Immunodeficiency Among HIV-positive Children in the Setting of Evolving Antiretroviral Therapy Initiation Policies: 6 Years of Pediatric CD4 Data from Zambézia Province, Mozambique

Final Report

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Executive Summary

Historically, antiretroviral therapy (ART) initiation was primarily based on immunologic (i.e., CD4 count [cells/mm3]) criteria, but this approach has been replaced with "Test and Start", wherein all HIV-positive persons are offered ART regardless of immune status. Evolving pediatric ART initiation policies have gradually been implemented in Zambézia Province, Mozambique. This evaluation describes the degree of and risk factors for immunodeficiency among children at the time of enrollment into HIV care and at ART start during a period of evolving ART initiation policies.

This retrospective evaluation used routinely collected data from electronic medical records of HIV-positive children enrolled in HIV services in Zambézia from 2012-2018. All children (5-14 years) with known enrollment dates, ART initiation dates, and CD4 data were included. Children <5 years of age were excluded due to a paucity of CD4 percentage data (71% missing). ART initiation policy periods correspond to implementation of evolving guidelines: in Period 1 (2012-2016), ART was recommended for those having a CD4 <350; during Period 2 (2016-2017), the CD4 threshold increased to <500, and implementation of Test and Start began in selected districts; in Period 3 (2017-2018), Test and Start was implemented province-wide. CD4 at enrollment was defined as the CD4 nearest enrollment, within two months before or after the enrollment date. CD4 at ART initiation was defined as the CD4 nearest the ART initiation date within the six months prior to and two months after ART initiation. These definitions were not mutually exclusive; CD4 at enrollment and at ART initiation could be the same. Severe immunodeficiency was defined as CD4 <200. For analysis of viral suppression among children starting ART, viral load data used for routine monitoring were considered only after August 2016 when routine viral load testing was introduced. Viral suppression was defined as any viral load result with less than 1,000 copies/ml.

Descriptive statistics were used to summarize temporal trends in CD4 and the proportion of children with severe immunodeficiency at the time of enrollment and ART initiation. Univariate and multivariable analyses were used to identify associations with severe immunodeficiency. Descriptive statistics were used to describe prevalence of ART initiation among ART eligible children. Univariate and multivariable analyses were used to measure associations with viral suppression; however, among the children who initiated ART *and* had valid CD4 data, 90% lacked viral load data, thereby limiting generalizability such that analyses of viral suppression stratified by degree of immunosuppression are not reported.

Among children 5-14 years old, 1,815/2,537 (72%) had CD4 data at enrollment and 1,922/2,569 (75%) had CD4 data at ART initiation. Median CD4 at enrollment and at ART initiation were similar across policy periods (495 vs. 477 in Period 1, 568 vs. 555 in Period 2, and 504 vs. 529 in Period 3). However, the proportion of children with severe immunodeficiency decreased over time (by calendar year and across policy periods). Severe immunodeficiency at enrollment decreased from 28% in 2013 to 15% in 2018 ($p< 0.001$ for trend) and at ART initiation decreased from 33% in 2013 to 15% in 2018 ($p< 0.001$ for trend). The proportion of children with severe immunodeficiency at enrollment was 20% in Period 1, 19% in Period 2, and 16% in Period 3 ($p=0.120$ for trend) and at ART initiation was 21% in Period 1, 17% in Period 2, and 15% in Period 3 ($p=0.003$ for trend). Children who initiated ART in Period 3 were significantly less likely

to have severe immunodeficiency at ART start compared to those in Period 1 (OR=0.67; 95%CI: 0.51-0.88). Older age (per year) was associated with severe immunodeficiency at enrollment (OR=1.13; 95%CI: 1.06-1.20) and at ART initiation (OR=1.14; 95%CI: 1.08-1.21). Those cared for at district capital facilities (*sedes*) were significantly less likely to have severe immunodeficiency than those at peripheral health facilities (non-*sedes*), at both enrollment (OR=0.72; 95%CI: 0.52-0.99) and ART initiation (OR=0.71; 95%CI: 0.51-0.97). Among the 2,526 children 5-14 years old for whom there was a valid CD4 count, we found that 38% (551/1,461) were eligible for ART during Period 1, 44% (147/332) during Period 2, and all were eligible via Test and Start in Period 3. Among those eligible, 10%, 4% and 1% did not initiate ART during Periods 1, 2 and 3, respectively. Among the subset of children who initiated ART and had valid CD4 data, 90% lacked viral load results within 6-12 months after ART initiation; however, among the subset of children with viral load results available, 113/189 (60%) had achieved viral suppression.

In conclusion, in this evaluation we described the degree of immunodeficiency and identified risk factors for enrolling in HIV care and starting ART with severe immunodeficiency among children during a period of evolving ART initiation policies in Zambézia Province, Mozambique. Our main findings were that in the setting of progressively more inclusive pediatric ART initiation guidelines, there were decreasing proportions of children with severe immunodeficiency at ART initiation, and that older children and those enrolled at peripheral health facilities were more likely to be severely immunodeficient. These findings suggest that more inclusive pediatric ART initiation guidelines have contributed to decreased prevalence of severe immunodeficiency at ART initiation, but additional resources and interventions are needed to further bolster services and ensure earlier diagnosis and treatment at lower-resourced clinics.

