	1.0	1.0		0
Sex				
Male	0.7 (0.4-1.4)	- -	-	-
Female	1.0	- -	-	-
BMI, kg/m^2				
Normal/low (<24.9)	1.0	1.0		0
High (>25.0)	2.8 (1.2-6.4)	3.7 (1.8-7.8)	1.31	2
Baseline VL, copies/ml				
≤100,000	1.0	1.0		0
>100,000	3.2 (1.5-7.1)	3.6 (1.8-7.0)	1.27	2
Time on therapy, months				
<7	4.2 (1.9-9.2)	4.2 (2.0-8.6)	1.43	3
7-12	2.0 (0.8-5.1)	2.9 (1.2-6.9)	1.07	2
>12	1.0	1.0		0
VL at failure, copies/ml				
≤10,000	1.0	1.0		0
10,001-100,000	7.3 (3.4-15.9)	6.3 (3.1-13.0)	1.85	4
>100,000	2.8 (1.1-7.2)	2.7 (1.2-6.1)	0.99	2
CD4 at screening, cells/mm^3				
≤100	1.8 (0.9-3.9)	- -	-	-
>100	1.0	- -	-	-
History of TB				
Yes	1.0	- -	-	-
No	1.8 (0.7-4.5)	- -	-	-
Treatment changed while on study				
Yes	0.4 (0.1-1.3)	- -	-	-
No	1.0	- -	-	-
Ever missed meds				
Yes	1.8 (0.9-3.7)	- -	-	-
No	1.0	- -	-	-
	Model 2 (without baseline VL) (n=290), AUROC=0.800			
	Full model OR (95% CI)	Reduced model OR (95% CI)	β^3	Predictor score[1]
Age, years				
≤30	1.8 (0.9-3.7)	1.8 (0.9-3.5)	0.59	1
>30	1.0	1.0		0
Sex				
Male	0.7 (0.3-1.3)	- -	-	-
Female	1.0	- -	-	-
BMI, kg/m^2				
Normal/low (<24.9)	1.0	1.0		0
High (>25.0)	2.5 (1.1-5.6)	3.2 (1.6-6.6)	1.18	2
Baseline VL, copies/ml				
≤100,000	- -	- -	-	-
>100,000	- -	- -	-	-
Time on therapy, months				
<7	3.9 (1.8-8.3)	4.3 (2.1-8.7)	1.45	3
7-12	1.9 (0.8-4.8)	3.1 (1.3-7.2)	1.13	2
>12	1.0	1.0		0

VL at failure, copies/ml						
≤10,000	1.0		1.0			0
10,001-100,000	7.4	(3.4-15.8)	6.3	(3.1-12.8)	1.85	4
>100,000	2.7	(1.1-6.8)	3.1	(1.4-7.0)	1.15	2
CD4 at screening, cells/mm^3						
≤100	2.6	(1.3-5.3)	2.2	(1.2-4.3)	0.81	2
>100	1.0		1.0			0
History of TB						
Yes	1.0		-	-	-	-
No	1.3	(0.6-3.2)	-	-	-	-
Treatment changed while on study						
Yes	0.4	(0.1-1.2)	-	-	-	-
No	1.0		-	-	-	-
Ever missed meds						
Yes	2.1	(1.0-4.1)	-	-	-	-
No	1.0		-	-	-	-

	Model 3 (without baseline VL or CD4) (n=260), AUROC=0.794					
	Full model OR (95% CI)		OR (95% CI)		β[4]	Predictor score[1]
Age, years						
≤30	1.6	(0.8-3.2)	1.7	(0.9-3.4)	0.53	1
>30	1.0		1.0			0
Sex						
Male	0.7	(0.4-1.4)	-	-	-	-
Female	1.0		-	-	-	-
BMI, kg/m^2						
Normal/low (<24.9)	1.0		1.0			0
High (>25.0)	2.3	(1.1-4.1)	2.7	(1.2-5.7)	0.98	2
Baseline VL, copies/ml						
≤100,000	-	-	-	-	-	-
>100,000	-	-	-	-	-	-
Time on therapy, months						
<7	3.6	(1.7-7.6)	3.7	(1.8-7.8)	1.32	3
7-12	1.9	(0.8-4.8)	2.1	(0.9- 5.2)	0.76	2
>12			1.0			0
VL at failure, copies/ml						
≤10,000	1.0		1.0			0
10,001-100,000	6.5	(3.1-13.5)	6.5	(3.1- 13.3)	1.87	4
>100,000	2.7	(1.1-6.6)	3.0	(1.2- 7.2)	1.10	2
CD4 at screening, cells/mm^3						
≤100	-	-	-	-	-	-
>100	-	-	-	-	-	-
History of TB						
Yes	1.0		-	-	-	-
No	1.3	(0.6-3.2)	-	-	-	-
Treatment changed while on study						
Yes	0.4	(0.1-1.3)	-	-	-	-
No	1.0		-	-	-	-
Ever missed meds						
Yes	2.1	(1.0-4.1)	1.8	(1.0-3.6)	0.61	1
No			1.0			0

[1]weighted; [2]constant = -3.94; [3]constant = -3.42; [4]constant = -3.11

CI, confidence interval; β, beta regression coefficient; BMI, body mass index; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; AUROC, area under receiver operating characteristic curve; VL, viral load

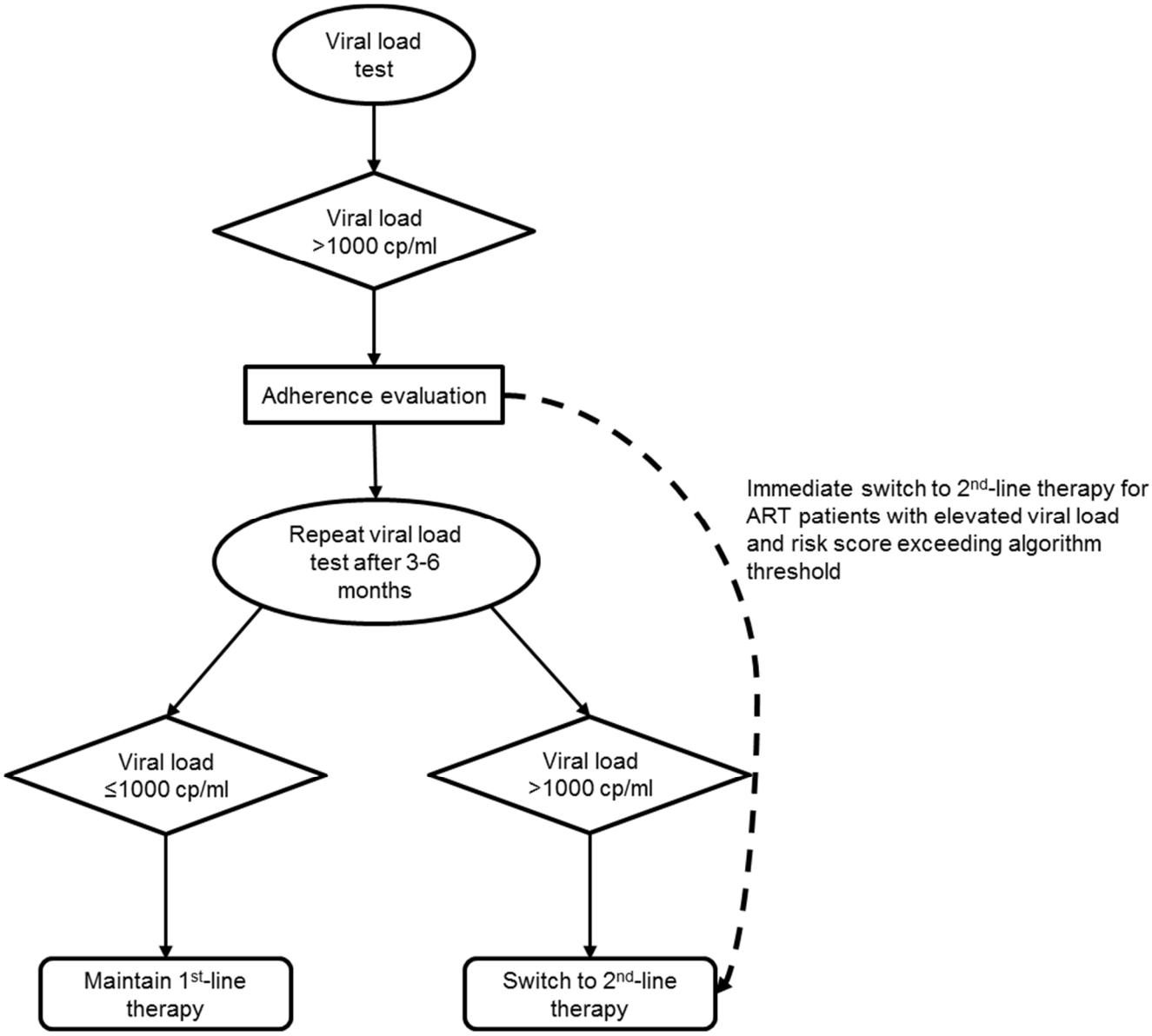
Table 6.3: Performance of resistance models and derived risk scores

