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Diagnosing Antiretroviral Treatment Failure In Resource-Limited Settings

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DIAGNOSING ANTIRETROVIRAL TREATMENT FAILURE IN RESOURCE-LIMITED SETTINGS

by

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EPIDEMIOLOGY

ABSTRACT

This dissertation had three central goals. The first was to review the existing literature for reports of virologic failure among adults initiating non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy in resource-limited settings. The second was to describe the epidemiology of virologic failure over the first 6 months of therapy in Lusaka, Zambia. The third was to examine the performance of different approaches for diagnosing virologic failure.

We performed a meta-analysis using data from previously published reports to create summary estimates for the prevalence of virologic failure and found that the lowest estimate of the 95% confidence interval was 10% or higher at all time points evaluated between 3 months and 2 years. We then examined data from one arm of a randomized trial of treatment monitoring strategies in Lusaka, Zambia and found the prevalence of virologic failure to be 6.7% (95% confidence interval, 4.9%-9.0%) at 6 months. We also found that poor adherence as determined from pharmacy attendance was associated with virologic failure (adjusted relative risk, 2.7; 95% confidence interval, 1.2-6.3).

We created an algorithm to predict virologic failure using regression modeling techniques. We used adherence and baseline anemia to estimate the probability of failure and classified any patient with an estimated probability $\geq$ 9% as a potential failure. The resulting predictive-score-based algorithm had a sensitivity of 24.4% (95% CI, 12.4%-40.3%) and a specificity of 91.7% (95% CI, 89.2%-93.8%). We algorithm recommended

by the World Health Organization had a sensitivity of 27.6% (95% CI, 12.7%-47.2%) and a specificity of 78.4% (95% CI, 74.4%-82.0%).

The results of this dissertation research indicate that antiretroviral treatment failure is a significant concern as 10% of patients have circulating viremia at any given time point between 3 months and 2 years. Furthermore, we found that only adherence was significantly associated with virologic failure and were unable to produce an algorithm for the diagnosis of virologic failure that performed well at 6 months. Although cost and technical requirements prevent routine virologic monitoring from being widely used in resource-limited settings, these results argue that efforts should be made to address this problem.

DEDICATION

This dissertation is dedicated to the memory of my beloved father, Terrell N. Cantrell, and to my dearest mother, Margaret B. Cantrell.

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AN EVALUATION OF DIFFERENT METHODS FOR DIAGNOSING ANTIRETROVIRAL TREATMENT FAILURE IN RESOURCE-LIMITED SETTINGS WHERE VIROLOGIC TESTING IS NOT AVAILABLE

LIST OF ABBREVIATIONS

3TC	lamivudine
ABC	abacavir
AIDS	acquired immune deficiency syndrome
ARR	adjusted relative risk
ART	antiretroviral therapy
AUC	area under the curve
BMI	body mass index
CD4	CD4+ T-lymphocyte
CI	confidence interval
D4T	stavudine
DDL	didanosine
EFV	efavirenz
FTC	emtricitabine
HAART	highly active antiretroviral therapy
HGB	hemoglobin
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
IDV	idinavir
IQR	interquartile range
LPV/r	lopinavir/ritonavir

LIST OF ABBREVIATIONS (Continued)

MPR	medication possession ratio
NFV	nelfinavir
NNRTI	non-nucleoside reverse transcriptase inhibitor
NPV	negative predictive value
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine
OAPR	odds of being affected given a positive result
PEPFAR	President’s Emergency Plan for AIDS Relief
PI	protease inhibitor
PPV	positive predictive value
RNA	ribonucleic acid
ROC	receiver operating characteristic
RR	relative risk
TNF	tenofovir
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children’s Fund
VL	viral load
WHO	World Health Organization
ZDV	zidovudine

INTRODUCTION

In the 25 years since the first reported case of what became known as acquired immune deficiency syndrome (AIDS), more than 25 million people have died of the disease, and the number of individuals living with the human immunodeficiency virus (HIV) worldwide has surpassed 33 million. In 2007, 2.1 million suffered AIDS related deaths, and 2.5 million were newly infected with HIV.[1] While these numbers are staggering, there is reason for encouragement. The advent of potent antiretroviral therapy (ART) has transformed the disease into a manageable, chronic condition in areas where antiretroviral drugs are available. The majority of persons living with HIV reside in low- and middle-income countries with 22.5 million (68%) living in sub-Saharan Africa. In the past few years, several initiatives, such as The Global Fund to Fight AIDS, Tuberculosis and Malaria, the World Bank's Global HIV/AIDS Program of Action, and the President's Emergency Plan for AIDS Relief (PEPFAR), have been launched to expand care and treatment of HIV infected individuals in resource-limited settings. The success of these efforts is reflected by the growing number of patients receiving ART worldwide. The World Health Organization (WHO), the Joint United Nations Programme on HIV/AIDS (UNAIDS), and the United Nations Children's Fund (UNICEF) estimate that nearly 3 million people were receiving ART in low- and middle-income countries by the end of 2007 with more than 2 million people receiving care in sub-Saharan Africa.[2]

As the number of people receiving ART in resource-limited settings rapidly increases, the focus of treatment efforts is shifting from emergency roll-out of services to

long-term, sustained care of patients. With this shift in focus, new public health challenges are emerging. Adherence to therapy is one challenge that has received significant attention. The ability to accurately quantify patient adherence so that individuals with poor adherence can be identified and intervened upon is one key to program success. Fortunately, reports have been encouraging. Carlucci et al have documented high levels of adherence for more than 80% of patients in their first months of ART in rural Zambia.[3] Mills et al performed a meta-analysis comparing reports of adherence in sub-Saharan Africa and North America and found that African patients displayed higher levels of adherence.[4] Program retention is another issue that has deservedly received attention. Programs that have successfully expanded treatment services in resource-limited settings are now struggling to retain patients enrolled into care. Rosen et al performed a systematic review of retention in sub-Saharan Africa and found that programs only retain about 60% of patients at 2 years with loss to follow-up being the single largest problem.[5]

An issue that has received considerably less attention is the identification of patients in whom ART has not successfully suppressed HIV. One potential reason this condition, known as antiretroviral treatment failure, has received less attention is that most developing world settings lack the resources and technical expertise to perform routine virologic monitoring. As a result there have been few reports detailing the prevalence and predictors of antiretroviral treatment failure in resource-limited settings. Although it has received less attention, the consequences of treatment failure, particularly the emergence of individual- and/or population-level drug resistance, are public health concerns.

Individual-level drug resistance develops when HIV replicates in the presence of non-suppressive therapy allowing mutations to accumulate resulting in HIV variants with

decreased drug susceptibility.[6-10] In resource-limited settings, the WHO currently recommends a first-line regimen consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI).[11] This regimen is recommended because of its efficacy, low cost relative to protease inhibitor-based regimens, and the availability of generic fixed dose combinations that do not need to be refrigerated. While these characteristics are advantageous for resource-limited settings, there is a drawback. As Clavel et al mention, a single mutation is generally sufficient to induce high levels of drug resistance to NNRTIs.[6] From a public health perspective, there is concern that the growing number of patients on NNRTI-based ART combined with the development of individual-level drug resistance may lead to the transmission of drug resistant strains of HIV with the potential for population-level drug resistance.[12-15] Bannister et al observed an 11.4% prevalence of transmitted drug-resistant HIV in the EuroSIDA cohort.[16] Little and colleagues observed that the transmission of drug resistant HIV in newly infected individuals residing in 10 North American cities increased from 3.4% to 12.4% from 1995 to 2000.[17]

Quantification of HIV ribonucleic acid (RNA) circulating in the plasma by molecular assay is the gold standard for monitoring the effectiveness of ART in the developed world.[9] The cost and technical requirements of the assay, however, prevent routine virologic monitoring from being widely used in resource-limited settings. As such, the development of cheaper and easier methods for identifying antiretroviral treatment failure need to be identified and tested. The WHO-recommended algorithm for the identification of antiretroviral treatment failure at 6 months post ART initiation uses immunologic criteria, specifically a persistent CD4+ T-lymphocyte (CD4) count below 100 cells/mm^3 by 6

months or a return of CD4 count to pre-therapy baseline or below, but few data are available on the performance of this algorithm.[11] Others have recommended algorithms that incorporate additional data such as adherence, clinical responses, and laboratory indices such as hemoglobin (HGB) response.[8,18-21] Colebunders et al proposed a new model for identifying antiretroviral treatment failure that uses clinical and treatment history, adherence information, and immunological measures, but the model has not been tested.[18]

This dissertation examines one of the emerging issues in the global fight against HIV, the diagnosis of antiretroviral treatment failure in resource-limited settings, through a series of three papers. The first paper reviews the existing literature for reports of virologic failure among adults initiating NNRTI-based ART in resource-limited settings and calculates summary estimates for the prevalence of virologic failure at months 3, 6, 12, 18, and 24. The second paper describes the epidemiology of virologic failure over the first 6 months of therapy among a cohort of adults initiating ART in one arm of a pragmatic, randomized trial of treatment monitoring strategies in Lusaka, Zambia. The third paper uses this same cohort to examine the performance of different approaches for diagnosing virologic failure including not only the WHO-recommended algorithm but also a predictive-score-based algorithm created using regression modeling techniques.

ANTIRETROVIRAL TREATMENT FAILURE AMONG ADULTS INITIATING NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR-BASED HIV THERAPY IN RESOURCE-LIMITED SETTINGS: A META-ANALYSIS

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ABSTRACT

BACKGROUND: As antiretroviral therapy (ART) programs in resource-limited settings progress from scale-up of services to long-term care of patients, identifying antiretroviral treatment failure among the large pool of patients receiving ART becomes even more important to programmatic success.

METHODS: We performed a review of the published literature to estimate the prevalence of antiretroviral treatment failure among adults initiating non-nucleoside reverse transcriptase inhibitor-based ART in resource-limited settings. We stabilized the variances of the individual proportions using a Freeman-Tukey transformation and then calculated pooled estimates using the DerSimonian-Laird random effects method. We assessed heterogeneity using the I^2 statistic.

RESULTS: Seventeen articles were identified representing 12 countries in resource-limited settings, 8 in sub-Saharan Africa, 3 in East Asia, and 1 in the Middle East. Using data from 8 articles that evaluated between 23 and 384 patients, we determined the prevalence of virologic failure at 6 months to be 13% (95% confidence interval [CI], 10%-17%; I^2, 49.7%). Using data from 6 articles that evaluated between 24 and 454 patients, we determined the prevalence of virologic failure at 12 months to be 15% (95% CI, 11%-21%; I^2, 70.9%). When examining the prevalence of antiretroviral treatment failure over time, the lower bound of the conservative 95% CI is 10% or higher at months 3, 6, 12, 18, and 24.

CONCLUSIONS: Our findings indicate that the prevalence of antiretroviral treatment is at least 10% between 3 months and 2 years. As the number of patients on ART in resource-limited setting continues to grow, the increasing reservoir of patients on non-

suppressive therapy is a public health concern. Resources should be dedicated to lowering this prevalence through the establishment of programs that limit the initiation of therapy to persons with proven adherence success, the creation of interventions to improve adherence once on therapy, the development of new and cheaper strategies to monitor the effectiveness of ART, and the expansion of virologic monitoring in resource-limited settings.

INTRODUCTION

In resource-limited settings, the rapid scale-up of treatment services for persons infected with the human immunodeficiency virus (HIV) has resulted in impressive decreases in morbidity and mortality.[1-5] The success of these scale-up efforts is reflected by the growing number of patients receiving antiretroviral therapy (ART). A recent report from the World Health Organization (WHO), the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the United Nations Children's Fund (UNICEF) indicates that nearly 3 million people were receiving ART in low- and middle-income countries by the end of 2007.[6] As treatment expansion shifts from emergency roll-out of services to sustained care or patients, new public health issues are emerging.

The long-term monitoring of patients on ART is one of these critical issues. In the developed world, the effectiveness of the ART regimen is generally determined by quantification of the amount of circulating HIV ribonucleic acid (RNA).[7] This approach is not employed in many resource-limited settings because of the associated cost and extensive technical requirements. Instead, crude clinical and immunologic markers are used to eva-

luate the effectiveness of treatment response.[8] One likely consequence of this approach is the potential for patients to remain on non-suppressive first-line therapy for prolonged periods, which may in turn result in antiretroviral drug resistance.[9,10] The development of resistance may compromise the efficacy of the second-line regimen at the individual level and of the first-line regimen at the population level.

Many countries use first-line ART regimens that contain two nucleoside reverse transcriptase inhibitors and one non-nucleoside transcriptase inhibitor (NNRTI) due to lower cost and the availability of generic fixed dose combinations.[8] Reports from large, programmatic cohorts indicate that less than 1% of patients are switched to second-line therapy over the first year of therapy.[11,12] In order to better understand the potential for the emergence of population-level drug resistance in the developing world, we must first understand if the observed switching rates reflect the overall prevalence of antiretroviral treatment failure in these settings. This review is designed to determine the prevalence of antiretroviral treatment failure among adults initiating NNRTI-based ART in resource-limited settings.

METHODS

Eligibility Criteria

For this analysis, we selected cohorts of ART naïve, HIV type 1 (HIV-1) positive adults initiating primarily NNRTI-based therapy in resource-limited settings, with virologic monitoring for treatment failure. We excluded articles that a) were not conducted in a low- or middle-income country as classified by the World Bank's analytical income cate-

gories;[13] b) did not report specific criteria for assessing virologic failure (e.g., specific viral load threshold); c) initiated <90% of patients on NNRTI-based regimens; d) enrolled <90% of patients who were ART naïve; e) included any intervention other than ART; or f) assessed only specific patient groups (e.g., children, pregnant women).

Search Strategy

We searched the PubMed database using a preselected list of terms to identify relevant publications. The terms used were ("human immunodeficiency virus" or "HIV" or "acquired immunodeficiency syndrome" or "AIDS") and ("antiretroviral therapy" or "ART" or "HAART") and ("Africa" or "Asia" or "Latin America" or "Caribbean" or "developing country" or "resource limited" or "resource poor" or "low income" or "middle income" or each low- and middle-income country listed separately) and ("viral load" or "failure" or "failed" or "switch" or "switched" or "suppressed" or "suppression"). These results were then limited to English language articles involving adult human subjects. Candidates for inclusion were identified from an initial review of publication abstracts and an examination of the references of select articles. Candidate publications were accessed and evaluated against the eligibility criteria.

