Misclassification of First-Line Antiretroviral Treatment Failure Based on Immunological Monitoring of HIV Infection in Resource-Limited Settings

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(See the editorial commentary by Sawe and McIntyre on pages 463–5)

Background. The monitoring of patients with human immunodeficiency virus (HIV) infection who are treated with antiretroviral medications in resource-limited settings is typically performed by use of clinical and immunological criteria. The early identification of first-line antiretroviral treatment failure is critical to prevent morbidity, mortality, and drug resistance. Misclassification of failure may result in premature switching to second-line therapy.

Methods. Adult patients in western Kenya had their viral loads (VLs) determined if they had adhered to first-line therapy for >6 months and were suspected of experiencing immunological failure (ie, their CD4 cell count decreased by ⩾25% in 6 months). Misclassification of treatment failure was defined as a ⩾25% decrease in CD4 cell count with a VL of <400 copies/mL. Logistic and tree regressions examined relationships between VL and 4 variables: CD4 T cell count (hereafter CD4 cell count), percentage of T cells expressing CD4 (hereafter CD4 cell percentage), percentage decrease in the CD4 T cell count (hereafter CD4 cell count percent decrease), and percentage decrease in the percentage of T cells expressing CD4 (hereafter CD4% percent decrease).

Results. There were 149 patients who were treated for 23 months; they were identified as having a ⩾25% decrease in CD4 cell count (from 375 to 216 cells/μL) and a CD4% percent decrease (from 19% to 15%); of these 149 patients, 86 (58%) were misclassified as having experienced treatment failure. Of 42 patients who had a ⩾50% decrease in CD4 cell count, 18 (43%) were misclassified. In multivariate logistic regression, misclassification odds were associated with a higher CD4 cell count, a shorter duration of therapy, and a smaller CD4% percent decrease. By combining these variables, we may be able to improve our ability to predict treatment failure.

Conclusions. Immunological monitoring as a sole indicator of virological failure would lead to a premature switch to valuable second-line regimens for 58% of patients who experience a ⩾25% decrease in CD4 cell count and for 43% patients who experience a ⩾50% decrease in CD4 cell count, and therefore this type of monitoring should be reevaluated. Selective virological monitoring and the addition of indicators like trends CD4% percent decrease and duration of therapy may systematically improve the identification of treatment failure. VL testing is now mandatory for patients suspected of experiencing first-line treatment failure within the Academic Model Providing Access to Healthcare (AMPATH) in western Kenya, and should be considered in all resource-limited settings.

In 2006, ~700,000 people worldwide who were infected with the human immunodeficiency virus (HIV) received antiretroviral therapy (ART) for the first time, and 2,015,000 overall received treatment in low- and middle-income countries, representing 28% of the 7.1 million people in need [1]. Most resource-limited countries use the standard first-line regimens that were recommended by the World Health Organization (WHO): zidovudine or stavudine plus lamivudine plus nevirapine or efavirenz [2]. With global access to ART increasing, it is expected that at least 5%–20% of patients will have their first-line regimens fail before 4 years of therapy, despite adequate adherence,

Received 25 November 2008; accepted 5 March 2009; electronically published 1 July 2009.

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Clinical Infectious Diseases 2009; 49:454–62

1058-4838/2009/4903-0023$15.00
DOI: 10.1086/600396

plasma drug levels, and treatment efficacy [3, 4]. Thus, among 700,000 patients treated, therapy will fail for up to 140,000 patients. Among those whose therapy will fail, it is reasonable to estimate that 35%–70% (up to 98,000 patients) will have drug-resistant HIV at time of treatment failure [5, 6]. This probably represents an underestimation, because the number of patients treated has increased and drug-resistance transmission has become more common [7].

The timing and accuracy of identifying treatment failure in resource-limited settings is fundamental but challenging. Delayed detection of treatment failure may increase drug toxicity, may lead to the accumulation of drug resistance–associated mutations (further limiting treatment options), and may result in increased morbidity and mortality [3, 8]. The misclassification of treatment failure could lead to the premature switch [9, 10] to and use of valuable second-line regimens, which are costly and may represent the last available regimen.

