BMJ Open

Improving Treatment of HIV Infected Ethiopian Children through Better Detection of Treatment Failure: Clinical Cohort Design and Baseline Characteristics

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013528
Article Type:	Cohort profile
Date Submitted by the Author:	19-Jul-2016
Complete List of Authors:	Tadesse, Birkneh Foster, Byron; University of Texas Health Science Center at San Antonio, Pediatrics Jerene, Degu; Management Sciences for Health, Health Programs Group, Addis Ababa, Ethiopia Ruff, Andrea; Johns Hopkins University Bloomberg School of Public Health
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Infectious diseases, Paediatrics
Keywords:	Children, HIV & AIDS < INFECTIOUS DISEASES, Antiretroviral therapy, Treatment Failure

Improving Treatment of HIV Infected Ethiopian Children through Better Detection of Treatment Failure: Clinical Cohort Design and Baseline Characteristics

Birkneh Tilahun Tadesse[1], Byron Alexander Foster[2], Degu Jerene[3], Andrea Ruff [4]

Affiliations: [1]Hawassa University Department of Pediatrics, [2]University of Texas Health Science Center at San Antonio, [3]Management Sciences for Health, Health Programs Group, Addis Ababa, Ethiopia, [4]Johns Hopkins University Bloomberg School of Public Health

Correspondence:

birknehtilahun@gmail.com

Abstract:

Introduction: Improving detection of treatment failure among children on first-line antiretroviral therapy (ART) is crucial to improving ART success among HIV infected children. The WHO guidelines for detection of first-line ART failure (ARTF) have low sensitivity and lead to a significant number of misclassifications in the absence of viral load testing. Developing a prediction rule which has a high sensitivity and is not dependent on viral load determination is imperative, especially in resource limited settings where a viral load is not readily available. This is the first study to evaluate the application of a prediction rule for the diagnosis of first-line ARTF in children in resource poor settings.

Methods: The study will assess the performance of the WHO criteria in detecting first-line ARTF and identify important clinical and immunological predictors to build a prediction rule which has a better accuracy than the current WHO guidelines to detect first-line ARTF. A prospective cohort study involving HIV-infected children on or initiating first-line ART will be conducted. From the cohort, distribution of several predictors among ARTF and non-ARTF groups will be analysed; likelihood ratios (LR) will be calculated to fit models of 'ARTF/ no ARTF' regressed on predictors. The Spiegelhalter method will be used to build a prediction rule for predicting first-line ARTF. Virological treatment failure will be taken as the gold standard.

Results: Expected outcomes will include performance of the current WHO guidelines for detection of first-line ARTF in children will be determined. Predictors of virological failure will be identified. A clinical-immunological prediction rule with area under the curve of >0.80 for first-line ARTF will be developed.

Conclusions: The successful completion of this study will allow for better targeting of viral-load testing to those at highest risk in a resource-poor setting and provide clinicians and policy-makers with a practical prediction rule.

Key Words: Children, HIV, ART, Treatment Failure

Strengths and Limitations of the Study:

The study will be the first pediatric scoring system for earlier detection of first line antiretroviral treatment failure in resource limited settings where viral load facility is limited or not accessible.

The prediction rule will be an important tool for targeting viral load measurement in settings where there is limited viral load facility and could be an alternative for earlier detection of treatment failure in settings where viral load is not accessible.

Though different mechanisms have been devised, nonadherence and loss to follow up could be challenges to in the study.

1. Introduction:

As of 2015, 1.8 million children less than 15 years of age were living with HIV among which 1.5 million were living in sub-Saharan Africa [1]. While use of ART has improved clinical and immunologic outcomes of children [2 3], issues of drug resistance and treatment failure have emerged.

Treatment failure is considered when a child who has taken ART for at least six months presents with clinical, immunological or virological evidence of unsuccessful treatment [4]. Time to treatment failure from initiation of ART appears to depend on several factors including genetics, adherence, previous ART exposure and age in children and can range from six months to many years [5-7].

Viral load testing is the gold standard for monitoring ART [8]. However, this test has been expensive and technically demanding [9], functionally prohibiting its use in resource-constrained settings [10 11]. In developing countries, viral load determination is not available as a diagnostic tool for detection of treatment failure in children. As part of the 90-90-90 plan, the current Ethiopian strategy for the testing, treatment and follow up of virological suppression, efforts are being made to make viral load testing available in the regional laboratories [12]. However, in remote areas and in settings where viral load determination is logistically difficult and not easily accessible, the WHO 2016 guidelines emphasize targeting viral load testing using clinical and immunologic parameters [13]. Therefore, in low resources settings, developing a clinical prediction rule which requires the use of fewer resources is important to prioritize viral load testing and target the available limited viral load facility.

Analyzing the clinical and immunological predictors used in the WHO criteria for detection of first-line ART failure (ARTF) in children and sorting them in their order of strength to predict treatment failure may lead to a more accurate prediction rule for detection of first-line ARTF. Predictors in the WHO criteria identified as strong will be used to build the new prediction rule. In the current study, using a thorough analysis of the possible clinical and immunological

predictors, we will be able to identify the strongest parameters that predict first-line ARTF in children. Ultimately, developing an accurate prediction rule for first-line ARTF will minimize the need for viral load determination. Consequently, this will decrease the cost and delay incurred by viral load determination and will decrease the duration of time children stay on a failing regimen and the resulting drug resistance, potentially decreasing the number of AIDS deaths among HIV infected children.

The objectives of this prospective cohort study are therefore three-fold: 1) Determine the performance of the WHO clinical criteria for detection of first-line ARTF in HIV infected Ethiopian children. 2) Identify important clinical and immunological predictors of first-line ARTF among HIV infected Ethiopian children. 3) Develop a practical, acceptable, and accurate clinical prediction rule to improve detection of first-line ARTF among HIV infected Ethiopian children. In this paper, we describe the basic design, recruitment and characteristics of the cohort.

2. Methods:

2.1. Study Design and Inclusion Criteria:

This prospective cohort study includes children under 18 years with a diagnosis of HIV based on WHO guidelines [4 14], who are eligible for or are already on first-line ART. Children who discontinue follow up before six months on ART, are on second line ART, or with ARTF at enrolment are excluded.

The study is being done in the Southern Nations Nationalities and Peoples Region (SNNPR) of Ethiopia, where in 2012, there were 27,200 children below 14 years of age with HIV, 12,258 of whom needed ART [15]. In SNNPR, pediatric ART services are provided in 22 hospitals. We enrolled participants in the first six months of the study at the following six established pediatric ART centers with the highest patient volume in SNNPR: Hawassa University; Yirgalem; Arba Minch; Sodo; Adare; and Hosana hospitals.

2.2. Baseline data:

On enrollment, through medical chart review and use of structured questionnaires, we collected demographics (age, gender, race) of the child; clinical data (age at diagnosis and ART initiation, history of prevention of mother to child transmission (PMTCT) exposure , nutritional status, WHO clinical stage, mode of transmission, current or past tuberculosis infection and current ART regimen; and laboratory parameters (leukocyte count, thrombocyte count, hemoglobin, CD4 count/percent, viral load).

2.3. Clinical Data:

Patients will be followed every six months for development of new symptoms using a structured symptom checklist. Growth parameters will be assessed using the WHO growth standards [16], along with assessment of age appropriate developmental milestones [4]. Body mass index z-score and other growth parameters are calculated using Epi Info (CDC, USA 2016). Adherence will be assessed using a visual analog scale (VAS) adherence [17].

2.4. Immunologic Data:

Complete blood count (CBC) and CD4 count will be checked every six months; CD4 percentage and percent change in CD4 count from previous record will be determined.

2.5. Virological Data:

We will determine viral load at baseline and every six months for two years. Viral load testing will be done using Abbott Real*Time* HIV-1 m2000rt *m2000sp* machine with calibration done before running every batch of samples. If the child has any acute illness, viral load determination will be deferred until infection resolves [4].

2.6. Patient Care:

During visits, decision to switch ART or other treatments will be made by the clinical staff and will not be directed by the study. Viral load testing results from the study will be reported to the treating clinician. Ethiopia is currently rolling out viral load testing to be done yearly across the country.

2.7. Assessing Performance of WHO guidelines:

In the course of follow up, patients will be followed for any evidence of treatment failure based on the WHO 2013 guidelines [4]. The proportion of children who are correctly identified by WHO guidelines will be checked against viral load.

2.8. WHO 2013 Definition of ARTF [4]:

Clinical: New or recurrent clinical events indicating advanced/severe immunodeficiency (WHO clinical stage 3 and 4 clinical condition with exception of Tuberculosis) after 6 months of effective treatment. *Immunologic*: Children younger than 5 years: persistent CD4 levels below 200 cells/mm^3 or <10%; and, older than 5 years: persistent CD4 levels below 100 cells/mm3. *Virological (Gold Standard):* Reduction in viral load less than 0.5 log10 copies/ml or less than threefold reduction from baseline after 1 month on first-line ART or sustained HIV RNA of more than 3.7 log10 copies/ml after 24 weeks of treatment.

