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**CD4 count recovery following initiation of HIV antiretroviral therapy in older childhood
and adolescence**

Short title: CD4 count after ART start in adolescence

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Abstract

Objective

To investigate CD4 cell count recovery following ART initiation in perinatally HIV-infected children diagnosed in later childhood.

Design

Observational prospective cohort study of newly diagnosed children aged 6-15 in Harare, Zimbabwe.

Methods

Participants were enrolled into a cohort at seven primary healthcare clinics between January 2013 and January 2015. ART was initiated according to national guidelines and CD4 counts were performed 6-monthly over 18 months. The relationship between CD4 count and time on ART was investigated using regression analysis with fixed (population) and random (individual) effects, and age at ART initiation as a covariate.

Results

Of the 307 participants who initiated ART, the median age at initiation was 11.7 years (interquartile range 9.6-13.8). The addition of an individual intercept and slope as random effects significantly improved the model fit compared to a fixed effects-only model. CD4 response (using a square root transformation) was best modelled using a 2-knot linear spline, with significant effects of time on ART and age at ART initiation. Younger children had a higher

CD4 count at ART initiation (-17.9 cells/mm³ per year of age), an accelerated increase during the first three months on ART (-38.9 cells/mm³ per year of age at day 84), and a sustained higher CD4 count.

Conclusions

Earlier ART initiation in older children is associated with accelerated CD4 count recovery and lasting immune reconstitution. Our findings support WHO guidance recommending ART initiation in all children, irrespective of disease stage and CD4 count.

Key words: adolescent; CD4 cell count; HIV; immune reconstitution

Introduction

Most children living with HIV acquired the infection perinatally. In the pre-ART era, HIV-positive infants in sub-Saharan Africa had a 50% risk of mortality before age two years [1]. However, as HIV epidemics matured, survival estimates were successively revised upwards, and it is estimated that about one-third of perinatally-infected infants have “slow progressing” HIV disease, with a median survival of at least 16 years without treatment [2]. Large numbers of children now present with undiagnosed perinatally-acquired HIV in older childhood and adolescence in sub-Saharan Africa[3], where over 80% of the world’s HIV-infected children live[4].

For infants, ART initiation regardless of CD4 count or disease stage substantially reduces mortality [5] and has been recommended since 2010[6]. In older children and adolescents, the evidence for reduced mortality is less clear. A systematic review of ART for children aged under 3 years found few studies [7], and the only trial in older children was underpowered and showed no mortality benefit of early initiation [8]. In the absence of clear evidence, and to simplify HIV treatment programmes, guidance for children aged ≥ 5 years followed that for adults, with ART initiation recommended for those with clinical WHO stage 3 or 4 disease, or low CD4 count (below 350 cells/mm³ in 2010 [6], with the threshold raised to 500 cells/mm³ in 2013 [9]).

More recently, the TEMPRANO and START trials showed that ART initiation before CD4 cell count dropped below 500 cells/mm³ reduced morbidity [10, 11] and the HPTN 052 trial showed that it also reduced sexual transmission [12]. As a result, the most recent WHO guidance (from 2016) recommends immediate ART initiation for all individuals living with HIV, irrespective of

CD4 count and disease stage [13]. Again, the guidance has also been applied to children even though based on evidence from trials conducted in adults.

There are concerns related to long-term toxicity and development of resistance due to poor adherence to ART, particularly for children who will be on ART a decade or more longer than adults initiating ART. Conversely, there are likely benefits of earlier ART initiation. The aim of this study is to estimate change in CD4 count over time in a cohort of children diagnosed with HIV in older childhood, and to assess whether age affected CD4 recovery.