In resource-rich settings, HIV-infected patients who receive ART are routinely monitored immunologically (ie, their CD4 T cell count is monitored) and virologically (ie, their viral load [VL] is monitored), according to existing guidelines [11]. This close monitoring allows accurate and early diagnosis of treatment failure. In resource-limited settings, monitoring is typically clinical and immunological, mainly as a result of financial and infrastructure constraints [12, 13]. These monitoring methods limit the diagnosis of virological failure, which predates immunological failure, allowing extended viral replication under drug pressure and development of drug resistance [14].

According to WHO guidelines, a decreasing CD4 T cell count (hereafter CD4 cell count) is considered a surrogate marker for treatment failure and should trigger a switch in ART, particularly if the CD4 cell count is <200 cells/μL [12]. Definitions of immunological treatment failure include (1) a CD4 cell count of <100 cells/μL after 6 months of therapy, (2) a return to, or a decrease below, the pretherapy CD4 cell count after 6 months of therapy, or (3) a >50% decrease from the on-treatment peak CD4 cell count [12]. Country-specific treatment guidelines are at times less restrictive [15], and decreases in the CD4 cell count of 25%–50% are regularly used in clinical practice to define treatment failure in resource-limited settings. Data on the accuracy of those methods in identifying treatment failure are lacking.

As a result of the partnership between the US Agency for International Development (USAID) and the Academic Model Providing Access to Healthcare (AMPATH), ART has been available in western Kenya since 2001. AMPATH is a joint initiative between the Moi University School of Medicine, the Moi Teaching and Referral Hospital, Indiana University, and Brown University [16]. The aim of our report is to increase clinicians' awareness of the potential consequences of immunological monitoring of HIV-infected patients undergoing treatment on the basis of data on patients from Moi Teaching and Referral Hospital in Eldoret, Kenya. We demonstrate that, in resource-limited settings, the use of both the current written guideline and the guideline in place may lead to a markedly incorrect identification of treatment failure that could result in an unnecessary switch from first- to second-line ART, and we examine potential improvements to this identification process.

METHODS

Patients and laboratory data. Our study was conducted during the period from May 2006 through March 2007 at Moi Teaching and Referral Hospital, the largest of 18 AMPATH clinics. As of August 2008, AMPATH has provided comprehensive clinical services to 80,909 HIV-infected patients. Of those, 16,792 adults were enrolled at Moi Teaching and Referral Hospital, 8547 (51%) of whom started ART. The supply of drugs was continuous and uninterrupted.

Adult patients attending Moi Teaching and Referral Hospital clinic during the study period had their VLs determined if (1) they were treated for >6 months with WHO-recommended first-line ART (zidovudine or stavudine plus lamivudine plus nevirapine or efavirenz), (2) they adhered to ART (ie, they self-reported taking >50% of their medication per month before the study visit), and (3) their therapy was considered to be a failure on the basis of consecutive decreases of ⩾25% in their CD4 cell count over 6 months. A decrease of ⩾25% in CD4 cell count with undetectable VL (<400 copies/mL) was defined as a misclassification of treatment failure. Our study was approved by the ethics committees of Lifespan and Moi University School of Medicine.

Measurements of CD4 cell count and VL were performed at the Moi Teaching and Referral Hospital AMPATH Reference Laboratory, where CD4 (FACSCaliber system; Becton Dickinson) and VL (Amplicor; Roche Molecular) assays are routinely performed and where adherence to good laboratory practices and external quality programs (United Kingdom National Quality Assessment Service and National Institutes of Health Department of AIDS Viral Quality Assurance Program) are maintained.