Project Background

The HIV pandemic continues to disproportionately affect vulnerable populations in resource-constrained settings. As of 2019, 38 million people were living with HIV globally, and more than two-thirds of those affected were in sub-Saharan Africa (SSA). Despite significant investments and widespread implementation of prevention of mother-to-child transmission (PMTCT) services, there are still 160,000 new pediatric HIV infections worldwide every year.[1] As is the case for HIV-positive adults, early initiation of antiretroviral therapy (ART) – well before progression to severe immunodeficiency/AIDS – is essential to ensure optimal health outcomes for HIV-positive children.[2-12]

Pediatric ART initiation guidelines have evolved over time in response to emerging evidence for improved outcomes with earlier ART initiation. Historically, decisions to initiate ART were based on clinical (i.e., World Health Organization [WHO] clinical stage 3 or 4) or immunologic (i.e., based on CD4+ T-cell count [CD4 count] cutoffs) criteria. In 2008, WHO updated guidelines to recommend ART initiation for all HIV-positive infants under 1 year of age, regardless of clinical or immunologic criteria.[13] In 2010, WHO expanded their age-based criteria to recommend ART initiation for all HIV-positive children less than 2 years of age,[14] and in 2013 they began recommending ART initiation for those less 5 years of age.[15] Aligned with adult

guidelines, ART initiation for children 5 years or older continued to be based on clinical and immunologic criteria until 2016.[16] Since 2016, WHO has recommended ART initiation for all HIV-positive persons (including children), regardless of clinical, immunologic, or age criteria, a strategy referred to as "Test and Start".[17] In Mozambique – a SSA country with an estimated HIV prevalence of 13% and a vertical transmission rate of approximately 5% – implementation of Test and Start began in a phased approach staring in July 2016.[18-20]

With support from the United States Government Centers for Disease Control and Prevention (CDC) and the President's Emergency Plan for AIDS Relief (PEPFAR), Vanderbilt University Medical Center (VUMC), through its subsidiary Friends in Global Health (FGH), has provided technical assistance in Zambézia Province, Mozambique since 2006. The Test and Start strategy commenced in Zambézia Province, starting first in the provincial capital of Quelimane in August 2016, followed by other large districts in April 2017, smaller districts as of November 2017, and all VUMC/FGH-supported districts as of February 2018.

Evaluation Purpose and Questions

ART program maturation has been accompanied by decreases in HIV-related immunodeficiency in global cohorts of children starting ART prior to 2013,[21,22] and more recently for adults in Mozambique,[23] but less is known about HIV-positive children receiving HIV care and treatment in Mozambique in recent years. The primary objectives of this evaluation were to describe the degree of immunodeficiency and to identify risk factors for enrolling in HIV care and starting ART with severe immunodeficiency among children during a period of evolving ART initiation policies in Zambézia Province, Mozambique. Secondary objectives were to: 1) report the proportion of HIV-positive children eligible for ART who started ART during each policy period; and, 2) describe rates of HIV viral suppression stratified by whether or not HIV-positive children had severe immunodeficiency at time of ART initiation.

Evaluation Design, Methods, and Limitations

Data Sources

An electronic Open Medical Record System (OpenMRS)™ is utilized at VUMC/FGH-supported health facilities to facilitate patient care and program monitoring and evaluation activities. Routinely collected, de-identified data were extracted from the OpenMRS database for this retrospective cohort analysis.

Study Settings

Children receiving HIV care and treatment at 107 health facilities in nine districts of Zambézia Province were included in this evaluation. Fifteen of the health facilities included in this evaluation were in Quelimane, the urban capital district of Zambézia Province, while the remaining health facilities were in rural districts. Each health facility offers comprehensive HIV services,

including clinical care, laboratory testing, and pharmacy services. Each district-level health system consists of one large central health facility/referral center and smaller peripheral health facilities.

Study Design and Definitions

This was a retrospective evaluation of routinely collected patient data. All HIV-positive, ART-naïve children (≥5 to <15 years) enrolled in HIV care at a VUMC/FGH-supported health facility from September 30, 2012 through September 30, 2018 were eligible for inclusion in this study. Those with known enrollment and ART initiation dates and documented absolute CD4 count data were included. ART initiation date was defined as the first ART pick-up at a health facility. Children less than 5 years of age were excluded due to a paucity of CD4 percentage data (71% missing), the standard by which immune status is assessed in this younger age group. Data of included children were captured from their date of enrollment to December 31, 2018.

CD4 count at enrollment was defined as the CD4 count nearest the date of enrollment into HIV care, within the range of 2 months before or after the enrollment date. *CD4 count at ART initiation* was defined as the CD4 count nearest the ART initiation date, within the range from 6 months prior to 2 months after ART initiation. These definitions were not mutually exclusive; CD4 count at enrollment and at ART initiation could be the same. *Severe immunodeficiency* was defined as a CD4 count <200 cells/mm^3.[24] CD4 counts documented as ≤0 (0.2%) or >3,500 cells/mm^3 (1.1%) were considered invalid and were deleted from the dataset.[25]

HIV viral suppression was determined based on the first viral load (VL) obtained within 6 to 12 months after ART initiation and was defined as a VL <1000 copies/ml. Since VL monitoring was not done as a part of routine clinical practice in this region until August 2016 (i.e., prior to August 2016, VL was only measured if there was suspected treatment failure), analyses considering VL were only performed in the period from August 2016 to study closure.

ART initiation "policy periods" corresponded to implementation of evolving pediatric ART initiation guidelines. In Period 1 (September 30, 2012 – July 31, 2016), ART was recommended for children ≥5 years of age who had a CD4 count <350 cells/mm^3 or a WHO clinical stage 3 or 4 defining condition. In Period 2 (August 1, 2016 – October 31, 2017), the CD4 count threshold increased to <500 cells/mm^3, and implementation of Test and Start began in Quelimane and Namacurra districts. In Period 3 (November 1, 2017 – September 30, 2018), Test and Start was implemented province-wide (**Table 1**).

When determining the proportion of HIV-positive children eligible for ART who started ART during each policy period, we utilized different approaches depending on the period. For children enrolled in Period 1, we identified the minimum CD4 count for each child within Period 1, and we considered a child eligible if the minimum CD4 count was <350 cells/mm^3. For Period 2, we considered children who enrolled in Period 2 and those who enrolled in Period 1 but had not yet initiated ART and were still in care during Period 2; we then identified the minimum CD4 count for each child within Period 2, and we used an ART eligibility cutoff of <500 cells/mm^3. In Period 3, we considered children who enrolled in Period 3 and those who enrolled in Periods 1 or 2 but had not yet initiated ART and were still in care during Period 3, and we considered all of these children to be ART eligible, since this was the era of Test and Start. Of note, WHO clinical

stage was not used to determine ART eligibility in this evaluation due to unreliable documentation of this variable in the dataset (see *Limitations* section for additional details).