Methods

Participants aged 6 to 15 years were recruited from seven public sector primary healthcare clinics in Harare, Zimbabwe between January 2013 and January 2015, following provider-initiated HIV testing and counselling. Those who tested HIV-positive were offered enrolment into a prospective cohort study. Exclusion criteria included living outside Harare or choosing not to seek HIV care at one of the study clinics. Participants were invited for follow-up visits at 3-monthly intervals for assessment of clinical status, up until 18 months post-enrolment. Within the cohort, participants who consented to home visits were enrolled into the ZENITH randomised controlled trial, reported elsewhere[14].

The guideline in use in January 2013 stated that children should be initiated on ART at a CD4 count below 350 cells/mm^3 or if they had WHO stage 3 or 4 HIV disease[6]. In March 2014 Zimbabwe adopted the new WHO guidelines [9], with a revised threshold for ART initiation of $500 \text{ CD4 cells/mm}^3$.

CD4 cell count was measured at 3 and 6 months post-initiation, and otherwise 6-monthly, with an Alere PIMA CD4 (Waltham, Massachusetts, USA) machine. Participant data were recorded

by a study nurse on paper clinical report forms and entered into an MS Access database using Cardiff TELEFORM Intelligent Character Optical Mark Recognition Software (Version 10.9). Statistical analyses were performed using R v3.4.1 and Stata v14.0 (StatCorp, Texas, USA).

To explore the relationship between the CD4 count and the explanatory variables, time on ART and age at ART initiation, we performed linear mixed-effects regression modelling of longitudinal data. Time was modelled using a linear spline. Splines provide a flexible way of modelling response curves over time using standard linear mixed modelling software. We used the simplest family of splines, in which the response curve is a continuous, piece-wise linear function. Comparison of nested models was made using the likelihood ratio test. A linear spline model was explored incorporating zero, one or two knots to determine which best fitted the data. The first knot was a priori positioned at time 0, due to the biological plausibility of a change in CD4 response at ART initiation. The optimal position for the second knot was ascertained by calculating the log-likelihood value for a knot on each day.

To build the model, we first used ordinary least squares regression, then added a random intercept and then a random slope. Next we added the spline terms one by one, then age at ART initiation, and finally an interaction between age and time. At each stage we used a likelihood ratio test to determine whether the increased complexity was justified.

The residuals were plotted against the fitted values for the CD4 response model to verify the assumptions of homogenous variance and normally distributed errors. As a sensitivity analysis we fitted the same linear spline model to a censored dataset which consisted only of data

between 100 days pre-initiation and 600 days post-initiation of ART. This was to ensure that the model was not unduly influenced by sparse data at the extreme ends of the follow-up period.

Ethical approval for the study was obtained from the Medical Research Council of Zimbabwe (MRCZ/A/1676) and the Ethics Committees of Harare City Health Services, the Biomedical Research and Training Institute (AP 108/2012) and the London School of Hygiene and Tropical Medicine (6305).

Results

Between 23 January 2013 and 23 January 2015, 385 children and adolescents were enrolled into the cohort, of whom 307 (79.7%) initiated ART during follow-up. Reasons for non-initiation were: No CD4 count below the threshold for ART initiation (350 cells/ μ l prior to March 2014, 500 cells/ μ l thereafter) (n=78); less than a month of follow-up before transferring out (n=5) or being lost to follow-up (n=1); only became eligible for ART at the last follow-up visit (n=4).

Participants were aged 6-15 years at enrolment and 6-17 years at ART initiation (Table 1). Of the 307 participants who initiated ART, about half were female (52.1%) and almost all (96.7%) acquired HIV perinatally, defined as a history of maternal or natural sibling HIV or death, and self-report of no sexual debut, blood transfusion or surgery[15]. There were a total of 1155 CD4 count test results, collected between 898 days prior to ART initiation and 1099 days post-initiation (median 149 days post-initiation, IQR -9 to 343), with a median follow-up per person of 516 days. Most participants (n=206; 67.1%) initiated ART within the first four weeks of enrolment. Following Zimbabwean national guidelines, all children aged <10 years or weighing

<25kg were initiated on AZT, all those weighing >35kg were initiated on TDF, and those in the middle range were initiated on either (including 10 on d4T).