Data Extraction

Data were extracted using a standardized form with the following data elements: country, program environment (urban or rural), program setting (public or private), cost to participant (fee or no fee), month and year enrollment began and ended, total number of patients, baseline characteristics of patients (age, sex, CD4+ T-lymphocyte (CD4) count,

and viral load), the number and percentage of patients initiating different ART regimens, criteria for diagnosing virologic failure, and the number and percentage of patients with virologic failure at months 3, 6, 12, 18, and 24. When articles reported failure using multiple definitions, we used the most stringent definition available. For example, if results for virologic failure defined as ≥50 copies/mL and ≥400 copies/mL were reported, we used the results with ≥50 copies/mL as the definition. If an article reported weeks instead of months, we used results at 12 weeks for 3 months, 24 weeks for 6 months, and 48 weeks for 12 months. If an article reported both an intention-to-treat analysis where patients who died or were lost to follow-up were classified as failing therapy and an on-treatment analysis where only patients evaluated for virologic failure were eligible to be classified as failing therapy, we used results from the on-treatment analysis. All data were extracted by a single investigator (RAC).

Statistical Analysis

To calculate the pooled proportion of patients identified as meeting the criteria for antiretroviral treatment failure, we first stabilized the variances of the raw proportions using the Freeman-Tukey transformation[14,15] method:

$$p = \sin^{-1}[\sqrt{r/(n+1)}] + \sin^{-1}[\sqrt{(r+1)/(n+1)}] \quad (1)$$

$$se = 1/(n+1) \quad (2)$$

where r is the number of patients experiencing virologic failure, n is the number of patients evaluated for virologic failure, p is the transformed proportion, and se is the variance of the transformed proportion. We estimated the proportion of the overall variation in failure that was attributable to between-study heterogeneity using the I^2 statistic.[16] We

believed that there would be a significant amount of heterogeneity between studies due to the varied locations, treatment protocols, virologic failure thresholds, and ART regimens, so we used the DerSimonian-Laird random effects method to pool the transformed proportions.[17,18] We then created a forest plot for virologic failure at 6 and 12 months with individual study proportions and their corresponding exact confidence intervals[19] along with the overall DerSimmonian-Laird pooled estimate. We also graphed the DerSimmonian-Laird pooled estimates of virologic failure over time. Analyses were conducted using Stata version 10.0 (StataCorp, College Station, Texas), SAS version 9.1.3 (SAS Institute, Cary, North Carolina), and R version 2.4.1 (http://www.r-project.org/).

RESULTS

Our initial search identified 802 articles. We excluded 399 publications by limiting the results to English language articles involving adult human subjects. We reviewed the 403 remaining abstracts and eliminated 336 as not being relevant to the topic of interest. A review of the references of the remaining 67 candidate articles identified an additional 7 candidates. We accessed these 74 articles and evaluated them against the eligibility criteria. Overall, we identified 17 articles for inclusion (FIGURE 1; TABLE 1). Eight of the articles reported virologic failure at 6 months, and six reported virologic failure at 12 months. These were included in an analysis presenting a summary measure of virologic failure at these time points. Four articles had information on failure at other specific time points and were included along with the other articles in an analysis presenting virologic failure over time. The remaining 5 articles were reports of virologic failure from a cross section of patients with varying amounts of time on ART.

Virologic Failure at 6 Months

We determined the prevalence of virologic failure at 6 months post ART initiation among ART naïve adults initiating NNRT-based therapy in resource-limited settings using data from 8 articles. These studies enrolled between 23 and 384 patients and were all conducted in sub-Saharan Africa between April 1999 and February 2004. The definition of virologic failure was a viral load measurement ≥400 copies/mL in 6 articles and ≥50 copies/mL in 2 articles. The combined analysis indicates that the prevalence of virologic failure at 6 months is 13% (95% CI, 10%-17%; I^2, 49.7%) (FIGURE 2).

Virologic Failure at 12 Months

Across the six studies with 12 month virologic outcomes, one was conducted in China and the remaining five were conducted in sub-Saharan Africa. These cohorts enrolled a between 23 and 526 patients and were conducted between June 2000 and June 2005. The definition of virologic failure was a viral load measurement ≥400 copies/mL in 4 articles and ≥50 copies/mL in 2 articles. The number of patients evaluated for failure at 12 months ranged from 24 to 454. The combined analysis indicates that the prevalence of virologic failure at 12 months is 15% (95% CI, 11%-21%; I^2, 70.9%) (FIGURE 2).

Virologic Failure Over Time

We examined the prevalence of virologic failure over time using the 8 articles in the previous analysis and an additional 4 articles with data on the prevalence of virologic failure at specific time points other than 6 or 12 months. The number of patients enrolled in all 12 articles ranged from 23 to 526. This analysis included a study set in Cambodia in addi-

tion to China and the 7 countries from sub-Saharan Africa represented in the previous analyses. The prevalence of virologic failure at 3 months was estimated to be 17% (95% CI, 10%-26%; I^2, 70.5%; studies, 5). As previously mentioned, the prevalence was 13% (95% CI, 10%-17%; I^2, 49.7%; studies, 8) at 6 months and 15% (11%-21%; I^2, 70.9%; studies, 6) at 12 months. The prevalence then increased to 27% (95% CI, 17%-37%; I^2, 77.7%; studies, 6) at 18 months and 24% (95% CI, 15%-36%; I^2, 77.4%; studies, 4) at 24 months (FIGURE 4). The lower bound of the conservative 95% CI was 10% or higher at every time point evaluated.

Cross-sectional Reports of Virologic Failure

We examined 5 studies that reported virologic failure data on a cross section of patients enrolled for varying amounts of time. One study in Thailand reported 14 failures among 327 patients (prevalence, 4%; 95% CI, 2%-7%) after a median of 19 months of ART (range, 6-42). In Tunisia, 10 failures were observed among 27 patients with data after 9 months of follow-up (prevalence, 37%; 95% CI, 19%-58%). In Malawi, 63 failures were observed among 397 patients (prevalence, 16%; 95% CI, 12%-20%) after a median of 9.5 months (interquartile range [IQR], 7.4-15.2). In Tanzania, 48 failures were observed in 150 patients (prevalence, 32%; 95% CI, 25%-40%) after a median of 12 months (range, 6-27). Finally, a study in Uganda reported 46 failures in 137 patients (prevalence, 34%; 95% CI, 26%-42%) after 38 weeks (IQR, 24-62).

DISCUSSION

Until recently, the global deployment of antiretroviral resources has focused on providing access to care for the millions of people living in with HIV in resource-constrained settings. The notable success of these programs has resulted in a growing pool of patients on therapy that require lifelong care and treatment. The transition from emergency scale-up to a sustained public health response offers new challenges in areas such as retention, adherence, and treatment failure.[20,21] We sought to add to this literature with a review of treatment failure in resource-limited settings. We found that 13% of ART naïve adults initiating NNRTI-based ART in resource-limited settings have detectable levels of virus after 6 months of therapy. The prevalence was 15% at 12 months and increased to more than 20% after 18 months. Most notably, the lowest estimate of the 95% CI was 10% at all time points evaluated between 3 months and 2 years.

The degree of heterogeneity in the reported prevalence of antiretroviral treatment failure was high across studies at all time points. This was expected and, we believe, appropriately controlled for by our analysis plan that involved stabilizing the variances of the individual estimates and using random-effects methods to create pooled proportions with conservative CIs. Another strength of this analysis was the restriction of articles to represent the current situation in the developing world. By limiting our analysis to programs using the current WHO-recommended first-line therapy, these results may be applicable to the multitude of programs operating under these conditions, particularly in sub-Saharan Africa. The primary limitation of our analysis is the potential for publication bias due to the reliance on publicly available reports. Because programs with access to

routine virologic testing may have greater resources all around, it is possible that these patients may experience less virologic failure or earlier switches to second-line therapy. This in turn could lead to a deceivingly low prevalence of treatment failure. It is also important to note that the estimates are cross-sectional in nature. We found a prevalence of 13% at 6 months and 15% at 12 months. Since this is not a cohort of patients being following longitudinally, one cannot conclude that the prevalence of virologic failure only increases 2% from 6 months to 12 months.

Our results are significantly higher than those documented in settings without virologic monitoring. For example, an analysis of 62 Medecins Sans Frontieres programs indicated that only 370 of 48,338 patients (0.8%) followed for a median of 20 months had been switched to a second-line regimen.[11] In Malawi, only 9 out of 967 patients (0.9%) followed for a median of 8.3 months (IQR, 5.5-3.1) had been switched.[12] With approximately 10% of patients on non-suppressive first-line therapy at any given time and reports of less than 1% being switched to second-line therapy in programmatic settings, the development and transmission of drug resistant strains is a concern. For comparison, Little and colleagues observed an increase in the transmission of drug resistant HIV in newly infected individuals from 3.4% to 12.4% between 1995 to 2000 when ART use was expanding in the States.[22]

In conclusion, we have found that more than 10% of patients are on non-suppressive therapy at any given time point from 3 months to 2 years. Clearly, interventions are needed to address this issue. Improving adherence is the most likely method to decrease

the prevalence of antiretroviral treatment failure. Development of better monitoring strategies should also be a priority. Where possible, resources should be allocated to the expansion to virologic testing, since it is the most accurate measure of viremia. Where resources are not available, however, development of reliable non-virologic algorithms for assessing treatment failure is clearly a scientific priority. If possible, the creation of a predictive model using a combination of baseline information and longitudinal measures of disease progression would be a useful low-cost alternative. Such an instrument would be an invaluable tool in resource-limited settings.

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Table 1. Description of publications included in a meta-analysis of antiretroviral treatment failure among adults initiating non-nucleoside reverse transcriptase inhibitor-based therapy in resource-limited settings

Reference	Location	Cost (in US$)	Virologic Failure Threshold (copies/mL)	Dates (month/year)	Sample Size
Publications with prevalence of virologic failure at specific time points					
Sub-Saharan Africa					
Wester et al,[23] 2005	Botswana	Free	≥400	04/01-01/02	153
Bisson et al,[24] 2006	Botswana	Free	≥400	04/99-02/04	384
Laurent et al,[25] 2008	Cameroon	Free	≥400	01/01-04/03	A,85 B,84
vanOosterhout et al,[26] 2005	Malawi	Pay	≥50	07/03-11/03	176
Idigbe et al,[27] 2005	Nigeria	$10/mo	≥400	02/02–04/02	50
Canestri et al,[28] 2007	Senegal	Free	≥50	06/00–04/01	40
Coetzee et al,[29] 2004	South Africa	Free	≥400	05/01–12/02	287
Baker et al,[30] 2007	Uganda	…	≥400	…	23
Kamya et al,[31] 2007	Uganda	Free	≥400	04/04-06/05	526
Oyugi et al,[32] 2004	Uganda	Pay	≥400	09/02-07/03	34
East Asia & Pacific					
Ferradini et al,[33] 2007	Cambodia	Free	≥40	10/02-05/03	416
Zhou et al,[34] 2007	China	…	≥50	04/03-05/05	27
Publications with cross-sectional data on virologic failure					
East Asia & Pacific					
Chaiwarith et al,[35] 2007	Thailand	Free	≥50	01/03-12/05	327
Middle East & North Africa					
Fethi et al,[36] 2005	Tunisia	…	≥400	01/03-09/03	27[¥]
Sub-Saharan Africa					
Ferradini et al,[12] 2006	Malawi	Free	≥400	08/02-10/03	1308
Ramadhani et al,[37] 2007	Tanzania	Free	≥400	06/05-08/05	150
Spacek et al,[38] 2006	Uganda	Pay	≥400	08/03-12/03	137

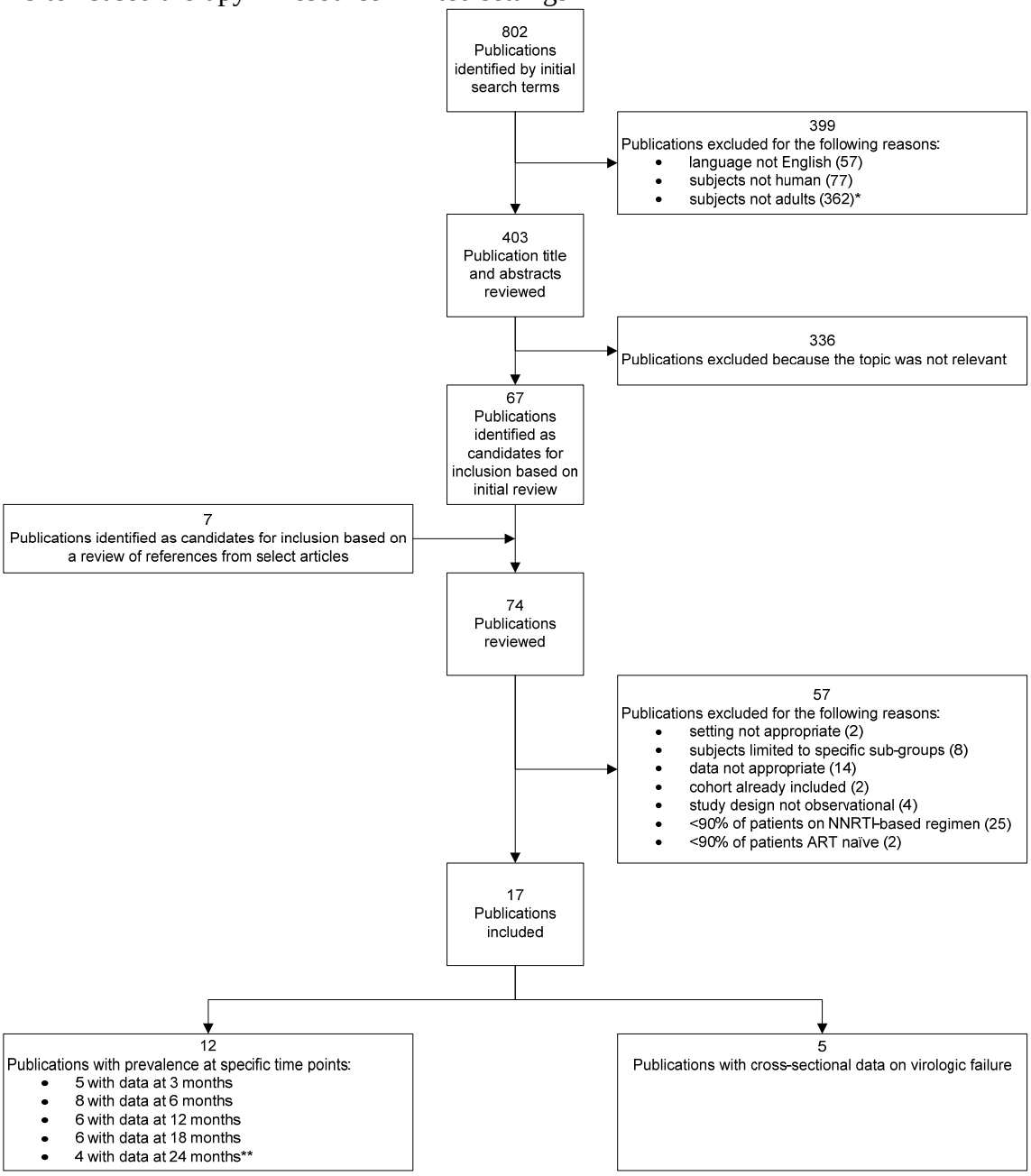
*median (IQR) [†]mean (SD) [¶]median (range) [€]mean (range)
[‡]mean [µ]median [¥]subgroup