Statistical Analyses

Descriptive statistics were used to summarize child, caregiver, and program characteristics. Temporal trends in CD4 counts and the proportion of children with severe immunodeficiency at enrollment in HIV care and at the time of ART initiation were also described and stratified by policy period and patient, caregiver, and program characteristics. Wilcoxon signed-rank test was used to compare CD4 counts between enrollment and ART initiation within each policy period, and the chi-squared test for trend was used to assess the proportion of children with severe immunodeficiency over time.

Univariate logistic regression models for each variable were built to assess its effect on severe immunodeficiency. Cases with missing data were omitted during the univariate analysis (**Appendix 1**). The likelihood ratio test was used to assess the statistical significance of each variable, while the difference between a level and the reference level within a variable was assessed by Wald test.

A multivariable logistic regression model was used to identify associations with severe immunodeficiency. Variables in the model were selected based on *a priori* hypotheses and included: policy period, district, health facility type (district referral center vs. peripheral health facility), sex, child's age, and parents' vital status. Inclusion of additional variables (e.g., parents' age) were considered but were excluded from the model due to excessive missingness and lack of statistical significance in univariate analyses ($p>0.05$). An interaction between policy period and age was also considered and included in the model. Calendar year was not included in the model due to a high correlation with policy period. Missing data for mother's and father's vital status were imputed by iterative chained equations using the mice R package,[26] with a total of 50 imputations, while accounting for all variables included in the multivariable regression model. The significance of each term in the model was assessed by using the univariate Wald test based on the pooled regression coefficient and standard error from 50 imputed datasets.

The proportion of children eligible for ART who initiated ART in each policy period was obtained through cross tabulation analysis between ART initiation status and eligibility. Viral suppression rates stratified by whether or not HIV-positive children had severe immunodeficiency at time of ART initiation were also described by cross tabulation. Univariate and multivariable logistic regression models were built to assess potential factors associated with viral suppression.

All statistical analyses were conducted using R statistical software 3.6.3.[27]

Limitations

We acknowledge several limitations for this analysis and evaluation. Firstly, we excluded children younger than 5 years of age from our analysis due to a paucity of CD4 percentage (the proportion of lymphocytes that are CD4+ T-cells) data; 69% of children in the cohort were less than 5 years of age and 71% of those had missing CD4 percentage data. CD4 percentage, as opposed to absolute CD4 count, is the standard by which immune status is assessed in this younger

age group. We attempted to circumvent this issue by calculating CD4 percentage when both absolute CD4 and lymphocyte counts were obtained on the same day, but even after systematically performing this exercise, there was still 71% missingness of CD4 percentage data. Therefore, it was determined that the sample of children less than 5 years of age would lack the external validity needed to extrapolate findings to the general population. This missingness led to a smaller sample size for our analyses, but there were still nearly 2,000 children older than 5 years of age from which we were able to generate generalizable results. This limitation also highlights important monitoring and evaluation and/or service delivery gaps; health facilities providing pediatric HIV care and treatment should be able to routinely obtain and document CD4 percentage results, and perhaps they did but data were not properly captured in the electronic database. Some might argue that in the era of Test and Start, CD4 monitoring is less important than it was in the past when immunologic criteria were used to determine ART eligibility; however, CD4 data are still very relevant for determining immune competence and risk for opportunistic infections. The reasons for these missing CD4 data are beyond the scope of this analysis, but one possible contributing factor might have been an "informal policy" at the time that allowed for ART initiation without CD4 count/CD4%. This issue certainly warrants further investigation.

An inherent limitation of retrospective observational studies is a relative inability to make causal inferences. However, by controlling for many important covariates in our multivariable model, we should have a high degree of confidence in identified associations with our outcomes of interest. There were, however, some factors that we had hoped to account for, but the data were either unavailable, had a high degree of missingness, or were non-informative. For example, we had planned to adjust for WHO clinical stage, but all of the children in this cohort were documented as WHO clinical stage I (i.e., there was no variation). The lack of informative WHO clinical stage data also limited our ability to accurately assess ART eligibility, since in Periods 1 and 2 both immunologic and clinical criteria were still being used to determine ART eligibility. While it seems unlikely that all of these children were truly asymptomatic (i.e., WHO clinical stage I), especially when considering nearly 20% of the cohort had severe immunodeficiency by CD4 criteria, it is not possible to verify the reasons for this assessment and/or documentation. This is another issue of clinical importance and warrants further investigation.

We had also hoped to gain more insights into the relationship between degree of immunodeficiency and HIV viral suppression, but VL monitoring was not incorporated into routine clinical practice in this region until 2016, and even after 2016 there was a very limited number of children with both VL and CD4 data from which to draw generalizable conclusions. This was likely due to VL monitoring tending to supplant CD4 monitoring in recent years.

It would have also been useful to know more about the mothers' and children's peripartum engagement in HIV care. One would expect that mothers who were aware of their HIV status and on ART throughout pregnancy and the period of breastfeeding would be more likely to have children with early and sustained engagement in HIV care. Similarly, one would expect that HIV-exposed infants who received post-partum antiretroviral prophylaxis would be more likely to have early engagement and sustained retention in HIV care. However, the data captured were insufficient to assess these hypotheses, primarily due to poor linkage or maternal and child records in the electronic database. Regardless, these factors are probably more important for younger children, while in this study we were only able to assess children 5 years of age and older, the vast

majority of whom were presumably perinatally infected, but missed earlier opportunities for HIV diagnosis and treatment. Altogether, this issue underscores the need for more complete clinical data and assessment of HIV-exposed infants.

