Predicting Virologic Failure Among HIV-1-Infected Children Receiving Antiretroviral Therapy in Tanzania: a Cross-Sectional Study

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Background: Many HIV care and treatment programs in resource-limited settings rely on clinical and immunologic monitoring of antiretroviral therapy (ART), but accuracy of this strategy to detect virologic failure (VF) among children has not been evaluated.

Methods: A cross-sectional sample of HIV-infected children aged 1–16 years on ART ≥6 months receiving care at a Tanzanian referral center underwent clinical staging, CD4 lymphocyte measurement, plasma HIV-1 RNA level, and complete blood count. Associations with VF (HIV-1 RNA ≥400 copies/mL) were determined utilizing bivariable and multivariate analyses; accuracy of current clinical and immunologic guidelines in identifying children with VF was assessed.

Findings: Of 206 children (median age 8.7 years, ART duration 2.4 years), 65 (31.6%) demonstrated VF at enrollment. Clinical and immunological criteria identified 2 (3.5%) of 57 children with VF on first-line therapy, exhibiting 3.5% sensitivity and 100% specificity. VF was associated with younger age, receipt of nevirapine vs. efavirenz-based regimen, CD4% < 25%, and physician documentation of maladherence ($P < 0.05$ on bivariable analysis); the latter 2 factors remained significant on multivariate logistic regression.

Interpretation: This study demonstrates poor performance of clinical and immunologic criteria in identifying children with virologic failure. Affordable techniques for measuring HIV-1 RNA level applicable in resource-limited settings are urgently needed.

Key Words: Africa, antiretroviral therapy, HIV, pediatrics, virologic failure

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INTRODUCTION

Access to antiretroviral therapy (ART) has rapidly expanded in resource-limited regions since 2004. In Tanzania, use of ART has increased from less than 1% of those meeting eligibility criteria in 2004 to 31% in 2007, including over 11,000 children.[1] With expanded access, programs in resource-limited settings are measuring reductions in morbidity and mortality similar to that achieved in high-income countries.[2–5]

However, substantial challenges for care and treatment programs remain; primary among them is limited availability of monitoring tools for measuring response to ART.[6,7] Plasma HIV-1 RNA level is central to ART management in high-income countries, but cost and technical challenges make such monitoring unavailable to the majority of patients in resource-poor settings such as Tanzania.[7,8] The World Health Organization (WHO) advocates a public health approach to ART, recognizing the potential role for plasma HIV-1 RNA testing but recommending clinical and immunological monitoring in most situations.[9,10] However, little work has been done to assess the sensitivity and specificity of clinical and immunologic criteria to predict virologic failure, and

we are aware of no other study evaluating these criteria in children.[11–16]

Effective monitoring methods may be particularly important to the long-term success of ART in resource-limited settings as there are limited options for second-line therapy and an increased risk of resistance mutations and subsequent second-line failure in patients who remain on failing regimens.[17–19] Thus, methods for accurately detecting virologic failure are urgently needed. This study evaluates the effectiveness of clinical and immunologic criteria to ascertain virologic failure in children and supplements currently recommended criteria in situations where plasma HIV-1 RNA testing is not available.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional cohort study with retrospective chart review enrolled HIV-infected children receiving medical care at the Child Centered Family Care Clinic (CCFCC) of the Kilimanjaro Christian Medical Centre (KCMC), a referral hospital in northern Tanzania. KCMC distributes ART through funding from the President's Emergency Fund for AIDS Relief, and was the first site in northern Tanzania to offer free ART to children, starting in January, 2005. Patients initially paid 1000 Tanzanian shillings (equivalent to approximately 0.78 USD by November 2009 exchange rates) at each clinic visit; this fee was waived in 2007 and pediatric HIV care has since been provided free of charge.

ART at the CCFCC is provided according to standards defined by the Tanzanian National Guidelines for the Management of HIV and AIDS.[20] First-line regimens are non-nucleoside reverse transcriptase inhibitor-based and consist of a combination of either nevirapine or efavirenz with zidovudine and lamivudine or stavudine and lamivudine. Second-line regimens are prescribed for children who meet immunologic and/or clinical criteria for regimen failure according to the Tanzanian National Guidelines. Second-line therapy consists of lopinavir/ritonavir with either abacavir and didanosine or zidovudine and lamivudine. Regimen changes between combinations of first-line medications, or changes to a protease inhibitor regimen due to toxicity or side effects from nevirapine and efavirenz, were still considered first line for the purposes of this analysis. The study was observational and any medication changes were at the discretion of clinicians.