Table 1. (Continued)

Reference	Characteristics of Population			
	Age	Female	Baseline CD4 Count (cells/mm^3)	Baseline Viral Load (log_{10} copies/mL)
Publications with prevalence of virologic failure at specific time points				
Sub-Saharan Africa				
Wester et al,[23] 2005	36 (30-42)*	59%	96 (33-165)*	5.6 (5.2-5.9)*
Bisson et al,[24] 2006	36(18-65)[€]	62%	103 (43-167)*	5.4 (4.8-5.9)*
Laurent et al,[25] 2008	36 (30-41)*	68%	152 (67-223)*	5.3 (4.8-5.6)*
	35 (29-41)*	65%	117 (68-188)*	5.2 (4.7-5.5)*
vanOosterhout et al,[26] 2005	39 (22-71)[¶]	55%	…	…
Idigbe et al,[27] 2005	35 (30-60)*	56%	260 (160-290)*	3.7 (2.6-4.8)*
Canestri et al,[28] 2007	36 (7)[†]	58%	133 (92)[†]	5.5 (0.4)[†]
Coetzee et al,[29] 2004	31 (28-37)*	70%	43 (13-94)*	5.2 (0.7)[†]
Baker et al,[30] 2007	33[‡]	48%	106	5.1(3.1-6.5)*
Kamya et al,[31] 2007	37 (8)[†]	69%	99 (24-165)*	5.3 (0.6)[†]
Oyugi et al,[32] 2004	35[‡]	71%	63.5[μ]	5.3[μ]
East Asia & Pacific				
Ferradini et al,[33] 2007	34 (30-38)*	41%	11 (3-60)*	…
Zhou et al,[34] 2007	34	37%	185[‡]	5.2[‡]
Publications with cross-sectional data on virologic failure				
East Asia & Pacific				
Chaiwarith et al,[35] 2007	37.8 (8)[†]	57%	108 (117)[†]	…
Fethi et al,[36] 2005	…	17%	236 (94)[†]	5.8 (0.3)[†]
Sub-Saharan Africa				
Ferradini et al,[12] 2006	35 (30-41)*	64%	112 (59-176)	…
Ramadhani et al,[37] 2007	41 (19-69)[¶]	73%	114 (1-628)[¶]	…
Spacek et al,[38] 2006	39 (8)[†]	54%	…	…

*median (IQR) [†]mean (SD) [¶]median (range) [€]mean (range)
[‡]mean [μ]median [¥]subgroup

Figure 1. Flow diagram for selection of publications included in a meta-analysis of antiretroviral treatment failure among adults initiating non-nucleoside reverse transcriptase inhibitor-based therapy in resource-limited settings

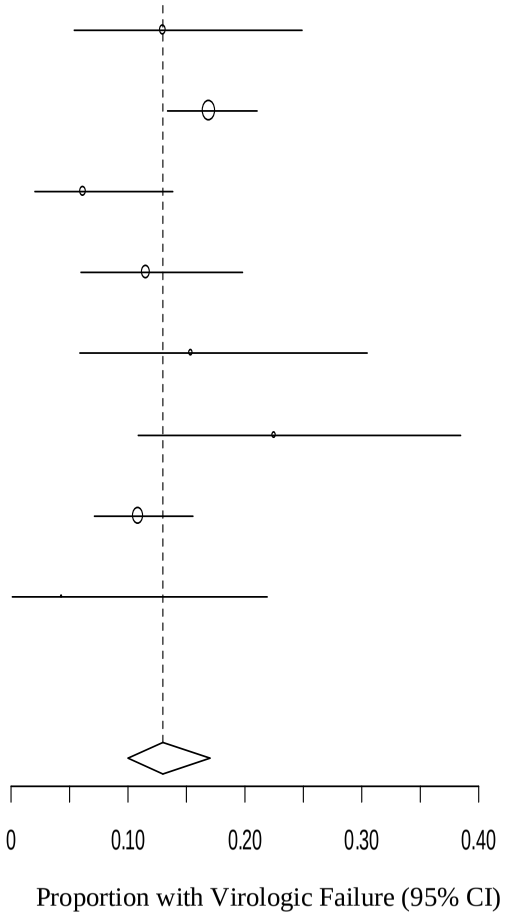


*numbers add up to more than 399 due to overlap between categories

**numbers add up to more than 12 due to overlap between categories

Figure 2. Proportion of patients with virologic failure at 6 months among adults initiating non-nucleoside reverse transcriptase inhibitor-based therapy in resource-limited settings

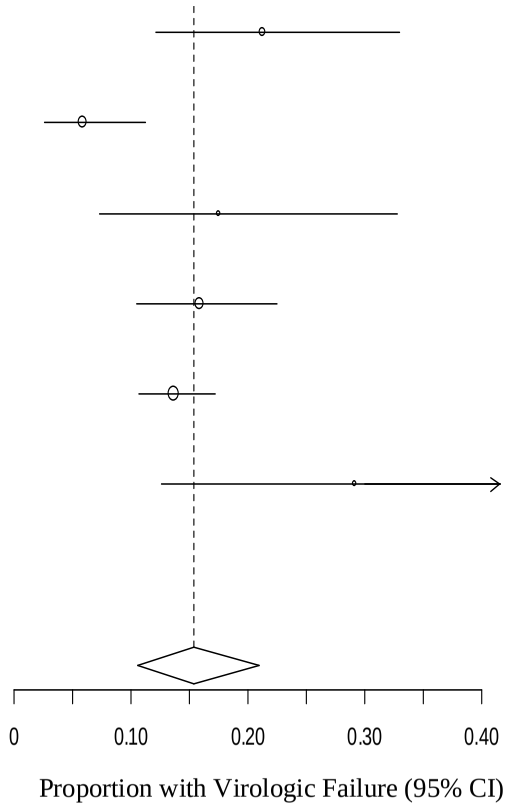
Reference (Location)	Failed	Total	Proportion with Virologic Failure (95% CI)
Wester et al,[23] 2005 (Botswana)	7	54	0.13 (0.05-0.25)
Bisson et al,[24] 2006 (Botswana)	65	384	0.17 (0.13-0.21)
Laurent et al,[25] 2008 (Cameroon)	5	81	0.06 (0.02-0.14)
vanOosterhout et al,[26] 2005 (Malawi)	11	95	0.12 (0.06-0.20)
Idigbe et al,[27] 2005 (Nigeria)	6	39	0.15 (0.06-0.31)
Canestri et al,[28] 2007 (Senegal)	9	40	0.23 (0.11-0.38)
Coetzee et al,[29] 2004 (South Africa)	25	231	0.11 (0.07-0.16)
Baker et al,[30] 2007 (Uganda)	1	23	0.04 (0.00-0.22)
Combined*			0.13 (0.10-0.17)



*combined estimate indicates pooled proportion as calculated by the DerSimmonian-Laird random effects method

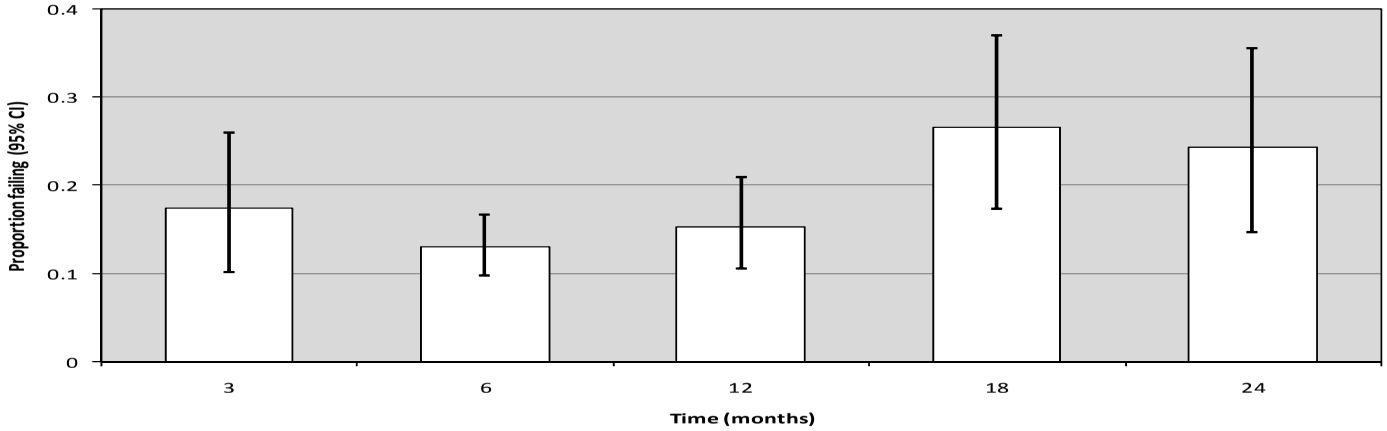
Figure 3. Proportion of patients with virologic failure at 12 months among adults initiating non-nucleoside reverse transcriptase inhibitor-based therapy in resource-limited settings

Reference (Location)	Failed	Total	Proportion with Virologic Failure (95% CI)
Wester et al,[23] 2005 (Botswana)	14	66	0.21 (0.12-0.33)
Laurent et al,[25] 2008 (Cameroon)	8	136	0.06 (0.03-0.11)
Canestri et al,[28] 2007 (Senegal)	7	40	0.18 (0.07-0.33)
Coetzee et al,[29] 2004 (South Africa)	25	158	0.16 (0.11-0.22)
Kamya et al,[31] 2007 (Uganda)	62	454	0.14 (0.11-0.17)
Zhou et al,[34] 2007 (China)	7	24	0.29 (0.13-0.51)
Combined*			0.15 (0.11-0.21)



*combined estimate indicates pooled proportion as calculated by the DerSimmonian-Laird random effects method

Figure 4. Proportion of patients with virologic failure over time among adults initiating non-nucleoside reverse transcriptase inhibitor-based therapy in resource-limited settings



	N	Prop. (95% CI)	N	Prop. (95% CI)	N	Prop. (95% CI)	N	Prop. (95% CI)	N	Prop. (95% CI)
Combined	400	0.17 (0.10-0.26)	947	0.13 (0.10-0.17)	878	0.15 (0.11-0.21)	355	0.27 (0.17-0.37)	878	0.24 (0.15-0.36)
Wester et al,[23] 2005 (Botswana)	77	0.19 (0.11-0.30)	54	0.13 (0.05-0.25)	66	0.21 (0.12-0.33)	55	0.33 (0.21-0.47)	…	...
Bisson et al,[24] 2006 (Botswana)	...	...	384	0.17 (0.13-0.21)	…	...	…	...	…	...
Laurent et al,[25] 2008 (Cameroon)	…	...	81	0.06 (0.02-0.14)	136	0.06 (0.03-0.11)	89	0.09 (0.04-0.17)	82	0.12 (0.06-0.21)
vanOosterhout et al,[26] 2005 (Malawi)	…	...	95	0.12 (0.06-0.20)	…	...	79	0.29 (0.19-0.40)	…	...
Idigbe et al,[27] 2005 (Nigeria)	39	0.21 (0.09-0.36)	39	0.15 (0.06-0.31)	…	...	…	...	…	...
Canestri et al,[28] 2007 (Senegal)	…	...	40	0.23 (0.11-0.38)	40	0.18 (0.07-0.33)	40	0.30 (0.17-0.47)	…	...
Coetzee et al,[29] 2004 (South Africa)	227	0.22 (0.17-0.28)	231	0.11 (0.07-0.16)	158	0.16 (0.11-0.22)	68	0.25 (0.15-0.37)	33	0.30 (0.16-0.49)
Baker et al,[30] 2007 (Uganda)	23	0.00 (0.00-0.15)	23	0.04 (0.00-0.22)	…	...	…	...	…	...
Ferradini et al,[33] 2007 (Cambodia)	…	...	…	...	…	...	…	...	346	0.20 (0.16-0.25)
Zhou et al,[39] 2007 (China)	…	...	…	...	24	0.29 (0.13-0.51)	24	0.42 (0.22-0.63)	24	0.46 (0.26-0.67)
Kamya et al,[31] 2007 (Uganda)	…	...	…	...	454	0.14 (0.11-0.17)	…	...	…	...
Oyugi et al,[32] 2004 (Uganda)	34	0.24 (0.11-0.41)	…	...	…	...	…	...	…	...

THE EPIDEMIOLOGY OF ANTIRETROVIRAL TREATMENT FAILURE IN LUSAKA, ZAMBIA

by

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Format adapted for dissertation

ABSTRACT

BACKGROUND: Owing to limited availability of viral load testing in programmatic African settings, few data are available on the prevalence and predictors of virologic failure in these populations.

METHODS: We performed routine monitoring of human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) viral load (VL) in one arm of a randomized trial of treatment monitoring strategies in Lusaka, Zambia. We defined virologic failure at 3 months as a HIV-1 VL $\geq$ 400 copies/mL with a decline from baseline of less than 1.0 $\log_{10}$ copies/mL. At 6 months, we defined virologic failure as a VL $\geq$ 400 copies/mL.