2.9. Derivation of scoring system:

In the cohort, subjects will be followed until virological failure occurs or at least until 24 months after initiation of first-line ART. We will identify cases of HIV-infected children who develop ARTF and age, duration of ART and sex matched controls among those who did not develop ARTF for at least 2 years after treatment commenced. Associations with clinical and laboratory predictors of ARTF will be done using these two groups. Parameters will include CD4 at baseline, CD4 change at 6 month, hemoglobin level, persistent thrombocytopenia, nutritional status at baseline, WHO staging at baseline, failure to thrive/growth faltering, PMTCT history, adherence using VAS and chronic gastroenteritis. Two by two tables will be created for each predictor. Bivariate analysis using chi square test and estimation of likelihood rations (LR) will be performed.

The Spiegelhalter method will be used to build a prediction rule [18]. A prediction rule will be developed using the composite of predictors with adjusted LR of more than 1.5 or less than 0.6. Log natural transformation will be done to the adjusted LR and will be used to build final score of predictors. A receiver operating characteristic (ROC) curve will be created; cut off point

on the ROC curve will be determined using the Zweig and Campbell formula as adapted by Lynen et al in Cambodia [19]; assuming the value of false positives to be the same as false negatives. A simplified diagnostic algorithm will be developed. Performance of the new prediction rule in detecting treatment failure will be compared with performance of the WHO guidelines.

2.10. Sample size:

To test the hypothesis that WHO criteria for detection of first-line ARTF have sensitivity of 10% and specificity of 40% [20] with precision of 5%; in a population with ARTF prevalence of 15% [5], a minimum of 960 participants will be required. Identification of important predictors to set up a prediction rule will require multiple sample size calculations for presumed predictors. The largest sample size is derived from history of maternal ART exposure. Considering prevalence of children who have ARTF to be 15% [5], with allocation ratio of cases to controls to be 0.2; and taking proportion of prior ART exposure in controls to be 11.5% based on a study in Mozambique [5 6 21], at 80% power and 5% level of significance, a sample size of 705 controls and 141 cases is needed to prove that the odds ratio for failing first-line ART is 2. To account for loss to follow up, we will include an additional 10%, therefore a minimum of 1056 children will be enrolled in this study. Matching controls by age and duration of ART will help address the heterogeneity of this population.

2.11. Data Quality Assurance:

Data will be entered electronically to be accessed at the hub institute through Redcap. There will be paper back up at each site. A one-day, intensive training was conducted with HIV clinic nurses on data abstraction, data and sample collection. The training included an overview of the study purpose and design, training in the electronic data entry system, a detailed walk-through of each form and practice sessions using sample charts for data abstraction and entry with direct feedback provided to each participant. Supervision visits will be made every three months.

Historical data on prior clinical staging, maternal ART prophylaxis and the child's age at diagnosis, previous treatment regimens and any changes will be abstracted from the child's chart by the nurses on the research team at time of enrollment.

2.12. Ethics and consent

Only volunteers are included in the study. Each participant receives written information about the study in their own local language and it is ensured that the consent is signed and dated by the parent or caregiver and the physician or ART nurse. Children older than 12 years also give assent. Ethical approvals have been obtained from the IRB of the regional health bureau and Hawassa University, College of Medicine and Health Sciences. Privacy is assured by removing patient identifiers including names and specific address.

3. Results:

From October 2015 through April 2016, 628 children have been enrolled from six different HIV treatment centers across southern Ethiopia.

3.1. Demographic Characteristics:

The mean age of the children at enrollment was 11.1 years and 47.6% of the children were female (Table 1). The median reported income was about $1 per day ($33/month) with 91.2% of caregivers reporting having a job with a wide range of reported occupations (Table 1). Of mothers in the cohort, 27.1% had no formal education, and 14.7% of fathers had no formal education. Only 48.0% of the children in this cohort had both parents alive and 71.1% of the caregivers providing data were HIV positive. In contrast, a minority of caregivers reported that the child had living siblings with HIV (15.0%).

3.2. *Clinical Characteristics*:

Overall, 81.4% of children were adequately nourished at enrollment by body mass index z-score using WHO criteria (Table 2). The vast majority of children were at WHO Clinical stage 1 at the time of enrollment into the cohort (88.6%), though only 20.6% were at stage 1 at the time of initiation of ART (Table 2) with a mean of 45 months between initiation of ART and enrollment in this study. Almost no children (0.8%) had a viral load done at initiation of their ART. Nearly half (42.6%) of children had some substitution of their ART drugs with 70.3% of those being due to a national guideline change where replacement of zidovudine or tenofovir for stavudine based regimens was done for all patients in Ethiopia.

About one quarter (24.7%) of children had ever been diagnosed with tuberculosis. Of those, 91.2% had a diagnosis of pulmonary tuberculosis. Only 45.6% of children had their HIV status disclosed to them as per the caregiver, and 23.9% of the caregivers reported being worried about stigma related to HIV (Table 2).

Adherence as assessed with the visual analog scale was high with 94.4% (SD=11.9) reporting adherence when asked over the past month, how often the child had taken their HIV medication. However, when asked how many doses over the past week the child had missed, 15.1% of caregivers reported missing at least 1 dose.

3.3. *Laboratory Characteristics*:

The enrolled children had a median CD4 count of 741 at enrollment with 3.1% having a value consistent with ARTF using immunologic criteria at the enrollment assessment (Table 3). Other laboratory parameters are shown in Table 3.

4. Discussion:

Currently, the use of viral load as a follow-up tool for the diagnosis of treatment failure is emphasized by the WHO [4]. On the other hand, recent WHO guidelines also recommended use of clinical and immunologic parameters to target the use of viral load testing for treatment monitoring and diagnosis of first line ARTF in settings where access to viral load is scarce [13]. In most resource limited settings and in communities living in remote places, the use of viral load as a point of care monitoring and diagnostic tool is difficult given the technical requirements of the procedure, the need for an efficient sample transport system, and the limited availability of the required reagents as is the case with the regional laboratory where the current cohort is located.

Performance of the WHO guidelines for detection of first line ARTF in children has been inadequately evaluated so far, and no local scoring system has been developed for use in HIV infected children who are on first line ART. Children are assumed to have different predictors of treatment failure because of the differences in mode of transmission, faster disease progression and previous exposure to ART for purpose of PMTCT [22 23]. Analysis of the predictors of first line ARTF and assessing the level of accurateness of the WHO criteria is of paramount importance for the development of such tools.

To our knowledge, this is the largest prospective, pediatric HIV cohort in Africa designed to identify a prediction rule using a composite of clinical and immunologic parameters to prioritize viral load testing for children on first line ART. Its prospective design allows for better data completeness and quality. However, challenges to the current cohort are multifaceted including poor adherence to and defaulting from care and treatment. Though we have devised strategies including better counselling and tracking systems to decrease the defaulter rate, it still remains to be a challenge because of the large geographic area served by each ART center.

Additionally, for information to be collected retrospectively including baseline laboratory values, clinical data and previous adherence, incomplete records pose a threat to data completeness and validity. The issue of adherence is particularly significant as changing to a second-line regimen due to poor adherence would likely not improve mortality and would potentially have consequences for emergence of resistant strains. While our method of using a visual analog scale for adherence has been validated in multiple settings, it has been found to overestimate adherence when compared with other methods such as automated assessment of bottle opening [24]. One potential adjunct to this study would be resistance testing of individual viral samples to assess resistance versus non-adherence though this is currently not part of the study protocol due to funding and resource constraints.

The prediction rule to be developed will be used in resource poor settings, especially remote areas with limited access to viral load testing for prioritization and treatment decision purposes. It will potentially decrease the HIV related mortality in children who would otherwise remain on a failing regimen because of lack of access to viral load testing. Additionally, it will help to investigate the effectiveness of ART in children. The potential of the prediction rule for over-diagnosis of ARTF and unnecessary switching will also be examined.

Funding

This project is supported by International AIDS Society, CIPHER Pediatric HIV Research Grant Program (Grant ID: 307-TAD).

Acknowledgment

We would like to thank Hawassa University for supporting the project. We also would like to acknowledge all the data collectors, children and parents for taking their time to be involved in the research.