Study inclusion criteria were aged 12 months to 16 years and treatment with ART for at least 6 months. Children less than 18 months at study enrollment had documentation of an HIV-1 RNA level >5000 copies per milliliter, CD4% < 25%, or a WHO Stage III or IV clinical event before ART initiation.

Informed consent was obtained from the parent or guardian for all participants and assent from the child if over 13 years of age. A standardized questionnaire was administered in Kiswahili by a social worker and by a physician to determine sociodemographic information and past medical history, including exposure to antiretroviral medications as part of prevention of maternal to child transmission programs, date of ART initiation, current ART regimen, medication changes, and tuberculosis treatment. Clinical staging was based on physician documentation of conditions included in the 2006 WHO Staging Criteria.[21] All post-ART CD4 lymphocyte measurements and complete blood count (CBC) results, pre-ART nadir CD4 lymphocyte measurement, and monthly height and weight measurements were recorded through chart review. Children who met combined clinical and immunologic recommendations for regimen change according to WHO guidelines (Table 1) were designated as failing therapy by clinical and immunologic criteria.[9,22]

Missed doses and treatment interruptions were assessed by physician documentation on the national standardized HIV care flow sheet—which is filled by clinicians monthly during follow up visits—and by asking the caregiver during the study enrollment visit to recall the last time a dose of medication was ever missed, how many doses were missed in the last month, and whether treatment had ever been stopped for a full day or more. Maladherence by physician history was defined as documentation on the HIV care flow sheet of at least 2 separate visits in which a family reported that the child missed more than 1 dose in the previous month, including any skipped or late clinic appointments resulting in delayed refill of medications. Family report of maladherence was defined as caregiver recall of more than 1 dose missed in the past month or any treatment interruption of at least 1 day. Disclosure of HIV status was assessed by questioning caregivers privately as to whether they had informed the child that she or he had HIV infection, that she or he had an illness (eg, of the blood or immune system) without ever specifying HIV, or that they had

TABLE 1. Summary of WHO Recommendations for Clinical and Immunological Monitoring of ART in Children in Areas Where CD4 Lymphocyte Count Is Available[9,22]

Clinical status at least 6 months post ART initiation		CD4 measurement(s) at least 6 months post ART initiation
Clinically well or new, relapsed or worsening WHO Stage I or II event(s)	→	Consider regimen change if two or more CD4 counts below age-related threshold for severe immunodeficiency,* but regimen change particularly recommended if CD4 declines further†
New, relapsed, or worsening WHO Stage III event(s)	→	Recommend regimen change if CD4 count below age-related threshold for severe immunodeficiency
New, relapsed, or worsening WHO Stage IV event(s)	→	One CD4 count below age-related threshold for severe immune deficiency is recommended but not necessary to advise regimen change

*Age-related threshold for severe immune deficiency CD4% <20% or CD4 <750 cells per milliliter (12–35 months), CD4% <20% or CD4 <350 cells per milliliter (36–59 months), CD4% <15% or CD4 <200 cells per milliliter (≥5 years).

†Regimen change particularly recommended if CD4% <15% (12–35 months of age), CD4% <10% (36–59 months), and CD4 <100 cells per milliliter (≥5 years).

never specifically discussed the child's illness. Analyses of disclosure were restricted to children aged 7 or older.

Laboratory Assessments

Five milliliters of whole blood were collected from each patient on the day of enrollment for CBC (Abbott Cell Dyn 3500 Hematology Analyzer, Abbott Diagnostics, Des Plaines, IL), CD4 lymphocyte measurement (FACSCalibur flow cytometry, Beckman, Dickinson, and Co., Franklin Lakes, NJ), and plasma HIV-1 RNA level (Abbott m2000 system, Abbott Molecular, Des Plaines, IL).[23] The lower limit of detection for plasma HIV-1 RNA PCR was 40 copies per milliliter for 600 uL samples and 150 copies per milliliter for 200 uL sample volumes.