Predictor	Model with baseline VL (n=290)			Model without baseline VL (n=290)			Model without baseline VL or CD4 (n=260)		
	Cutoff	Sens (%)	Spec (%)	Cutoff	Sens (%)	Spec (%)	Cutoff	Sens (%)	Spec (%)
Unrestricted (RLS & non-RLS)									
Model*	0.657	22.7	98.1	0.640	22.7	97.2	0.741	13.4	98.4
Weighted risk score	≥9	28.0	96.7	≥9	16.0	98.6	≥9	14.7	98.1
Restricted (RLS only)									
Model*	0.653	28.0	97.2	0.697	18.7	98.1	0.691	14.9	98.4
Weighted risk score	≥9	26.0	97.4	≥9	10.0	99.5	≥9	14.0	99.5

*Cutoff values for the models are thresholds derived by summing the beta coefficients and converting to a probability

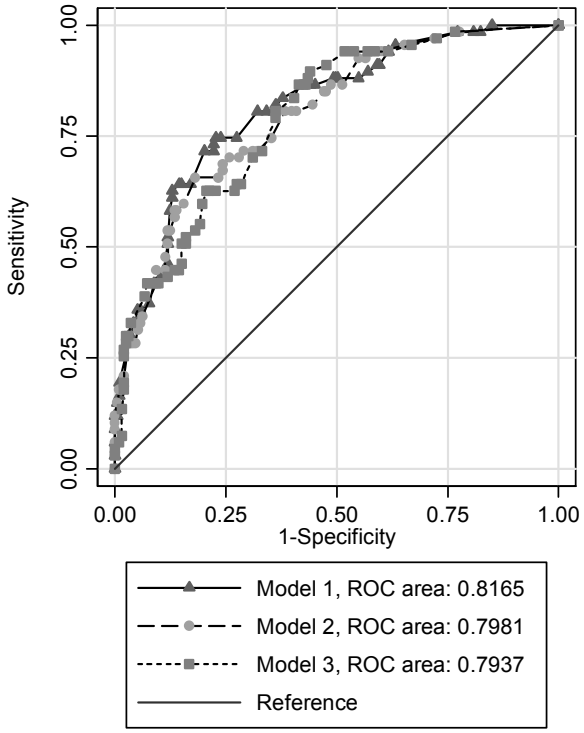
RLS, resource-limited setting; Sens, sensitivity; Spec, specificity; VL, viral load

Figure 6.1: WHO viral load testing strategy for treatment failure



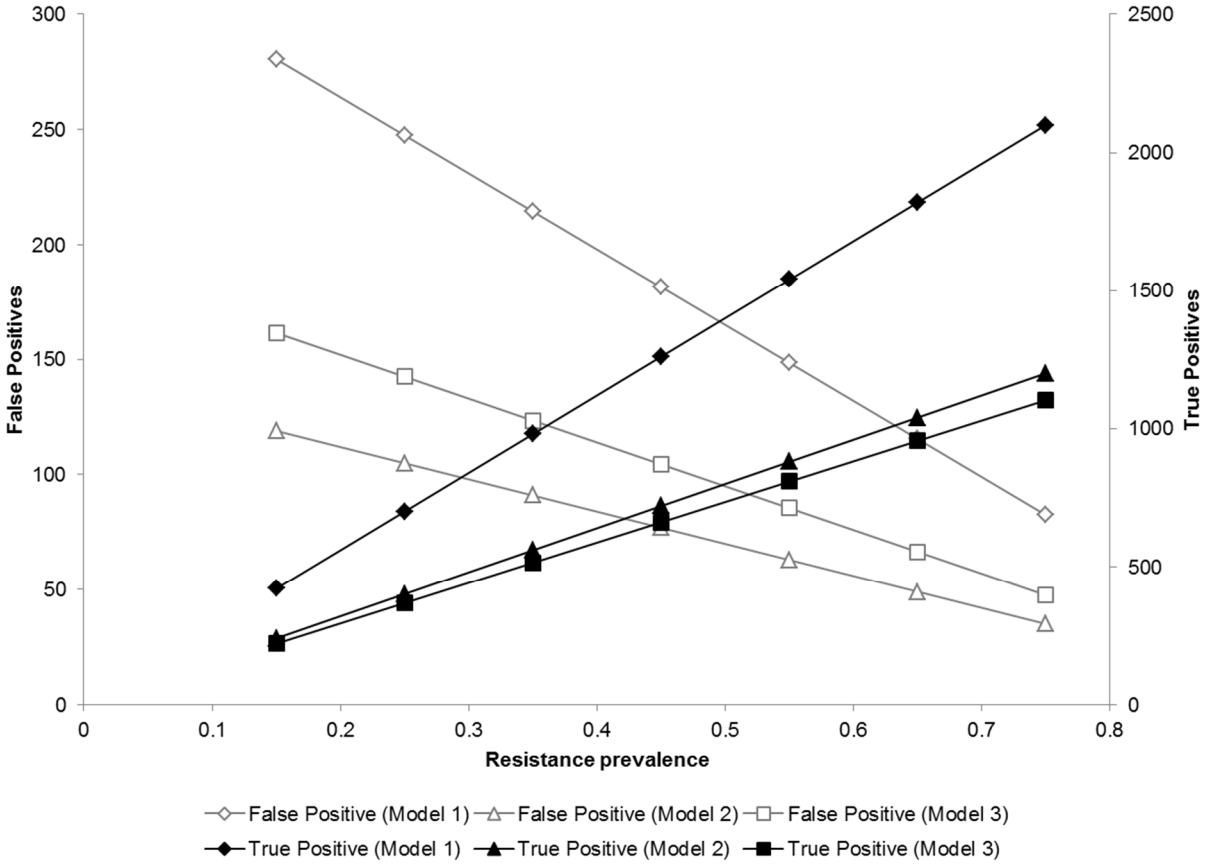
Caption: Figure 6.1: Patients eligible for viral load testing may be tested using plasma-based assays or dried blood spots [8]. For plasma assays, a viral load >1000 copies/ml prompts an evaluation of adherence to antiretroviral therapy and targeted adherence counseling if deficiencies in adherence are observed. The viral load test is repeated 3 to 6 months later (confirmatory test). Patient management is dictated by results of this second test – patients with confirmed elevated (>1000 copies/ml) viral loads are switched to second-line therapy. The dashed arrow represents implementation of the risk score algorithm. Patients with a risk score exceeding the predefined algorithm threshold would be switched immediately to second-line therapy.

Figure 6.2: Receiver operating characteristic (ROC) curves for Models 1-3.