Data analysis. Data collected included age, sex, current and past ARTs, current (time point 2) and past (time point 1 [~6 months prior to time point 2]) CD4 cell counts and CD4 cell percentage, and current VL (time point 2). Derived variables included percentage decrease in absolute CD4 cell count, percent change in proportion of T cells expressing CD4 (hereafter CD4 cell percentage) between time points 1 and 2, duration of ART, duration of time between time points 1 and 2, and duration of time between the CD4 cell count at time point 2 and VL at time point 2. The percentage of patients whose

Table 1. Patient Demographic, Treatment, and Laboratory Characteristics

Characteristic	Patients whose CD4 cell count decreased ⩾25%			Patients whose CD4 cell count decreased ⩾50%[a]		
	All (n = 149)	With a VL of >400 copies/mL (n = 63)	With a VL of <400 copies/mL (n = 86)	All (n = 42)	With a VL of >400 copies/mL (n = 24)	With a VL of <400 copies/mL (n = 18)
No. (%) of patients who received ART						
D4T, 3TC, NVP	102 (68)	41 (65)	61 (71)	29 (69)	15 (62)	14 (78)
D4T, 3TC, EFV	34 (23)	15 (24)	19 (22)	11 (26)	7 (29)	4 (22)
AZT, 3TC, NVP	10 (7)	5 (8)	5 (6)	1 (2)	1 (4)	0 (0)
AZT, 3TC, EFV	3 (2)	2 (3)	1 (1)	1 (2)	1 (4)	0 (0)
Duration of treatment, mean (range), months	23 (8–61)	25	21	24 (9–61)	25	22
Duration of time between CD4 cell counts, mean (range), months	6 (5–9)	7	6	7 (5–9)	7	6
Duration of time between CD4 and VL testing (range), months	2 (0–3)	1	2	1 (0–3)	1	2
Age, mean (range), years	39 (18–70)	38 (18–59)	39 (18–70)	38 (18–70)	37 (18–50)	41 (28–70)
Percentage of female patients	58	57	59	55	42	72
CD4 cell count at time point 1, mean (range), cells/μL	375 (17–1428)	308	425	391 (17–1428)	303	508
CD4 cell count at time point 2, mean (range), cells/μL	216 (6–666)	166	251	151 (6–544)	116	198
CD4 cell count decrease, mean (range), cells/μL	160 (9–884)	140	174	240 (9–884)	187	311
CD4 cell count percentage decrease, mean (range)	42 (25–88)	45	40	62 (50–88)	61	62
CD4 cell percentage at time point 1, mean (range)	19 (1–59)	17	20	18 (1–59)	15	23
CD4 cell percentage at time point 2, mean (range)	15 (1–41)	13	17	12 (1–41)	10	15
CD4 percent decrease, mean (range)	3 (−9 to 27)	4	3	6 (−5 to 27)	6	7
CD4% percent decrease, mean (range)	17 (−38 to 82)	22	13	31 (−38 to 82)	35	26
VL, mean (range), copies/mL	…	70,485 (503–1,096,191)	…	…	114,035 (503–1,096,191)	…

NOTE. All patients were still on their first-line regimens at the time of sample acquisition. ART, antiretroviral therapy; AZT, zidovudine; D4T, stavudine; EFV, efavirenz; NVP, nevirapine; 3TC, lamivudine; VL, viral load.

[a] Patients with a ⩾50% decrease in CD4 cell count were a subset of patients with a ⩾25% decrease.

Table 2. Viral Load (VL) Detectability According to CD4 Cell Count

CD4 cell count	No. of patients	No. (%) of patients with a VL of <400 copies/mL
0–99 cells/μL	29	12 (41)
100–199 cells/μL	47	21 (45)
200–349 cells/μL	47	31 (66)
⩾350 cells/μL	26	22 (85)
Total	149	86 (58)

treatment was misclassified as failing was the main outcome variable, computed across levels of 4 immunological variables: CD4 cell count and CD4 cell percentage at time point 2 and CD4 cell count decrease and CD4 cell percentage change between time points 1 and 2. We fit logistic regression models to examine univariate and multivariate relationships between undetectable VL, CD4 cell count, and CD4 cell percentage at time point 2 and percentage change in CD4 cell count and CD4 cell percentage between time points 1 and 2. Sex, age, time lag between CD4 cell count and VL, and duration of ART were examined in a univariate analysis and included in the multivariate model to adjust for potential unexplained variation. We checked model fit using the Hosmer and Lemeshow goodness-of-fit test, deviance residuals, and leverage statistics [17].