Enrollment laboratory testing was performed at the KCMC Biotechnology Laboratory, which participates in External Quality Assurance programs including United Kingdom National External Quality Assurance Scheme, College of American Pathologists, and the AIDS Clinical Trials Group Viral Quality Assurance Program. Prior CD4 lymphocyte measurements and CBC results abstracted from charts were performed at a variety of clinical laboratories, including KCMC Clinical Laboratory and other district hospitals in Tanzania. Study-related laboratory assessments were performed on the day of (CBC and CD4 lymphocyte count) or within 2 weeks of (HIV-1 RNA level) sample receipt and were made immediately available to clinicians to assist with clinical care.

Statistical Analysis

Statistical analyses were conducted using Stata software, version 10.0 (StataCorp, College Station, TX). The primary outcome was virologic failure, defined as HIV-1 RNA level $\geq$400 copies per milliliter. Participant characteristics at time of study enrollment were stratified by HIV-1 RNA level $<$ or $\geq$400 copies per milliliter and compared using Pearson χ^2 and Wilcoxon rank-sum tests as appropriate. Bivariable analyses were performed to identify associations with virologic failure for all enrolled children. Both bivariable analyses and multivariate logistic regression were performed on select variables to identify predictors of first-line failure. The number of variables in the multivariate model was limited to 1 per 10 virologic failure events, and variables were preselected by an expert in the field blinded to preliminary results to limit overfitting in the model.[24] Performance of currently recommended clinical and immunologic criteria in identifying children with virologic failure was assessed using sensitivity, specificity, and positive and negative predictive values. Receiver operating characteristic (ROC) curves were plotted for the currently recommended clinical and immunological criteria and those criteria in combination with significant predictors from the logistic regression model. Areas under ROC curves for the resulting monitoring strategies were compared using STATA 10.0 rocgold. Standard errors for areas under ROC curves were calculated using the Hanley method, and Sidak method was used to adjust for multiple comparisons.

Research Ethics

The study protocol was approved by an Institutional Review Board of Duke University Medical Center, the Kilimanjaro Christian Medical Centre Research Ethics Committee, and the Tanzania National Institute for Medical Research National Medical Research Coordinating Committee.

Role of the Funding Source

Study sponsors played no role in study design, data collection or analysis, article writing, or submission for publication.

RESULTS

Study Population

Two hundred six children were enrolled between October 2008 and June 2009, representing 84% of the clinic population eligible for the study (Fig. 1). Of the 38 eligible children who were not enrolled, 8 declined participation and 8 came to clinic without a parent or guardian. In 4 cases, the caretaker came alone to collect medications, and in 2 cases, the parent or guardian did not speak Kiswahili. The remaining 16 children attended clinic sporadically and were never selected on their visits. Enrollment was closed after there was less than 1 eligible child in attendance per week for greater than 1 week of clinic.

Characteristics of the 206 participants at the time of study enrollment are described in Table 2. The median [intraquartile range (IQR)] age was 8.1 (5.4–11.2) years, and median (IQR) duration of ART before enrollment was 2.4 (1.1–3.3) years. Five children (2.4%) (ranging from 1.3–2.9 years of age) had received single-dose nevirapine for prevention of perinatal transmission of HIV. At enrollment, 182 participants (88.3%) were receiving a non-nucleoside reverse transcriptase inhibitor. Nevirapine-based ART was being taken by 121 children (58.7%), 71 (34.5%) in combination with zidovudine and lamivudine and 50 (24.3%) in combination with stavudine and lamivudine. Efavirenz-based ART was being taken by 61 children (29.6%), 57 (27.7%) in combination with zidovudine and lamivudine and 4 (1.9%) in combination with stavudine and lamivudine. Twenty-four children (11.6%) were using protease inhibitor–based ART, and 23 (95.8%) of those 24 were receiving it as second-line therapy. The remaining 183 children were on first-line ART.