Caption: Figure 6.2: The area under an ROC curve is a measure of model performance. Specifically, the area measures discrimination – in this case the ability of the predictive model to correctly classify persons with and without resistance. Model 1, in which we assumed that viral load and CD4 cell counts from time of treatment initiation were available, performed the best, and had an area under the ROC curve of 0.8165. In Model 2, when viral load from treatment initiation was excluded as an eligible predictor, performed slightly less well (area under ROC curve of 0.7981). Finally, in Model 3, we assumed that neither viral load nor CD4 cell counts from time of treatment initiation were available. This model performed the poorest of all three models evaluated, with an area under the ROC curve of 0.794 – although this difference was not statistically significant and may not be clinically meaningful.

Figure 6.3: Number of false positive and true positive results in hypothetical cohort of 10,000 ART patients with elevated viral load at varied resistance prevalence estimates



Caption: Figure 6.3: Using the sensitivities and specificities for each risk score at the defined threshold, we generated the number of false positives and true positives that would be expected among a 10,000-person cohort of patients with an initially elevated viral load. We evaluated these outcomes at varying levels of ART resistance. As the prevalence of resistance increases, the positive predictive value of the risk scores also improves.

CHAPTER 7: DISCUSSION

When used appropriately, ART suppresses viral replication, decreases HIV-associated morbidity and mortality, and reduces HIV transmission [7, 42-47]. The scale-up of ART in resource-limited settings has been remarkable. Increased ART access is especially impressive in sub-Saharan Africa, from fewer than two million persons on treatment in 2006 to nearly 10 million in 2013 [37, 48]. The individual and public health benefits of ART are contingent on virological suppression, which requires routine VL monitoring to determine whether patients are on the right medication. Despite extraordinary advances in ART coverage, only a fraction of the millions of African patients receiving ART have access to routine VL monitoring [27, 28].

The goal of this dissertation was to address the overarching question of how to design and implement effective, efficient, and feasible VL monitoring strategies in resource-limited settings. Specifically, we: (1) assessed the programmatic and clinical outcomes of DBS for VL monitoring in the sub-Saharan African context (Aim 1); (2) examined provider-perceived barriers and facilitators to VL monitoring implementation (Aim 2); and (3) developed a risk score to identify persons with ART resistance and improve VL monitoring efficiency (Aim 3). Taken together, these studies may inform strategies for successful and sustainable VL monitoring scale-up in sub-Saharan Africa. Our studies also expose important gaps in knowledge, providing direction for future research towards the long-term objective of optimizing ART use and outcomes in resource-limited settings.

Summary of findings

Aim 1: DBS for VL monitoring

Our first study evaluated use of DBS for VL monitoring at five district hospitals in Malawi. VL monitoring using DBS was feasible and effective when implemented by ART providers. More than 99% of the VL results were returned from the central laboratory to the enrolling clinic, and nearly 80% of participants received their VL results within 3 months of testing. Among the participants with confirmed elevated VL, 92.6% initiated second-line therapy, and 91% were switched within one year of their first high VL. Eligible participants were rapidly transitioned to second-line therapy: >50% of participants initiated second-line therapy the same day that they received confirmatory VL results. Compared to other VL monitoring programs in the region, we observed faster initiation of second-line therapy after confirmation of virological failure [91].

Among the 1,498 ART patients enrolled, only 88 (5.9%) were failing with an elevated VL (>5,000 copies/ml) at baseline. Similar failure rates (6.6%) have been observed using DBS elsewhere in Malawi [92]. Both younger age and increased time on ART were associated with increased risk of treatment failure in multivariable models, but clinical symptoms were not. The relationship between age and failure is frequently associated with inadequate adherence, perhaps linked to behavioral challenges unique to adolescence [201]. However, most participants in our study initiated ART post-adolescence. This increased risk of failure among young adults indicates the need for adherence interventions targeted specifically to this group, who may experience different barriers to ART adherence than their younger or older counterparts. Participants on ART >4 years were also considerably more likely to be failing compared to participants on therapy 1-4 years (RR 1.70). Higher failure rates with longer ART exposure time is not surprising, but should be considered when initiating VL monitoring programs; specifically, catch-up testing coverage needs to include persons with extended ART exposure. Clinical symptoms were not

associated with increased risk of failure, emphasizing shortcomings of relying on clinical staging for predicting virological failure [8, 16, 81].

Current WHO guidelines advocate repeat VL testing for persons with an elevated VL after 3 to 6 months [8]. Approximately one-third of participants with elevated VLs resuppressed on confirmatory testing, mirroring resuppression rates elsewhere in the region [26, 177, 178]. These resuppression rates emphasize the importance of adherence counseling after VL testing, especially among persons with elevated VLs [25]. In our study, there was no difference in resuppression rates for participants retested 0-3 months after baseline testing compared to 3-6 months after baseline testing. In contrast, participants who had 3-6 months between receipt of results and confirmatory testing were more likely to resuppress compared to those with 0-3 months between receipt of results and confirmatory testing. Our findings suggest that the critical period for resuppression is not the time between tests, but rather the time between receipt of results and confirmatory testing. We explore barriers to returning results in Aim 2, and address the broader question of confirmatory testing, and opportunities to avoid the delays and expenses associated with confirmatory testing, in Aim 3.

Aim 2: Provider perceived barriers and facilitators to VL monitoring

Our second study interviewed providers involved in the DBS VL monitoring study (Aim 1). Providers identified a complex set of interconnected barriers and facilitators to VL monitoring that fit within the framework of a modified social ecological model. Providers emphasized their desire for improved ART monitoring strategies. Providers also described patients craving information regarding their VL and the frustration on the part of both provider and patient with restrictive MOH eligibility criteria. Although many providers pled for expanding VL monitoring eligibility to include all persons on ART, the most salient provider-perceived barrier to VL monitoring implementation was the pressure of work associated with

monitoring activities. The work burden was exacerbated by inefficient data management systems, highlighting a critical interaction between provider-, facility-, and system-level factors described in the SEM. Although centralized testing increases VL monitoring access in remote settings, challenges with data management accentuate the drawbacks associated with a centralized laboratory model. Lack of integration between laboratory and clinical systems complicated the process for alerting providers when results were available, and these communication gaps were intensified by poor facility connectivity.

Provider empowerment emerged as an unexpected facilitator of VL monitoring. For many providers, the DBS study was the first time they used an objective marker of ART response to guide clinical management. Providers' knowledge of a patient's virological status increased confidence in adherence counseling and clinical decision making. Emphasizing provider empowerment during VL scale-up activities may improve providers' willingness to adopt additional clinical duties and counseling responsibilities. Based on our results, VL monitoring can modify provider behavior, and should be presented as a tool to help providers improve the quality of HIV care they deliver to patients.

Aim 3: Resistance risk score among patients with high VL

As described in Aim 1, rapidly identifying persons who will not resuppress on confirmatory testing, namely those with resistance, may improve clinical outcomes and save resources. In our final study, we developed a risk score that successfully identified persons with resistance among those with elevated VLs. To facilitate eventual clinical implementation of this risk score, we only used parameters likely to be available to providers in resource-limited settings. We developed three model iterations, each more restrictive than the previous in terms of assumptions regarding the information that a provider may have on hand: the first model assumed the provider had information on a patient's VL and CD4 cell count at time of ART initiation; the second model removed baseline VL as an eligible

predictor; and the third model removed both baseline VL and CD4 cell count from the pool of eligible predictors. All three resulting risk scores performed well – most >98% specific.

Higher VL at ART initiation, higher VL at time of failure, shorter therapy duration, and higher BMI were all associated with increased risk of resistance in bivariable models. The risk scores derived from multivariable models retained predictive power, with high AUROCs (0.813, 0.797, and 0.802, for risk score 1, 2, and 3, respectively). Model performance was comparable when the study population was restricted to persons from resource-limited settings. The sensitivity for the three models ranged from 10.0%-26.0%, and specificity ranged from 97.4%-99.5%. High specificity comes at the cost of reduced sensitivity; however, the low sensitivity is less concerning as these "misses" represent someone with resistance proceeding with the current standard-of-care confirmatory test for virological failure [8].