To augment our primary findings from logistic regression analysis, in which our main objective was to characterize predictors of misclassification, we conducted regression tree analysis [18] as an exploratory tool for subdividing the sample according to important clinical predictors of viral failure among patients with a ⩾25% decrease in CD4 cell count. The regression tree provides a useful sample partition that is based on cut points for key predictors of viral failure, and it suggests a possible starting point for the development of a validated clinical rule and for the identification of features of important subpopulations based on clinical and immunological indicators. To fit this tree, we used a maximum depth of 3 splits requiring at least 40 observations per split. Recursive splits were chosen such that each successive split maximized reduction in impurity within the data set.

To aid in model interpretation, estimated misclassification rates were computed for key subgroups defined by CD4 cell count and duration of therapy at time point 2, by CD4 cell count decrease between time points 1 and 2, and by decrease in the percentage of T cells expressing CD4 (hereafter CD4% percent decrease) between time points 1 and 2. These variables were chosen on the basis of significance in the multivariate logistic regression model and on the basis of variables selected by regression tree analysis. Statistical analyses were performed using statistical package R, version 2.6.1 [19].

RESULTS

One hundred and forty-nine patients fit the inclusion criteria—with a ⩾25% decrease in CD4 cell count within a median of 6 months between time points 1 and 2—and were identified as having had ART that potentially failed. Table 1 shows the demographic, treatment, and laboratory characteristics of all patients with a ⩾25% or ⩾50% decrease in CD4 cell count. No major differences were observed between the 2 groups. Average duration of ART was 23 months. Average duration of time between time points 1 and 2 was 6 months, which is less frequent than in resource-rich settings but customary in Kenya. CD4 cell counts decreased from an average of 375 to 216 cells/μL, CD4 cell percentages decreased from 19% to 15%. Good Spearman rank correlation was observed between CD4 cell counts and CD4 cell percentages for both time points (time point 1, $\rho = 0.75$; time point 2, $\rho = 0.72$). The Spearman rank correlation between percent change in CD4 cell count and percent changed in CD4 cell percentage was weak ($\rho = 0.3$). Patients with no immunological failure were not part of the study.

Of 149 patients whose ART was identified as failing as a result of a ⩾25% decrease in CD4 cell count, 86 (58%) were misclassified (95% binomial confidence interval [CI], 50%–66%; table 1). Of the 86 misclassified patients, 33 (38%) had a CD4 cell count of <200 cells/μL at time point 2. Of 42 patients whose ART was identified as failing as a result of a ⩾50% decrease in CD4 cell count, 18 (43%) were misclassified (95% binomial CI, 28%–59%; table 1). Of those 18 misclassified patients, 12 (67%) had a CD4 cell count of <200 cells/μL at time point 2. Of 7 patients with persistent CD4 cell counts of <100 cells/μL, 3 (43%) had an undetectable VL (95% binomial CI, 16%–84%). Table 2 shows VL detectability by CD4 cell count, table 3 shows VL detectability by CD4 cell percentage, table 4 shows VL detectability by CD4 cell count percent decrease, and table 5 shows VL detectability by CD4% percent change; all of the values in these tables were obtained upon identification of treatment failure. It was observed that a higher number of patients were misclassified with higher CD4 cell counts and percent values and with smaller percent changes in CD4 cell

Table 3. Viral Load (VL) Detectability According to CD4 Cell Percentage

CD4 cell percentage	No. of patients	No. (%) of patients with a VL of <400 copies/mL
0–9	31	11 (35)
10–19	79	46 (58)
20–29	34	25 (74)
⩾30	5	4 (80)
Total	149	86 (58)

Table 4. Viral Load (VL) Detectability According to CD4 Cell Count Percent Decrease

CD4 count percent decrease	No. of patients	No. (%) of patients with a VL of <400 copies/mL
25–34	59	39 (66)
35–44	36	22 (61)
45–54	26	12 (46)
>54	28	13 (46)
Total	149	86 (58)

count and CD4 cell percentage. The changes in CD4 cell count and the changes in CD4 cell percentage were discordant in 37 (25%) of 149 patients (table 5). VL data were not available for nonstudy patients.