Virologic Failure

Sixty-five children (31.6%) demonstrated virologic failure (HIV-1 RNA $\geq$400 copies/mL) (Fig. 1). Of the participants experiencing failure, 57 (87.7%) were on first-line therapy and the remaining 8 (12.3%) were on second-line therapy. Compared with children with virologic suppression, children with virologic failure were significantly younger [7.7 (4.5–10.1) versus 9.0 (6.0–11.5) years; $P = 0.02$], and were more likely to be taking a nevirapine-based regimen versus any other regimen [odds ratio (OR) = 1.9; $P = 0.04$] and versus an efavirenz-based regimen (OR = 2.4; $P = 0.02$) (Table 2). A history of WHO Stage IV disease (OR = 2.2; $P = 0.01$) was also associated with virologic failure in this cohort. Among

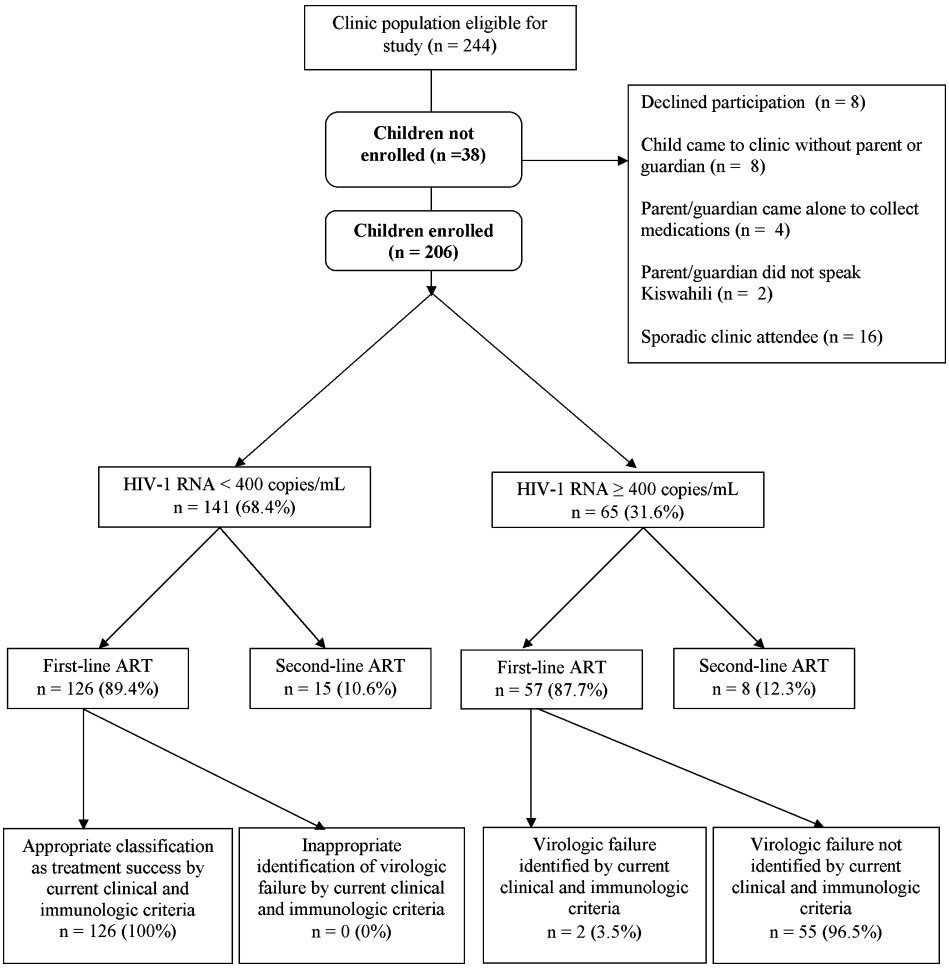

FIGURE 1. Study flow.

those ever treated for tuberculosis, children with virologic failure were more likely to have taken antituberculosis therapy and ART simultaneously (OR = 2.4; $P = 0.04$) as opposed to sequentially.

A greater proportion of children with virologic failure had ever received care at an HIV care center in addition to KCMC (OR = 2.1; $P = 0.04$) and had a history of at least 1 adult on ART in the household (OR = 2.0; $P = 0.03$). Physician documentation of maladherence (OR = 3.2; $P < 0.01$) was found more frequently among participants with virologic failure, whereas family report of missed doses was not significantly associated. Full disclosure of HIV status to children aged 7 and older was protective against virologic failure (OR = 0.26, $P = 0.02$).