The square root transformation of CD4 count resulted in a more symmetrical data distribution than either the raw values or a log transformation (Figure 1), and was the selected outcome measure. A random intercept model was a much better fit than a fixed-effects model, with a likelihood ratio test result of 660.1 ($p < 0.001$). A model with a random intercept and slope was slightly better than a random intercept alone (LR test=28.8, $p < 0.001$). The best-fitting linear spline model of CD4 count results incorporated two knots, the first fixed *a priori* at 0 days after ART initiation and the second, established using log-likelihood values, at 84 days post-initiation (Supplementary Figure 1, <http://links.lww.com/QAD/B311>). Compared to a 1-knot model the likelihood ratio χ^2 value was 198.4 ($p < 0.001$). Age at ART initiation improved the fit both as a main effect (LR test=24.8, $p < 0.001$) and again as an interaction effect of age at ART initiation with time (LR test=13.5, $p = 0.004$).

The final model showed that CD4 count decreased over time until ART initiation, then increased until the second knot at day 84, and then remained steady (Table 2). CD4 count was significantly associated with age as a fixed effect. On the day of initiation, one additional year of age at ART initiation was associated with a 17.9-point decrease in CD4 cell count. Over time the effect of age on CD4 count increased. For example, after 84 days on ART, each one year increase in age was associated with a decrease in CD4 count of 38.9 cells/ μ l. On day 84 post-initiation, the estimated average CD4 count was 696 cells/ μ l for a child who started ART aged 6 years; 518

cells/ μ l for a child who started ART aged 10 years; and 332 cells/ μ l for age at ART initiation of 15 years (Figure 2).

Before ART initiation, the interaction effect of age at initiation was statistically significant but negligible in its effect. Once participants were on ART the trajectories diverged, with younger children gaining a greater rise in CD4 count (a difference in square root of CD4 of 0.005, $p=0.01$).

For the sensitivity analysis, 120 outlier observations at the extreme ends of the observation period were dropped, and the models were refitted (Supplementary Table 1, <http://links.lww.com/QAD/B311>). Results were similar (Supplementary Figure 2, <http://links.lww.com/QAD/B311>). Residuals of both models were unbiased and homoscedastic.

Discussion

Among children and adolescents living with HIV in Zimbabwe, CD4 count increased in the first three months following ART initiation, and then stabilised. The first three months of ART appear to be a key window for immune reconstitution in children. Immune reconstitution in children is qualitatively and mechanistically different from adults, probably due to greater involvement of the naïve T-cell pool and a more active thymus gland[16]. T-cells are formed in bone marrow and migrate to the thymus where they mature. From childhood, the thymus begins to atrophy, although it still produces some new T cells throughout life. Adults have a biphasic response to ART, with a rapid increase in memory CD4 cells followed by a slower, smaller increase in naïve CD4 cells[17]. In contrast, children demonstrate peripheral CD4 repopulation through increased

thymic naïve T cell output, with only a small increase in memory CD4 cells [17, 18]. For this reason, the reference curves for CD4 count response in the first 9 months of ART, based on data from Europe [18], cannot be applied to children.

Children who initiated ART at a younger age showed a greater immediate benefit, which was sustained over time. Among adults, older age has also been associated with poorer CD4 response to ART [18], but not to the extent observed among children in this study. In our study, older participants had lower CD4 counts at ART initiation, and poorer immunological recovery in the critical 3-month period after initiation. As a result, the difference in CD4 count between age groups was greater in participants who were stable on ART than among those not yet initiated on ART. The missed opportunity for recovery in older children and adolescents had long-lasting consequences, as they remained at a lower average CD4 count to the end of follow-up.