Table 1: Baseline demographic characteristics of enrolled patients across all six sites

Age at enrollment, years, mean (SD)	11.1 (3.6)
Sex, % female (n)	47.6% (296)
Age at Diagnosis, years, mean (SD)	4.7 (3.7)
Family size, mean (SD)	5.5 (19.9)
Monthly income in US dollars, median (IQR)	33 (19-70)
Ethnicity, % of reporting (n)	
Amhara	19.2% (120)
Oromo	12.5% (78)
Sidama	12.9% (81)
Wolaita	24.6% (154)
Tigray	1.9% (12)
Gurage	4.5% (28)
Silte	0.8% (5)
Gamo	6.9% (43)
Other	16.8% (105)
Occupation of caregivers, % of reporting (n)	
Farmers	10.1% (63)
Government employees	19.1% (119)
Merchants	20.4% (127)
Daily Laborers	21.8% (136)
Other	19.9% (124)
Jobless	8.8% (55)
Mother's education, % of reporting (n)	
No school	27.1% (168)
Attended some school	23.4% (145)
Completed primary education	22.7% (141)
Completed secondary education	12.4% (77)
Completed tertiary education	10.8% (67)
Read and write, no school	3.5% (22)
Father's education, % of reporting (n)	
No school	14.7% (89)
Attended some school	17.1% (103)
Completed primary education	23.0% (139)
Completed secondary education	23.5% (142)
Completed tertiary education	19.0% (115)
Read and write, no school	2.6% (16)
Child lives with, % reporting (n)	
Mother and Father	28.2% (176)
Mother only	35.7% (223)
Father only	12.2% (76)
Other relative	19.1% (119)
Other	4.8% (30)
Parents alive, % both alive	48.0% (299)
Caregiver HIV positive, % yes	71.1% (443)
Caregiver has HIV healthcare, % yes	71.3% (440)
Caregiver on ART, % yes	69.7% (434)
Other siblings with HIV, % yes	15.0% (94)
Other siblings who died of HIV, % yes	9.4% (59)

* Not all percentages reflect total of 628 subjects as some with missing or unknown values, ART = anti-retroviral treatment, SD = standard deviation

Table 2. Clinical characteristics of the cohort of HIV positive children at enrollment

Height, z-score, mean (SD)	-1.02 (1.45)
BMI, z-score, mean (SD)	-0.90 (1.26)
% malnourished (BMI z-score <2)	17.4 (100)
% adequate nourishment (-2>BMI z-score <2)	81.4 (468)
% obese (BMI z-score >2)	1.2 (7)
Child took ARV prophylaxis after birth, % yes (n)	1.1% (7)
Clinical Stage at initiation of ART, % of total (n)	
Stage 1	20.6% (129)
Stage 2	35.1% (220)
Stage 3	37.1% (232)
Stage 4	7.2% (45)
Clinical stage at enrollment, % of total (n)	
Stage 1	88.6% (552)
Stage 2	6.9% (43)
Stage 3	4.0% (25)
Stage 4	0.5% (3)
Viral load at initiation, % yes (n)	0.8% (5)
Substitution of ART drugs, % yes (n)	42.6% (253)
Reason for substitution, % of those with substitution (n)	
Toxicity or side effects	14.7% (39)
Tuberculosis co-infection	0.8% (2)
National Guideline Change	70.3% (187)
Total ART duration, months, mean (SD)	45.4 (35.8)
ART Regimen, % of total (n)	
AZT, 3TC, EFV	13.5% (84)
AZT, 3TC, NVP	45.9% (286)
D4T, 3TC, EFV	5.5% (34)
D4T, 3TC, NVP	20.4% (127)
TDF, 3TC, EFV/NVP	9.6% (60)
AZT,D4T, 3TC, PI	0.3% (2)
ABC, 3TC, NVP/EFV/PI	4.8% (30)
Adherence of caregiver if taking ART over past month by VAS, mean (SD)	93.7% (12.3)
Adherence of child reported by caregiver over past month by VAS, mean (SD)	94.4% (11.9)
Tuberculosis, % ever diagnosed (n)	24.7% (154)
Type of TB diagnosed, % with ever TB diagnosis (n)	
Pulmonary	91.2% (134)
Disseminated	3.4% (5)
Lymph node	4.8% (7)
Currently prescribed PCP prophylaxis, % yes (n)	32.6% (202)
Caregiver worried about stigma, % yes (n)	23.9% (150)
Child's HIV status disclosed to them, % yes (n)	45.6% (283)

SD = standard deviation, BMI = body mass index, ART = anti-retroviral treatment, TB = tuberculosis, PCP = pneumocystis jirovecii, ARV= Antiretroviruses

CD4 Count at baseline, median (IQR)	741 (517, 1014)
CD4 Count, % above treatment failure threshold by age (n)	
Less than 5 years old, CD4 ≥200, % of tested (n)	100 (32)
5 years or older, CD4 ≥100, % of tested (n)	96.9 (558)
WBC, median (IQR)	6.3 (4.9, 8.0)
Total lymphocyte count, median (IQR)	3.4 (2.3, 36.1)
Hemoglobin, median (IQR)	13.4 (12.4, 14.6)
Platelet count, median (IQR)	314 (253, 377)
Urinalysis, % normal (n)	95.7% (517)
BUN, median (IQR)	7.5 (5, 11.5)
Cr, median (IQR)	0.52 (0.40, 0.70)
SGOT, median (IQR)	32 (25, 39)
SGPT, median (IQR)	22 (16, 31)

Table 3. Baseline laboratory characteristics at enrollment of the cohort of HIV positive children

IQR = interquartile range, WBC = white blood cell count, BUN = blood urea nitrogen, Cr = creatinine, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic pyruvic transaminase

Contribution Statement: BTT conceived the idea. BT and BAF developed the letter of intent (LOI). BTT, BAF, DJ, AR all contributed with the full proposal development, writing, analysis and editing of the manuscript.

Conflict of Interest: All the authors declare that they have no conflict of interest.

References:

1. UNAIDS: AIDSInfo 2015. Available at: http://aidsinfo.unaids.org/ [Accessed on July 3, 2016].
2. Musoke PM, Mudiope P, Barlow-Mosha LN, et al. Growth, immune and viral responses in HIV infected African children receiving highly active antiretroviral therapy: a prospective cohort study. BMC Pediatr 2010;**10**:56.
3. Verweel G, van Rossum AM, Hartwig NG, et al. Treatment with highly active antiretroviral therapy in human immunodeficiency virus type 1-infected children is associated with a sustained effect on growth. Pediatrics 2002;**109**.
4. WHO. Consolidated Guidelines for the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for Public Health Approach. Geneva: WHO; 2013. Available at: http://appswhoint/iris/bitstream/10665/85321/1/9789241505727_engpdf?ua=1 2013.
5. Bacha T, Tilahun B, Worku A. Predictors of treatment failure and time to detection and switching in HIV-infected Ethiopian children receiving first line anti-retroviral therapy. BMC Infectious Diseases 2012;**12**(1):1-8.
6. Workneh N, Girma T, Woldie M. Immunologic and clinical outcomes of children on HAART: a Retrospective cohort analysis at Jimma University specialized hospital. Ethiop J Health Sci 2009;**19**.
7. Peacock-Villada E, Richardson BA, John-Stewart GC. Post-HAART outcomes in pediatric populations: comparison of resource-limited and developed countries. Pediatrics 2011;**127**(2):e423-41.
8. Hammer SM, Saag MS, Schechter M, et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society--USA panel. Topics in HIV medicine : a publication of the International AIDS Society, USA 2006;**14**(3):827-43.
9. Fiscus SA, Cheng B, Crowe SM, et al. HIV-1 viral load assays for resource-limited settings. PLoS medicine 2006;**3**(10):e417.
10. Calmy A, Ford N, Hirschel B, et al. HIV viral load monitoring in resource-limited regions: optional or necessary? Clin Infect Dis 2007;**44**(1):128-34.
11. Schooley RT. Viral load testing in resource-limited settings. Clin Infect Dis 2007;**44**(1):139-40.
12. UNAIDS. 90-90-90 An ambitious treatment target to help end the AIDS epidemic. http://wwwunaidsorg/sites/default/files/media_asset/90-90-90_en_0pdf 2014;**(Accessed on June 20, 2016)**.
13. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach - Second edition. http://wwwwhoint/hiv/pub/arv/arv-2016/en/ June 2016;**(Accessed on June 20, 2016)**.
14. WHO. Antiretroviral therapy for HIV infection in infants and children: towards universal access. Recommendations for Public Health Approach. Geneva: WHO. Available at: http://whqlibdocwhoint/publications/2010/9789241599801_engpdf?ua=1 2010.
15. Institute EHaNR. HIV Related Estimates and Projections for Ethiopia-2012. Addis Ababa, Ethiopia. Available from: http://wwwunaidsorg/sites/default/files/en/media/unaids/contentassets/documents/data-and-analysis/tools/spectrum/Ethiopia2012reportpdf 2012.
16. WHO. Child Growth Standards. Switzerland, Geneva. Available from: http://wwwwhoint/childgrowth/standards/en/
17. Amico KR, Fisher WA, Cornman DH, et al. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. J Acquir Immune Defic Syndr 2006;**42**(4):455-9.
18. Seymour DG, Green M, Vaz FG. Making better decisions: construction of clinical scoring systems by the Spiegelhalter-Knill-Jones approach. BMJ (Clinical research ed) 1990;**300**(6719):223-6.
19. Lynen L, An S, Koole O, et al. An algorithm to optimize viral load testing in HIV-positive patients with suspected first-line antiretroviral therapy failure in Cambodia. J Acquir Immune Defic Syndr 2009;**52**(1):40-8.
20. Westley BP, DeLong AK, Tray CS, et al. Prediction of treatment failure using 2010 World Health Organization Guidelines is associated with high misclassification rates and drug resistance among HIV-infected Cambodian children. Clin Infect Dis 2012;**55**(3):432-40.
21. Vaz P, Augusto O, Bila D, et al. Surveillance of HIV drug resistance in children receiving antiretroviral therapy: a pilot study of the World Health Organization's generic protocol in Maputo, Mozambique. Clin Infect Dis 2012;**54 Suppl 4**:S369-74.