In univariate logistic regression analyses (table 6), odds of misclassification of virologic failure were associated with (1) a higher CD4 cell count at time point 2 (odds ratio [OR], 1.80 per 100-cell difference; 95% CI, 1.32–2.47); (2) a smaller percent decrease in CD4 cell count (OR, 1.27 per 10% decrease 95% CI, 1.01–1.60); (3) a higher CD4 cell percentage at time point 2 (OR, 1.08 per 1-unit difference; 95% CI, 1.03–1.14); (4) a smaller percent decrease in CD4 cell percentage (OR, 1.21 per 10% decrease; 95% CI, 1.04–1.41); and (5) a shorter duration of therapy (OR, 1.03 per month difference of therapy; 95% CI, 1.002–1.07).

We fit a multivariate logistic regression analysis (table 7) that included CD4 variables, duration of therapy, age, sex, CD4-VL time lag, and regimen of treatment that presumably failed. The model showed that a misclassification of viral failure was associated with a higher CD4 cell count (OR, 2.13 per 100-cell increase; 95% CI, 1.22–3.72) and a shorter duration of therapy (OR, 1.06 per month; 95% CI, 1.02–1.11), with statistically significant associations. The model also suggested that smaller decreases in CD4 cell percentage are associated with misclassification (OR, 1.22 per 10% smaller decrease; 95% CI, 0.98–1.54). Model diagnostics described in Methods indicated a good model fit.

Exploratory regression tree analyses largely reinforced results found with multivariate logistic regression and suggested possible interactions among covariates in their ability to distinguish patients whose treatment truly failed from those who were misclassified. Splits in the tree occurred at (1) CD4 cell count of 268 cells/μL at presumed treatment failure; (2) duration of therapy of 19.5 months; and (3) CD4% percent decrease of 14%. Tables 8 and 9 show the misclassification rates for different subpopulations. The interaction between the first tree split (CD4 cell counts) and the other second tree split (duration of therapy) and their prediction of treatment failure misclassification are shown in table 8. The interaction between the first tree split (CD4 cell counts) and the second tree split (percent decline in CD4%) and their prediction of treatment failure misclassification are shown in table 9. Estimates predicted by the multivariate model as well as the observed data are shown.

Among patients with a CD4 cell count of <200 cells/μL who were on therapy for >24 months, 21% were misclassified (table 8). A much greater misclassification rate (77%) was seen for patients with a CD4 cell count of >200 cells/μL who were on therapy for <18 months. Considering the CD4% percent decrease (table 9) among patients with a CD4 cell count of <200 cells/μL, only 37% of patients with >14% CD4% percent decrease were misclassified, compared with 61% of patients with no CD4% percent decrease. Among patients with a CD4 cell count of >200 cells/μL, the misclassification rates were high (>64%) regardless of CD4% percent change.

We conducted sensitivity analyses to account for potential limitations due to lack of confirmatory CD4 measures and due to a VL detection limit of 400 copies/mL, compared with 50 copies/mL. We used simulation techniques to generate a modified data set and compared regression model estimates from modified data to estimates obtained using primary data. Bootstrap resampling was used to compare regression coefficients [20]. Sensitivity analyses indicated that our main findings would not have been different had we been able to obtain confirmatory CD4 cells counts and that our results are robust to the effect of a more sensitive VL assay (data available upon request).

DISCUSSION

We present results from patients in western Kenya of treatment failure misclassification based on immunological monitoring, the predominant practice in resource-limited settings. Our findings indicate that immunological monitoring as a sole indicator of virological failure may lead to misclassification and potentially to a premature switch to valuable second-line regimens in 58% of patients whose treatment was considered to have failed on the basis of a ⩾25% decrease in CD4 cell count and in 43% of patients whose treatment was considered to have failed on the basis of a ⩾50% decrease in CD4 cell count.