Resistance rates in the trial (25.9%) were lower than observed in sub-Saharan Africa based cohorts, where rates of resistance are as high as 70% [14, 23, 198-200]. A higher prevalence of resistance would favor use of the risk score, increasing the score's positive predictive value. Assuming 55% resistance among patients with an elevated VL, we demonstrated that in a hypothetical cohort of 10,000 ART patients, >1,500 would be appropriately classified as resistant and switched immediately, with only 150 false positives. False positives may result in prematurely switching a patient to second-line therapy, which could have substantial person- and system-wide consequences. Modeling the consequences of delayed second-line initiation versus premature treatment switches may help elucidate the trade-offs at varying score "switch" thresholds.

Summary of contributions

Our studies identified programs that reliably identified virological failure (Aim 1), are feasible in the resource-limited ART clinical setting (Aim 2), and equip providers with point-

of-care algorithms that facilitate rapid treatment change for patients with ART resistance (Aim 3). Together, these findings bring us closer towards our longer-term goal of optimizing ART use and improving the quality of ART management and HIV care delivered in resource-limited settings.

Implications for policy and practice

Increasing access to VL monitoring is an urgent global health priority, and essential to achieve and sustain the individual and public health benefits of ART. Our findings may inform the development and implementation of VL monitoring algorithms in resource-limited settings. Specifically, we identified opportunities to reduce resource utilization and improve scale-up logistics.

Decreasing the cost of VL testing may be essential to increase coverage in resource-limited settings. Pooling specimens may be one cost-effective alternative to individual VL testing, especially given observed low failure rates [92]. Another strategy to improve efficiency, reduce cost, and improve outcomes of VL monitoring may be incorporating a risk score algorithm to distinguish patients harboring resistance from patients needing focused adherence counseling. At the appropriate threshold, the risk score may avoid unnecessary expenses associated with confirmatory testing.

Because patients enrolled under targeted monitoring criteria have substantially higher baseline virological failure rates, one scale-up strategy could be to limit monitoring to patients showing clinical signs of failure. Although such a strategy would increase VL monitoring efficiency by reducing the number of eligible patients and containing costs, our results highlight two issues with this approach. First, this strategy misses a substantial number of failing patients who do not show clinical signs of failure and who are at increased risk of poor clinical outcomes and forward transmission. Second, provider interviews demonstrated irritation with the already restrictive milestone-based monitoring. Further

restrictions may be met with provider resistance. Indeed, a catch-up approach, in which everyone on ART for more than 2 years is eligible for VL testing could be a more effective and acceptable approach to VL program scale-up.

Our studies identified two key system-level strategies that may contribute to more successful and sustainable VL monitoring program implementation. The first is the need to modify ART clinic staffing models. Findings from both Aims 1 and 2 demonstrate the need for more mid-tier providers. Though lower-tier providers, such as HSAs, were able to assist in DBS specimen collection, the time-consuming work of adherence counseling remained with the higher level providers, such as nurses and clinical officers. Incorporating lay adherence counselors could fill an important human resources gap. Peer support and lay counselors have been a central part of HIV testing and counseling scale-up in sub-Saharan Africa [202, 203]. This cadre of counselors would be especially useful if their training focused on intensive adherence counseling after VL testing – by far one of the most time-intensive components of VL monitoring. The second system-level strategy to improve VL monitoring would focus resources on bolstering laboratory and clinic data management systems. Both Aims 1 and 2 identified gaps in data management systems contributing to delayed result return, delayed referral for second-line therapy, and general frustration of providers and patients. Future POC tests would facilitate immediate delivery of results and may address some of the data management system shortcomings. However, in the interim, it is imperative that policymakers commit resources to improving software and communication capacity between central laboratories and district hospitals.

Knowledge gaps and opportunities for future research

Aim 1 is one of the first studies to assess use of DBS for VL monitoring in sub-Saharan Africa that included an effectiveness endpoint, specifically, patient initiation of second-line ART. Despite attempts to mimic “real-world” conditions, elements of our

evaluation may not be replicated outside the study setting. Providers were aware of the data collection procedures, which may have influenced referral rates. Monitoring and evaluation activities after VL scale-up should use time-to-second-line initiation as an indicator of program effectiveness.

Resistance testing for persons with elevated VL is cost-prohibitive and generally not available in resource-limited settings. An important next step to our research includes assessment of ART resistance among patients in the DBS study who were eligible for second-line ART. Testing confirmatory DBS specimens will help identify underlying prevalence of resistance in this ART patient population, providing important surveillance data, and quantifying missed opportunities for targeted adherence interventions. Understanding prevalence of resistance in even this small cohort of patients may be important to justify larger resistance surveillance efforts coupled with monitoring scale-up – informing practice patterns in terms of counseling and second-line regimen drug selection.

DBS may systematically overestimate VL compared to the referent standard plasma tests, and this bias needs to be considered in interpreting clinical and programmatic implications of our results [197]. Although not the focus of this study, the limitations of DBS, namely VL overestimation and poor specificity below 5,000 copies/ml, have important implications for health system costs and operations. Our estimated failure rate doubled (12%) if the failure threshold definition was lowered from 5,000 copies/ml to 1,000 copies/ml – the WHO threshold for failure on plasma specimens [8]. At this failure rate, pooling specimens may no longer be a cost-effective option. Given the additional costs associated with second-line ART, future research should continue to explore the appropriate VL failure threshold for DBS. Although a deviation from WHO guidelines, the Malawi monitoring model may be a suitable alternative to dealing with the unreliability of DBS at lower VLs [140]. The Malawi model specifies repeat testing for anyone with VL >1,000 copies/ml, classifying these persons as a “potential failure” and providing intensive adherence support for at least

3 months. In this algorithm, patients are switched to second-line therapy only after a VL >5,000 copies/ml. Modeling the consequences of this algorithm in terms of resuppression rates and missed failures could inform international guidelines for use of DBS for VL monitoring.

Results from our second study provide unique insight into provider perceptions of VL monitoring. However, perspectives from the small number of providers engaged in DBS study activities may not be generalizable to all ART providers in Malawi or sub-Saharan Africa. Perceived barriers and facilitators are influenced by clinic-specific staffing models and quality of clinic infrastructure, factors that are likely to vary across settings. Furthermore, our provider interviews were conducted in the context of an ongoing public health evaluation of DBS for VL monitoring, and not as part of a government-sponsored VL monitoring program. Despite efforts to minimize extra study-specific tasks, we are unable to tease out what barriers may have been influenced by the provider responsibilities due to the DBS parent study. Ongoing monitoring and evaluation of program scale-up in Malawi and elsewhere should engage providers to examine emergence of additional themes to those observed in our study. Future research also should interview patients to further elucidate patient comprehension and patient-perceived barriers or facilitators that could impact uptake.

Improved patient retention may be an important secondary benefit of VL monitoring. Higher perceived quality of care and nurse satisfaction may improve retention of patients in ART care [204, 205]. Indeed, our second study highlights provider empowerment as related to improved care delivery. As such, an important research opportunity would be to examine changes in patient retention before and after VL monitoring. We know that VL monitoring contributes to virological resuppression rates, the final stage of the HIV treatment cascade (see **Figure 2.3**), but keeping patients engaged in care may be equally important to improving ART outcomes.

Our third study developed a risk score with the ART provider in mind. The risk score performed well and is a promising opportunity to quickly transition patients with resistance to more effective regimens – improving ART morbidity and mortality outcomes. External validation with larger and more diverse patient populations is important to confirm model performance in non-trial settings. Moreover, a model that could reliably distinguish clinical features indicative of NRTI resistance as distinct from NNRTI resistance would help guide second-line regimen selection. An exciting potential data source for external validation of the risk score algorithm would be using data and leftover DBS specimens from participants described in Aim 1. A protocol outlining resistance testing for these specimens is under review at local institutional review boards and, pending approval, may commence in spring 2015.