Ethical Considerations and Assurances

This data use and evaluation plan were approved the Vanderbilt University Medical Center Institutional Review Board (170970), the Institutional Research Ethics Committee for Health of Zambézia Province (*Comité Institucional de Bioética para Saúde – Zambézia*; 16-CIBS-Z-18), and the Associate Director for Science of the Center for Global Health, U.S. Centers for Disease Control and Prevention (CGH 2016-163a).

All data included in this analysis were de-identified programmatic data. The electronic databases outlined in the *Methods* section were stored on password protected and encrypted servers at FGH. De-identified data were extracted from these secure databases and sent via secure file transfer to relevant key personnel (i.e., the biostatisticians).

Deviations from Scope of Work (SOW)/Protocol

There were no significant deviations from the proposed concept note. However, as detailed in the *Limitations* section, there were some variables that we were unable to assess/adjust for in our analyses due to the data not being available, excessive missingness, or because they were non-informative (e.g., WHO clinical stage and tuberculosis co-infection).

Data Quality Assurance

Programmatic data used in this evaluation were subject to routine data verification processes conducted by trained members of FGH's Monitoring and Evaluation (M&E) team and was stored securely on password-protected databases at district and provincial level offices. The performance of the program indicators was monitored by health facility staff. All subsequent indicators were collected and internally reported monthly by the Health Information Systems (HIS) team, following the regular reporting period for program data.

Findings

Baseline Characteristics

Among 8,266 children <15 years of age (31% of whom were ≥5 to <15 years of age) receiving HIV care and treatment from VUMC/FGH-supported health facilities in Zambézia, there were 26,551 CD4 measurements obtained during the study period. Among ART-naïve children ≥5

to <15 years of age, 1,815/2,537 (72%) and 1,922/2,569 (75%) children had valid absolute CD4 count data available at enrollment into HIV care and at ART initiation, respectively.

At enrollment into HIV care, the median age was 8.5 years (interquartile range [IQR]: 6.5-10.8). Fifty-eight percent were female, but there were no differences between males and females in terms of missingness of CD4 data. Among the 980 (54%) for whom parents' vital status was known, both parents were alive for 56% of the children, only the mother was alive for 18%, only the father was alive for 15%, and 11% were orphans/both parents were deceased. One-third of the cohort was from Quelimane district, the urban capital of Zambézia Province, while the remaining children were from the eight other rural districts from which we collected pediatric CD4 data. Approximately one-quarter of children were enrolled in care at a district capital health facility, while 73% were enrolled at peripheral health facilities within the respective districts. Fifty-four percent of children were enrolled in HIV care during Period 1, 14% during Period 2, and 32% in Period 3 (**Table 2**).

CD4 Count Trends

Median CD4 count at enrollment into HIV care and at ART initiation were similar at 504 cells/mm3 (IQR: 277-798) and 501 cells/mm3 (IQR: 275-809), respectively (**Table 2**). Median CD4 counts at enrollment and at ART initiation were also similar across policy periods (495 vs. 477 cells/mm3 in Period 1 (p=0.418), 568 vs. 555 cells/mm3 in Period 2 (p=0.925), and 504 vs. 529 cells/mm3 in Period 3 (p=0.421)). However, the proportion of children with severe immunodeficiency (i.e., CD4 count <200 cells/mm3) decreased over time (by calendar year and across policy periods). Severe immunodeficiency at enrollment decreased from 28% in 2013 to 15% in 2018 (p< 0.001 for trend) and at ART initiation decreased from 33% in 2013 to 15% in 2018 (p< 0.001 for trend). As shown in **Figure 1A** and **Table 3**, the proportion of children with severe immunodeficiency at enrollment was 20% in Period 1, 19% in Period 2, and 16% in Period 3 (p=0.120 for trend) and at ART initiation was 21% in Period 1, 17% in Period 2, and 15% in Period 3 (p=0.003 for trend).

ART Initiation Relative to ART Eligibility

In Period 1, 551 (38%) of 1,461 children were eligible for ART. Among those who were ART eligible in Period 1, 10% of them did not start ART, and 89%, 0.4%, and 0.7% initiated ART in Period 1, Period 2, and Period 3, respectively. In Period 2, 147 (44%) of 332 children (inclusive of those who enrolled in Period 1 but had not yet started ART) were eligible for ART. Among those who were ART eligible in Period 2, 4% of them did not start ART, and 94% and 2% initiated ART in Period 2 and Period 3, respectively. In Period 3, there were 845 children (inclusive of those who enrolled in Periods 1 and 2 but had not yet started ART) all of whom were ART eligible in the setting of Test and Start. In Period 3, only 1% of children did not start ART, and 99% were initiated on ART.

Univariate Analyses: associations with severe immunodeficiency

In univariate analyses (**Table 3**), older age (p<0.001 at enrollment and at ART initiation) and receiving care at a peripheral health facility (p=0.041 at enrollment; p=0.037 at ART initiation) were associated with severe immunodeficiency. Having a living mother was protective against severe immunodeficiency at enrollment in HIV care (p=0.003) and at ART initiation (p=0.005). When considering parents' vital status simultaneously (**Figure 1B**), compared to orphans (i.e., both mother and father were deceased), children for whom both parents were alive were less likely to have severe immunodeficiency at the time of enrollment into HIV care (OR=0.56; 95% CI: 0.35-0.93) and at ART initiation (OR=0.62; 95% CI: 0.39-1.03). Parents' age was not associated with severe immunodeficiency at enrollment (mother's age, p=0.196; father's age, p=0.900) or at ART initiation (mother's age, p=0.113; father's age, p=0.790).