Study enrollment CD4% < 25% (OR = 2.9, $P < 0.01$) and CD4 lymphocyte count <200 cells per milliliter (OR undefined, $P < 0.01$) were both associated with virologic failure in this cohort. For children with chart-abstracted CD4 lymphocyte measurements available in addition to study values (Table 3), a decline to below pre-ART CD4% nadir after greater than 6 months of ART was associated with virologic failure (OR undefined, $P = 0.04$), whereas a decline to below pre-ART nadir CD4 lymphocyte count (assessed among children ≥5 years at ART start) was not. A single decline in CD4 lymphocyte count by >30% between consecutive tests (OR = 2.1, $P = 0.05$) or 2 declines in CD4 lymphocyte count by >10% over consecutive tests (OR = 3.5, $P = <0.01$) were significantly associated with virologic failure among the 159 children aged ≥5 years at study enrollment. A decline in CD4% from post-treatment peak to study CD4% was not significantly associated with virologic failure.

In evaluating virologic failure on first-line therapy (Table 4), bivariable analysis of endpoints independently selected before data analysis revealed enrollment CD4% < 25% (OR = 3.2; $P < 0.01$) to be significantly predictive of failure and physician documentation of maladherence (OR = 2.3; $P = 0.07$) trended toward significance. On multivariate analysis, both CD4% < 25% (OR = 3.7; $P < 0.01$) and physician documentation of maladherence (OR = 5.0; $P = 0.01$) were significantly predictive of virologic failure. Family reported adherence, a decline from post-treatment peak CD4%, and recent change in weight-for-age Z score were not predictive of failure in this group.

Analyses with virologic failure defined as ≥1000 copies per milliliter [found in 60 (29%) participants] revealed few differences across all assessments. Care at another center in addition to KCMC was no longer significant. Physician report of maladherence was significant in both bivariable (OR = 2.4,

TABLE 2. Demographic and Clinical Characteristics at Enrollment of Children Receiving ART at the Kilimanjaro Christian Medical Centre in Moshi, Tanzania, October 2008–June 2009. Participants Stratified by Virologic Failure (HIV-1 RNA ≥400 Copies/mL)

Characteristic	All patients n = 206	HIV-1 RNA <400 n = 141	HIV-1 RNA ≥400 n = 65	*P**
Male sex	98 (47.6)	68 (48.2)	30 (46.2)	0.78
Age in years, median (IQR)	8.7 (5.4–11.2)	9.0 (6.0–11.5)	7.7 (4.5–10.1)	**0.02**
Transferred or care in >1 center†	37 (18.0)	20 (14.2)	17 (26.2)	**0.04**
Duration of ART in years, median (IQR)	2.4 (1.1–3.3)	2.4 (1.2–3.3)	2.1 (1.1–3.4)	0.96
Current ART Regimen				
Nevirapine-based	121 (58.7)	76 (53.9)	45 (69.2)	**0.04**
Efavirenz-based	61 (29.6)	49 (34.8)	12 (18.5)	**0.02**
Protease inhibitor-based	24 (11.6)	16 (11.4)	8 (12.3)	0.82
Prior medication change‡				
None	158 (76.7)	109 (77.3)	49 (75.4)	0.60
1 change	44 (21.4)	29 (20.7)	15 (23.1)	0.72
2 changes	10 (4.8)	8 (5.7)	2 (3.1)	0.51
Perinatal nevirapine exposure	5 (2.4)	1 (0.7)	4 (6.2)	**0.04**
Caregiver change in past year	24 (12.2)	18 (13.3)	6 (9.8)	0.64
Mother deceased	89 (43.2)	66 (46.8)	23 (35.4)	0.17
Father deceased	69 (33.5)	50 (35.5)	19 (29.2)	0.43
Primary caregiver not biologic parent	94 (45.6)	67 (47.5)	26 (40.0)	0.37
No. of children <18 years in household, median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)	0.73
Time to clinic in hours, median (IQR)	1.5 (0.8–2.0)	1.5 (0.8–2.0)	1.5 (1.0–2.5)	0.98
Cost of travel to clinic in USD, median (IQR)§	1.8 (0.9–3.8)	1.9 (0.9–3.8)	1.8 (0.9– 3.1)	0.60
Disclosure of HIV status				
Child ≥7 years aware‖	48 (36.9)‖	40 (72.3)‖	8 (16.7)‖	**0.02**
All others in household ≥13 years aware	110 (53.7)	73 (51.8)	37 (57.8)	0.45
Seropositivity in household				
HIV-infected but no ART	21 (10.2)	16 (11.4)	5 (7.7)	0.62
At least one adult on ART	88 (42.7)	53 (37.6)	35 (53.8)	**0.03**
No one else with HIV in Household	87 (42.2)	67 (47.5)	20 (30.8)	**0.02**
Maladherence				
Family report	46 (22.3)	29 (20.6)	17 (26.2)	0.45
Physician documentation	31 (15.3)	14 (10.1)	17 (26.6)	**<0.01**
Tuberculosis status				
Ever treated	108 (52.4)	73 (51.8)	35 (53.8)	0.52
TB and ART taken Simultaneously	59 (54.1)	35 (47.3)	24 (68.6)	**0.04**
WHO Stage				
Stage II	29 (14.1)	22 (15.6)	7 (10.8)	0.40
Stage III	118 (57.3)	86 (61.0)	32 (49.2)	0.13
Stage IV	56 (27.2)	31 (22.0)	25 (38.5)	**0.01**
Weight-for-age Z-score, median (IQR)	−1.4 (−2.2–−0.5)	−1.4 (−2.3–−0.5)	−1.2 (−2.2–−0.3)	0.37
Pre-ART CD4%, median (IQR)	12 (6–14)	12 (6–14)	11 (6–11)	0.90
Enrollment laboratory values				
CD4%, median (IQR)	28 (21–34)	30 (24–35)	25 (16–28)	**<0.01**
CD4% < 25%	66 (33.2)	35 (25.6)	31 (50.0)	**<0.01**
CD4 count, median (IQR)	834 (543–1154)	926 (658–1162)	615 (437–1151)	**<0.01**
CD4 count <200 cells/mL	4 (2.0)	0 (0.0)	4 (6.3)	**<0.01**
Hemoglobin, median (IQR)	12.0 (11.1–12.8)	12.1 (11.4–12.8)	11.8 (10.9–12.6)	0.11
MCV, median (IQR)	94 (86–102)	95 (86–102)	92 (83–101)	0.16
Platelet count, median (IQR)	346 (289–411)	347 (292–417)	332 (268–400)	0.12