Our analysis demonstrated that the incorporation of information on CD4 cell counts at time of presumed treatment

Table 5. Viral Load (VL:) Detectability According to CD4% Percent Change

CD4% percent	No. of patients	No. (%) of patients with a VL of <400 copies/mL
≥0% increase	37	24 (65)
1%–14% decrease	46	31 (67)
15%–29% decrease	27	16 (59)
>29% decrease	39	15 (38)
Total	149	86 (58)

Table 6. Univariate Logistic Regression Analysis of the Misclassification of Treatment Failure

Variable	OR of misclassification (95% CI)	*P*
CD4 cell count, per 100-cell increase	1.80 (1.30–2.43)	<.001
% decrease in CD4 cell count, per 10% smaller decrease	1.27 (1.02–1.60)	.04
Change in CD4 cell count, per 100-unit larger decrease	1.31 (0.95–1.81)	.10
CD4 cell percentage, per 1-unit increase	1.08 (1.03–1.14)	.003
% change in CD4 cell percentage, per 10% smaller decrease	1.21 (1.03–1.41)	.02
Change in CD4 cell percentage, per 1-unit smaller decrease	1.02 (0.96–1.09)	.49
Duration of therapy, per less month of therapy	1.02 (1.003–1.07)	.04
Age, per year older	1.02 (0.98–1.06)	.38
Sex, female vs. male	1.09 (0.56–2.12)	.86
Duration of time between CD4 and VL testing, per month	1.49 (0.97–2.31)	.07
ART[a]		
3TC, D4T, EFV	0.85 (0.38–1.89)	.69
3TC, AZT, NVP	0.67 (0.18–2.51)	.56
3TC, AZT, EFV	0.34 (0.03–3.96)	.39

NOTE. ART, antiretroviral therapy; AZT, zidovudine; CI, confidence interval; D4T, stavudine; EFV, efavirenz; NVP, nevirapine; OR, odds ratio; 3TC, lamivudine; VL, viral load.

[a] Compared with therapy using 3TC, D4T, and NVP.

failure, duration of therapy, and trends in CD4 cell percentage may improve one's ability to predict treatment failure. Patients with a higher CD4 cell count, patients who received ART for a shorter duration of time, patients with a smaller CD4% percent decrease may be more likely to have their therapy misclassified as failing. In resource-limited settings in which testing is limited or in which there is no second-line regimen available, these results, which need to be validated in larger studies, may assist one in determining VL testing guidelines once it is presumed that there is treatment failure. In our study, there was a high likelihood of virological failure of therapy if the patient had a CD4 cell count of <200 cells/μL and was on therapy for >20 months; there was a low likelihood of failure of therapy if the patient had a CD4 cell count of <300 and >200 cells/μL and was on therapy for <12 months, or if the patient had a CD4 cell count of >300 cells/μL with <14% CD4% percent decrease.

Previous discussions on the potential risks of immunological monitoring in resource-limited settings have appropriately focused on the accumulation of drug resistance and its consequences [8]. Our report highlights the potential risk of misclassifying treatment as failure. The utility of CD4 cell counts

Table 7. Multivariate Logistic Regression Analysis of the Misclassification of Treatment Failure

Variable	OR of misclassification (95% CI)	*P*
CD4 cell count, per 100-cell increase	2.13 (1.22–3.72)	.009
% decrease in CD4 cell count, per 10% smaller decrease	0.88 (0.63–1.24)	.48
CD4 cell percentage, per 1-unit increase	0.98 (0.90–1.07)	.73
% change in CD4 cell percentage, per 10% smaller decrease	1.23 (0.98–1.54)	.07
Duration of therapy, per less month of therapy	1.06 (1.02–1.11)	.002
Age, per year older	1.03 (0.98–1.08)	.26
Sex, female vs. male	1.04 (0.45–2.41)	.93
Duration of time between CD4 and VL testing, per month	1.28 (0.77–2.12)	.34
ART[a]		
3TC, D4T, EFV	0.81 (0.32–2.07)	.66
3TC, AZT, NVP	0.62 (0.11–3.38)	.58
3TC, AZT, EFV	0.47 (0.03–8.74)	.62

NOTE. ART, antiretroviral therapy; AZT, zidovudine; CI, confidence interval; D4T, stavudine; EFV, efavirenz; NVP, nevirapine; OR, odds ratio; 3TC, lamivudine; VL, viral load.

[a] Compared with therapy using 3TC, D4T, and NVP.