Multivariable Analyses: associations with severe immunodeficiency

In multivariable analyses (**Table 4**), children who enrolled in HIV care during Period 3 trended toward lower odds of severe immunodeficiency at enrollment (OR=0.80; 95% CI: 0.61-1.06), and were significantly less likely to have severe immunodeficiency at ART initiation (OR=0.67; 95% CI: 0.51-0.88) compared to Period 1. With each year increase in age there was 13% increased odds of enrollment into HIV care with severe immunodeficiency (OR=1.13; 95% CI: 1.06-1.20) and 14% increased odds of ART initiation with severe immunodeficiency (OR=1.14; 95% CI: 1.08-1.21). Those enrolled in care at a district referral center were less likely to have severe immunodeficiency than those enrolled at peripheral health facilities, both at enrollment (OR=0.72; 95% CI: 0.52-0.99) and at ART initiation (OR=0.71; 95% CI: 0.51-0.97). Receiving care in the district of Gilé was also associated with lower odds of enrollment in HIV care (OR=0.21; 95% CI: 0.06-0.77) and initiating ART (OR=0.18; 95% CI: 0.05-0.66) with severe immunodeficiency; however, children in Gilé only accounted for 3% of the cohort and <1% of cases of severe immunodeficiency, so the explanation for this finding is uncertain and we cannot exclude the possibility that this was due to the small sample size in this district. While not statistically significant, there was a trend toward males being more likely to enroll in HIV care (OR=1.26; 95% CI: 0.99-1.61) and initiate ART (OR=1.26; 95% CI: 0.99-1.60) with severe immunodeficiency compared to females. Compared to orphans, there was a non-statistically significant trend toward children with a living mother and father being less likely to enroll in care (OR=0.67; 95% CI: 0.43-1.04) and initiate ART (OR=0.72; 95% CI: 0.45-1.15) with severe immunodeficiency.

Viral Suppression

The viral load data for current analysis are from August 1, 2016 to December 31, 2018. Among children who initiated ART in the time frame (i.e., one year before August 1, 2016 to six months before December 31, 2018) that were eligible for viral load testing within current study period, 775/1,516 (51%) (432 [56%] girls and 343 [44%] boys) had a viral load test done for

routine monitoring (i.e., test done within 6 to 12 months after ART initiation), of whom 330 (43%) were virally suppressed. The median VL was 3,570 (IQR: 19 – 54,368). The viral suppression rates were not significantly different between the policy periods, but did significantly differ by geographic setting/locale, namely they were 53% virally suppressed in urban compared to 35% in rural districts (p<0.001). Girls were more frequently virally suppressed than boys (48% vs. 36%; p=0.001), and older children were more frequently virally suppressed than younger children (63% versus 39%; p<0.001) (**Table 5**). The results of a multivariable logistic model analyzing *all* children with a viral load results available, irrespective of CD4 data availability and adjusting for policy period, sex, age, urban vs. rural status, and *sede* vs. non-*sede,* are reported in **Table 6.** Older age was associated with 12% higher odds of viral suppression (per 1-year increase; OR=1.12; 95% CI: 1.08-1.16). Males had significantly lower odds of viral suppression (OR=0.67; 95%CI: 0.50-0.91) compared to females. Children residing in urban settings were significantly more likely to be virally suppressed (OR=1.74; 95% CI: 1.15-2.66) compared to those in rural settings. Policy Period was not associated with viral suppression.

Among the 1,922 children who initiated ART *and* had valid CD4 data, 90% lacked viral load data within the window of 6 to 12 months after ART initiation. Therefore, univariate and multivariable analyses for this subgroup are not reported.

Tables and Figures

Table 1: Pediatric antiretroviral therapy (ART) initiation policy periods. There was phased implementation of ART initiation guidelines across districts over time, with evolution from immune-based criteria (CD4 count) to Test and Start.

District	Period 1 ART for ≥5 years of age with CD4 count <350 cells/mm^3 or WHO clinical stage 3 or 4 Sept 30, 2012 – July 31, 2016	Period 2 ART for ≥5 years of age with CD4 count <500 cells/mm^3 or WHO clinical stage 3 or 4 Roll-out of Test & Start Aug 1, 2016 – Oct 31, 2017	Period 3 Test & Start (all districts) Nov 1, 2017 – Sept 30, 2018
Alto Molòcué			Test & Start
Gilé			Test & Start
Ile*			Test & Start
Inhassunge			Test & Start
Maganja da Costa			Test & Start
Mocubela			Test & Start
Namacurra**		Test & Start	Test & Start
Pebane			Test & Start
Quelimane		Test & Start	Test & Start

Note: Periods in which immune-based criteria (CD4 count) were used to determine eligibility for ART initiation are indicated in grey.

* For Ile, Test and Start was implemented February 1, 2018.
** For Namacurra, Test and Start was implemented April 11, 2017.

Table 2: Child, caregiver, and program characteristics at enrollment in HIV care and at the time of antiretroviral therapy (ART) initiation.

	Enrollment (N=1815)	**ART Initiation** (N=1922)
	For continuous variables, median [interquartile range] are shown. For categorical variables, frequency (percentage) are shown.	
CD4 count (cells/mm^3)	504 [277-798]	501 [275-809]
<200 (severe immunodeficiency)	335 (18%)	357 (19%)
≥200 (not severe)	1480 (82%)	1565 (81%)
Age (years)	8.5 [6.5-10.8]	8.4 [6.5-10.7]
5-9	1203 (66%)	1266 (66%)
10-14	612 (34%)	656 (34%)
Sex		
Female	1061 (58%)	1115 (58%)
Male	754 (42%)	807 (42%)
Mother's age (years)	30 [25-35]	30 [25-35]
Missing *	697 (38%)	733 (38%)
Father's age (years)	36 [30-42]	36 [30-42]
Missing	948 (52%)	1001 (52%)
Parents' vital status		
Both mother and father alive	547 (56%)	589 (57%)
Only mother alive	179 (18%)	181 (17%)
Only father alive	147 (15%)	150 (14%)
Both mother and father deceased	107 (11%)	118 (11%)
Missing	835 (46%)	884 (46%)
District		
Alto Molòcué (rural)	88 (5%)	93 (5%)
Gilé (rural)	58 (3%)	60 (3%)
Ile (rural)	88 (5%)	88 (5%)
Inhassunge (rural)	164 (9%)	176 (9%)
Maganja da Costa (rural)	185 (10%)	190 (10%)
Mocubela (rural)	170 (9%)	173 (9%)
Namacurra (rural)	314 (17%)	339 (18%)
Pebane (rural)	162 (9%)	176 (9%)
Quelimane (urban)	586 (32%)	627 (33%)
Policy period		
Period 1	981 (54%)	1004 (52%)
Period 2	253 (14%)	273 (14%)
Period 3	581 (32%)	645 (34%)
Year		
2013	201 (11%)	160 (8%)
2014	248 (14%)	257 (13%)
2015	262 (14%)	283 (15%)
2016	323 (18%)	371 (19%)
2017	333 (18%)	364 (19%)
2018	448 (25%)	487 (25%)
Health facility type		
District referral center	492 (27%)	514 (27%)
Peripheral facility	1323 (73%)	1408 (73%)