Data are number (%) of patients unless otherwise indicated. IQR, interquartile range; ART, antiretroviral therapy; USD, United States dollars; SD, standard deviation; MCV, mean corpuscular volume.

*Significance tests for comparisons between HIV-1 RNA < or ≥ 400 copies/ml based on Pearson's chi-square for categorical patient characteristics and Wilcoxon rank-sum for continuous characteristics with skewed distributions.

†Identifies children who have ever received care at another HIV treatment center besides KCMC.

‡Medication change due to toxicity, drug interaction, or simplification of regimen, NOT due to suspected treatment failure.

§Based on currency conversion 1 USD = 1300 Tanzanian shillings.

‖Denominator =130 children equal to or older than 7 years; 54 with HIV-1 RNA<400 and 28 with HIV-1 RNA ≥ 400 copies/mL.

TABLE 3. Immunologic Associations With Virologic Failure after Initiation of ART

All participants	N with finding/N with available data (%)	HIV-1 RNA < 400	HIV-1 RNA ≥400	P*
Study CD4% less than post-treatment peak	119/203 (58.6)	79/140 (56.4)	40/63 (63.5)	0.63
Any CD4% less than pre-treatment nadir†	2/44 (4.6)	0/29 (0.0)	2/15 (13.3)	**0.04**
Participants ≥5 years old at ART start				
Any CD4 count less than pre-treatment nadir†	19/140 (13.6)	12/102 (11.6)	7/38 (18.4)	0.40
Decline in CD4 count by >30% over two consecutive tests	46/157 (29.3)	29/113 (24.8)	18/44 (40.9)	**0.05**
Two declines in CD4 count >10% over three consecutive tests‡	21/136 (15.4)	10/99 (10.1)	11/37 (29.7)	**<0.01**

*Significance tests for comparisons between HIV-1 RNA < or ≥ 400 copies/ml based on Pearson's chi-square.
†After 6 months of ART.
‡Only one child over age 5 had a consective decline in both CD4 count and percent; this child had HIV-1 RNA >400 copies/mL.

$P = 0.04$) and multivariate analyses (OR = 4.1, $P = 0.01$) of virologic failure on first-line therapy.

Strategies for Identifying Virologic Failure

Currently recommended clinical and immunologic criteria demonstrated 3.5% sensitivity in this cohort, correctly classifying 2 of 57 subjects (3.5%) with virologic failure on first-line ART (Table 5). All 126 participants with plasma HIV-1 RNA <400 copies per milliliter were correctly identified by these criteria as not requiring regimen change.