22. Greig JE, du Cros PA, Mills C, et al. Predictors of raised viral load during antiretroviral therapy in patients with and without prior antiretroviral use: a cross-sectional study. PloS one 2013;**8**(8):e71407.
23. Kellerman SE, Ahmed S, Feeley-Summerl T, et al. Beyond prevention of mother-to-child transmission: keeping HIV-exposed and HIV-positive children healthy and alive. AIDS 2013;**27 Suppl 2**:S225-33.
24. Muller AD, Jaspan HB, Myer L, et al. Standard measures are inadequate to monitor pediatric adherence in a resource-limited setting. AIDS and behavior 2011;**15**(2):422-31.

BMJ Open

Improving Treatment of HIV Infected Ethiopian Children through Better Detection of Treatment Failure: Clinical Cohort Design and Baseline Characteristics

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013528.R1
Article Type:	Protocol
Date Submitted by the Author:	27-Oct-2016
Complete List of Authors:	Tadesse, Birkneh Foster, Byron; University of Texas Health Science Center at San Antonio, Pediatrics Jerene, Degu; Management Sciences for Health, Health Programs Group, Addis Ababa, Ethiopia Ruff, Andrea; Johns Hopkins University Bloomberg School of Public Health
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Infectious diseases, Paediatrics
Keywords:	Children, HIV & AIDS < INFECTIOUS DISEASES, Antiretroviral therapy, Treatment Failure

Improving Treatment of HIV Infected Ethiopian Children through Better Detection of Treatment Failure: Clinical Cohort Design and Baseline Characteristics

Birkneh Tilahun Tadesse[1], Byron Alexander Foster[2], Degu Jerene[3], Andrea Ruff [4]

Affiliations: [1]Hawassa University Department of Pediatrics, [2]University of Texas Health Science Center at San Antonio, [3]Management Sciences for Health, Health Programs Group, Addis Ababa, Ethiopia, [4]Johns Hopkins University Bloomberg School of Public Health

Correspondence:

birknehtilahun@gmail.com

Abstract*:*

Introduction: Improving detection of treatment failure among children on first-line antiretroviral therapy (ART) is crucial to improving ART success among HIV infected children. The WHO guidelines for detection of first-line ART failure (ARTF) have low sensitivity and lead to a significant number of misclassifications in the absence of viral load testing. Developing a prediction rule which has a high sensitivity and is not dependent on viral load determination is imperative, especially in resource limited settings where a viral load is not readily available. This is the first study to evaluate the application of a prediction rule for the diagnosis of first-line ARTF in children in resource poor settings.

Methods: The study will assess the performance of the WHO criteria in detecting first-line ARTF and identify important clinical and immunological predictors to build a prediction rule which has a better accuracy than the current WHO guidelines to detect first-line ARTF. A prospective cohort study involving HIV-infected children on or initiating first-line ART will be conducted. From the cohort, distribution of several predictors among ARTF and non-ARTF groups will be analysed; likelihood ratios (LR) will be calculated to fit models of 'ARTF/ no ARTF' regressed on predictors. The Spiegelhalter method will be used to build a prediction rule for predicting first-line ARTF. Virological treatment failure will be taken as the gold standard.

Results: Expected outcomes will include performance of the current WHO guidelines for detection of first-line ARTF in children will be determined. Predictors of virological failure will be identified. A clinical-immunological prediction rule with area under the curve of >0.80 for first-line ARTF will be developed.

Conclusions: The successful completion of this study will allow for better targeting of viral-load testing to those at highest risk in a resource-poor setting and provide clinicians and policy-makers with a practical prediction rule.

Key Words: Children, HIV, ART, Treatment Failure

Strengths and Limitations of the Study:

The study will be the first pediatric scoring system for earlier detection of first line antiretroviral treatment failure in resource limited settings where viral load facility is limited or not accessible.

The prediction rule will be an important tool for targeting viral load measurement in settings where there is limited viral load facility and could be an alternative for earlier detection of treatment failure in settings where viral load is not accessible.

Though different mechanisms have been devised, nonadherence and loss to follow up could be challenges to in the study.

1. Introduction:

As of 2015, 1.8 million children less than 15 years of age were living with HIV among which 1.5 million were living in sub-Saharan Africa [1]. While use of ART has improved clinical and immunologic outcomes of children [2 3], issues of drug resistance and treatment failure have emerged.

Treatment failure is considered when a child who has taken ART for at least six months presents with clinical, immunological or virological evidence of unsuccessful treatment [4]. Time to treatment failure from initiation of ART appears to depend on several factors including genetics, adherence, previous ART exposure, transmitted HIV resistance, and age in children; and it can occur from six months to many years after initiation of therapy[5-7]. In Ethiopia, immunological treatment failure has been reported to be 4-17.5% among adults[8-10], and in children who are on first line ART, 5.9% clinical and 6.7% immunologic treatment failure were reported [5]. In the absence of viral load monitoring, treatment failure is diagnosed using immunologic and clinical parameters following the WHO guidelines [4].

Viral load testing is the gold standard for monitoring ART [11]. However, this test has been expensive and technically demanding [12], functionally prohibiting its use in resource-constrained settings [13 14]. In developing countries, viral load determination is not available as a diagnostic tool for detection of treatment failure in children. As part of the 90-90-90 plan, the current Ethiopian strategy for the testing, treatment and follow up of virological suppression, efforts are being made to make viral load testing available in the regional laboratories [15]. However, in remote areas and in settings where viral load determination is logistically difficult and not easily accessible, the WHO 2016 guidelines emphasize targeting viral load testing using clinical and immunologic parameters [16]. Therefore, in low resources settings, developing a clinical prediction rule which requires the use of fewer resources is important to prioritize viral load testing and target the available limited viral load facility.

Analyzing the clinical and immunological predictors used in the WHO criteria for detection of first-line ART failure (ARTF) in children and sorting them in their order of strength based on likelihood ratios (LR) to predict treatment failure may lead to a more accurate prediction rule for detection of first-line ARTF. Predictors in the WHO criteria identified as strong will be used to build the new prediction rule. In the current study, using a thorough analysis of the possible clinical and immunological predictors, we will be able to identify the strongest parameters that predict first-line ARTF in children. Ultimately, developing an accurate prediction rule for first-line ARTF will minimize the need for viral load determination. Consequently, this will decrease the cost and delay incurred by viral load determination and will decrease the duration of time children stay on a failing regimen and the resulting drug resistance, potentially decreasing the number of AIDS deaths among HIV infected children.

The objectives of this prospective cohort study are therefore three-fold: 1) Determine the performance of the WHO clinical criteria for detection of first-line ARTF in HIV infected Ethiopian children. 2) Identify important clinical and immunological predictors of first-line ARTF among HIV infected Ethiopian children. 3) Develop a practical, acceptable, and accurate clinical prediction rule to improve detection of first-line ARTF among HIV infected Ethiopian children. In this paper, we describe the basic design, recruitment and characteristics of the cohort.

2. Methods:

2.1. Study Design and Inclusion Criteria:

This prospective cohort study includes children under 18 years with a diagnosis of HIV based on WHO guidelines [4 17], who are eligible for or are already on first-line ART. Based on the Ethiopian guideline for ART care in children, all children infected with HIV are initiated on ART. Children who discontinue follow up before six months on ART, are on second line ART, or with ARTF at enrolment are excluded.

The study is being done in the Southern Nations Nationalities and Peoples Region (SNNPR) of Ethiopia, where in 2012, there were 27,200 children below 14 years of age with HIV, 12,258 of whom needed ART [18]. In SNNPR, pediatric ART services are provided in 22 hospitals. We enrolled participants in the first six months of the study at the following six established pediatric ART centers with the highest patient volume in SNNPR: Hawassa University; Yirgalem; Arba Minch; Sodo; Adare; and Hosana hospitals. Data will be recorded on structured and pretested questionnaires (Supplementary S1).

2.2. Baseline data:

On enrollment, through medical chart review and use of structured questionnaires, we collected demographics (age, gender, race) of the child; clinical data (age at diagnosis and ART initiation, history of prevention of mother to child transmission (PMTCT) exposure , nutritional status, WHO clinical stage, mode of transmission, current or past tuberculosis infection and current ART regimen; and laboratory parameters (leukocyte count, thrombocyte count, hemoglobin, CD4 count/percent, viral load).

2.3. Clinical Data:

Patients will be followed every six months (when they come for their routine follow up as recommended by WHO guidelines) for development of new symptoms using a structured symptom checklist. Growth parameters will be assessed using the WHO growth standards [19], along with assessment of age appropriate developmental milestones [4]. Body mass index z-score and other growth parameters are calculated using Epi Info (CDC, USA 2016). Adherence will be assessed using a visual analog scale (VAS) adherence by trained nurse data collectors [20], and self-report on missed doses in the last week and month.