* "Missing" category appears only when there are missing values for a variable.

Table 3: Univariate analysis of associations with severe immunodeficiency at enrollment in HIV care and at the time of antiretroviral therapy (ART) initiation.

	Enrollment		ART initiation	
	Severe immunodeficiency n/N (%)	**p-value***	**Severe immunodeficiency n/N (%)**	**p-value***
Age (years)		<0.001		<0.001
5-9	190/1203 (16%)		206/1266 (16%)	
10-14	145/612 (24%)		151/656 (23%)	
Sex		0.090		0.120
Female	182/1061 (17%)		194/1115 (17%)	
Male	153/754 (20%)		163/807 (20%)	
District		0.097		0.066
Alto Molòcué	17/88 (19%)		19/93 (20%)	
Gilé	3/58 (5%)		3/60 (5%)	
Ile	18/88 (21%)		18/88 (21%)	
Inhassunge	35/164 (21%)		37/176 (21%)	
Maganja da Costa	36/185 (20%)		38/190 (20%)	
Mocubela	32/170 (19%)		34/173 (20%)	
Namacurra	49/314 (16%)		55/339 (16%)	
Pebane	27/162 (17%)		27/176 (15%)	
Quelimane	118/586 (20%)		126/627 (20%)	
Setting		0.206		0.235
Urban	118/586 (20%)		126/627 (20%)	
Rural	217/1229 (18%)		231/1295 (18%)	
Policy period		0.271		0.009
Period 1	192/981 (20%)	(0.120 for	212/1004 (21%)	(0.003
Period 2	48/253 (19%)	trend)	46/273 (17%)	for trend)
Period 3	95/581 (16%)		99/645 (15%)	
Year		0.001		<0.001
2013	56/201 (28%)	(<0.001	52/160 (33%)	(<0.001
2014	55/248 (22%)	for trend)	58/257 (23%)	for trend)
2015	42/262 (16%)		56/283 (20%)	
2016	48/323 (15%)		54/371 (15%)	
2017	66/333 (20%)		66/364 (18%)	
2018	68/448 (15%)		71/487 (15%)	
Health facility type		0.041		0.037
District referral center	76/492 (15%)		80/514 (16%)	
Peripheral facility	259/1323 (20%)		277/1408 (20%)	

* P-values from likelihood ratio tests are reported, except where p-values from chi-squared tests for trend in proportions are noted in parentheses.

Table 4: Multivariable analysis of associations with severe immunodeficiency at enrollment in HIV care and at the time of antiretroviral therapy (ART) initiation.

	Enrollment	**ART Initiation**
	OR (95% CI)	**OR (95% CI)**
Policy period		
Period 1	reference	reference
Period 2	1.02 (0.70-1.51)	0.80 (0.55-1.17)
Period 3	0.80 (0.61-1.06)	0.67 (0.51-0.88)
Age (per 1-year increase)	1.13 (1.06-1.20)	1.14 (1.08-1.21)
Sex		
Female	reference	reference
Male	1.26 (0.99-1.61)	1.26 (0.99-1.60)
Health facility type		
Peripheral facility	reference	reference
District referral center	0.72 (0.52-0.99)	0.71 (0.51-0.97)
Parents' vital status		
Both mother and father dead	reference	reference
Only mother alive	0.69 (0.40-1.19)	0.87 (0.52-1.44)
Only father alive	0.91 (0.55-1.51)	0.97 (0.57-1.66)
Both mother and father alive	0.67 (0.43-1.04)	0.72 (0.45-1.15)
District		
Alto Molòcué	reference	reference
Gilé	0.21 (0.06-0.77)	0.18 (0.05-0.66)
Ile	1.06 (0.50-2.27)	0.95 (0.45-1.99)
Inhassunge	1.00 (0.52-1.95)	0.84 (0.44-1.59)
Maganja da Costa	0.93 (0.48-1.80)	0.83 (0.44-1.57)
Mocubela	0.79 (0.39-1.57)	0.69 (0.35-1.35)
Namacurra	0.69 (0.37-1.30)	0.61 (0.33-1.12)
Pebane	0.75 (0.38-1.50)	0.60 (0.31-1.17)
Quelimane	0.81 (0.43-1.52)	0.71 (0.39-1.29)

NOTE: An interaction between age and policy period was accounted for in the model

Table 5. Univariate analysis of associations with viral suppression.

	Children with VL available (n=775) Virally suppressed (n, %)	p-value
Age		**<0.001**
5-9	251/650 (38.6%)	
10-14	79/125 (63.2%)	
Sex		**0.001**
Female	206/432 (47.7%)	
Male	124/343 (36.2%)	
District		**0.002**
Alto Molocue	13/29 (44.8%)	
Gile	10/25 (40%)	
Ile	11/30 (36.7%)	
Inhassunge	16/39 (41%)	
Maganja da Costa	17/70 (24.3%)	
Mocubela	15/43 (34.9%)	
Namacurra	49/143 (34.3%)	
Pebane	20/57 (35.1%)	
Quelimane	179/339 (52.8%)	
Setting		**<0.001**
Urban	179/339 (52.8%)	
Rural	151/436 (34.6%)	
Policy Period		0.135
Period 1	33/74 (44.6%)	
Period 2	74/202 (36.6%)	
Period 3	223/499 (44.7%)	
Health Facility Type		**<0.001**
District referral	77/231 (33.3%)	
Peripheral	253/544 (46.5%)	

Table 6: Multivariable analysis of associations with viral suppression.