By incorporating physician documentation of maladherence and CD4% < 25% into current monitoring strategies, sensitivity in detecting failure increased to 57.1%, with a decrease in specificity to 69.6%. Thirty-two of 57 children (56.1%) with first-line failure were correctly identified with this method. However, 38 of 126 children (30.2%) with virologic suppression were incorrectly classified as experiencing virologic failure. The area under the ROC curve for currently recommended clinical and immunologic criteria was 0.52 [standard error (SE) = 0.05]. Including CD4% <25% increased the area under ROC curve to 0.64 (SE = 0.05) and including both predictors produced an area of 0.63 (SE = 0.05).

DISCUSSION

As antiretroviral access continues to expand in resource-limited settings, monitoring response to therapy is becoming an increasingly critical issue. Virologic and immunologic testing are central to guidelines for monitoring ART in resource-replete settings, and plasma HIV-1 RNA testing has been shown to be cost-effective and improve patient outcomes in resource-rich countries.[25] With only 1 second-line regimen available in many resource-limited areas, timely detection of virologic failure is likely to be crucial in preventing resistance mutations that could jeopardize the long-term effectiveness of currently available ART.[17–19] Clinical and immunological monitoring of children in resource-limited settings may be even more challenging than in adults, given children's baseline increased risk of infections, the normal age-related decline in CD4 lymphocyte counts, and frequent lack of availability of CD4%. Yet in our cohort and in others in sub-Saharan Africa, children seem to be at high risk for virologic failure, with published prevalence ranging from 19.7% in KwaZulu-Natal, South Africa, to 50% in Cote d'Ivoire.[5,26] Therefore, close monitoring of response to ART may be particularly important in children, especially given the goal of lifelong therapy from infancy through adulthood.

Several clinical and immunologic features correlated with virologic failure in our cohort have been identified in other studies, including CD4% <25%,[4] younger age at ART initiation,[3,27] and use of nevirapine vs. efavirenz-based ART.[2,28] The superiority of efavirenz-based regimens has been suggested by cohort studies in adults,[29,30] but clinical trial data are not available for children. Furthermore, efavirenz is not currently recommended for children less than 3 years of age, a group that seems to be at higher risk for virologic

TABLE 4. Predictors of Virologic Failure (HIV-1 RNA ≥ 400 Copies/mL) In a Pediatric Tanzanian Cohort After at Least Six Months of First-line ART (n = 183)

Variable	Bivariate analysis		Multivariate analysis*	
	Unadjusted OR (95% CI)	P	Adjusted OR (95% CI)	P
Maladherence				
Family report	1.0 (0.48–2.2)	0.95	0.6 (0.21–1.8)	0.37
Physician documentation	2.3 (0.95–5.6)	0.07	5.0 (1.4–17.3)	0.01
Drop in CD4% from peak	1.4 (0.71–2.6)	0.34	1.7 (0.76–3.9)	0.19
Enrollment CD4% < 25%	3.2 (1.7–6.3)	<0.01	3.7 (1.7–8.4)	<0.01
Weight-for-age growth velocity†	1.1 (0.61–1.9)	0.78	1.0 (0.56–1.9)	0.92
Hemoglobin at enrollment	0.98 (0.95–1.0)	0.04	0.98 (0.96–1.0)	0.51

CI, confidence interval.
*Controls for age, time on ART, and gender.
†Represents change in weight-for-age Z-score over the past 6 clinic visits (mean 6.5 months, standard deviation 3.0).

TABLE 5. Performance Characteristics of Strategies for Predicting Virologic Failure (HIV-1 RNA ≥ 400 Copies/mL) in a Pediatric Tanzanian Cohort on First-line ART

Strategy	Area under ROC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Currently recommended clinical and immunologic criteria	0.52 (0.49–0.54)	3.5 (0.4–12.1)	100 (97.1–100)	100 (15.8–100)	69.6 (62.4–76.2)
Current criteria plus physician documentation of maladherence	0.57 (0.51–0.63)	23.2 (13.0–36.4)	90.4 (83.8–94.9)	52.0 (31.3–72.2)	72.4 (64.7–79.3)
Current criteria plus CD4% < 25%	0.64 (0.56–0.71)	50.9 (37.3–64.4)	77.0 (68.6–84.0)	50.0 (36.6–63.4)	77.6 (69.3–84.6)
Current criteria, documented maladherence, and CD4% < 25%	0.63 (0.56–0.71)	57.1 (43.2–70.3)	69.6 (60.7–77.5)	45.7 (33.7–58.1)	78.4 (69.6–85.6)

ROC, receiver operating characteristic; CI, confidence interval.

failure. Use of nevirapine-based regimens in children aged less than 3 years may have contributed to our finding that a history of receipt of ART in conjunction with antituberculosis therapy was associated with virologic failure. This merits further exploration in additional studies.