For this cohort low CD4 count at baseline was a risk factor for other adverse outcomes, including increased risk of hospital admission and mortality, as shown previously [19]. Of 13 children who died during follow-up, 11 had a CD4 count below 350 cells/ μ l at enrolment [20]. There are two possible causes of the lower CD4 count at baseline and poorer immune recovery of adolescents compared with younger children. Firstly, among perinatally-infected children, older age corresponds to later diagnosis. Children who were older at ART initiation had therefore been immunosuppressed for a longer period. Secondly, adolescents may have limited capacity for rapid immunological recovery, as thymic involution has already advanced but a memory T-cell reserve is not yet established. HIV-infected children have a smaller thymus than HIV-exposed but uninfected children at age 3 months [21], and early thymic involution is associated with rapid disease progression [22]. Delayed ART initiation in children may thus have a dual effect. As

well as prolonging the duration of untreated infection, it also raises the age of ART initiation, and so limits the child's capacity for immunological recovery. This would explain why a year's delay in a child has more effect on CD4 count than a year's delay for an adult.

Our results are comparable to other studies exploring CD4 cell count reconstitution following ART initiation in children. Recent studies of HIV-infected children starting ART in Uganda, Zimbabwe [23] and Europe [24] have described a non-linear model for CD4 reconstitution, with a rapid rise in CD4 cell count reaching a plateau which varied by age. However, a quarter of children did not demonstrate this asymptotic immune reconstitution. In these studies, the investigators fitted a non-linear mixed-effects model which predicted higher long-term CD4 counts for children starting ART at a younger age and with a higher initial count [23, 24], and the trajectory of increase was steeper in younger children. This is consistent with our finding that age at ART initiation was associated with both the level of CD4 cell count at ART initiation and the rate of change in CD4 cell count after ART initiation. The cohort from Uganda and Zimbabwe, part of the ARROW trial, benefited from a longer follow-up (median 4 years) with CD4 count measured every 12 weeks [23].

Strengths of this study include the prospective cohort design, and the fact that participants received care in the public sector, making the findings more generalisable. The use of splines revealed that CD4 recovery is concentrated within a short 3-month period, and the use of interaction and random slopes allowed for the effect of age on CD4 count to change over time. Longer-term follow-up of cohorts are needed to measure the effects of ART toxicity or resistance development, and to understand the long-term CD4 response as children grow up. Failure to

achieve long-term CD4 cell count reconstitution is associated with poor clinical outcomes. Perinatally infected cohorts in the USA had a higher rate of advanced disease-defining events and mortality at lower CD4 counts, up to age 30[25]. Even small CD4 count deficits are associated with increased risk of cardiovascular disease, stroke and cancer in adult HIV patients[26,27]. Additionally, CD4 cell count is only one element of immunological health. A study of children and adolescents in Kenya found that after ART initiation CD4 cell count quickly recovered to levels normal for the HIV negative population, but CD4:CD8 ratio and plasma soluble CD14 did not[28].

A recent review of the benefits and risks of universal ART for children concluded that there is evidence of reduced morbidity, improvement of growth and better immune recovery [29]. However, risks remain in terms of drug toxicity, development of resistance due to poor adherence, and the challenges of implementation, especially in resource-limited settings. This study adds to the evidence on immunological decline and reconstitution in children, and provides clinical evidence to support the policy of universal ART in older children and adolescents to maximise the potential gains from treatment.

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Table 1: Characteristics of study participants (N=307)

Variable	Categories	N (%)
N		307 (100)
Gender	Male	147 (47.9)
	Female	160 (52.1)
Age at ART initiation (years)	6-9	98 (31.9)
	10-13	146 (47.6)
	14-17	63 (20.5)
CD4 count at enrolment (cells/\squarel)	0-99	49 (16.0)
	100-199	37 (12.1)
	200-349	89 (29.0)
	350-499	66 (21.5)
	500+	66 (21.5)
Time from enrolment to ART initiation	Up to 4 weeks	206 (67.1)
	>4 weeks to 1 year	80 (26.1)
	>1 year	21 (6.8)
ART regimen at initiation	TDF/3TC/EFV	66 (25.3%)
	TDF/3TC/NVP	62 (23.8%)