For any risk score to be successful, it is critical that policymakers and providers reach consensus regarding the appropriate threshold above which a patient should be switched immediately to second-line ART. Such a threshold must consider the person and public health tradeoffs of false positives (i.e., premature treatment switch) and false negatives (i.e., delayed second-line initiation). Modeling the consequences of alternative risk score switch thresholds may help clarify the ideal cutoff. Importantly, tradeoffs, and thus appropriate thresholds, may vary by population. For example, providers may be more willing to “risk” false positive results in HIV-infected pregnant women given the importance of viral suppression at time of delivery to prevent vertical transmission. Acceptable true positive-to-false positive ratios also may differ depending on anticipated time-to-referral, as the patient and public health benefits of immediate switching may be greater in settings where there are extensive delays in confirmatory testing and second-line initiation [85, 91]. Future modeling endeavors would make these tradeoffs explicit and could inform risk score utilization.

Conclusions

Virological testing is recommended by the WHO as the preferred method for monitoring response to ART and identifying treatment failure [8]. Increased access to VL monitoring is critical to fully realize the health benefits of ART and achieve the transmission reduction potential of treatment as prevention. Unfortunately, implementation of VL monitoring in resource-limited settings has been hampered by prohibitively expensive and complex laboratory technologies. We demonstrated not only the feasibility, but also the effectiveness of VL monitoring using DBS at district hospitals in Malawi. The barriers to scale-up were frequently system-driven, but are surmountable obstacles if the MOH and external funders commit sufficient and sustained resources to consumables, infrastructure, and personnel training. In addition to informing Malawi MOH officials, our findings provide timely insight into opportunities for VL monitoring implementation strategies in other sub-Saharan African countries.

APPENDIX 1: CASE REPORT FORMS (DBS STUDY)

ADHERENCE ASSESSMENT (ADH)

DBS: Adherence assessment report form (ADH)

Date of Visit: |___|___| / |___|___|___| / |___|___| (DD/MMM/YY)

Visit Number:

☐ (0)Visit 1 ☐ (1) Visit 2 ☐ (2)Visit 3 ☐ (3)Visit 4 ☐ (8)Other

Participant Identification Number (Site Code - ART Clinic Number):

|___|___|___|___| –|___|___|___|___|___|___|___|___|___|___|

---

1. What regimen is the patient on? (pills/day)
   - ☐ (0) 1A (d4T/3TC/NVP) (2pills)
   - ☐ (1) 2A (AZT/3TC/NVP) (2 pills)
   - ☐ (2) 3A (d4T/3TC + EFV) (3 pills)
   - ☐ (3) 4A (AZT/3TC + EFV) (3 pills)
   - ☐ (4) 5A (TDF/3TC/EFV) (1 pill)
   - ☐ (5) 6A (TDF/3TC + NVP) (3 pills)
   - ☐ (6) 7A (TDF/3TC+LPV/r) (5 pills)
   - ☐ (7) 8A (AZT/3TC+LPV/r) (5 pills)
   - ☐ (8) Other_____

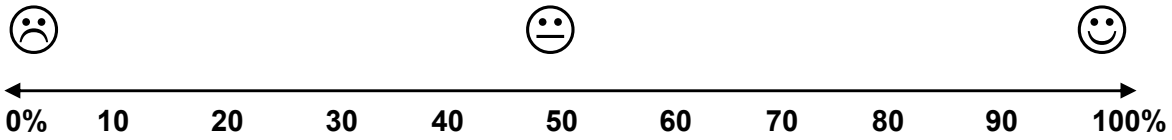
2. The next section asks about medications that you may have missed taking over the last week. We know that it is hard for most people to take their medicine perfectly all the time.

	HOW MANY DOSES DID YOU MISS…?				
HIV drug[1]	Yesterday	Day before yesterday (2 days ago)	3 days ago	4 days ago	In the last week
	____doses	____doses	____doses	____doses	____doses
	____doses	____doses	____doses	____doses	____doses

3. In the last 4 weeks, when was the last time you missed taking any of your anti-HIV medications?
   - ☐ (1) Within the past week
   - ☐ (2) 1-2 weeks ago
   - ☐ (3) 2-4 weeks ago
   - ☐ (0) never skipped medications in the last 4 weeks

[1] If patient is on 1A, 2A, OR 5A, write these codes for "HIV drug." Otherwise, write in the specific pills that a patient takes on separate lines. For example, for patients on 3A, the first line would refer to d4T/3TC doses missed, and the second would be EFV.

4. Now think about the last 30 days. Please point on the line showing the number that is your best guess about how much [Medicine] you have taken in the past 30 days. 0% means you have taken no [Medicine] (sad face), 50% means you have taken half of your [Medicine], and 100% means you have taken every single dose of [Medicine] (smiley face).
   PROVIDER: Mark line where patient indicates.



5. The last time you missed a tablet, which of these describes the reason why?
   Choose all that apply.

a.	Travelling and left pills at home/took insufficient doses with me	☐ (1) Yes	☐ (0) No
b.	I did not want person(s) nearby to see me taking the medication	☐ (1) Yes	☐ (0) No
c.	I was trying to avoid side effects	☐ (1) Yes	☐ (0) No
d.	I felt healthy	☐ (1) Yes	☐ (0) No
e.	Ran out of pills	☐ (1) Yes	☐ (0) No
f.	I do not believe the medicines are beneficial	☐ (1) Yes	☐ (0) No
g.	Felt depressed/overwhelmed	☐ (1) Yes	☐ (0) No
h.	Other_____	☐ (1) Yes	☐ (0) No
i.	Not Applicable – no missed tablets	☐ (1) Yes	☐ (0) No

6. What was the date of the patient's last visit at which they were provided ART?
   |__|__|/|__|__|__|/|__|__| (DD/MMM/YY) ☐ (88) Unknown

7. How many days' worth of therapy was given to this patient at their last visit?
   |__|__|__| ☐ (88) Unknown

8. Did patient bring remaining pills at this visit?
   Yes ☐(1) → Indicate number of pills |__|__|__| → → (End of form)
   No ☐(0) Indicate reasons
   - ☐(0) Forgot
   - ☐(1) Drugs were finished
   - ☐(2) Drugs were stolen
   - ☐(3) Not explained
   - ☐(4) Other

CLINICAL EVENTS (CLIN)

DBS: Clinical Events report form (CLIN)

Date of Visit: |___|___| / |___|___|___| / |___|___|___|___| (DD/MMM/YYYY)

Visit Number:

☐ (0) Visit 1 ☐ (2) Visit 3

Participant Identification Number (Site Code - ART Clinic Number):

|___|___|___|___| –|___|___|___|___|___|___|___|___|___|___|

Complete the following question for each participant. Be sure to complete each question.

At this visit, is the patient presenting with any of the following?

	Condition	Yes	No	If yes: New	Ongoing	
1	Herpes zoster	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
2	Papular Pruritic Eruption	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
3	Unexplained chronic diarrhea (>1 month)	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
4	Unexplained persistent fever	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
5	Moderate unexplained weight loss	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
6	Oral candidiasis	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
7	Esophageal candidiasis	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
8	Pulmonary tuberculosis (TB)	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
9	Extra pulmonary TB	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
10	Pneumonia	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
11	Cryptococcal meningitis	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
12	Other:_____	☐ (1)	☐ (0)	☐ (1)	☐ (0)	
13	Kaposi's sarcoma	☐ (1)	☐ (0)	☐ (1)	(Stable) ☐ (0)	Progressive ☐ (2)

ENROLLMENT FOR DBS (VST 1)

DBS: Demographics and 1st DBS (VST1)

Date of Visit: |___|___| / |___|___|___| / |___|___|___|___| (DD/MMM/YYYY)

Participant Identification Number (Site Code - ART Clinic Number):
|___|___|___|___| -|___|___|___|___|___|___|___|___|___|___|

Questions are asked at the patient's **first** visit.