	All children with VL available (n=775) OR (95% CI)	p-value
Age (per 1-year increase)	1.12 (1.08 - 1.16)	**<0.001**
Sex		
Female	Reference	
Male	0.67 (0.50 - 0.91)	**0.011**
Setting		
Rural	Reference	
Urban	1.74 (1.15 - 2.66)	**0.010**
Health Facility Type		
Peripheral	Reference	
District referral	0.95 (0.63 – 1.43)	0.796
Policy Period		
Period 1	Reference	
Period 2	0.87 (0.48 - 1.60)	0.657
Period 3	0.83 (0.50 - 1.41)	0.493

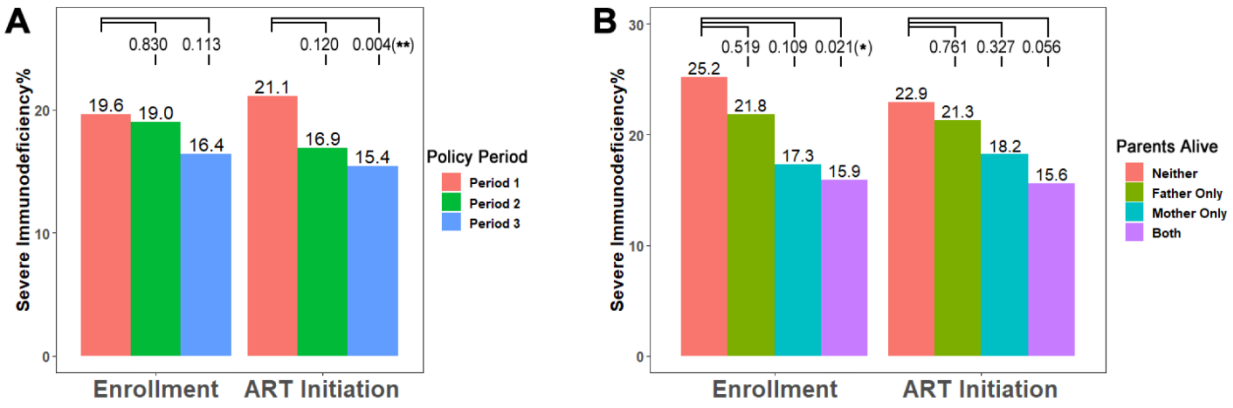

Figure 1. Prevalence of severe immunodeficiency at enrollment or ART initiation stratified by (**A**) policy periods, or (**B**) parents' vital status. P values of Wald test in the univariate logistic model are shown above the bars. The statistical significance is highlighted by ** ($p<0.01$) and * ($p<0.05$).

Conclusions and Recommendations

In this evaluation we described the degree of immunodeficiency and identified risk factors for enrolling in HIV care and starting ART with severe immunodeficiency among children over a period of evolving ART initiation policies in Zambézia Province, Mozambique. Our main findings were that in the setting of progressively more inclusive pediatric ART initiation guidelines, there were decreasing proportions of children with severe immunodeficiency at ART initiation, and that older children and those enrolled at peripheral health facilities were more likely to be severely immunodeficient. These findings suggest that more inclusive pediatric ART initiation guidelines have contributed to decreased prevalence of severe immunodeficiency at ART initiation, but additional resources and interventions are needed to further bolster services and ensure earlier diagnosis and treatment at lower-resourced clinics. It was anticipated that the prevalence of severe immunodeficiency would decrease over time and across policy periods, continuing the trend previously reported from low- and middle-income countries (LMIC) prior to 2013,[21,22] but it is difficult to know whether this improvement was attributable to specific ART initiation policy changes. However, the fact that we found a non-significant trend in severe immunodeficiency at enrollment in HIV care across policy periods and a significant difference in severe immunodeficiency at ART initiation across policy periods suggests that policy changes may have played a causal role in the observed improvements. Regardless, it is notable that we observed a decrease in severe immunodeficiency at ART initiation from 33% in 2013 to 15% in 2018. Similar LMIC cohorts reported 58-66% of children starting ART with severe immunodeficiency in 2010,[22] and 42-64% in 2013;[21] however, inclusion of children younger than 5 years of age in these cohorts limits comparability.

Median CD4 counts and the proportion of children with severe immunodeficiency, overall, were similar at enrollment and at ART initiation, which suggests that delays in ART initiation were not the sole cause for starting ART with severe immunodeficiency. It seems that many children who start ART with severe immunodeficiency do so after entering care already severely immunodeficient. This is consistent with our finding that older children, most of whom were probably perinatally infected and had been living with untreated HIV for years,[28] were more likely to have severe immunodeficiency both at enrollment and at ART initiation. The association between older age and starting ART at a late stage of disease has also been reported in other studies,[22,29] indicating that barriers to early diagnosis and treatment of HIV-positive children persist. Unfortunately, we did not capture high quality data from children younger than 5 years of age to better understand this relationship, but it stands to reason that earlier diagnosis of HIV and improved linkage to HIV care and treatment would further decrease the proportion of children entering into care and starting ART with severe immunodeficiency.

We also found that children enrolled at district capital health facilities were less likely to have severe immunodeficiency at enrollment and at ART initiation. Other studies have also found that pediatric HIV service delivery and outcomes are worse in more remote clinical settings.[30-33] Nevertheless, great progress has been made in recent years to bolster and decentralize both community- and facility-based services provided to HIV-affected populations.[29,34-42] These findings support the importance of these investments and suggest that even more should be done to support pediatric HIV service delivery at peripheral sites.