Table 8. Proportion of Patients Whose Antiretroviral Therapy Was Misclassified as a Failure, Stratified by CD4 Cell Count and Duration of Therapy

	Proportion of patients	
Duration of therapy	With CD4 cell count of ≤200 cells/μL	With CD4 cell counts of >200 cells/μL
≤18 months	55% (63%, 22/35)	77% (77%, 20/26)
>18 and ≤24 months	46% (39%, 7/18)	75% (64%, 9/14)
>24 months	26% (21%, 5/24)	67% (72%, 23/32)

NOTE. Entries in each bin are P1% (P2%, n/N), where P1% is the mean of the predicted probability of misclassification from the multivariate model and observed data, P2% is the observed percent misclassification, n is the number misclassified, and N is the total number of observations in the stratum.

to predict virological failure is limited. Previous studies have shown significant VL–CD4 cell count discordance in the range of 10%–30% [3, 21–26], making it difficult to use this measure to predict virological failure. It was also shown that associations between CD4 cell count and percentage are not simple [27] and may be affected by time, age, sex, and ethnic origin [28, 29]. It has been debated whether CD4 cell percentage may be a better surrogate for disease progression than CD4 cell count [23, 30, 31]. The majority of studies were performed in resource-rich settings, in early disease stages, and were focused on when to start ART.

Ledergerber et al. [32] examined the correlation between CD4 slopes and VL, but patients in resource-rich settings were highly experienced with treatment. Similarly, in British Columbia, Moore et al. [33] evaluated the clinical utility of CD4 parameter changes in terms of their ability to help one identify virologic suppression, demonstrating significant (21%–25%) misclassification of treatment response, predicting potential problematic immunological monitoring in resource-limited settings. Mee et al. [10] showed that WHO clinical and immunologic criteria poorly predict virologic failure after 1-year treatment in South Africa, where VL testing is available. Results presented here from Kenya verify and extend those predictions. Moreover, we found that CD4% percent decrease over time may better predict virologic failure, although verification through further studies is needed. Previous studies in Australia [34] and the United States [35] showed that this measure had significant prognostic value in determining AIDS-free survival time. To fully develop a decision rule and to assess the magnitude of failure misclassification, VL data are also required from a cohort not meeting immunological failure criteria.

In a recent study, Phillips et al. [36] used a computer simulation model to compare different HIV outcomes that resulted from different monitoring strategies, to determine when to switch treatment. They concluded that the benefits of VL (or CD4) testing over clinical monitoring, with regard to morbidity and mortality and relative to cost, are only modest. We believe the real-life results presented here from a resource-limited setting, where selective VL testing is feasible, are not directly comparable to the simulation study. Two assumptions from that study are not consistent with our patient sample. First, the model assumed immunological monitoring to be an accurate predictor to VL failure, with a misclassification rate of only 12%–19% among patients with a recent CD4 cell count decrease; by contrast, our data show misclassification rates ranging from 43% to 58%. Second, the simulation model only included patients with a CD4 cell count of <200 cells/μL upon regimen switch; that population comprises only 51% of our sample. Thus, the consequences of an early switch due to a misclassification of treatment failure—longer duration of second-line regimen and increased drug resistance, morbidity, mortality, and costs—may not be accurately estimated by Phillips et al. [37]. A more recent cost-effective analysis supports this reasoning [38].

The concern about switching too early rather than too late is less widely held. Treatment failure is commonly diagnosed either clinically, immunologically, or virologically [12]. Delayed switching may lead to increased drug resistance, morbidity, and mortality [3, 8, 39] and limited subsequent treatment options [40]. Biologically, virological failure occurs earlier, followed by immunological failure, then clinical failure. Diagnostically, the reverse is true in resource-limited settings, because of availability. VL is the most sensitive, informative way to identify treatment failure and is used in resource-rich settings. Immunological monitoring is different from clinical monitoring but is sequentially closest to virological failure. Early switching, the risk of which is presented here, may lead to premature presumption of virological failure and a switch to a second-line, potentially final regimen in resource-limited settings. In those settings, clinicians and patients must aim to maximize yield from available regimens and to maintain their adequate usage.