1. Sex of Respondent ☐ (1) Male ☐ (0) Female

2. a. Age of respondent in years |___|___| → [*If known, skip to 2c]* ☐ (88) Unknown
   b. Year of birth if age unknown |___|___|___|___| birth year
   c. Is the respondent over 18 years old? (Born before 1995)
   ☐ (1) Yes ☐ (0) No →**Not Eligible**

3. How many months has the respondent been on ARVs?
   ☐ (0) 6 months (+/- 1 month)
   ☐ (1) 2 years (+/- 3months)
   ☐ (2) 4 years (+/- 3months)
   ☐ (3) Every 2 years thereafter (6, 8, 10, etc +/- 3months) _____
   ☐ (4) None of the above

4. Does the patient show clinical signs of failure? ☐ (1) Yes ☐ (0) No

***If Question #3 = "None of the above" and patient is not showing signs of clinical failure* → Not Eligible**

5. How specimen was collected (check all that apply)?
   ☐ (0) Finger stick only ☐ (1) Finger stick and venous draw ☐ (2) Venous draw only

Visit 2: VL RESULT DELIVERY (VST2)

DBS: VL Result Delivery (VST2)

Date of Visit: |___|___| / |___|___|___| / |___|___|___|___| (DD/MMM/YYYY)

Participant Identification Number (Site Code - ART Clinic Number):
|___|___|___|___| -|___|___|___|___|___|___|___|___|___|___|

Complete the following items at the **second** visit (~1 month after first visit). If viral load result is <5,000 copies/ml the results is considered ***undetectable***.

1. Were viral load results delivered at Visit 2?

   ☐ (0) No (Stop & *Complete rest of form when results are delivered*) ☐ (1) Yes

2. Viral load results: |___|___|___|___|___|___|___| (copies/ml) ☐ (8) Not detectable

3. Date viral load results delivered to patient:

   |___|___| / |___|___|___| / |___|___|___|___| (DD/MMM/YYYY)

4. Is viral load > 5,000 copies/ml? ☐ (0) No ☐ (1) Yes → **Instruct to return for 2nd DBS in 2 months**

To be completed by study officials only:

☐ (88) Not delivered

Visit 3: SECOND DBS (VST3)

DBS: Collection of 2nd DBS (VST3)

Date of Visit: |___|___| / |___|___|___| / |___|___|___|___| (DD/MMM/YYYY)

Participant Identification Number (Site Code - ART Clinic Number):
|___|___|___|___| -|___|___|___|___|___|___|___|___|___|___|

The following questions should be asked of all participants who had an initially elevated viral load and who require a second dried blood spot (DBS).

1. Has the respondent accepted or declined confirmatory testing?
   ☐ (1) Accepted ☐ (0) Declined

2. Does the patient show clinical signs of failure? ☐ (1) Yes ☐ (0) No
3. How specimen was collected (check all that apply)?
   ☐ (0) Finger stick only ☐ (1) Finger stick and venous draw ☐ (2) Venous draw only

Assess patient understanding of the meaning of viral load with the following question. Read aloud both response options to the patient and mark whichever the patient chooses ***even if it is incorrect***

4. For most people, if you take all of your medications your viral load will:
   ☐ (0) Go up (be higher)
   ☐ (1) Go down (be lower)

Visit 4: VL RESULT DELIVERY/REFERRAL (VST4)

DBS: VL Result Delivery and Referral (VST4)

Date of Visit: |___|___| / |___|___|___| / |___|___|___|___| (DD/MMM/YYYY)

Participant Identification Number (Site Code - ART Clinic Number):
|___|___|___|___| -|___|___|___|___|___|___|___|___|___|___|

Complete questions #1-5 at time of visit according to indicated skip patterns. For patients with elevated viral load and referral for 2nd line (question #3 and #4 → "Yes"), complete **Treatment Follow-up (TXFU)** *form up to 4 weeks later or when 2nd-line treatment initiation is confirmed.*

Questions are asked of all respondent's at the **fourth** visit (~1 month after third visit). If viral load result is <5,000 copies/ml the results is considered ***undetectable***.

1. Were viral load results delivered at Visit 4?

   ☐ (0) No (Stop & *Complete rest of form when results are delivered*) ☐ (1) Yes

2. Viral load results: |___|___|___|___|___|___|___| (copies/ml)

   ☐ (8) Not detectable

3. Date viral load results delivered to patient: |___|___| / |___|___|___| / |___|___|__|__|
   (DD/MMM/YYYY)

4. Is viral load ≥5,000 copies/ml? ☐ (1) Yes *(go to question #5)* ☐ (0) No → **End of Form**

5. Was patient referred for 2nd line therapy?

   ☐ (1) Yes → **End of Form** ☐ (0) No *(go to question #6)*

6. Which of the following reasons describe why patient was not referred:

	Yes	No
a. Patient feels well	☐ (1)	☐ (0)
b. No money for transport	☐ (1)	☐ (0)
c. Patient is concerned about 2nd line therapy side effects	☐ (1)	☐ (0)
d. Patient refused referral (no additional reason provided)	☐ (1)	☐ (0)
e. Patient refused (other) (reason:_____)	☐ (1)	☐ (0)
f. Patient is unreliably adherent to 1st line therapy	☐ (1)	☐ (0)
g. Provider did not offer referral (reason:_____)	☐ (1)	☐ (0)

To be completed by study officials only:

☐ (88) Not delivered

TREATMENT FOLLOW-UP: (TXFU)

DBS: Treatment follow-up (TXFU)

Date of form completion: |___|___| / |___|___|___| / |___|___|___|___| DD/MMM/YYYY)

Participant Identification Number (Site Code - ART Clinic Number):
|___|___|___|___| −|___|___|___|___|___|___|___|___|___|___|

This form to be completed up to 4 weeks later or when 2nd-line treatment initiation is confirmed.

1. Is initiation on 2nd line therapy known? ☐ (1) Yes *(go to question #2)* ☐ (0) No → **End of Form**
2. Date on which the patient was started on 2nd line therapy:
   |___|___| / |___|___|___| / |___|___| ___|___| (DD/MMM/YYYY)

STUDY TERMINATION: (TERM)

DBS: Termination (TERM)

Date of form completion: |___|___| / |___|___|___| / |___|___|___|___| DD/MMM/YYYY)

Participant Identification Number (Site Code - ART Clinic Number):
|___|___|___|___| -|___|___|___|___|___|___|___|___|___|___|

This form to be completed only when a participant informs study staff that they will be terminating their participation in the study. In the event of a participant having died, this information may be communicated by a guardian or other reliable source.

1. Why has this participant been terminated from the study?

☐ (0) Died → date of death, if known |___|___| / |___|___|___| / |___|___| ___|___|
(DD/MMM/YYYY)

☐ (1) Moved away

☐ (2) Other _____

APPENDIX 2: IN-DEPTH INTERVIEW GUIDE

Date of Interview |___|___| / |___|___|___| / |___|___| (DD/MMM/YY)

Interviewer Number |___|___|___|

Clinic: ☐(6)Ntcheu ☐(5)Dowa ☐(4)Ntchisi ☐(3)Malamulo ☐(2)Mulanje ☐(1)Neno

How many months elapsed since enrolled in study (#): |___| months

Position of interviewee ☐(2) Clinician ☐(1) Nurse ☐(9) Other _____
Length of time at that role (in years): |___|___|

[Read or paraphrase to participant after obtaining informed consent:]

Thank you for agreeing to talk with me about your participation in this study. I would like to hear your thoughts on any challenges and successes you encountered by incorporating dried blood spot (DBS) for evaluation of viral load in the clinic.