2.4. Immunologic Data:

Complete blood count (CBC) and CD4 count will be checked every six months during their routine follow up as recommended by WHO guidelines; CD4 percentage and percent change in CD4 count from previous record will be determined.

2.5. Virological Data:

We will determine viral load at baseline and every six months for two years. Viral load testing will be done using Abbott Real*Time* HIV-1 m2000rt *m2000sp* machine with calibration done before running every batch of samples. If the child has any acute illness, viral load determination will be deferred until infection resolves [4].

2.6. Patient Care:

During visits, decision to switch ART or other treatments will be made by the clinical staff and will not be directed by the study. Viral load testing results from the study will be reported to the treating clinician. Ethiopia is currently rolling out viral load testing to be done yearly across the country.

2.7. Assessing Performance of WHO guidelines:

In the course of follow up, patients will be followed for any evidence of treatment failure based on the WHO 2013 guidelines [4]. The proportion of children who are correctly identified by WHO guidelines will be checked against viral load.

2.8. WHO 2013 Definition of ARTF [4]:

Clinical: New or recurrent clinical events indicating advanced/severe immunodeficiency (WHO clinical stage 3 and 4 clinical condition with exception of Tuberculosis) after 6 months of effective treatment. *Immunologic*: Children younger than 5 years: persistent CD4 levels below 200 cells/mm^3 or <10%; and, older than 5 years: persistent CD4 levels below 100 cells/mm3. *Virological (Gold Standard):* HIV RNA of more than 1000 copies/ml on at least two measurements after 24 weeks of treatment. When viral load becomes more than 1000 copies/ml, a repeat viral load will be done in three to six months after adherence support is given by trained nurses working in the ART clinics.

2.9. Derivation of scoring system:

In the cohort, subjects will be followed until virological failure occurs or at least until 24 months after initiation of first-line ART. We will identify cases of HIV-infected children who

develop ARTF and age, duration of ART and sex matched controls among those who did not develop ARTF for at least 2 years after treatment commenced. Associations with clinical and laboratory predictors of ARTF will be done using these two groups. Parameters will include CD4 at baseline, CD4 change at 6 month, hemoglobin level, persistent thrombocytopenia, nutritional status at baseline, WHO staging at baseline, failure to thrive/growth faltering, PMTCT history, adherence using VAS, duration on ART, mode of HIV infection and chronic gastroenteritis. Two by two tables will be created for each predictor. Bivariate analysis using chi square test and estimation of LR will be performed. Patients will also be disaggregated by duration on ART during the analysis.

The Spiegelhalter method will be used to build a prediction rule [21]. A prediction rule will be developed using the composite of predictors with adjusted LR of more than 1.5 or less than 0.6. Log natural transformation will be done to the adjusted LR and will be used to build final score of predictors. A receiver operating characteristic (ROC) curve will be created; cut off point on the ROC curve will be determined using the Zweig and Campbell formula as adapted by Lynen et al in Cambodia [22]; assuming the value of false positives to be the same as false negatives. A simplified diagnostic algorithm will be developed. Performance of the new prediction rule in detecting treatment failure will be compared with performance of the WHO guidelines.

2.10. Sample size:

To test the hypothesis that WHO criteria for detection of first-line ARTF have sensitivity of 10% and specificity of 40% [23] with precision of 5%; in a population with ARTF prevalence of 15% [5], a minimum of 960 participants will be required. Identification of important predictors to set up a prediction rule will require multiple sample size calculations for presumed predictors. The largest sample size is derived from history of maternal ART exposure. Considering prevalence of children who have ARTF to be 15% [5], with allocation ratio of cases to controls to be 0.2; and taking proportion of prior ART exposure in controls to be 11.5% based on a study in

Mozambique [5 6 24], at 80% power and 5% level of significance, a sample size of 705 controls and 141 cases is needed to prove that the odds ratio for failing first-line ART is 2. To account for loss to follow up, we will include an additional 10%, therefore a minimum of 1056 children will be enrolled in this study. Matching controls by age and duration of ART will help address the heterogeneity of this population.

2.11. Data Quality Assurance:

Data will be entered electronically to be accessed at the hub institute through Redcap. There will be paper back up at each site. A one-day, intensive training was conducted with HIV clinic nurses on data abstraction, data and sample collection. The training included an overview of the study purpose and design, training in the electronic data entry system, a detailed walk-through of each form and practice sessions using sample charts for data abstraction and entry with direct feedback provided to each participant. Supervision visits will be made every three months to check the quality of clinical and immunological data collection. Refresher trainings for the data collectors and supervisors is given based on the observed deficiencies. Viral load determination is done following standard operating procedures and during each run, three controls are done to control quality.

Historical data on prior clinical staging, maternal ART prophylaxis and the child's age at diagnosis, previous treatment regimens and any changes will be abstracted from the child's chart by the nurses on the research team at time of enrollment.

2.12. Ethics and consent

Only volunteers are included in the study. Each participant receives written information about the study in their own local language and it is ensured that the consent is signed and dated by the parent or caregiver and the physician or ART nurse. Children older than 12 years also give assent. Participants will be given compensation for travel and lunch for visitations done for the purpose of the study and all the costs related to the laboratory tests requested for the purpose

of research are covered by the study. Ethical approvals have been obtained from the IRB of the regional health bureau and Hawassa University, College of Medicine and Health Sciences. Privacy is assured by removing patient identifiers including names and specific address.

3. Results:

From October 2015 through April 2016, 628 children have been enrolled from six different HIV treatment centers across southern Ethiopia.

3.1. Demographic Characteristics:

The mean age of the children at enrollment was 11.1 years and 47.6% of the children were female (Table 1). The median reported income was about $1 per day ($33/month) with 91.2% of caregivers reporting having a job with a wide range of reported occupations (Table 1). Of mothers in the cohort, 27.1% had no formal education, and 14.7% of fathers had no formal education. Only 48.0% of the children in this cohort had both parents alive and 71.1% of the caregivers providing data were HIV positive. In contrast, a minority of caregivers reported that the child had living siblings with HIV (15.0%).

3.2. Clinical Characteristics:

Overall, 81.4% of children were adequately nourished at enrollment by body mass index z-score using WHO criteria (Table 2). The vast majority of children were at WHO Clinical stage 1 at the time of enrollment into the cohort (88.6%), though only 20.6% were at stage 1 at the time of initiation of ART (Table 2) with a mean of 45 months between initiation of ART and enrollment in this study. Almost no children (0.8%) had a viral load done at initiation of their ART. Nearly half (42.6%) of children had some substitution of their ART drugs with 70.3% of

those being due to a national guideline change where replacement of zidovudine or tenofovir for stavudine based regimens was done for all patients in Ethiopia.

About one quarter (24.7%) of children had ever been diagnosed with tuberculosis. Of those, 91.2% had a diagnosis of pulmonary tuberculosis. Only 45.6% of children had their HIV status disclosed to them as per the caregiver, and 23.9% of the caregivers reported being worried about stigma related to HIV (Table 2).

Adherence as assessed with the visual analog scale was high with 94.4% (SD=11.9) reporting adherence when asked over the past month, how often the child had taken their HIV medication. However, when asked how many doses over the past week the child had missed, 15.1% of caregivers reported missing at least 1 dose.

3.3. Laboratory Characteristics:

The enrolled children had a median CD4 count of 741 at enrollment with 3.1% having a value consistent with ARTF using immunologic criteria at the enrollment assessment (Table 3). Other laboratory parameters are shown in Table 3.

4. Discussion:

Currently, the use of viral load as a follow-up tool for the diagnosis of treatment failure is emphasized by the WHO [4]. On the other hand, recent WHO guidelines also recommended use of clinical and immunologic parameters to target the use of viral load testing for treatment monitoring and diagnosis of first line ARTF in settings where access to viral load is scarce [16]. In most resource limited settings and in communities living in remote places, the use of viral load as a point of care monitoring and diagnostic tool is difficult given the technical requirements of the procedure, the need for an efficient sample transport system, and the limited availability of the required reagents as is the case with the regional laboratory where the current cohort is located.

Performance of the WHO guidelines for detection of first line ARTF in children has been inadequately evaluated so far, and no local scoring system has been developed for use in HIV infected children who are on first line ART. Children are assumed to have different predictors of treatment failure because of the differences in mode of transmission, faster disease progression and previous exposure to ART for purpose of PMTCT [25 26]. Analysis of the predictors of first line ARTF and assessing the level of accurateness of the WHO criteria is of paramount importance for the development of such tools.

To our knowledge, this is the largest prospective, pediatric HIV cohort in Africa designed to identify a prediction rule using a composite of clinical and immunologic parameters to prioritize viral load testing for children on first line ART. Its prospective design allows for better data completeness and quality. However, challenges to the current cohort are multifaceted including poor adherence to and defaulting from care and treatment. Though we have devised strategies including better counselling and tracking systems to decrease the defaulter rate, it still remains to be a challenge because of the large geographic area served by each ART center.