RESULTS: Between December 1, 2006 and October 1, 2007, 759 patients enrolled in the routine virologic monitoring arm of the trial and initiated antiretroviral therapy (ART). The median age was 34 years (interquartile range [IQR], 29-40), and 447 (58.9%) were female. The median baseline VL was 5.3 $\log_{10}$ copies/mL (IQR, 4.8-5.7). Of 689 patients with an evaluation of virologic failure at 3 months, 24 were failing for a prevalence of 3.5% (95% confidence interval [CI], 2.2%-5.1%). Only poor adherence as measured by pharmacy attendance (medication possession ratio [MPR] < 80%) was associated with virologic failure at 3 months (relative risk [RR] 6.9; 95% CI, 3.0-16). Of 652 patients with a viral load measure at 6 months, 44 were failing for a prevalence of 6.7% (95% CI, 4.9%-9.0%). Poor adherence was also associated with virologic failure at 6 months (adjusted relative risk [ARR], 2.7; 95% CI, 1.2-6.3). A borderline increase in risk for failure at 6 months was noted for severe anemia (hemoglobin [HGB] <8 g/dL) (ARR, 2.5; 95% CI, 0.9-6.6) and high viral burden (VL > 100,000 copies/mL) at baseline (ARR, 1.8; 95% CI, 0.8-3.7).

CONCLUSIONS: In an urban, resource-limited setting, the prevalence of virologic failure was low at both 3 and 6 months, and poor adherence as determined by a simple, inexpensive, pharmacy-based adherence measure was a much better predictor of failure than any baseline measure of disease burden or longitudinal measure of poor response to therapy.

INTRODUCTION

In 2007, an estimated 33.2 million people were living with the human immunodeficiency virus (HIV), and an estimated 2.1 million died from AIDS,[1] yet there is reason for encouragement. The advent of combination antiretroviral therapy (ART) is transforming this disease into a manageable chronic illness in areas where therapy is accessible.[2,3] Several initiatives have been established to deliver ART to resource-limited settings through local governments by international donors such as the President's Emergency Plan for AIDS Relief (PEPFAR), the Global Fund To Fight AIDS, Tuberculosis and Malaria, and the World Bank's Global HIV/AIDS Program of Action.[4-6] As the accelerated scale-up of HIV care and treatment continues in the developing world, the number of patients on ART will continue to grow.

In the developed world, the effectiveness of ART is typically monitored by molecular assays to quantify plasma viral load of HIV ribonucleic acid (RNA). In resource-limited settings like sub-Sahara Africa, where 68% of the global total of people with HIV reside,[1] cost and infrastructure prevent virologic monitoring from being widely available. Treatment failure, which is characterized by the incomplete suppression of HIV replication in

the face of antiretroviral medication, usually leads to increased viral drug resistance among circulating HIV populations. As more individuals start ART in resource-limited settings, it is essential to understand the incidence and predictors of treatment failure. If not, the impressive decreases in morbidity and mortality seen thus far[7-12] could be off-set by the long-term consequences of treatment failure, particularly population-level drug resistance.[13,14] Our aim was to quantify the prevalence of virologic failure at 3 and 6 months after the initiation of ART and identify factors associated with virologic failure to better inform care of these patients in resource-limited settings.

METHODS

The Viral Load Study

In December 2006, a cluster-randomized trial of ART monitoring strategies began in Lusaka, Zambia. In this trial, we randomly allocated six control clinics to use the current standard of care and six intervention clinics to use the standard of care enhanced with routine viral load (VL) testing. The study was designed to demonstrate a 33% reduction in mortality at 18 months after the initiation of ART. Patients were recruited between December 1, 2006 and May 15, 2008 from clinics providing HIV care and treatment through the Zambian national program.[7,8] Patients meeting the following inclusion criteria were eligible to participate: documented HIV type 1 (HIV-1) infection, 18 years of age or greater, eligibility for ART by Zambian national guidelines (defined as having either a CD4+ T-lymphocyte (CD4) count less than 200 cells/mm^3, WHO Stage IV disease, or WHO Stage III disease and CD4 cell count less than 350 cells/mm^3), and initiating ART on the same day as providing informed consent and baseline blood collection. Patients

were not allowed to participate if they met any of the following exclusion criteria: receipt of more than 7 days (cumulative) of prior ART except the use of zidovudine (ZDV) and/or single dose nevarapine (NVP) for prevention of mother-to-child transmission, any condition that would interfere with adherence to the study protocol including any serious illness requiring referral to the hospital, and participation in another research protocol offering routine VL testing. After a routine evaluation consisting of a demographic profile, a medical history, a physical examination including clinical WHO staging, and a laboratory evaluation including CD4 testing with a Beckman Coulter Epics XL and FlowCARE PLG CD4 reagents (Fullerton, CA), patients were evaluated against the inclusion/exclusion criteria. All laboratory tests were performed at a central laboratory certified by the National Institutes of Health, Division of AIDS.

The trial had a pragmatic design. With the exception of the intervention monitoring strategy, HIV care follows guidelines set forth by the Zambian Ministry of Health and is provided by ministry personnel in the normal programmatic setting.[15,16] ART is provided free of charge, and first line therapy consists of one non-nucleoside reverse transcriptase inhibitor (NNRTI), specifically NVP or efavirenz (EFV), and two nucleoside reverse transcriptase inhibitors (NRTI), specifically lamivudine (3TC) with ZDV or stavudine (D4T). In July 2007, the list of first line NRTIs was expanded to include tenofovir (TNF) and emtricitabine (FTC). Preservation of the first line NNRTI-based regimen occurs via single drug substitutions of the NRTI segment of the regimen with other NRTIs, such as abacavir (ABC) and didanosine (DDL), when indicated. If necessary, a switch to second-line therapy may be prescribed and involves changing the NNRTI portion of the regimen

to a protease inhibitor (PI), such as lopinavir/ritonavir (LPV/r), nelfinavir (NFV), or idinavir (IDV). Routine clinical follow-up occurs every 3 months, and CD4 testing is performed every 6 months. Patients are encouraged to identify a friend or family member to serve as an adherence supporter who is authorized to collect drugs at pharmacy appointments when the patient is unable. Information from each visit, including the date of the next scheduled visit, is recorded in Smartcare, the Zambian national electronic medical record system.[17] Each study site also employs a clinic assistant who is responsible for knowing where patients in the trial live and is dispatched to find patients that are more than 5 days late for an appointment.

Virologic Failure

Participants at the intervention sites receive routine virologic monitoring at months 0, 3, 6, 12, and 18 using a Roche Cobas Amplicor with Roche PCR Amplicor Monitor v1.5 Reagents (Basel, Switzerland), which has a lower limit of detection of 400 copies/mL of HIV-1 RNA. We defined virologic failure at 3 months as a VL measurement $\geq$ 400 copies/mL and a decline from baseline of less than 1.0 $\log_{10}$ copies/mL. At 6 months, we defined virologic failure as a VL measurement $\geq$ 400 copies/mL. We used a window of 45 to 134 days for the 3 month visit and 135 to 225 days for the 6 month visit. Since treatment failure can result from opportunistic infections, poor adherence, or viral resistance, a standardized Ministry of Health algorithm is used to rule out infection and/or adherence problems prior to switching patients to a second-line regimen. According to the algorithm, when patients present with treatment failure, staff investigate and treat any active infection and, if poor adherence is suspected, order intensive adherence support (four

weeks of weekly adherence counseling and medication provision). Once these issues have been addressed, the viral load measurement is repeated. Patients meeting criteria for treatment failure are switched to a second-line regimen.

Explanatory Variables

Adherence was estimated using pharmacy refill data to determine the percentage of days on therapy a patient is known to have medication on hand. This simple, objective measure of adherence is an adaptation of the medication possession ratio (MPR)[18,19] and is calculated by dividing the number of days late for pharmacy refills by total days on therapy and subtracting this percentage from 100%. This calculation uses pharmacy refill data from ART initiation until the determination of failure. Since the pharmacy technician usually dispenses an extra three day supply of drugs, a three-day grace period was factored into the calculation. We categorized the resulting MPR as it has been reported in previous studies, optimal ($\geq$ 95%), suboptimal (80–94.9%), and poor (< 80%).[20,21] Creatinine clearance was estimated using the Cockcroft-Gault formula[22] and renal insufficiency categorized using published clinical guidelines from the U.S. National Kidney Foundation's Kidney Disease Outcome Quality Initiative, normal ($\geq$ 90 mL/min) and abnormal (< 90 mL/min).[23] Severe anemia was defined as having a hemoglobin (HGB) concentration less than 8 g/dL as reported in previous studies.[7,8] We used thresholds defined in the National Institutes of Health, Division of AIDS Toxicity Table for Grading Adverse Events to define abnormal liver function.[24,25] Other baseline measures including body mass index (BMI), CD4 count and VL were divided into conventional categories based on established thresholds from the published literature.[7,26] We also examined clinical and

immunologic information available at the six month evaluation of failure. We defined negative weight responses, negative CD4 responses, and negative HGB responses as any weight loss, any decrease in CD4 count, and any decrease in HGB, respectively. Finally, we examined opportunistic infections defined as any new or recurrent WHO stage III or IV condition occurring in the 6 month window.

Statistical Analysis

The study data set was locked for analysis on May 15, 2008 and included patients enrolled at intervention sites with the opportunity to have an evaluation of virologic failure at 6 months (i.e., enrolled by October 1, 2007). When examining failure at 3 months, we limited analyses to patients with virologic monitoring data in the 3 month window and excluded patients who died, withdrew or were lost to follow-up prior to this period. When examining failure at 6 months, we limited the analysis to patients with virologic monitoring data in the 6 month window and excluded patients who died, withdrew, were lost to follow-up, or were failing prior to this period. We excluded patients that were failing at 3 months from the 6 month analyses because they were either switched to a second-line regimen or had undergone intensive adherence counseling and were, consequently, not representative of the general patient population who do not have access to virologic monitoring at three months. We calculated the prevalence of virologic failure at both 3 and 6 months with corresponding 95% confidence intervals using the exact method for binomial proportions.[27] We assessed the normality assumption of continuous medical and demographic variables using the Shapiro-Wilk test[28] and evaluated associations using a Student's t-test or a Wilcoxon rank-sum test, as appropriate. Associations involving dicho-

tomous/categorical variables were evaluated with the Pearson Chi-square test or Fisher's exact test, as appropriate. All p-values were two-sided. Crude relative risks with 95% confidence intervals were calculated using the Mantel Haensel estimator[29-31] or logit estimator,[32] as appropriate. Because the prevalence of virologic failure at 6 months was greater than 5% and all patients have approximately equal follow-up times, we used modified Poisson regression with robust error variances[33,34] to calculate adjusted relative risks (ARR) with conservative 95% confidence intervals. Adjusted models included any covariate with a p-value below 0.20 in crude analyses. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, North Carolina). The study protocol and consent forms were approved by the National Institutes of Health, Division of AIDS, the institutional review board of The University of Alabama at Birmingham, and the research ethics committee of The University of Zambia.

RESULTS

Baseline Characteristics

Between December 1, 2006 and May 15, 2008, 1,974 patients were enrolled in the Viral Load Study. Of the 989 patients enrolled in the intervention arm, 759 (77%) were enrolled by October 1, 2007 and were thus eligible for this analysis. Among those eligible, the median age was 34 years (IQR, 29-40), and 447 (58.9%) were female. The median baseline CD4 count was 143 cell/mm^3 (IQR, 83-204), and the median baseline VL was 5.3 $\log_{10}$ copies/mL (IQR, 4.8-5.7). The median HGB concentration was 10.9 g/dL (IQR, 9.6-12.2), and 46 (6.2%) patients were categorized as having severe anemia (TABLE 1).

Factors Associated with Virologic Failure at 3 Months

Of the 759 patients enrolled for greater than six months, 27 (4%) died and 1 withdrew prior to the 3 month window. Of the remaining 731 active patients, 689 (94%) had a VL measurement in the 3 month window and were included in the analysis of failure at 3 months (FIGURE 1). The 42 (5%) of patients without a baseline or 3 month VL measurement were less likely to report having identified an adherence supporter (90.5% vs. 98.4%, $p<0.01$), were more likely to be severely anemic (14.3% vs. 5.1%, $p=0.01$), were more likely to have a BMI below 16 kg/m^2 (17.1% vs. 5.5%, $p<0.01$), but did not otherwise differ from the 689 patients with a viral load according to key demographic and medical characteristics (data not shown).

Of the 689 patients evaluated at 3 months, 24 had a detectable VL that did not decrease at least 1.0 $\log_{10}$ copies/mL from baseline and were categorized as failing for a prevalence of 3.5% (95% CI, 2.2%-5.1%). Only poor adherence as measured by pharmacy attendance was associated with virologic failure at 3 months (ARR, 6.9; 95% CI, 3.0-16). The following characteristics were not associated with virologic failure at 3 months: age, sex, or baseline VL, CD4 count, adherence support, WHO stage, HGB, BMI, creatinine clearance, alanine aminotransferase, or anti-tuberculosis therapy (data not shown).

Factors Associated with Virologic Failure at 6 Months

Of the 731 patients active at the start of the 3 month window, 17 (2%) died, 19 (3%) withdrew or were lost to follow-up, and 24 (3%) were failing prior to the 6 month window. Of the remaining 671 active patients, 652 (97%) had a VL measurement in the 6

month window and were included in the analysis of failure at 6 months (FIGURE 1). Patients without a 6 month VL were more likely to be severely anemic (15.8% vs. 4.4%, p=0.02) but did not otherwise differ from the patients with a viral load according to key demographic and medical characteristics (data not shown).

Of the 652 patients evaluated at 6 months, 44 had a detectable VL and were thus categorized as failing for a prevalence of 6.7% (95% CI, 4.9%-9.0%). Poor adherence was also associated with virologic failure at 6 months (ARR, 2.7; 95% CI, 1.2-6.3). A borderline increase in risk for virologic failure at 6 months was noted for patients with severe anemia (ARR, 2.5; 95% CI, 0.9-6.6) and a baseline VL above 100,000 copies/mL (ARR, 1.8; 95% CI, 0.8-3.7). The following characteristics were not associated with virologic failure at 6 months: age, sex, or baseline CD4 count, adherence support, WHO stage, BMI, creatinine clearance, alanine aminotransferase, and anti-tuberculosis therapy (TABLE 2).

Overall, 11 patients had an incident or recurrent WHO stage III or IV condition during the 6 month window. There was 1 incident case of a bacterial infection, 5 incident cases of tuberculosis, 4 recurrent cases of tuberculosis, and 1 recurrent case of persistent weight loss. One patient with an incident condition did not have a 6 month viral load, but among the remaining 643 patients evaluated at 6 months, virologic failure was not more likely among patients with an incident or recurrent WHO stage III or IV condition (0%) when compared to patients without (2%, $p > 0.99$).