We want your honest opinions about what you think is good and what you think could be improved—there are no right or wrong answers, what we want to know is what you think and believe. Your responses are completely confidential. Please do not hesitate to tell us any information, whether good or bad, because this information will be used to improve the implementation of using dried blood spots for viral load in the future.

Section 1: Sample collection

1. Did your clinic always have all the supplies and reagents necessary to collect samples when desired? ☐(1)Yes ☐(0)No
   a. [If not, probe what issues with supplies they had, especially regarding the DBS card, and how long elapsed before they were able to take samples again]

2. How many staff members are trained to obtain venous blood draws? |___|___|
   a. Finger sticks? |___|___|

3. [For sites where tests are repeated with venous draws] Was staff ever a limitation in gaining venous draws from qualifying patients? ☐(1)Yes ☐(0)No

   a. If so, how often? ☐(3)Daily ☐(2)Weekly ☐(1)Monthly ☐(9)Other _____

4. How often did the finger stick draw need to be repeated due to difficulty obtaining the sample? ☐(3)Daily ☐(2)Weekly ☐(1)Monthly ☐(9)Other _____

5. [For sites with venous draws] How often did the venous draw need to be repeated due to difficulty obtaining the sample?

   ☐(3)Daily ☐(2) Weekly ☐(1) Monthly ☐(9) Other _____

6. How much time did sample collection add to a clinic visit (minutes)? |___|___|

    a. What are your thoughts on the extra time devoted to collecting these samples?

7. Were there issues with storing the samples until transport? ☐(1)Yes ☐(0)No

    a. If so, what kind of issues?

8. Were there issues with transporting the sample to central laboratories? ☐(1)Yes ☐(0)No

    a. If so, what kind of issues?

Section 2: Results

1. What was the expected turn-around time (days)? |__|__|
2. What was the real turn-around time for results (days)? |__|__|
3. Were there any issues that affected turn-around time in receiving results? ☐(1)Yes ☐(0)No

    a. If so, what kind of issues?

4. What are your thoughts regarding the SMS system for receiving results?
5. Did any patients not receive results as expected? ☐(1)Yes ☐(0)No

    a. When/if patients did not receive results as expected, what were the reasons?

6. After receiving the results, were you always able to implement the changes in care as indicated? ☐(1)Yes ☐(0)No ☐(8) Not sure

    a. If not, what difficulties arose?

Section 3: Acceptability

1. Did you encounter any resistance from patients to complete these tests? ☐(1)Yes ☐(0)No

    a. If so, what kind?

2. How long did it take before your site became comfortable using this system?

    |__|__| ☐(3) Days ☐(2) Weeks ☐(1) Months ☐(9) Other _____

3. What does your site think about this system?

☐(5) Like it a lot ☐(4) Like it ☐(3) Indifferent ☐(2) Dislike it ☐(1) Dislike it a lot

a. Why so?

4. Outside of this study, would you use this system in your current setting?

   ☐(1)Yes ☐(0)No

   a. If not, why so?

5. What specific aspects of this system did you appreciate the most?

6. What specific aspects of this system did you dislike the most?

7. What modifications would you suggest to improve the use of this system?

APPENDIX 3: SUPPLEMENTARY TABLES (AIM 3)

Table I: Sensitivity Analysis Adjusted Odds Ratios and Risk Scores of NRTI/NNRTI resistance (Time on therapy, <7mo, 7-24mo, >24mo)

Predictor	Model 4 (with baseline VL) (n=290), AUROC=0.815			
	Full model OR (95% CI)	Reduced OR (95% CI)	β^2	Predictor score[1]
Age, years				
≤30	2.1 (1.0-4.5)	2.0 (1.0-4.0)	0.71	1
>30	1.0	1.0		0
Sex				
Male	0.7 (0.3-1.4)	- -	-	-
Female	1.0	- -	-	-
BMI, kg/m^2				
Normal/low (<24.9)	1.0	1.0		0
High (>25.0)	2.9 (1.3-6.5)	3.9 (1.9-8.1)	1.35	3
Baseline VL, copies/ml				
≤100,000	1.0	1.0		0
>100,000	3.2 (1.5-7.1)	3.7 (1.9-7.2)	1.30	3
Time on therapy, months				
<7	5.3 (1.8-15.6)	5.6 (2.1-15.1)	1.72	3
7-24	2.0 (0.7-5.8)	2.6 (1.0-7.1)	0.96	2
>24	1.0	1.0		0
VL at failure, copies/ml				
≤10,000	1.0	1.0		0
10,001-100,000	7.1 (3.3-15.3)	6.0 (3.0-12.3)	1.80	4
>100,000	2.7 (1.0-7.0)	2.6 (1.2-5.9)	0.97	2
CD4 at screening, cells/mm^3				
≤100	1.9 (0.9-3.9)	- -	-	-
>100	1.0	- -	-	-
History of TB				
Yes	1.0	- -	-	-
No	1.8 (0.7-4.6)	- -	-	-
Treatment changed while on study				
Yes	0.4 (0.1-1.3)	- -	-	-
No	1.0	- -	-	-
Ever missed meds				
Yes	2.0 (1.0-4.0)	- -	-	-
No	1.0	- -	-	-
	Model 5 (without baseline VL) (n=290), AUROC=0.794			
	Full model OR (95% CI)	Reduced model OR (95% CI)	β^3	Predictor score[1]
Age, years				
≤30	1.8 (0.9-3.6)	1.8 (0.9-3.4)	0.57	1
>30	1.0	1.0		0
Sex				
Male	0.7 (0.3-1.3)	- -	-	-
Female	1.0	- -	-	-
BMI, kg/m^2				
Normal/low (<24.9)	1.0	1.0		0
High (>25.0)	2.5 (1.1-5.6)	3.3 (1.6-6.7)	1.20	2
Baseline VL, copies/ml				
≤100,000	- -	- -	-	-
>100,000	- -	- -	-	-
Time on therapy, months				
<7	4.9 (1.7-14.0)	5.7 (2.1-15.1)	1.73	3
7-24	1.9 (0.7-5.5)	2.7 (1.0-7.3)	1.0	2

>24	1.0		1.0			0
VL at failure, copies/ml						
≤10,000	1.0		1.0			0
10,001-100,000	7.1	(3.4-15.2)	5.9	(3.0-11.9)	1.78	4
>100,000	2.7	(1.1-6.7)	3.1	(1.4-6.8)	1.12	2
CD4 at screening, cells/mm^3						
≤100	2.7	(1.3-5.4)	2.3	(1.2-4.3)	0.82	2
>100	1.0		1.0			0
History of TB						
Yes	1.0		-	-	-	-
No	1.4	(0.6-3.3)	-	-	-	-
Treatment changed while on study						
Yes	0.4	(0.1-1.1)	-	-	-	-
No	1.0		-	-	-	-
Ever missed meds						
Yes	2.2	(1.1-4.3)	-	-	-	-
No	1.0		-	-	-	-

	Model 6 (without baseline VL or CD4) (n=260), AUROC =0.794					
	Full model OR (95% CI)		OR (95% CI)		β[4]	Predictor score[1]
Age, years						
≤30	1.5	(0.8-3.1)	1.7	(0.8-3.4)	0.52	1
>30	1.0		1.0			0
Sex						
Male	0.7	(0.4-1.4)	-	-	-	-
Female	1.0		-	-	-	-
BMI, kg/m^2						
Normal/low (<24.9)	1.0		1.0			0
High (>25.0)	2.3	(1.1-5.1)	2.7	(1.3-5.7)	0.99	2
Baseline VL, copies/ml						
≤100,000	-	-	-	-	-	-
>100,000	-	-	-	-	-	-
Time on therapy, months						
<7	4.4	(1.6-12.3)	4.7	(1.7-13.0)	1.55	3
7-24	1.8	(0.7-5.1)	2.0	(0.7-5.5)	0.69	1
>24	1.0		1.0			0
VL at failure, copies/ml						
≤10,000	1.0		1.0			0
10,001-100,000	6.3	(3.0-13.1)	6.3	(3.1-12.9)	1.84	4
>100,000	2.7	(1.1-6.5)	2.9	(1.2-7.1)	1.07	2
CD4 at screening, cells/mm^3						
≤100	-	-	-	-	-	-
>100	-	-	-	-	-	-
History of TB						
Yes	1.0		-	-	-	-
No	1.4	(0.6-3.2)	-	-	-	-
Treatment changed while on study						
Yes	0.4	(0.1-1.3)	-	-	-	-
No	1.0		-	-	-	-
Ever missed meds						
Yes	2.2	(1.1-4.3)	2.0	(1.0-3.8)	0.68	1
No	1.0		1.0			0