Additionally, for information to be collected retrospectively including baseline laboratory values, clinical data and previous adherence, incomplete records pose a threat to data completeness and validity. The issue of adherence is particularly significant as changing to a second-line regimen due to poor adherence would likely not improve mortality and would potentially have consequences for emergence of resistant strains. While our method of using a visual analog scale for adherence has been validated in multiple settings, it has been found to overestimate adherence when compared with other methods such as automated assessment of bottle opening [27]. One potential adjunct to this study would be resistance testing of individual viral samples to assess resistance versus non-adherence though this is currently not part of the study protocol due to funding and resource constraints.

The prediction rule to be developed will be used in resource poor settings, especially remote areas with limited access to viral load testing for prioritization and treatment decision purposes. It will potentially decrease the HIV related mortality in children who would otherwise remain on a failing regimen because of lack of access to viral load testing. Additionally, it will help to investigate the effectiveness of ART in children. The potential of the prediction rule for over-diagnosis of ARTF and unnecessary switching will also be examined.

Funding

This project is supported by International AIDS Society, CIPHER Pediatric HIV Research Grant Program (Grant ID: 307-TAD).

Acknowledgment

We would like to thank Hawassa University for supporting the project. We also would like to acknowledge all the data collectors, children and parents for taking their time to be involved in the research.

Table 1: Baseline demographic characteristics of enrolled patients across all six sites

Age at enrollment, years, mean (SD)	11.1 (3.6)
Sex, % female (n)	47.6% (296)
Age at Diagnosis, years, mean (SD)	4.7 (3.7)
Family size, mean (SD)	5.5 (19.9)
Monthly income in US dollars, median (IQR)	33 (19-70)
Ethnicity, % of reporting (n)	
Amhara	19.2% (120)
Oromo	12.5% (78)
Sidama	12.9% (81)
Wolaita	24.6% (154)
Tigray	1.9% (12)
Gurage	4.5% (28)
Silte	0.8% (5)
Gamo	6.9% (43)
Other	16.8% (105)
Occupation of caregivers, % of reporting (n)	
Farmers	10.1% (63)
Government employees	19.1% (119)
Merchants	20.4% (127)
Daily Laborers	21.8% (136)
Other	19.9% (124)
Jobless	8.8% (55)
Mother's education, % of reporting (n)	
No school	27.1% (168)
Attended some school	23.4% (145)
Completed primary education	22.7% (141)
Completed secondary education	12.4% (77)
Completed tertiary education	10.8% (67)
Read and write, no school	3.5% (22)
Father's education, % of reporting (n)	
No school	14.7% (89)
Attended some school	17.1% (103)
Completed primary education	23.0% (139)
Completed secondary education	23.5% (142)
Completed tertiary education	19.0% (115)
Read and write, no school	2.6% (16)
Child lives with, % reporting (n)	
Mother and Father	28.2% (176)
Mother only	35.7% (223)
Father only	12.2% (76)
Other relative	19.1% (119)
Other	4.8% (30)
Parents alive, % both alive	48.0% (299)
Caregiver HIV positive, % yes	71.1% (443)
Caregiver has HIV healthcare, % yes	71.3% (440)
Caregiver on ART, % yes	69.7% (434)
Other siblings with HIV, % yes	15.0% (94)
Other siblings who died of HIV, % yes	9.4% (59)

* Not all percentages reflect total of 628 subjects as some with missing or unknown values, ART = anti-retroviral treatment, SD = standard deviation

Table 2. Clinical characteristics of the cohort of HIV positive children at enrollment

Height, z-score, mean (SD)	-1.02 (1.45)
BMI, z-score, mean (SD)	-0.90 (1.26)
% malnourished (BMI z-score <2)	17.4 (100)
% adequate nourishment (-2>BMI z-score <2)	81.4 (468)
% obese (BMI z-score >2)	1.2 (7)
Child took ARV prophylaxis after birth, % yes (n)	1.1% (7)
Clinical Stage at initiation of ART, % of total (n)	
Stage 1	20.6% (129)
Stage 2	35.1% (220)
Stage 3	37.1% (232)
Stage 4	7.2% (45)
Clinical stage at enrollment, % of total (n)	
Stage 1	88.6% (552)
Stage 2	6.9% (43)
Stage 3	4.0% (25)
Stage 4	0.5% (3)
Viral load at initiation, % yes (n)	0.8% (5)
Substitution of ART drugs, % yes (n)	42.6% (253)
Reason for substitution, % of those with substitution (n)	
Toxicity or side effects	14.7% (39)
Tuberculosis co-infection	0.8% (2)
National Guideline Change	70.3% (187)
Total ART duration, months, mean (SD)	45.4 (35.8)
ART Regimen, % of total (n)	
AZT, 3TC, EFV	13.5% (84)
AZT, 3TC, NVP	45.9% (286)
D4T, 3TC, EFV	5.5% (34)
D4T, 3TC, NVP	20.4% (127)
TDF, 3TC, EFV/NVP	9.6% (60)
AZT,D4T, 3TC, PI	0.3% (2)
ABC, 3TC, NVP/EFV/PI	4.8% (30)
Adherence of caregiver if taking ART over past month by VAS, mean (SD)	93.7% (12.3)
Adherence of child reported by caregiver over past month by VAS, mean (SD)	94.4% (11.9)
Tuberculosis, % ever diagnosed (n)	24.7% (154)
Type of TB diagnosed, % with ever TB diagnosis (n)	
Pulmonary	91.2% (134)
Disseminated	3.4% (5)
Lymph node	4.8% (7)
Currently prescribed PCP prophylaxis, % yes (n)	32.6% (202)
Caregiver worried about stigma, % yes (n)	23.9% (150)
Child's HIV status disclosed to them, % yes (n)	45.6% (283)

SD = standard deviation, BMI = body mass index, ART = anti-retroviral treatment, TB = tuberculosis, PCP = pneumocystis jirovecii, ARV= Antiretroviruses

CD4 Count at baseline, median (IQR)	741 (517, 1014)
CD4 Count, % above treatment failure threshold by age (n)	
Less than 5 years old, CD4 ≥200, % of tested (n)	100 (32)
5 years or older, CD4 ≥100, % of tested (n)	96.9 (558)
WBC, median (IQR)	6.3 (4.9, 8.0)
Total lymphocyte count, median (IQR)	3.4 (2.3, 36.1)
Hemoglobin, median (IQR)	13.4 (12.4, 14.6)
Platelet count, median (IQR)	314 (253, 377)
Urinalysis, % normal (n)	95.7% (517)
BUN, median (IQR)	7.5 (5, 11.5)
Cr, median (IQR)	0.52 (0.40, 0.70)
SGOT, median (IQR)	32 (25, 39)
SGPT, median (IQR)	22 (16, 31)

Table 3. Baseline laboratory characteristics at enrollment of the cohort of HIV positive children

IQR = interquartile range, WBC = white blood cell count, BUN = blood urea nitrogen, Cr = creatinine, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic pyruvic transaminase

Contribution Statement: BTT conceived the idea. BT and BAF developed the letter of intent (LOI). BTT, BAF, DJ, AR all contributed with the full proposal development, writing, analysis and editing of the manuscript.

Conflict of Interest: All the authors declare that they have no conflict of interest.

References:

1. Dagnew M, Yismaw G, Gizachew M, et al. Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. *BMC research notes* 2013;6:283. doi: 10.1186/1756-0500-6-283
2. Musoke PM, Mudiope P, Barlow-Mosha LN, et al. Growth, immune and viral responses in HIV infected African children receiving highly active antiretroviral therapy: a prospective cohort study. *BMC Pediatr* 2010;10:56. doi: 10.1186/1471-2431-10-56 [published Online First: 2010/08/10]
3. Verweel G, van Rossum AM, Hartwig NG, et al. Treatment with highly active antiretroviral therapy in human immunodeficiency virus type 1-infected children is associated with a sustained effect on growth. *Pediatrics* 2002;109 doi: 10.1542/peds.109.2.e25
4. WHO. Consolidated Guidelines for the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for Public Health Approach. Geneva: WHO; 2013. *Available at: http://appswhoint/iris/bitstream/10665/85321/1/9789241505727_engpdf?ua=1* 2013
5. Bacha T, Tilahun B, Worku A. Predictors of treatment failure and time to detection and switching in HIV-infected Ethiopian children receiving first line anti-retroviral therapy. *BMC Infectious Diseases* 2012;12(1):1-8. doi: 10.1186/1471-2334-12-197
6. Workneh N, Girma T, Woldie M. Immunologic and clinical outcomes of children on HAART: a Retrospective cohort analysis at Jimma University specialized hospital. *Ethiop J Health Sci* 2009;19
7. Peacock-Villada E, Richardson BA, John-Stewart GC. Post-HAART outcomes in pediatric populations: comparison of resource-limited and developed countries. *Pediatrics* 2011;127(2):e423-41. doi: 10.1542/peds.2009-2701 [published Online First: 2011/01/26]
8. Ayalew MB, Kumilachew D, Belay A, et al. First-line antiretroviral treatment failure and associated factors in HIV patients at the University of Gondar Teaching Hospital, Gondar, Northwest Ethiopia. *HIV/AIDS (Auckland, NZ)* 2016;8:141-6. doi: 10.2147/hiv.s112048 [published Online First: 2016/09/14]
9. Teshome W, Tefera A. Detection of immunological treatment failure among HIV infected patients in Ethiopia: a retrospective cohort study. *BMC immunology* 2015;16:55. doi: 10.1186/s12865-015-0120-1 [published Online First: 2015/09/18]
10. Teshome Yimer Y, Yalew AW. Magnitude and Predictors of Anti-Retroviral Treatment (ART) Failure in Private Health Facilities in Addis Ababa, Ethiopia. *PloS one* 2015;10(5):e0126026. doi: 10.1371/journal.pone.0126026 [published Online First: 2015/05/07]
11. Hammer SM, Saag MS, Schechter M, et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society--USA panel. *Topics in HIV medicine : a publication of the International AIDS Society, USA* 2006;14(3):827-43. [published Online First: 2006/10/05]
12. Fiscus SA, Cheng B, Crowe SM, et al. HIV-1 viral load assays for resource-limited settings. *PLoS medicine* 2006;3(10):e417. doi: 10.1371/journal.pmed.0030417 [published Online First: 2006/10/13]
13. Calmy A, Ford N, Hirschel B, et al. HIV viral load monitoring in resource-limited regions: optional or necessary? *Clin Infect Dis* 2007;44(1):128-34. doi: 10.1086/510073 [published Online First: 2006/12/05]
14. Schooley RT. Viral load testing in resource-limited settings. *Clin Infect Dis* 2007;44(1):139-40. doi: 10.1086/510090 [published Online First: 2006/12/05]
15. UNAIDS. 90-90-90 An ambitious treatment target to help end the AIDS epidemic. *http://wwwunaidsorg/sites/default/files/media_asset/90-90-90_en_0pdf* 2014;(Accessed on June 20, 2016)
16. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach - Second edition. *http://wwwwhoint/hiv/pub/arv/arv-2016/en/* June 2016;(Accessed on June 20, 2016)
17. WHO. Antiretroviral therapy for HIV infection in infants and children: towards universal access. Recommendations for Public Health Approach. Geneva: WHO. *Available at: http://whqlibdocwhoint/publications/2010/9789241599801_engpdf?ua=1* 2010
18. Institute EHaNR. HIV Related Estimates and Projections for Ethiopia-2012. Addis Ababa, Ethiopia. *Available from:*

http://wwwunaidsorg/sites/default/files/en/media/unaids/contentassets/documents/data-and-analysis/tools/spectrum/Ethiopia2012reportpdf 2012

19. WHO. Child Growth Standards. Switzerland, Geneva. *Available from:* http://wwwwhoint/childgrowth/standards/en/
20. Amico KR, Fisher WA, Cornman DH, et al. Visual analog scale of ART adherence: association with 3-day self-report and adherence barriers. *J Acquir Immune Defic Syndr* 2006;42(4):455-9. doi: 10.1097/01.qai.0000225020.73760.c2 [published Online First: 2006/07/01]
21. Seymour DG, Green M, Vaz FG. Making better decisions: construction of clinical scoring systems by the Spiegelhalter-Knill-Jones approach. *BMJ (Clinical research ed)* 1990;300(6719):223-6. [published Online First: 1990/01/27]
22. Lynen L, An S, Koole O, et al. An algorithm to optimize viral load testing in HIV-positive patients with suspected first-line antiretroviral therapy failure in Cambodia. *J Acquir Immune Defic Syndr* 2009;52(1):40-8. doi: 10.1097/QAI.0b013e3181af6705 [published Online First: 2009/06/25]
23. Westley BP, DeLong AK, Tray CS, et al. Prediction of treatment failure using 2010 World Health Organization Guidelines is associated with high misclassification rates and drug resistance among HIV-infected Cambodian children. *Clin Infect Dis* 2012;55(3):432-40. doi: 10.1093/cid/cis433 [published Online First: 2012/04/28]
24. Vaz P, Augusto O, Bila D, et al. Surveillance of HIV drug resistance in children receiving antiretroviral therapy: a pilot study of the World Health Organization's generic protocol in Maputo, Mozambique. *Clin Infect Dis* 2012;54 Suppl 4:S369-74. doi: 10.1093/cid/cis006 [published Online First: 2012/05/11]
25. Greig JE, du Cros PA, Mills C, et al. Predictors of raised viral load during antiretroviral therapy in patients with and without prior antiretroviral use: a cross-sectional study. *PloS one* 2013;8(8):e71407. doi: 10.1371/journal.pone.0071407 [published Online First: 2013/08/24]
26. Kellerman SE, Ahmed S, Feeley-Summerl T, et al. Beyond prevention of mother-to-child transmission: keeping HIV-exposed and HIV-positive children healthy and alive. *AIDS* 2013;27 Suppl 2:S225-33. doi: 10.1097/QAD.0000000000000107
27. Muller AD, Jaspan HB, Myer L, et al. Standard measures are inadequate to monitor pediatric adherence in a resource-limited setting. *AIDS and behavior* 2011;15(2):422-31. doi: 10.1007/s10461-010-9825-6 [published Online First: 2010/10/19]

Enrolment baseline

Study ID (Initials of the hospital and MRN) _____

Study ID (Hospital Initials and MRN) _____

Date of enrollment _____
(YYYY-MM-DD)

Upload the patient's consent form

CONTACT INFORMATION & DEMOGRAPHIC DATA

First Name _____

Father's Name _____

Address (Region, Wereda, Kebele)

_____

Phone number _____

Date of birth _____

Child's age at enrollment (years) _____
(age at enrollment in years)

What is the family size? _____

What is the average monthy income for the family? _____

Does the family have external support?
- ◯ Yes
- ◯ No

If the family has external support, what source is it?
- ◯ Government
- ◯ NGO
- ◯ Charity
- ◯ Other

What is the occupation of the caregivers?
- ◯ Farmers
- ◯ Jobless
- ◯ Governement Employees
- ◯ Merchants
- ◯ Daily Laborors
- ◯ Other

What is the educational status of the mother?
- ◯ Didn't attend School
- ◯ Attended some school, din't complete primary
- ◯ Read/Write, no formal school
- ◯ Completed Primary Education
- ◯ Competed Secondary Education
- ◯ Completed Tertiary Education

What is the educational status of the Father?
- ◯ Didn't attend School
- ◯ Attended some school, din't complete primary
- ◯ Read/Write, no formal school
- ◯ Completed Primary Education
- ◯ Competed Secondary Education
- ◯ Completed Tertiary Education

Are you worried about stigma and dicrimination at your locality because of HIV?
◯ Yes
◯ No

Are there other siblings who have HIV?
◯ Yes
◯ No

Are there other siblings who died of HIV?
◯ Yes
◯ No

Date of HIV diagnosis of the child
_____

Was DNA PCR done?
◯ Yes
◯ No

What was the DNA PCR result?
◯ Positive
◯ Negative
◯ Unknown

What was the mode of feeding during early childhood?
◯ Exclusive breast feeding for 6 months then complementary
◯ Exclusive Formula Feeding
◯ Mixed
◯ I don't remember/don't know

Ethnicity

◯ Amhara ◯ Oromo ◯ Sidama ◯ Wolaita ◯ Tigray ◯ Gurage ◯ Silte ◯ Gamo ◯ Other

If other for the above question, describe.
_____

What is the sex of the child?
◯ Female
◯ Male

CLINICAL CONDITION

Height
_____
(height/length in cm (xxx,x cm))

Weight
_____
(xx,x kilograms)

Mid upper arm circumference
_____

BMI
_____

Head Circumference (up to 5 years)
_____
(xx,x cm)

Pitting Edema
◯ Yes
◯ No

Hair change (reddish/greyish, sparse, plaquable)
◯ Yes
◯ No

Visible severe wasting
◯ Yes
◯ No

Kwashiorkor Dermatosis
◯ Yes
◯ No

Did the child take ART prophylaxis immediately after birth?
◯ Yes
◯ No
◯ Unknown

What was given? _____

For how long did s/he take the prophylaxis? _____

CHILD ART INFO

When was ART initiated (date)? _____

What was the age of the child at ART initiation? _____

What is the ART regimen?
◯ AZT, 3TC, EFV
◯ AZT, 3TC, NVP
◯ D4T, 3TC, EFV
◯ D4T, 3TC, NVP
◯ TDF, 3TC, EFV/NVP
◯ AZT/D4T, 3TC, PI
◯ ABC, 3TC, NVP/EFV/PI

WHO stage at ART start (from record/card)
◯ WHO Stage 1
◯ WHO Stage 2
◯ WHO Stage 3
◯ WHO Stage 4

What was the CD4 Percent at Initiation ? _____

What was the absolute CD4 count at initiation? _____

Was Viral Load done at Initiation or before?
◯ Yes
◯ No

If Viral Load was done, what was the value? _____

How long after diagnosis was ART initiated? _____

Duration on ART (in months) _____

Was there any substitution of ART drugs?
◯ Yes
◯ No

What was the substitution that was done? _____

What was the reason for substitution?
◯ Toxicity and drug side effects
◯ Tuberclosis Co-infection
◯ National Guideline Change
◯ Other