Secondary Analysis Using Data on Clinical and Immunologic Responses

Not all patients had clinical and immunologic information at 6 months, so we performed a secondary analysis limited to those patients who did to evaluate the relationship between virologic failure at 6 months and changes from baseline for weight, CD4 count, and HGB concentration. Of the 652 patients evaluated at 6 months, 590 (90%) had information on weight change, 515 (85%) had information on CD4 response, and 543 (83%) had information on HGB response. Virologic failure was not more likely among patients missing information versus patients not missing information for weight response (5% vs. 7%, p=0.79), CD4 response (10% vs. 6%, p=0.07), or HGB response (9% vs. 6%, p=0.27). In our analysis of the subset of patients with clinical and immunologic information available, patients who were failing versus not failing did not have a significantly different weight response (3.0 kg vs. 2.5 kg, p=0.60), CD4 response (96 cells/mm^3 vs. 109 cells/mm^3, p=0.60), or HGB response (1.1 g/dL vs. 1.2 g/dL, p=0.62). Furthermore, virologic failure at 6 months was not associated with losing weight (ARR, 1.0; 95% CI, 0.5-2.1), a decrease in CD4 count (ARR, 1.6; 95% CI, 0.7-3.6), or a decrease in HGB concentration (ARR, 0.6; 95% CI, 0.3-1.7) (TABLE 3).

DISCUSSION

In an urban, resource-limited setting, we observed a low prevalence of virologic failure at both 3 and 6 months post ART initiation and found that a simple, inexpensive measure of adherence was strongly associated with virologic failure. We also found a borderline association between virologic failure at 6 months and both elevated viral load and severe anemia at baseline but no association between failure at 6 months and negative changes

in weight, CD4 count, or HGB concentration over that time period. In our setting, the MPR adherence measure was a much better predictor of failure than any baseline measure of disease burden or longitudinal measure of disease progression.

Our prevalence of virologic failure at 6 months was similar to other reports from developing world settings in which patients initiated NNRTI-based regimens. The NNRTI response trial reported the prevalence of failure at 6 months of 9.8% among women initiating NNRTI-based regimens in Thailand, Kenya, and Zambia in 2005 and 2006.[35] In Thailand, an efficacy trial of the NNRTI-based regimen D4T, 3TC, and NVP observed results similar to ours at both 3 and 6 months.[36] Our prevalence was, however, less than the 24.9% observed by the Antiretroviral Therapy in Low-Income Countries (ART-LINC) Collaboration[37] and the 20.8% that Tan and colleagues observed in an urban HIV clinic Alabama.[38] Both the ART-LINC report and the Alabama report describe cohorts initiating ART in the late 1990s with a significant proportion of patients initiating PI-based regimens. Differences in potency, tolerability and adherence between NNRTI-based regimens available today and PI-based regimens available in the late 1990s could account for differences in the incidence of failure. Another important point to consider is that patients who agree to be part of a trial may be more likely to adhere to therapy than patients followed as part of a programmatic cohort. Although we would expect this Hawthorne effect to be mitigated by our study's liberal inclusion criteria and pragmatic study design, it could account for our lower observed prevalence of failure.

We found that a simple, objective, inexpensive adherence measure was associated with virologic failure. Others have reported a relationship between MPR measures of adherence and virologic suppression.[21,26,39-44] However, our observations demonstrate that MPR adherence measurements are associated with an increased risk for virologic failure as early as 3 months as opposed to after 6 months[26,39] or initial viral suppression.[43] We also found that increased viral burden and severe anemia at baseline were potential predictors of failure at 6 months. Though insignificant, our estimate of baseline viral burden was comparable to other reports.[26,39,45] To our knowledge, no other report has examined baseline HGB levels and risk for virologic failure, but others have reported an association between anemia and an increased risk for death[7,46]

We did not find an association between longitudinal measures of poor response to ART and failure. Others, however, have found an association between clinical and immunologic changes and disease progression and death. A report out of the EuroSIDA cohort found that current HGB was more predictive of progression to AIDS or death than hemoglobin measured at baseline[47] while Rajasekaran and colleagues found that negative changes in HGB, total lymphocytes, and weight were all predictive of immunologic and/or clinical failure after 12 months on therapy.[48] As disease progression and death follows virologic failure, it would make sense that if these measures predicted death after 12 months on ART, they might predict failure at 6 months. A possible explanation for our negative finding is that some patients are simply clinically non-responsive. The ART-LINC Collaboration,[37] the HIV/AIDS Drug Treatment Program of the British Columbia Centre for Excellence in HIV/AIDS,[49] and Tan and colleagues in Alabama[38] reported that 19.0%,

15.4% and 8.7% of patients initiating ART, respectively, received less than a 50 cells/mm^3 increase in CD4 count even though their viral load was successfully suppressed.

The primary strength of this analysis was the pragmatic study design of the trial. Nested within the context of the Zambian national ART program, care was provided primarily by clinical officers and nurses using standard protocols.[7] It is encouraging that within this real world setting, we observed a low prevalence of virologic failure at 3 and 6 months and adherence rates comparable to the United States.[20] Also noteworthy was the fact we were able to use the Zambian electronic medical record system[17] as the data collection tool for this study. Since this instrument is able to generate facility-level reports from real time data, any lessons learned from this study can be incorporated into future reports to inform patient care.

The primary limitation of this analysis involves the use of the MPR to estimate ART adherence. The MPR does not actually measure the amount of medication ingested but represents the best case scenario. The possible overestimation of adherence by the MPR may lead to misclassification of exposure status. We would, however, expect this misclassification to be nondifferential with respect to the outcome of interest. There is little reason to believe that the amount of medication ingested by two people with the same MPR would differ between a patient who is failing treatment and a patient who is not failing treatment. Patients who ingest fewer pills, failing therapy or not, would most likely wait until all of the medication was finished before presenting for a refill and would

have an MPR to reflect this experience. Another potential limitation of this analysis is our reliance upon a single viral load measurement. Without a confirmatory measure, we may have incorrectly classified people as failing who only experienced an intermittent spike or "blip" in viral load. Since this only happens among patients that are not truly failing treatment, the misclassification may be differentially associated with high levels of adherence. The net result of this type of misclassification is, however, to categorize more adherent patients as failing and bias the results towards the null. Finally, the lack of resistance testing is a limitation that prevents knowing for certain if the observed failure is due to viral resistance and requires a regimen switch. Future studies should include viral drug resistance testing to be able to examine this issue.

In summary, these results suggest that ART delivery in an urban, resource-limited setting results in low rates of virologic failure and that an MPR adherence measure is associated with failure as early as 3 months after initiating ART. While these results are encouraging, further research is needed to determine if it is possible to create a predictive model for virologic failure using routine clinical, immunological, and adherence-based measures.

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Table 1. Baseline characteristics of ART naïve adults enrolled in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia (December 1, 2006 – October 1, 2007)

		N	Value
Age, median years (Q1, Q3)		759	34 (29, 40)
Sex	Female	447	58.9%
	Male	312	41.1%
Viral load, median $\log_{10}$ copies/mL (Q1, Q3)		756	5.3 (4.8, 5.7)
	$\leq$100,000 copies/mL	249	32.9%
	>100,000 copies/mL	507	67.1%
Adherence support	No	20	2.6%
	Yes	739	97.4%
CD4 count, median cells/mm^3 (Q1, Q3)		746	143 (83, 204)
	$\geq$ 200 cells/mm^3	195	26.1%
	50 – 199 cells/mm^3	451	60.5%
	< 50 cells/mm^3	100	13.4%
WHO stage	I or II	205	28.4%
	III	448	62.0%
	IV	70	9.7%
Hemoglobin, median g/dL (Q1, Q3)		741	10.9 (9.6, 12.2)
	$\geq$ 8.0 g/dL	695	93.8%
	< 8.0 g/dL	46	6.2%
Body mass index, median kg/m^2 (Q1, Q3)		738	19.9 (18.0, 22.3)
	$\geq$ 16 kg/m^2	684	92.7%
	< 16 kg/m^2	54	7.3%
Creatinine clearance, median mL/min (Q1, Q3)		737	115 (93, 140)
	Normal	579	78.6%
	Abnormal	158	21.4%
Alanine aminotransferase, median U/L (Q1, Q3)		739	19.0 (13.0, 27.0)
	Normal	712	96.3%
	Abnormal	27	3.7%
Anti-tuberculosis therapy	No	644	84.8%
	Yes	115	15.2%
Antiretroviral regimen	ZDV + 3TC + NVP	347	45.7%
	ZDV + 3TC + EFV	41	5.4%
	D4T + 3TC + NVP	289	38.1%
	D4T + 3TC + EFV	43	5.7%
	TDF + FTC + NVP	9	1.2%
	TDF + FTC + EFV	29	3.8%
	TDF + 3TC + NVP	1	0.1%

Table 2. Relative risk of virologic failure at 6 months for ART naïve adults enrolled in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia (December 1, 2006 – October 1, 2007)

		Virologic Failure at 6 months (excluding deaths, withdrawals, lost to follow-up, and failures at 3 months)	
Characteristic*	Group	Crude RR (95% CI)	Adjusted RR (95% CI) n=632
Viral load	≤100,000 copies/mL	1.0	1.0
	>100,000 copies/mL	2.0 (1.0 - 4.1)	1.8 (0.8 - 3.7)
Adherence	Optimal	1.0	1.0
	Sub-optimal	1.2 (0.5 - 2.6)	0.9 (0.3 - 2.1)
	Poor	2.8 (1.2 - 6.2)	2.7 (1.2 - 6.3)
Sex	Female	1.0	
	Male	0.9 (0.5 - 1.6)	
Adherence support	No	1.0	
	Yes	0.7 (0.1 - 4.4)	
CD4 count	≥200 cells/mm^3	1.0	
	50-199 cells/mm^3	0.9 (0.5 - 1.9)	
	<50 cells/mm^3	1.4 (0.6 - 3.4)	
WHO stage	I or II	1.0	
	III	0.7 (0.4 - 1.4)	
	IV	1.0 (0.4 - 2.7)	
Hemoglobin	≥8 g/dL	1.0	1.0
	<8 g/dL	2.3 (0.9 - 6.1)	2.5 (0.9 - 6.6)
Body mass index	≥16 kg/m^2	1.0	
	<16 kg/m^2	0.4 (0.1 - 3.0)	
Creatinine clearance	Normal	1.0	
	Abnormal	1.2 (0.6 - 2.3)	
Alanine aminotransferase	Normal	1.0	
	Abnormal	0.6 (0.1 - 4.3)	
Anti-tuberculosis therapy	No	1.0	
	Yes	1.1 (0.5 - 2.4)	
Age	Per 10 years	0.9 (0.6 - 1.4)	

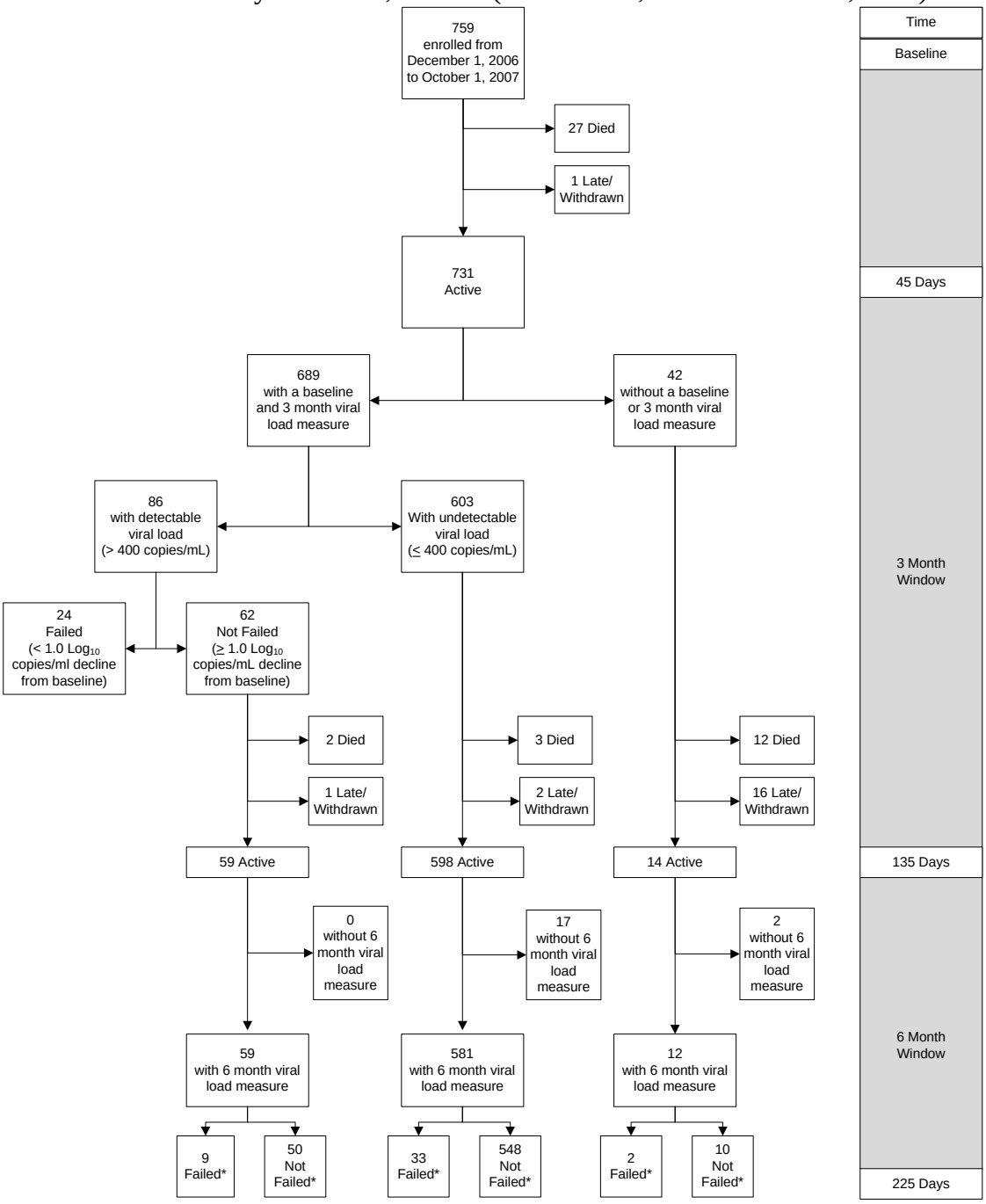
* All characteristics except adherence are determined at ART initiation. Adherence is calculated using pharmacy refill data from ART initiation until evaluation of failure (See Methods).