	TDF/3TC/ATV	1 (0.4%)
	AZT/3TC/EFV	32 (12.3%)
	AZT/3TC/NVP	90 (34.5%)
	d4T/3TC/EFV	3 (1.2%)
	d4T/3TC/NVP	7 (2.7%)
	Unknown	46
Number of CD4 count test results	1	35 (11.4)
	2	30 (9.8)
	3	50 (16.3)
	4	66 (21.5)
	5	111 (36.2)
	6-7	15 (4.9)

Table 2: Results of linear spline model of square root of CD4 count over time around ART initiation (N=1155 observations)

Parameter	Value (95% CI)	SE	p-value
Days pre-ART	-0.02 (-0.04, 0.00)	0.01	0.08
0-84 days post-ART	0.13 (0.09, 0.17)	0.02	<0.01
>84 days post-ART	-0.11 (-0.15, -0.08)	0.02	<0.1
Age at ART initiation (years)	-0.54 (-0.84, -0.25)	0.15	<0.01
Interaction of days pre-ART with age at ART initiation	0.00 (-0.00, 0.00)	0.00	0.74
Interaction of 0-84 days post-ART with age at ART initiation	-0.01 (-0.01, -0.00)	0.00	0.01
Interaction of >84 days post-ART with age at ART initiation	0.00 (0.00, 0.01)	0.00	0.01
Intercept	22.38 (18.97, 25.79)	1.74	<0.01

Figure 1: Square root of CD4 count over time since ART initiation with LOWESS smoothing (N=1155 observations)

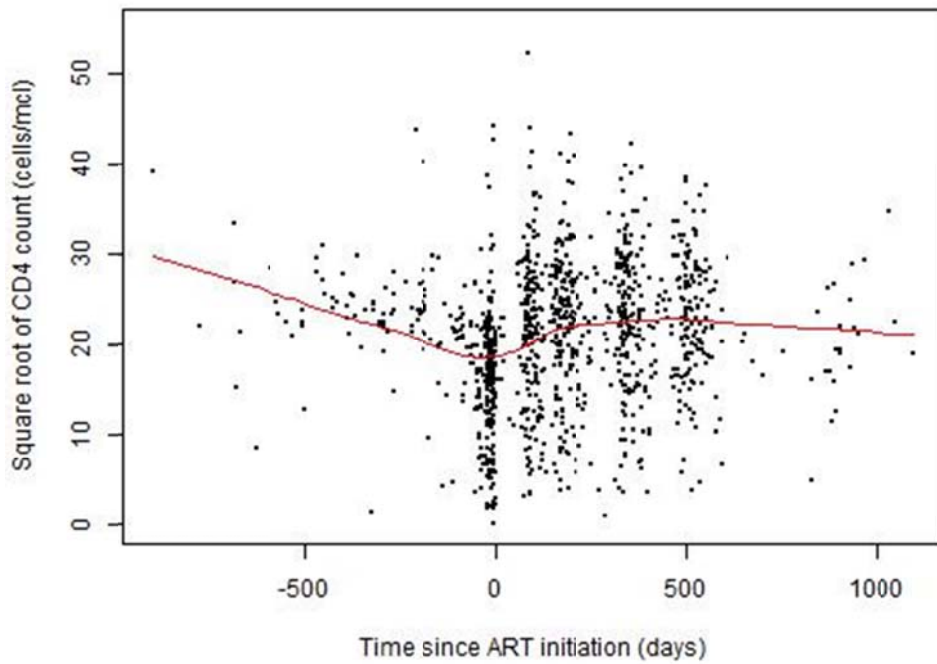


Figure 2: Fitted CD4 count over time since ART initiation, by age at ART initiation

(N=1155 observations)