[1]weighted; [2]constant = -4.23; [3]constant = -3.67; [4]constant = -3.34

CI, confidence interval; β, regression coefficient; BMI, body mass index; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; AUROC, area under receiver operating characteristic curve; VL, viral load

Table II: Sensitivity Analysis Adjusted Odds Ratios and Risk Scores of NRTI/NNRTI resistance (Time on therapy, <7mo, ≥7mo)

Predictor		Model 7 (with baseline VL) (n=290), AUROC=0.802					
		Full model OR (95% CI)		Reduced OR (95% CI)		β^2	Predictor score[1]
Age, years							
	≤30	2.2	(1.0-4.6)	2.1	(1.1-4.1)	0.74	1
	>30	1.0		1.0			0
Sex							
	Male	0.7	(0.3-1.4)	-	-	-	-
	Female	1.0		-	-	-	-
BMI, kg/m^2							
	Normal/low (<24.9)	1.0		1.0			0
	High (>25.0)	2.8	(1.2-6.2)	3.8	(1.8-7.8)	1.33	2
Baseline VL, copies/ml							
	≤100,000	1.0		1.0			0
	>100,000	3.2	(1.5-6.9)	3.6	(1.8-7.1)	1.29	3
Time on therapy, months							
	<7	3.2	(1.6-6.4)	2.8	(1.5-5.2)	1.03	2
	≥7	1.0		1.0			0
VL at failure, copies/ml							
	≤10,000	1.0		1.0			0
	10,001-100,000	7.3	(3.4-15.6)	6.3	(3.1-12.7)	1.84	4
	>100,000	2.7	(1.0-6.9)	2.7	(1.2-5.9)	0.98	2
CD4 at screening, cells/mm^3							
	≤100	1.9	(0.9-3.9)	-	-	-	-
	>100	1.0		-	-	-	-
History of TB							
	Yes	1.0		-	-	-	-
	No	1.9	(0.8-4.8)	-	-	-	-
Treatment changed while on study							
	Yes	0.4	(0.1-1.2)	-	-	-	-
	No	1.0		-	-	-	-
Ever missed meds							
	Yes	2.0	(1.0-3.9)	-	-	-	-
	No	1.0		-	-	-	-
		Model 8 (without baseline VL) (n=290), AUROC=0.786					
		Full model OR (95% CI)		Reduced model OR (95% CI)		β^3	Predictor score[1]
Age, years							
	≤30	1.8	(0.9-3.7)	1.9	(1.0-3.6)	0.62	1
	>30	1.0		1.0			0
Sex							
	Male	0.7	(0.3-1.3)	-	-	-	-
	Female	1.0		-	-	-	-
BMI, kg/m^2							
	Normal/low (<24.9)	1.0		1.0			0
	High (>25.0)	2.5	(1.1-5.5)	3.3	(1.7-6.7)	1.20	2
Baseline VL, copies/ml							
	≤100,000	-	-	-	-	-	-

>100,000	-	-	-	-	-	-
Time on therapy, months						
<7	3.0	(1.6-5.9)	2.7	(1.5-5.0)	1.01	2
≥7	1.0		1.0			0
VL at failure, copies/ml						
≤10,000	1.0		1.0			0
10,001-100,000	7.3	(3.5-15.6)	6.2	(3.1-12.3)	1.82	4
>100,000	2.6	(1.1-6.5)	3.0	(1.4-6.6)	1.10	2
CD4 at screening, cells/mm^3						
≤100	2.7	(1.3-5.4)	2.2	(1.2-4.2)	0.79	2
>100	1.0		1.0			0
History of TB						
Yes	1.0		-	-	-	-
No	1.5	(0.6-3.4)	-	-	-	-
Treatment changed while on study						
Yes	0.4	(0.1-1.1)	-	-	-	-
No	1.0		-	-	-	-
Ever missed meds						
Yes	2.2	(1.1-4.3)	-	-	-	-
No	1.0		-	-	-	-

	Model 9 (without baseline VL or CD4) (n=260), AUROC =0.787					
	Full model OR (95% CI)		OR (95% CI)		β[4]	Predictor score[1]
Age, years						
≤30	1.6	(0.8-3.2)	1.7	(0.9-3.5)	0.55	1
>30	1.0		1.0			0
Sex						
Male	0.7	(0.4-1.4)	-	-	-	-
Female	1.0		-	-	-	-
BMI, kg/m^2						
Normal/low (<24.9)	1.0		1.0			0
High (>25.0)	2.3	(1.1-5.1)	2.7	(1.3-5.7)	0.98	2
Baseline VL, copies/ml						
≤100,000	-	-	-	-	-	-
>100,000	-	-	-	-	-	-
Time on therapy, months						
<7	2.8	(1.5-5.4)	2.8	(1.5-5.4)	1.04	2
≥7	1.0		1.0			0
VL at failure, copies/ml						
≤10,000	1.0		1.0			0
10,001-100,000	6.5	(3.1-13.5)	6.4	(3.1-13.2)	1.86	4
>100,000	2.6	(1.1-6.3)	2.8	(1.2-6.8)	1.04	2
CD4 at screening, cells/mm^3						
≤100	-	-	-	-	-	-
>100	-	-	-	-	-	-
History of TB						
Yes	1.0		-	-	-	-
No	1.4	(0.6-3.3)	-	-	-	-
Treatment changed while on study						
Yes	0.4	(0.1-1.2)	-	-	-	-
No	1.0		-	-	-	-

Ever missed meds						
Yes	2.2	(1.1-4.3)	2.0	(1.0-3.8)	0.68	1
No	1.0		1.0			0

[1]weighted; [2]constant = -3.56; [3]constant = -2.97; [4]constant = -2.86

CI, confidence interval; β, regression coefficient; BMI, body mass index; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; ROC, receiver operating characteristic; VL, viral load

Table III: Sensitivity analysis performance of resistance models and derived risk scores

Predictor	Model with baseline VL (n=290)			Model without baseline VL (n=290)			Model without baseline VL or CD4 (n=260)		
	Cutoff	Sens (%)	Spec (%)	Cutoff	Sens (%)	Spec (%)	Cutoff	Sens (%)	Spec (%)
<7mo, 7-24mo, >24mo									
Model*	0.656 (4)	17.3	97.7	0.614 (5)	22.7	97.2	0.635 (6)	23.9	97.9
Weighted risk score	≥10[1]	34.7	95.3	≥9[2]	16.0	98.1	≥9[3]	14.7	98.1
<7mo vs ≥7mo									
Model*	0.656 (7)	17.3	97.7	0.634 (8)	18.7	97.2	0.646 (9)	23.9	97.9
Weighted risk score	≥9[4]	26.7	95.8	≥8[5]	13.3	98.1	≥9[6]	24.0	97.2

*Cutoff values for the models are thresholds derived by summing the beta coefficients and converting to a probability

[1]Range of scores for this model was 0-14; [2]Range of scores for this model was 0-12; [3]Range of scores for this model was 0-11; [4]Range of scores for this model was 0-12; [5] Range of scores for this model was 0-11; [6]Range of scores for this model was 0-10

Mo, months; RLS, resource-limited setting; VL, viral load

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