Table 3. Relative risk of virologic failure at 6 months for different longitudinal measures of disease progression among ART naïve adults enrolled in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia (December 1, 2006 – October 1, 2007)

		Virologic Failure at 6 months (excluding deaths, withdrawals, lost to follow-up, and failures at 3 months)		
Characteristic	Group	Failures	Crude RR (95% CI)	Adjusted RR* (95% CI)
Change in weight at 6 months	≥0 kg	32 of 440 (7.3%)	1.0	1.0
	<0kg	9 of 150 (6.0%)	0.8 (0.4 - 1.7)	1.0 (0.5 - 2.1)
Change in CD4 count at 6 months	≥0 cells/mm^3	23 of 432 (5.3%)	1.0	1.0
	<0 cells/mm^3	7 of 83 (8.4%)	1.6 (0.7 - 3.6)	1.6 (0.7 - 3.6)
Change in hemoglobin at 6 months	≥0 g/dL	29 of 423(6.9%)	1.0	1.0
	<0 g/dL	5 of 120 (4.2%)	0.6 (0.2 - 1.5)	0.6 (0.3 - 1.7)

*Adjusted for adherence, baseline viral load and hemoglobin.

Figure 1: Progress of ART naïve adults enrolled in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia (December 1, 2006 – October 1, 2007)



* Failure is defined as > 400 copies/mL unless otherwise indicated

AN EVALUATION OF DIFFERENT METHODS FOR DIAGNOSING ANTIRETROVIRAL TREATMENT FAILURE IN RESOURCE-LIMITED SETTINGS WHERE VIROLOGIC TESTING IS NOT AVAILABLE

by

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ABSTRACT

BACKGROUND: The World Health Organization (WHO) recommends using clinical and immunologic indicators to monitor the effectiveness of antiretroviral therapy (ART) in resource-limited settings where virologic monitoring is not available.

METHODS: Using data from one arm of a large pragmatic randomized trial of treatment monitoring strategies in Lusaka, Zambia, we evaluated the performance of the WHO-recommended algorithm and other approaches for monitoring treatment including a predictive-score-based algorithm created through regression modeling techniques. We calculated the sensitivity, specificity, positive predictive value, negative predictive value, total misclassification value, and false negative value of all models and the associated 95% confidence intervals (CI) using the exact method for binomial proportions.

RESULTS: Between December 1, 2006 and October 1, 2007, 759 patients enrolled in the routine virologic monitoring arm of the trial. Of these, 652 (90%) were evaluated for virologic failure at 6 months, and 44 were found to be failing (prevalence, 6.7%; 95% CI, 4.9%-9.0%). The WHO-recommended algorithm for the detection of failure at 6 months had a sensitivity of 27.6% (95% CI, 12.7%-47.2%), a specificity of 78.4% (95% CI, 74.4%-82.0%), and a total misclassification value of 24.5% (95% CI, 20.8%-28.5%). Our new, predictive-score-based algorithm used adherence and baseline anemia to estimate the probability of failure and classified patients with an estimated probability $\geq$ 9% as a potential failure. This algorithm had a sensitivity of 24.4% (95% CI, 12.4%-40.3%), a specificity of 91.7% (95% CI, 89.2%-93.8%), and a total misclassification value of 12.6% (95% CI, 10.1%-15.5%).

CONCLUSIONS: In an urban, resource-limited setting, neither the WHO-recommended algorithm nor our new, predictive-score-based algorithm performed well at 6 months. As scale-up of antiretroviral treatment in resource-limited setting continues and the population of patients started on ART without access to routine virologic monitoring increases, the growing number of patients falsely identified as failing therapy will require more resources to monitor and treat while the growing number of patients on non-suppressive therapy may have dramatic long-term consequences with public health implications, particularly population-level drug resistance.

INTRODUCTION

As expansion of care and treatment for persons infected with the human immunodeficiency virus (HIV) in resource-limited setting continues,[1-3] appropriate monitoring of antiretroviral therapy (ART) response in this ever growing pool of patients is a significant public health concern. Quantification of HIV type 1 (HIV-1) ribonucleic acid (RNA) circulating in the plasma by molecular assay is the gold standard for monitoring the effectiveness of ART in the developed world.[4] The cost and technical requirements of the assay, however, prevent routine virologic monitoring from being widely used in resource-limited settings. As a result, surrogate markers are used to create clinical and/or immunological algorithms to identify patients who may be failing. Inaccurate algorithms may fail to detect individuals with incomplete viral suppression, a scenario that could lead to decline of CD4+ T-lymphocyte (CD4) cells, appearance of opportunistic infections, and emergence of viral drug resistance.[5-8] As the reservoir of patients with resistance mutations increases, there is a very real danger of population-level drug resistance.

While acknowledging that there is a limited amount of data on which to base recommendations, the World Health Organization (WHO) advocates using clinical and immunologic indicators to monitor the effectiveness of ART in resource-limited settings where virologic monitoring is not available. The WHO-recommended algorithm to identify patients who are failing at 6 months is based on immunologic criteria, specifically a persistent CD4 count below 100 cells/mm^3 or a decrease in CD4 count at 6 months.[9] Others have recommended algorithms that incorporate additional data such as adherence, clinical responses, and laboratory indices such as hemoglobin (HGB) response.[6,10-13] In this analysis, we sought to identify an optimal screening test for diagnosing antiretroviral treatment failure by evaluating the diagnostic accuracy of the existing WHO-recommended algorithm and new algorithms created using additional clinical and immunological indicators.

METHODS

The Viral Load Study

We examined 6 month virologic results from patients in the Viral Load Study, a pragmatic, cluster randomized trial of ART monitoring strategies in Lusaka, Zambia. Between December 1, 2006 and May 15, 2008, the trial enrolled adult patients ($\geq$18 years or age) with documented HIV-1 infection who were eligible for ART by Zambian national guidelines. Candidates were excluded if they had received more than 7 days cumulative prior combination ART or had any condition that would interfere with adherence to the study protocol.

Patients in the six clinics that were randomly allocated to the control arm of the trial received the current standard of care[14-16] consisting of a routine baseline examination and scheduled follow-up visits. The baseline evaluation consists of a) a questionnaire detailing the patient's demographic profile and medical history, b) a physical examination complete with WHO clinical staging, and c) a laboratory evaluation including CD4 testing with a Beckman Coulter Epics XL and FlowCARE PLG CD4 reagents (Fullerton, CA). Routine follow-up visits occur every 3 months with CD4 testing every 6 months.

Patients in the six clinics that were randomly allocated to the intervention arm of the trial received the current standard of care enhanced with routine viral load testing at months 0, 3, 6, 12, and 18 using a Roche Cobas Amplicor with Roche PCR Amplicor Monitor v1.5 Reagents (Basel, Switzerland) with a lower limit of detection of HIV-1 RNA viral load (VL) of 400 copies/mL. For this analysis, we focused on virologic outcomes at 6 months (window of 135 to 225 days). We defined virologic failure at 6 months as a VL $\geq$ 400 copies/mL. We calculated the prevalence of failure at 6 months with 95% confidence intervals (CI) using the exact method for binomial proportions.[17]

Analysis Cohort

This analysis is limited to patients in the intervention arm of the trial with virologic monitoring data available at 6 months. We excluded patients who were enrolled after to September 30, 2007 because they had not been enrolled long enough to have a 6 month evaluation. We excluded patients who were determined to be failing at 3 months (window of 45 to 134 days). We defined failure at 3 months as a VL $\geq$ 400 copies/mL and a decline

from baseline of less than 1.0 $\log_{10}$ copies/mL. These patients were excluded because they had been intervened upon and were not representative of patients without access to virologic monitoring at 3 months. We also excluded patients who died, withdrew, or were lost to follow-up prior to the 6 month evaluation of failure.

Algorithms to Predict Virologic Failure

In order to evaluate WHO-recommended algorithm for determining treatment failure, we created variables for having a CD4 count <100 cells/mm^3 at the evaluation of failure and having a negative CD4 response between ART initiation and the evaluation of failure[9] and assessed the performance of each variable alone and in combination. Since others have recommended using additional data to predict failure,[6,10-13] we created algorithms that included adherence and variables for two other routinely collected measures, weight and HGB. We defined a negative weight response as weight loss between ART initiation and the evaluation of failure and a negative HGB response as a decrease in HGB concentration between ART initiation and the evaluation of failure. We created a variable for adherence based on pharmacy attendance using a variation of the medication possession ratio (MPR).[18,19] A description of this variable has been reported elsewhere.[20] This simple, objective measure uses pharmacy refill data from ART initiation until the determination of failure and is calculated by dividing the number of days a patient has pills on hand by the total number of days a patient has been on therapy. We defined poor adherence as an MPR below 80% as reported in other studies.[20,21] We used these variables alone and in combination with the WHO-recommended algorithm to create 7 additional algorithms for the prediction of failure at 6 months.

We also used regression modeling techniques to create a predictive-score-based algorithm for virologic failure. First, we examined the association of baseline measures and the previously described longitudinal measures with failure at 6 months in bivariate analyses. We defined severe anemia as HGB concentration <8 g/dL and categorized body mass index (BMI) and CD4 count using established thresholds from the published literature.[14] We examined adherence based on pharmacy attendance as both a continuous variable and as a categorical variable with poor adherence defined as <80% as previously noted.[20,21] We estimated creatinine clearance using the Cockcroft-Gault formula[22] and defined abnormal creatinine clearance as <90 mL/min.[23,24] We defined abnormal liver function as an alanine aminotransferase $\geq$62.5 U/L based on pre-existing toxicity guidelines from the Division of AIDS, National Institutes of Health.[25,26] We used the Shapiro-Wilk test to assess normality of continuous variables and used a Student's t-test or a Wilcoxon rank-sum test to evaluate associations, as appropriate.[27] We used a Pearson Chi-square test or Fisher's exact test to evaluate categorical associations, as appropriate. All p-values were two-sided. Modified Poisson regression[28,29] was used to develop an equation for prediction of failure at 6 months. Risk factors included in the model were covariates with a p-value below 0.20 in crude analyses. We used the -2 log-likelihood ratio test to evaluate the overall significance of the final mathematical model. We used the resulting predictive equation to estimate of the risk of virologic failure at 6 months for each patient. To evaluate the performance of this new, predictive-score-based algorithm, we constructed a receiver-operating characteristic (ROC) curve using different probability cut-points and calculated the area under the curve (AUC) with 95% CIs via 1,000 bootstrap simulations using unrestricted random sampling with replacement.[30,31] We eva-

luated two different criterion to determine the optimal cut-point from the ROC curve, specifically the Youden Index[32] and the shortest distance to the upper left hand corner. The Youden Index chooses the cut-point that maximizes the sum of the sensitivity and the specificity and is calculated by equation (1).

$$J = (Sensitivity + Specificity - 1) \quad (1)$$

The cut-point with the shortest distance to the upper left hand corner minimizes *d* in equation (2).

$$d = \sqrt{(1 - Sensitivity)^2 + (1 - Specificity)^2} \quad (2)$$

Performance Indicators

To evaluate the performance of the different algorithms, we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), misclassification value, and false negative value with 95% confidence intervals.[17,33] Sensitivity was calculated as [true positives / (true positives + false negatives)] × 100. Specificity was calculated as [true negatives / (true negatives + false positives)] × 100. Positive predictive value (PPV) was calculated as [true positives / (true positives + false positives)] × 100. Negative predictive value (NPV) was calculated as [true negatives / (true negatives + false negatives)] × 100. The total misclassification value represents the percentage of patients that were incorrectly classified by the algorithm and was calculated as [(false positives + false negatives) / total patients screened] × 100. The false negative value was calculated as [false negatives / total patients screened] × 100. We also examined the odds of being affected given a positive result (OAPR) which is the ratio of the number of affected to unaffected individuals among those with positive results and was calculated as [true posi-

tives / false positives]. An OAPR below 1 indicates that the algorithm identifies more false positives than true positives.

The data were collected using Smartcare, the Zambian national electronic medical record system.[34] All data were entered into two separate databases by different data associates. These databases were compared, and any discrepancies were corrected, as necessary. The dataset was locked for analysis on May 15, 2008. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, North Carolina). The institutional review board of The University of Alabama at Birmingham, the research ethics committee at The University of Zambia, and the National Institutes of Health, Division of AIDS approved the study protocol and consent forms.

RESULTS

Description of Cohort

Between December 1, 2006 and May 15, 2008, 1,974 patients were enrolled in the Viral Load Study. Of the 989 patients enrolled in the routine virologic monitoring arm, 230 (23.3%) were enrolled after September 30, 2007 and were not eligible for this analysis. Of the remaining 759 patients, 44 (5.8%) died, 20 (2.6%) withdrew or were lost to follow-up, 24 (3.2%) were deemed to be failing at 3 months, and 19 (2.5%) did not have a viral load measurement in the 6 month window. This left 652 patients in the analysis cohort. Virologic failure was detected in 44 of these patients (prevalence, 6.7%; 95% CI, 4.9%-9.0%). Patients who were failing were more likely to have poor adherence as determined from pharmacy attendance as compared to patients that were not failing (13.6%

vs. 4.6%, p=0.03) and were more likely to have a baseline viral load above 100,000 copies/mL (79.5% vs. 64.9%, p=0.05). There was a suggestion that patients who were failing were more likely to have severe anemia at baseline compared to patients who were not failing (9.8% vs. 4.0%, p=0.10). There was no association between failure and other baseline characteristics or longitudinal measures of disease progression including decreases in weight, CD4 count, and HGB (TABLE 1).

Performance of Different Algorithms

Not all patients had concurrent CD4 results available at the 6 month evaluation of failure. Of the 652 patients evaluated at 6 months, 142 (21.8%) were missing this information. Patients missing concurrent CD4 information were more likely to fail 10.6% vs. 5.7% (p=0.04) than patients not missing this information. The treatment monitoring algorithm recommended by the WHO to detect antiretroviral treatment failure at 6 months had a sensitivity of 27.6% (95% CI, 12.7%-47.2%), a specificity of 78.4% (95% CI, 74.4%-82.0%), a total misclassification value of 24.5% (95% CI, 20.8%-28.5%), a false negative value of 4.1% (95% CI, 2.6%-6.2%), and an OAPR of 0.08 among patients with data available. Because missing this immunologic information was associated with virologic failure, we performed a sensitivity analysis assuming that the WHO-recommended algorithm correctly categorized all 142 of these patients. In this analysis, the sensitivity and specificity of the WHO-recommended algorithm were higher but not significantly different than observed (sensitivity, 52.3%; 95% CI, 36.7%-67.5%; specificity, 82.9%; 95% CI, 79.7%-85.8%).