Table 9. Proportion of Patients Whose Antiretroviral Therapy Was Misclassified as a Failure, Stratified by CD4 Cell Count and CD4% Percent Decrease.

	Proportion of patients	
CD4% percent change	With CD4 cell count of ≤200 cells/μL	With CD4 cell counts of >200 cells/μL
≤0	56% (61%, 14/23)	80% (71%, 10/14)
0–14	52% (38%, 5/13)	75% (79%, 26/33)
>14	35% (37%, 15/41)	64% (64%, 16/25)

NOTE. Entries in each bin are P1% (P2%, n/N), where P1% is the mean of the predicted probability of misclassification from the multivariate model and observed data, P2% is the observed percent misclassification, n is the number misclassified, and N is the total number of observations in the stratum.

By considering the financial and infrastructure constraints, we recognize the fact that, although desirable, selective virological monitoring may not be instantly achievable. These results suggest the need to reconsider recommendations on immunological monitoring in resource-limited settings. We found that CD4 cell percentage changes may be important to improve accuracy in identifying treatment failure. This is acceptable considering the lower variability of CD4 cell percentages, compared with CD4 cell counts [41], and in light of previous findings suggesting that CD4 cell percentages may have better predicting value [23, 30, 31, 35]. However, because of unknown differences in variability among different global populations [28], these findings must be evaluated in populations beyond ours, to determine generalizability.

There are several limitations to our observations. First, VL and CD4 verification were not available. These represent real-life conditions in resource-limited settings, as acknowledged in the WHO treatment guidelines [12]. The sensitivity analyses conducted demonstrate that our results are robust to variations in CD4 measurement and to the use of a VL assay having a lower limit of detection. Second, the time interval between CD4 and VL measurement is important for their correlation assessment. Here, the average interval was low (2 months; range, 0–3 months), but they were not simultaneously drawn. Although it is reasonable to assume that the consequence is minimal, it is unclear how this may have affected results. Third, in addition to marked analytic variation in CD4 cell counts [41], additional factors, such as seasonal variations and concurrent diseases [29], were not taken into account and may influence CD4 variation.

In summary, immunological monitoring may lead to delayed identification of virological failure and also to premature erroneous conclusions. In all cases, clinical judgment should be included in decision making [12]. There is considerable, explainable heterogeneity in virologic failure for those with a $\geqslant$25% decrease in CD4 cell count. There is an urgent need for agreement on defining treatment failure and for standardized identification. Although this is not a randomized clinical trial and outcome data are lacking, these results are significant to the care of HIV-infected patients in resource-limited settings. Clinicians should take these results into account when considering switching patients to valuable second-line ART. These results or the lack of VL testing capability should by no means delay ART initiation or expansion. In those circumstances, the development of tools to better predict virological failure, as initiated here, is essential.

Within AMPATH, mandatory VL testing is now practiced for each HIV-infected patient whose ART is thought have failed. Similar measures should be considered in other resource-limited settings where VL testing is available. In settings where VL testing is not feasible, further research and considerations of incorporating additional variables to better recognize treatment failure are urgently needed.

Acknowledgments

We thank Drs. H.N.K. Arap Mengech, Fabian Esamai, Sylvester Kimaiyo, William Tierney, and Joe Mamlin for leadership and support; Drs. Charles Carpenter, Timothy Flanigan, Winston Nyandiko, Barbara Van Der Pol, John Sidle, and Kara Wools-Kaloustian for their help and review of the manuscript; Drs. John Coffin and Kenneth Mayer for mentorship; Dr. Bharat Ramratnam, Mr. Paul Park, and Mr. Kip Augustine for assistance; and Ms. Janet O'Connell for all the help and support.

Financial support. This research was funded by R.K.'s developmental grant P30 AI42853 from the Lifespan/Tufts/Brown Center for AIDS Research (a program funded by the National Institutes of Health) and supported in part by a grant to the USAID-AMPATH Partnership from the US Agency for International Development as part of the President's Emergency Plan for AIDS Relief.

Potential conflicts of interest. All authors: no conflicts.

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