While we did not find a statistically significant relationship between sex and severe immunodeficiency in our analyses, after adjusting for other important characteristics there was a trend toward males being more likely to have severe immunodeficiency than females. We also found that males were less likely to be virally suppressed, but the external validity of this finding is uncertain considering the very limited sample size for our viral suppression analysis. We know of barriers to engagement of adult males in HIV services in LMIC,[43-45] and in a recent study we found that men in Zambézia were more likely to initiate ART with severe immunodeficiency.[23] Taken together with the fact that only 42% of children in this cohort were male, there are possibly sex-based disparities that need to be addressed to ensure timely access to pediatric HIV care and treatment for both boys and girls.

This evaluation also revealed an important signal about the role that parents and caregivers play in the care and treatment of HIV-positive children. In univariate analysis, we found that having a living mother was protective against severe immunodeficiency, and in our multivariable analysis we found a non-significant trend toward those with both a living mother and father being less likely to have severe immunodeficiency compared to orphans. Other studies have also linked maternal health and mortality to pediatric outcomes in the context of HIV exposure and infection,[46-48] and others have emphasized the vulnerability of HIV-affected orphans,[49-51] but less is known about the causal pathway between parents' vital status and children's risk for HIV-associated immunodeficiency. Nonetheless, it appears that providing optimal care for HIV-positive children also includes efforts to ensure optimal health for and engagement of parents. And, when children are orphaned, additional resources and caregiver support may be necessary to ensure timely diagnosis and treatment of their HIV-positive children.

The evaluation showed that only 51% of the children had a viral load test done within the period 6-12 months after ART initiation. Of those, 43% who had their first routine viral load monitoring done were virally suppressed. This seems to be in line with provincial-level program data, where in June 2020, 45% of children (0-14 years of age) had a viral load below 1,000 copies/ml. More girls were virally suppressed than boys, and more children in urban areas were virally suppressed. Importantly, older children had a high viral suppression rate which is important as this group enters adolescence, with some evidence suggesting adolescents are more at risk for not receiving viral load testing.[52]

In conclusion, implementation of progressively more inclusive pediatric ART initiation guidelines was associated with decreasing proportions of children with severe immunodeficiency at ART initiation. However, considering that 15% of HIV-positive children in 2018 commenced ART with severe immunodeficiency, there is still much work to be done. It seems that additional efforts and resources are needed to ensure early diagnosis and treatment of HIV-positive children, especially at lower-resourced peripheral clinics. Additionally, more research is needed to understand and improve outcomes for vulnerable subgroups of children, namely orphans and males.

Dissemination plan

This concept was developed in collaboration by the Ministry of Health (MOH) and this evaluation was a collaborative partnership between the MOH, the CDC, the provincial health

directorate (DPS-Zambézia) and VUMC/FGH investigators. VUMC/FGH, who has led the analysis for this evaluation, will share English and Portuguese versions of the recently completed final results, including lessons learned and potential future directions, with provincial- and national-level MOH authorities. Wider dissemination will be in the form of an accepted poster presentation at the INTEREST 2020 virtual conference in December 2020, as well as a peer-reviewed publication that is being finalized for submission to a high-impact public health journal.

Appendices

Approved evaluation SOW/protocol

The approved concept note is submitted along with this final report for reference.

Data collection instruments/tools

Not applicable.

Informed consent

There was no consent form necessary for use of data for this evaluation, as only routinely collected, de-identified, programmatic data was included in the analysis for this evaluation. Waiver of consent did not adversely affect the rights nor welfare of the patients whose data was included in the evaluation.

Biosketches

Not applicable.

Conflict of interest statement

The collaborators in this evaluation have no conflicts of interest to declare.

Evaluation costs

Not applicable.

Results or Logical Framework

Not applicable.

Appendix 1: Summary of variables, missingness, and p-values for assessing univariate effect. Cases with missing data were omitted during the univariate analysis; only the "valid#" of cases for each variable were included in univariate analysis, and p-values reflect this restricted analysis.

Variable	At Enrollment (N = 1815)				At ART Initiation (N = 1922)			
	Valid#	Valid%	Missing%	p	Valid#	Valid%	Missing%	p
Age	1815	100	0	<0.001	1922	100	0	<0.001
Sex	1815	100	0	0.090	1922	100	0	0.120
Policy Period	1815	100	0	0.271	1922	100	0	0.009
Calendar Year	1815	100	0	0.001	1922	100	0	0.000
District	1815	100	0	0.097	1922	100	0	0.066
Urban (vs. Rural)	1815	100	0	0.206	1922	100	0	0.235
Health Facility Type (Sede vs. Non-Sede)	1815	100	0	0.041	1922	100	0	0.037
Age of Father	867	47.77	52.2	0.900	921	47.92	52.1	0.790
Age of Mother	1118	61.6	38.4	0.196	1189	61.86	38.1	0.113
Mother HIV Test Result	867	47.77	52.2	0.543	919	47.81	52.2	0.352
Father HIV Test Result	548	30.19	69.8	0.286	588	30.59	69.4	0.082
Mother HIV Treatment	634	34.93	65.1	0.008	679	35.33	64.7	0.012
Father HIV Treatment	471	25.95	74.1	0.238	501	26.07	73.9	0.083
Mother Alive	1301	71.68	28.3	0.004	1392	72.42	27.6	0.008
Father Alive	1115	61.43	38.6	0.121	1181	61.45	38.6	0.103
ARV Exposure from Mother	794	43.75	56.3	0.801	844	43.91	56.1	0.506
ARV Exposure after Birth	718	39.56	60.4	0.787	765	39.8	60.2	0.457
History of Breastfeeding	545	30.03	70.0	0.499	566	29.45	70.6	0.483
Weight	655	36.09	63.9	0.593	686	35.69	64.3	0.565
Height	530	29.2	70.8	0.218	554	28.82	71.2	0.167

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