When we added adherence to the WHO-recommended algorithm, sensitivity and specificity were not appreciably improved, 37.5% (95% CI, 21.1%-56.3%) and 73.6% (95% CI, 69.5%-77.5%), respectively. Adding negative weight response, negative HGB response, or the combination of adherence, negative weight response, and negative HGB response to the WHO-recommended algorithm resulted in marginal increases in sensitivity and significant decreases in specificity. Of all the non-predictive-score-based algorithms evaluated, only the one using adherence alone had specificity above 90%. Unfortunately, sensitivity of this algorithm was low, 13.6% (95% CI, 5.2%-27.4%). The adherence alone algorithm did, however, have the lowest misclassification value, 10.4% (95% CI, 8.2%-13.0%) and the highest OAPR, 0.20 (TABLE 2).

Creation of Predictive-Score-Based Algorithm

We created a model for failure at 6 months using modified Poisson regression. The final model included presence of severe anemia at baseline (p=0.07) and adherence as determined from pharmacy refill data (p<0.01) and resulted in equation (3) to estimate the probability of failure.

$$P(\textit{failure}) = \exp\left(-2.94292 + ((100 - \text{MPR}) \times 0.02498) + (0.90291 \times (\text{Anemia}))\right) \qquad (3)$$

where MPR is a continuous variable with values ranging from 0 to 100 and anemia is a dichotomous variable for the presence of severe anemia at baseline with a value of 1 yes and 0 for no (TABLE 3). Using equation (3), the probability of failure for a given patient can be estimated. For example, the risk of failure for a patient who did not have severe anemia at baseline and had perfect adherence at the 6 month evaluation of failure is estimated to be 5.3%; whereas the risk of failure for a patient who did not have severe ane-

mia at baseline and had only 70% adherence at the 6 month evaluation of failure is estimated to be 11.2% (TABLE 4).

After assigning each patient a predicted probability of failure using equation (3), we classified patients as potential failures using different probability cut-points and constructed an ROC curve (FIGURE 1). This curve had an AUC value of 0.58 (95% CI, 0.50-0.67). The minimum distance to the upper left corner criterion identified 6% as the ideal cut-point while the Youden Index identified 9% (TABLE 5). Since setting the cut-point at 6% would flag virtually all patients as failures, we decided to use the Youden Index cut-point. This new, predictive-score-based algorithm with a threshold of 9% has a sensitivity of 24.4% (95% CI, 12.4%-40.3%), a specificity of 91.7% (95% CI, 89.2%-93.8%), a total misclassification value of 12.6% (95% CI, 10.1%-15.5%), a false negative value of 4.9% (95% CI, 3.3%-6.9%), and an OAPR of 0.20.

DISCUSSION

In this urban, resource-limited setting, the WHO-recommended algorithm for the prediction of virologic failure did not perform well at 6 months. We were able to create a new algorithm for the prediction of virologic failure that had better specificity than the WHO-recommended algorithm with comparable sensitivity, but both misclassified many patients and identified more false positives than true positives. Our inability to produce a model with an acceptable AUC (>0.70),[35] a good combination of sensitivity (>80%) and specificity (>95%),[36] or even an OAPR above one demonstrates that clinical and/or im-

munologic monitoring is not a reliable substitute for virologic monitoring of early treatment outcomes.

Since cost and difficulty of routine virologic monitoring prevent it from being widely used in resource-limited settings, programs are left using risk factors to flag patients as suspected failures. Others have discussed the limitations of using risk factors as prognostic tools and noted that a given risk factor must have a much stronger association with an outcome than normally seen in etiologic research if it is to be a worthwhile screening test.[36,37] We attempted to use a predictive-score-based algorithm that combined risk factors and were still unable to produce an adequate screening test.

Using inaccurate algorithms to flag patients as suspected failures has many drawbacks. First, they flag too many patients (both false positives and false negatives) thus draining resources by causing more work for an already taxed workforce. Second, some flagged patients will unnecessarily be switched to second-line therapy, which is generally much more expensive in most settings. Finally, as these algorithms will undoubtedly miss patients that are actually failing, population-level drug resistance remains a public health concern. Phillips and colleagues used mathematical modeling techniques to examine the long-term consequences of using clinical criteria in place of immunological or virological monitoring and concluded that there would be no detrimental effects on survival or development of resistance.[38] Contrary to their conclusion, however, the authors note that clinical and immunological monitoring strategies had appreciably higher percentages of life-years with resistance when compared to virological monitoring. Further, the authors

did not account for retention problems in resource-limited settings where every year a significant proportion of patients become lost to follow-up and add to the pool of infected individuals with potentially resistant virus.[39]

Given that virologic monitoring may not be currently possible in many settings, research should continue in an attempt to identify other approaches to monitor the effectiveness of ART. It is possible that the WHO-recommended algorithm may perform better beyond 6 months with the recommended addition of 2 more criteria, specifically a 50% fall from the on-treatment peak CD4 count or the incidence/recurrence of select WHO stage III conditions (e.g. pulmonary tuberculosis or severe bacterial infections) or any WHO stage IV condition after 6 months,[9] but Chaiwarith and colleagues reported results comparable to ours using the algorithm beyond 6 months.[40] As a single risk factor is unlikely to perform well,[36,37] further research using predictive-score-based multiple regression models with data from several time points is warranted.

The pragmatic design of the Viral Load Study was a major strength of this analysis. HIV care followed guidelines set forth by the Zambian Ministry of Health, took place in government clinics, and was provided by existing personnel.[14-16] In this context, we were able to gauge the performance of the WHO-recommended algorithm (and others) in a programmatic setting. The use of the Zambian electronic medical record system as the data collection tool was also a strength.[34] We did not need to collect any data that were not routinely collected to perform this analysis. Further, had the predictive-score-based algorithm produced a reliable screening test, the predictive equation could be easily pro-

grammed into the Zambian electronic medical record system and used to flag patients as potential failures.

The primary limitation of this analysis is missing data. We were unable to evaluate the WHO-recommended algorithm for 142 (21.8%) patients because they lacked concurrent CD4 information at the 6 month evaluation of failure. It is possible that clinicians acted contrary to the protocol and ordered a viral load but not a CD4 count among patients suspected of failure. In the unlikely event that the WHO-recommended algorithm performed perfectly in all of these patients, our sensitivity analysis suggests that it still would be inadequate since the sensitivity did not reach 80% and the specificity did not reach 95%.

Other limitations include the use of the MPR to estimate ART adherence and the reliance on a single viral load measure to evaluate failure. The MPR represents the best case scenario and likely overestimates the true amount of medication ingested thus leading to a potential misclassification of exposure. This potential misclassification is most likely nondifferential with respect to virologic failure as all patients are likely to wait until the medication bottle is empty before returning to the pharmacy, regardless of whether or not they are failing. Without a confirmatory viral load measurement, patients experiencing an intermittent spike or “blip” in viral load may have been misclassified as failing. Unfortunately, the misclassification of healthy patients as failures would lower the sensitivity of a reliable screening test by increasing the number of false positives. While this issue is problematic, it is unlikely to account for the poor performance of the WHO-recommended algorithm. If 10% of the false positives were actually true positives, the

sensitivity of the WHO-recommended algorithm would only increase from 27.6% to 30.8%.

In summary, these results suggest that clinical and/or immunologic algorithms are not a reliable substitute for virologic monitoring at 6 months post ART initiation. While necessary in the absence of virologic monitoring, every effort should be made to make viral load monitoring available in resource-limited settings. This should be a priority at all levels and will require substantial investment of resources both to develop newer, cheaper assays and to build local laboratory and human resource capacity. Not to do so risks the development of population-level drug resistance and could threaten the remarkable early effectiveness of the ART roll-out effort.[14,41-43]

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Table 1. Characteristics by virologic failure status at 6 months among patients in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia

		Patients with virologic failure at 6 months		Patients without virologic failure at 6 months		
		N	Value	N	Value	p
Age, median years (Q1, Q3)		44	34 (27, 41)	608	34 (30, 40)	0.22
Sex	Female	28	63.6%	364	59.9%	0.62
	Male	16	36.4%	244	40.1%	
Viral load, median $\log_{10}$ copies/mL (Q1, Q3)		44	5.4 (5.1, 5.6)	606	5.3 (4.8, 5.6)	0.11
	≤100,000 copies/mL	9	20.5%	213	35.1%	0.05
	>100,000 copies/mL	35	79.5%	393	64.9%	
Adherence support	No	1	2.3%	9	1.5%	0.51
	Yes	43	97.7%	599	98.5%	
CD4 count, median cells/mm^3 (Q1, Q3)		42	134 (77, 205)	598	150 (87, 205)	0.52
	≥ 200 cells/mm^3	11	26.2%	159	26.6%	0.65
	50 – 199 cells/mm^3	24	57.1%	368	61.5%	
	< 50 cells/mm^3	7	16.7%	71	11.9%	
WHO stage	I or II	15	34.9%	166	28.8%	0.57
	III	23	53.5%	355	61.6%	
	IV	5	11.6%	55	9.5%	
Hemoglobin, median g/dL (Q1, Q3)		41	11.2 (10.0, 12.2)	593	11.0 (9.7, 12.3)	0.87
	≥ 8.0 g/dL	37	90.2%	569	96.0%	0.10
	< 8.0 g/dL	4	9.8%	24	4.0%	
Body mass index, median kg/m^2 (Q1, Q3)		43	20.4 (18.9, 22.1)	592	20.2 (18.2, 22.6)	0.94
	≥ 16 kg/m^2	42	97.7%	559	94.4%	0.72
	< 16 kg/m^2	1	2.3%	33	5.6%	
Creatinine clearance, median mL/min (Q1, Q3)		44	116 (98, 135)	586	115 (95, 141)	0.86
	Normal	34	77.3%	469	80.0%	0.66
	Abnormal	10	22.7%	117	20.0%	
Alanine aminotransferase, median U/L (Q1, Q3)		44	19.0 (13.0, 29.0)	589	19.0 (13.0, 27.0)	0.85
	Normal	43	97.7%	567	96.3%	>0.99
	Abnormal	1	2.3%	22	3.7%	
Anti-tuberculosis therapy	No	37	84.1%	518	85.2%	0.84
	Yes	7	15.9%	90	14.8%	
Adherence (MPR)	≥ 80%	38	86.4%	578	95.1%	0.03
	< 80%	6	13.6%	30	4.9%	
Weight change, median kg		41	2.0 (0.0, 6.0)	549	2.0 (-0.5, 5.0)	0.63
	≥0 kg	32	78.0%	408	74.3%	0.60
	<0 kg	9	22.0%	141	25.7%	
Hemoglobin change, median g/dL		34	1.0 (0.1, 1.9)	509	1.3 (0.1, 2.5)	0.40
	≥0 g/dL	29	85.3%	394	77.4%	0.28
	<0 g/dL	5	14.7%	115	22.6%	
CD4 count change, median cells/mm^3		30	83 (20, 165)	485	96 (27, 168)	0.44
	≥ 0 cells/mm^3	23	76.7%	409	84.3%	0.30
	< 0 cells/mm3	7	23.3%	76	15.7%	
Absolute CD4 count, median cells/mm^3		32	237 (155, 321)	506	234 (153, 340)	0.89
	≥ 100 cells/mm^3	30	93.8%	454	89.7%	0.76
	< 100 cells/mm3	2	6.3%	52	10.3%	

Table 2. Performance indicators of different algorithms for predicting virologic failure at 6 months among patients in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	OAPR	Misclassification Value (95% CI)	False Negative Value (95% CI)
Delta CD4 count <50 cells/mm^3	23.3 (9.9 - 42.3)	84.3 (80.8 - 87.5)	8.4 (3.5 - 16.6)	94.7 (92.1 - 96.6)	0.09	19.2 (15.9 - 22.9)	4.5 (2.9 - 6.6)
Absolute CD4 count <100 cells/mm^3	6.3 (0.8 - 20.8)	89.7 (86.7 - 92.2)	3.7 (0.5 - 12.7)	93.8 (91.3 - 95.8)	0.04	15.2 (12.3 - 18.6)	5.6 (3.8 - 7.9)
WHO algorithm (Delta CD4 count <50 cells/mm^3 or absolute CD4 count <100 cells/mm^3)	27.6 (12.7 - 47.2)	78.4 (74.4 - 82.0)	7.1 (3.1 - 13.6)	94.7 (92.0 - 96.7)	0.08	24.5 (20.8 - 28.5)	4.1 (2.6 - 6.2)
Adherence MPR below 80%	13.6 (5.2 - 27.4)	95.1 (93.0 - 96.6)	16.7 (6.4 - 32.8)	93.8 (91.6 - 95.6)	0.20	10.4 (8.2 - 13.0)	5.8 (4.2 - 7.9)
Weight decrease	22.0 (10.6 - 37.6)	74.3 (70.4 - 77.9)	6.0 (2.8 - 11.1)	92.7 (89.9 - 95.0)	0.06	29.3 (25.7 - 33.2)	5.4 (3.7 - 7.6)
HGB decrease	14.7 (5.0 - 31.1)	77.4 (73.5 - 81.0)	4.2 (1.4 - 9.5)	93.1 (90.3 - 95.4)	0.04	26.5 (22.9 - 30.4)	5.3 (3.6 - 7.6)
WHO algorithm plus adherence MPR below 80%	37.5 (21.1 - 56.3)	73.6 (69.5 - 77.5)	8.5 (4.5 - 14.4)	94.7 (92.0 - 96.8)	0.09	28.6 (24.8 - 32.7)	3.8 (2.4 - 5.9)
WHO algorithm plus HGB decrease	50.0 (29.9 - 70.1)	59.1 (54.5 - 63.5)	6.3 (3.4 - 10.6)	95.5 (92.5 - 97.6)	0.07	41.4 (37.0 - 45.9)	2.6 (1.4 - 4.4)
WHO algorithm plus weight decrease	45.2 (27.3 - 64.0)	53.5 (48.9 - 58.1)	6.0 (3.3 - 9.9)	93.7 (90.1 - 96.3)	0.06	47.0 (42.6 - 51.5)	3.4 (2.0 - 5.4)
WHO algorithm plus adherence MPR below 80% or HGB decrease or weight decrease	65.6 (46.8 - 81.4)	38.8 (34.4 - 43.4)	6.7 (4.2 - 10.0)	94.4 (90.2 - 97.2)	0.07	59.5 (55.1 - 63.8)	2.2 (1.1 - 3.8)

Table 3. Estimated regression coefficients, standard errors, p-values, relative risks, and confidence intervals for factors in a modified Poisson regression model for virologic failure at 6 months among patients in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia

Variable	Estimated regression coefficient	Estimated robust standard errors	p	Estimated relative risk	95% CI for relative risk
Intercept	-2.94292	0.17746	<0.0001	-	-
Adherence[¥]	0.02498	0.00854	0.0034	1.03	1.01-1.04
Severe anemia[¶]	0.90291	0.49423	0.0677	2.47	0.94-6.50

[¥] Relative risk for adherence is per 1 unit decrease from 100%.
[¶] Relative risk for severe anemia is for patients with a baseline hemoglobin concentration below 8.0 g/dL compared to patients with a baseline hemoglobin concentration 8.0 g/dL or above.