We found a significant association between virologic failure and physician documentation of maladherence but not with family-reported maladherence. The lack of correlation between family report and virologic outcomes is consistent with prior studies.[2,28] In our setting, the finding underscores the importance of the use of the national HIV care flow sheet at each visit and optimized attention to adherence and vigilance for treatment failure among families with any physician documentation of missed doses.

Full disclosure of HIV status by caregivers to their school-aged children (in this case, we defined school-age as 7 years and older) was associated with a protective effect against virologic failure. These data suggest that techniques to improve rates of caregiver disclosure might improve virologic outcomes in children. The finding that having another adult on ART in the household was associated with virologic failure was surprising. Poorer health or economic status among caretakers receiving ART compared with non-HIV–infected caretakers or those who were at early stages of HIV infection may have been responsible for this association but were not assessed in our study.

Currently recommended clinical and immunologic criteria demonstrated poor performance in our cohort, identifying very few participants with virologic failure. Their excellent specificity avoided unnecessary treatment changes for children who were virologically suppressed, but low sensitivity left 96.5% of patients with virologic failure on failing first-line regimens. The addition of 2 factors highly correlated with virologic failure in our multivariate analysis markedly reduced the specificity of the model and, if used clinically, would risk over ascertainment of virologic failure. Although we analyzed only a limited number of prespecified factors in our multivariate model, use of other factors that were significantly associated with virologic failure on bivariable analysis would be unlikely to improve the sensitivity of clinical and immunologic detection to a clinically meaningful level. For example, rapid or repeated declines in CD4 count among children aged 5 or older were associated with but were not found exclusively among children with virologic failure.

There are several limitations to this study. The cross-sectional nature of the study design did not allow for multiple plasma HIV-1 RNA measurements, and blips in HIV-1 RNA level are known to occur.[31] However, analysis of our data points with an outcome of ≥1000 copies per milliliter did not substantially change findings. The study design also did not allow us to assess whether the sensitivity of current guidelines changes with the length of time a child experiences unsuppressed viremia nor could we assess the degree to which long-term clinical outcomes and response to second-line therapy are impacted by use of clinical and immunologic versus virologic monitoring strategies. In addition, the low number of children meeting clinical guidelines for failure at study enrollment may have been influenced by death, loss to follow-up of clinic attendees, and use of second-line therapy. Further, retrospective laboratory measurements abstracted from charts were performed in a variety of clinical laboratories, some of which do not adhere to the same rigorous quality control standards as the KCMC Biotechnology Laboratory where all enrollment laboratories were performed. Although this lack of consistency in laboratory capacity does represent a limitation, it is more reflective of real-world circumstances under which most children in resource-limited settings receive HIV-related care. Finally, because the WHO guidelines were written specifically to address first-line failure, our multivariate model included only children on first-line therapy.

Despite these limitations, this study also has important strengths. Our cohort has taken ART for a mean of 2.4 years, which is substantially longer than many current studies examining virologic outcomes in children. Detailed socio-demographic and clinical information was collected on the cohort, and differences between subjects on failing and suppressive regimens may direct future studies examining underlying reasons for virologic failure. Furthermore, as far as we are aware, this study is the first to examine the effectiveness of currently recommended clinical and immunologic criteria in monitoring children for virologic failure on ART.

CONCLUSIONS

This study has demonstrated poor performance of currently recommended clinical and immunologic criteria in detecting virologic failure among children in a resource-limited setting. Resistance mutations and subsequent second-line

failure shown to develop in patients remaining on failing regimens represent a barrier to long-term effectiveness of ART, which makes evaluation and improvement of the current clinical and immunologic monitoring strategy a critical issue.[18] Ultimately, there is an urgent need for affordable techniques for measuring plasma HIV-1 RNA level that could be applied in resource-limited settings.

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