If other for the above question, describe. _____

CAREGIVER INFO

Who is the caregiver for the child/ who is the child living with?
- ◯ Mother and Father
- ◯ Mother
- ◯ Father
- ◯ Relative or Sibling
- ◯ Foster care
- ◯ Grandmother
- ◯ Other

Describe if 'other' for the above question: _____

Are biological parents alive?
- ◯ Yes both alive
- ◯ Yes- only mother alive
- ◯ Yes- only father alive
- ◯ No, both died

Is the caregiver HIV positive?
- ◯ Yes
- ◯ No

Are caregivers on HIV care follow up?
- ◯ Yes
- ◯ No
- ◯ Unknown

Is caregiver on ART?
- ◯ Yes
- ◯ No

What is the ART regimen that the caregiver is taking? _____

Was the mother taking prophylaxis during pregnancy?
- ◯ Yes
- ◯ No
- ◯ Unknown

If yes, what was she taking (please check with the mothers records)? _____

What is the level of adherence of the caregiver to care and treatment?
0% means they have taken none
50% means they have taken half
100% means they have taken every single dose

0% 50% 100%

(Place a mark on the scale above)

Ask the patient: over the past month, how often has your child taken their HIV medication?

0% means they have taken none
50% means they have taken half
100% means they have taken every single dose

0% 50% 100%

(Place a mark on the scale above)

How many doses of their HIV medication has your child missed over the past week? _____

Is the child currently prescribed PCP prophylaxis (bactrim/dapson)?
- ◯ Yes
- ◯ No

Over the past month, how often have you taken your PCP prophylaxis?

0% means they have taken none
50% means they have taken half
100% means they have taken every single dose

0% 50% 100%

(Place a mark on the scale above)

How many doses of their PCP prophylaxis has your child missed over the last week? _____

If caregiver is on ART, was he/she diagnosed to have treatment failure at some point? (please check records if needed)

- ◯ Yes
- ◯ No
- ◯ Unknown

TUBERCLOSIS INFORMATION

Was the child diagnosed for TB previously

- ◯ Yes
- ◯ No

What was the mode diagnosis of TB?

- ◯ Symptoms only
- ◯ Symptoms and CXR
- ◯ CXR only
- ◯ Sputum positive
- ◯ gene-expert
- ◯ other

Has the child taken INH prophylaxis?

- ◯ Yes
- ◯ No

Was there TB treatment before initation of ART?

- ◯ Yes
- ◯ No

What type of TB was diagosed?

- ◯ Pulmonary
- ◯ Disseminated TB
- ◯ Lymphnode TB
- ◯ CNS TB
- ◯ Pleural TB
- ◯ Other

HOSPITALIZATION

Was the child hospitalized in the past?

- ◯ Yes
- ◯ No

If s/he was hospitalized, what was the reason for hospitalization? (be specific) _____

If s/he was hospitalized, How many times? _____

DRUG SIDE-EFFECTS (only document yes, if the symptoms appear after initiation of ART and clinicans ascribe it to the drugs)

Skin Rash	◯ Yes ◯ No
Nausea or vomiting	◯ Yes ◯ No
feeling generally unwell or extremely tired	◯ Yes ◯ No
muscle or joint ache	◯ Yes ◯ No
swelling of the eye, lips, mouth or face	◯ Yes ◯ No
yellowing of the skin or eyes	◯ Yes ◯ No
Dark urine	◯ Yes ◯ No
Lipodystrophy	◯ Yes ◯ No
Lipid abnormalities and the heart	◯ Yes ◯ No
Anemia	◯ Yes ◯ No
Appetite loss	◯ Yes ◯ No
stomach pain	◯ Yes ◯ No
bloating	◯ Yes ◯ No
Insulin resistance and diabetes	◯ Yes ◯ No
Insomnia	◯ Yes ◯ No
Dizziness, confusion, and sleeping problems	◯ Yes ◯ No
Kidney damage (nephrotoxicity)	◯ Yes ◯ No
Lactic acidiosis	◯ Yes ◯ No
Liver damage (hepatotoxicity	◯ Yes ◯ No
Pancreas damage (pancreatitis)	◯ Yes ◯ No

Peripheral neuropathy (nerve damage)	◯ Yes ◯ No
Diarrhoea	◯ Yes ◯ No
Headache	◯ Yes ◯ No
osteoporis, osteopenia	◯ Yes ◯ No
Thrombocytopenia	◯ Yes ◯ No

OPPORTUNISTIC INFECTIONS (for this section, use the chart record for the past 6 months as a reference - indicate if the patient has had any of the effects or symptoms at any time in the past 6 months)

Asymptomatic	◯ Yes ◯ No
Unexplained persistent hepatosplenomegaly	◯ Yes ◯ No
Persistent generalised lymphadenopathy	◯ Yes ◯ No
Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, sinusitis, tonsillitis)	◯ Yes ◯ No
Herpes zoster	◯ Yes ◯ No
Linear gingival erythema	◯ Yes ◯ No
Recurrent oral ulceration	◯ Yes ◯ No
Papular pruritic eruption	◯ Yes ◯ No
Fungal nail infections	◯ Yes ◯ No
Extensive wart virus infection	◯ Yes ◯ No
Extensive molluscum contagiosum	◯ Yes ◯ No
Unexplained persistent parotid enlargement	◯ Yes ◯ No
Unexplained moderate malnutrition not adequately responding to standard therapy	◯ Yes ◯ No
Unexplained persistent diarrhea (14 days or more)	◯ Yes ◯ No

Unexplained persistent fever (above 37.5C, intermittent or constant, for longer than one 1 month)	◯ Yes ◯ No
Persistent oral candidiasis (after first 6 weeks of life)	◯ Yes ◯ No
Oral hairy leukoplakia	◯ Yes ◯ No
Lymph node tuberculosis	◯ True ◯ False
Pulmonary tuberculosis	◯ True ◯ False
Severe recurrent bacterial pneumonia	◯ True ◯ False
Acute necrotizing ulcerative gingivitis or periodontitis	◯ True ◯ False
Unexplained anemia	◯ True ◯ False
Symptomatic lymphoid interstitial pneumonitis (defined as a syndrome of fever, cough, and dyspnea, with bibasilar pulmonary infiltrates consisting of dense interstitial accumulations of lymphocytes and plasma cells)	◯ True ◯ False
Chronic HIV-associated lung disease, including bronchiectasis	◯ True ◯ False
Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy	◯ True ◯ False
Pneumocystis (jirovecii) pneumonia	◯ True ◯ False
Recurrent severe bacterial infections (such as empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia)	◯ True ◯ False
Chronic herpes simplex infection (orolabial or cutaneous of more than 1 month's duration or visceral at any site)	◯ True ◯ False
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)	◯ True ◯ False
Extrapulmonary tuberculosis	◯ True ◯ False
Kaposi sarcoma	◯ True ◯ False
Cytomegalovirus infection (retinitis or infection of other organs with onset at age more than 1 month)	◯ True ◯ False

Central nervous system toxoplasmosis (after the neonatal period)	◯ True ◯ False
HIV encephalopathy	◯ True ◯ False
Extrapulmonary cryptococcosis, including meningitis	◯ True ◯ False
Disseminated nontuberculous mycobacterial infection	◯ True ◯ False
Progressive multifocal leukoencephalopathy	◯ True ◯ False
Chronic cryptosporidiosis (with diarrhoea)	◯ True ◯ False
Chronic isosporiasis	◯ True ◯ False
Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis, penicilliosis)	◯ True ◯ False
Lymphoma (cerebral or B-cell non-Hodgkin)	◯ True ◯ False
HIV-associated nephropathy or cardiomyopathy	◯ True ◯ False

BASELINE LABS

Viral Load_Baseline	_____
Absolute CD4 count at Baseline	_____ (The CD4 at Baseline)
CD4 percent at baseline	_____
CD8 count	_____
CD4/CD8 Ratio	_____
White Cell Count	_____
Total Lymphocyte Count	_____
Hemoglobin	_____
Platelet Count	_____
Hepatitis B virus surface antigen	◯ Positive ◯ Negative
HCV antibody?	◯ Positive ◯ Negative
Stool Examination	_____

Urine Analysis	◯ Normal ◯ Hematuria (RBC positive) ◯ Trace proteinuria ◯ Proteinuria +1 and above ◯ Pyuria seen
Creatinine	_____
BUN	_____
SGOT	_____
SGPT	_____
WHO Stage	◯ WHO stage 1 ◯ WHO stage 2 ◯ WHO stage 3 ◯ WHO stage 4

DISCLOSURE

Has the child's HIV status been disclosed to them?	◯ Yes ◯ No
Has the caregiver disclosed their HIV status to anyone?	◯ Yes ◯ No
Whom did the caregiver disclose to?	◯ partner ◯ relatives ◯ their child