Table 4. Estimated probability of failure for select levels of adherence and baseline anemia in a modified Poisson regression model for virologic failure at 6 months among patients in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia

MPR	Severe Anemia at Baseline	ln(Risk)*	Risk	Relative Risk
100	No	-2.9429	5.3%	1.0
98	No	-2.8930	5.5%	1.1
96	No	-2.8430	5.8%	1.1
94	No	-2.7930	6.1%	1.2
92	No	-2.7431	6.4%	1.2
90	No	-2.6931	6.8%	1.3
88	No	-2.6432	7.1%	1.3
86	No	-2.5932	7.5%	1.4
84	No	-2.5432	7.9%	1.5
82	No	-2.4933	8.3%	1.6
80	No	-2.4433	8.7%	1.6
78	No	-2.3934	9.1%	1.7
76	No	-2.3434	9.6%	1.8
74	No	-2.2934	10.1%	1.9
72	No	-2.2435	10.6%	2.0
70	No	-2.1935	11.2%	2.1
100	Yes	-2.0400	13.0%	2.5

* Log risk is calculated using the coefficients from the modified Poisson regression equation.
ln(Risk) = -2.94292 + ((100-MPR)*0.02498) + (0.90291*(Severe Anemia))
MPR is a continuous variable with values ranging from 0 to 100
Severe anemia is a dichotomous variable with a value of 1 for yes and 0 for no

Table 5. Performance indicators of different cut-points for a new algorithm to predict virologic failure at 6 months among patients in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia

Predicted probability for declaring failure	Relative risk for failure*	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
6%	1.1	34.1 (20.1 - 50.6)	76.7 (73.1 - 80.1)	9.2 (5.1 - 15.0)	94.4 (92.0 - 96.3)
7%	1.3	29.3 (16.1 - 45.5)	82.1 (78.8 - 85.1)	10.2 (5.4 - 17.1)	94.4 (92.0 - 96.2)
8%	1.5	26.8 (14.2 - 42.9)	88.2 (85.3 - 90.7)	13.6 (7.0 - 23.0)	94.6 (92.3 - 96.3)
9%	1.7	24.4 (12.4 - 40.3)	91.7 (89.2 - 93.8)	16.9 (8.4 - 29.0)	94.6 (92.4 - 96.3)
10%	1.9	19.5 (8.8 - 34.9)	92.9 (90.5 - 94.8)	16.0 (7.2 - 29.1)	94.3 (92.2 - 96.1)
11%	2.1	19.5 (8.8 - 34.9)	93.6 (91.3 - 95.4)	17.4 (7.8 - 31.4)	94.4 (92.2 - 96.1)
12%	2.3	17.1 (7.2 - 32.1)	94.6 (92.5 - 96.3)	17.9 (7.5 - 33.5)	94.3 (92.1 - 96.0)
13%	2.5	17.1 (7.2 - 32.1)	95.1 (93.1 - 96.7)	19.4 (8.2 - 36.0)	94.3 (92.1 - 96.0)
14%	2.7	7.3 (1.5 - 19.9)	98.7 (97.4 - 99.4)	27.3 (6.0 - 61.0)	93.9 (91.7 - 95.6)
15%	2.8	7.3 (1.5 - 19.9)	98.7 (97.4 - 99.4)	27.3 (6.0 - 61.0)	93.9 (91.7 - 95.6)
16%	3.0	4.9 (0.6 - 16.5)	98.8 (97.6 - 99.5)	22.2 (2.8 - 60.0)	93.8 (91.6 - 95.5)
17%	3.2	2.4 (0.1 - 12.9)	99.0 (97.8 - 99.6)	14.3 (0.4 - 57.9)	93.6 (91.4 - 95.4)
18%	3.4	2.4 (0.1 - 12.9)	99.0 (97.8 - 99.6)	14.3 (0.4 - 57.9)	93.6 (91.4 - 95.4)
19%	3.6	2.4 (0.1 - 12.9)	99.2 (98.0 - 99.7)	16.7 (0.4 - 64.1)	93.6 (91.4 - 95.4)
20%	3.8	2.4 (0.1 - 12.9)	99.3 (98.3 - 99.8)	20.0 (0.5 - 71.6)	93.6 (91.4 - 95.4)

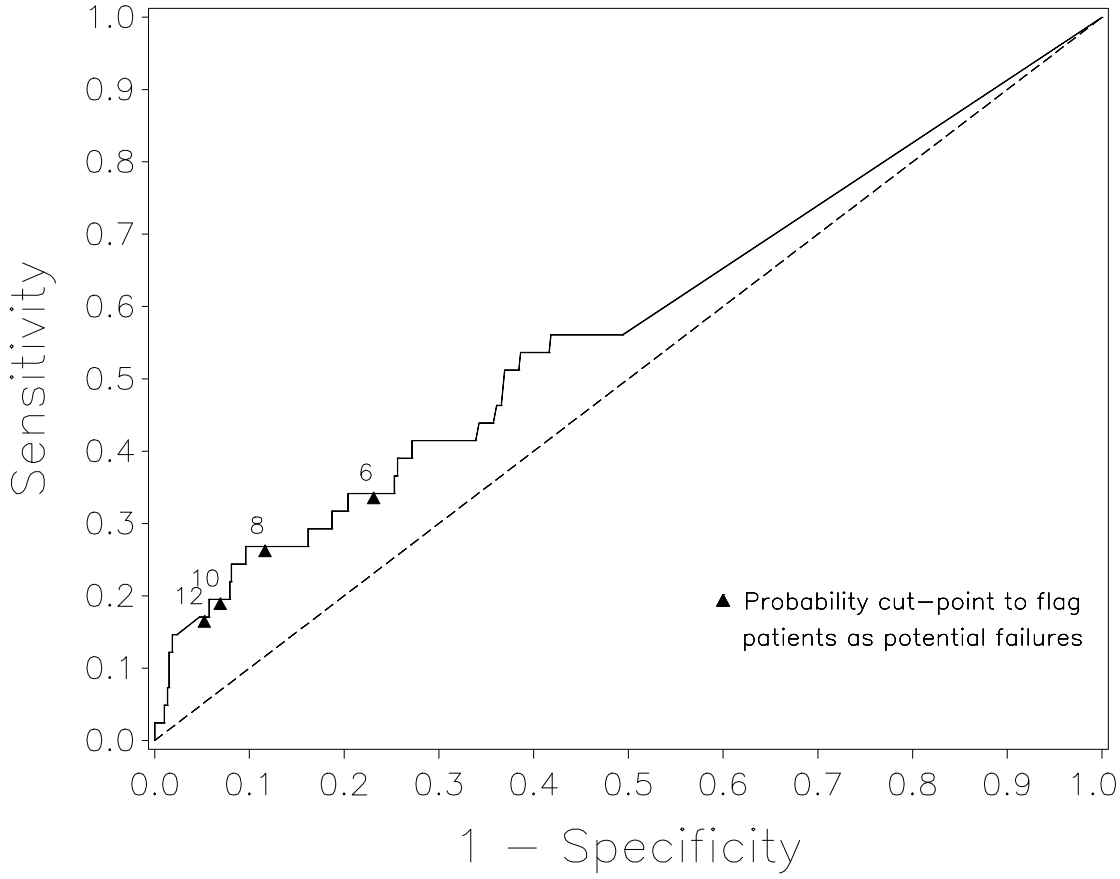
* The relative risk for failure is compared to someone without severe anemia at baseline and with 100% adherence at the 6 month evaluation who has a probability of failure of 5.3%.

Table 5. (Continued)

Predicted probability for declaring failure	Relative risk for failure*	OAPR	Misclassification Value (95% CI)	False Negative Value (95% CI)	Youden Index	Distance to (0,1)
6%	1.1	0.10	26.0 (22.6 - 29.6)	4.3 (2.8 - 6.1)	0.11	0.70
7%	1.3	0.11	21.3 (18.2 - 24.7)	4.6 (3.1 - 6.5)	0.11	0.73
8%	1.5	0.16	15.8 (13.0 - 18.8)	4.7 (3.2 - 6.7)	0.15	0.74
9%	1.7	0.20	12.6 (10.1 - 15.5)	4.9 (3.3 - 6.9)	0.16	0.76
10%	1.9	0.19	11.8 (9.4 - 14.6)	5.2 (3.6 - 7.2)	0.12	0.81
11%	2.1	0.21	11.2 (8.9 - 13.9)	5.2 (3.6 - 7.2)	0.13	0.81
12%	2.3	0.22	10.4 (8.1 - 13.1)	5.4 (3.7 - 7.4)	0.12	0.83
13%	2.5	0.24	9.9 (7.7 - 12.5)	5.4 (3.7 - 7.4)	0.12	0.83
14%	2.7	0.38	7.3 (5.4 - 9.6)	6.0 (4.3 - 8.1)	0.06	0.93
15%	2.8	0.38	7.3 (5.4 - 9.6)	6.0 (4.3 - 8.1)	0.06	0.93
16%	3.0	0.29	7.3 (5.4 - 9.6)	6.2 (4.4 - 8.3)	0.04	0.95
17%	3.2	0.17	7.3 (5.4 - 9.6)	6.3 (4.5 - 8.5)	0.01	0.98
18%	3.4	0.17	7.3 (5.4 - 9.6)	6.3 (4.5 - 8.5)	0.01	0.98
19%	3.6	0.20	7.1 (5.2 - 9.4)	6.3 (4.5 - 8.5)	0.02	0.98
20%	3.8	0.25	6.9 (5.1 - 9.2)	6.3 (4.5 - 8.5)	0.02	0.98

* The relative risk for failure is compared to someone without severe anemia at baseline and with 100% adherence at the 6 month evaluation who has a probability of failure of 5.3%.

Figure 1. Receiver operating characteristic curve for a new model to predict virologic failure at 6 months among patients in the routine virologic monitoring arm of the Viral Load Study in Lusaka, Zambia



CONCLUSIONS

With the growing number of patients starting ART in resource-limited settings,[2] the problem of diagnosing antiretroviral treatment failure in areas without access to routine virologic monitoring is one of the emerging issues in public health. We performed a meta-analysis using data from previously published reports to create summary estimates for the prevalence of virologic failure in resource-limited settings. We found that 13% of ART naïve adults initiating NNRTI-based ART had detectable viremia at 6 months of therapy. This prevalence remained consistent at 15% at 12 months and increased to more than 20% after 18 months. Our most intriguing finding, however, was that the lowest estimate of the conservative 95% confidence interval was 10% or higher at all time points evaluated between 3 months and 2 years. Clearly, the proportion of patients with detectable virus is significant and the raw numbers will increase as successful programs continue to initiate more and more patients on ART.

Having identified that virologic failure is a relatively common outcome among patients initiating therapy in resource-limited settings, we used data from one arm of a pragmatic, randomized trial of treatment monitoring strategies in Lusaka, Zambia to identify factors associated with virologic failure. As non-adherence is known to be associated with virologic failure, we were particularly interested in evaluating our simple, inexpensive MPR measure of adherence. We found that this metric was strongly associated with virologic failure at both 3 and 6 months. We also found baseline viral load and presence of severe anemia to be associated with a borderline increased risk of virologic failure at 6

months. We were also interested in determining what longitudinal measures of disease progression, if any, were associated with virologic failure. Unfortunately, negative changes in weight, CD4 count, or HGB concentration were not associated with virologic failure.

We created a predictive-score-based algorithm for diagnosing virologic failure at 6 months using factors found to be associated with failure in the descriptive epidemiology study, specifically adherence and baseline anemia. We evaluated the performance of this algorithm as well as the WHO-recommended algorithm and found neither to perform particularly well at 6 months. The predictive-score-based algorithm did not have an acceptable AUC to be considered a useful screening tool. That neither the predictive-score-based algorithm nor the WHO-recommended algorithm had a good combination of sensitivity (>80%) and specificity (>95%) suggested that clinical and/or immunologic monitoring is not a reliable substitute for virologic monitoring of early treatment outcomes.

The results of this dissertation research have policy implications for resource-limited settings. While acknowledging that the results of the randomized trial will provide more information on the utility of virologic monitoring in resource-limited settings, these results indicate that current algorithms are not effective monitoring tools, particularly at 6 months. It is feasible that clinical and immunological criteria will be more useful at time points beyond 6 months, and efforts such as this should continue in an attempt to identify an effective algorithm. As routine virologic monitoring is not currently feasible in most of the developing world, these results argue that efforts should be made to address this deficiency. Whether through the development of newer, cheaper assays or through the improvement of local laboratory and human resource capacity, these efforts

will require a substantial investment of resources. Failure to make this investment is dangerous as the development and transmission of drug resistant virus could jeopardize the remarkable success of the global ART treatment effort.[22-25]

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APPENDIX

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM INSTITUTIONAL REVIEW BOARD APPROVAL LETTER

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines. The Assurance became effective on November 24, 2003 and expires on February 14, 2009. The Assurance number is FWA00005960.

Principal Investigator: CANTRELL, RONALD

Co-Investigator(s):

Protocol Number: **X060810003**

Protocol Title: *Diagnosing Antiretroviral Treatment Failure in Resource-Limited Settings*

The IRB reviewed and approved the above named project on 08/21/07. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 8-21-07

Date IRB Approval Issued: 08/21/07

HIPAA Waiver Approved?: N/A

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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