Age and CD4 Count at Initiation of Antiretroviral Therapy in HIV-Infected Children: Effects on Long-term T-Cell Reconstitution

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Background. Effective therapies and reduced AIDS-related morbidity and mortality have shifted the focus in pediatric human immunodeficiency virus (HIV) from minimizing short-term disease progression to maintaining optimal long-term health. We describe the effects of children's age and pre–antiretroviral therapy (ART) CD4 count on long-term CD4 T-cell reconstitution.

Methods. CD4 counts in perinatally HIV-infected, therapy-naive children in the Paediatric European Network for the Treatment of AIDS 5 trial were monitored following initiation of ART for a median 5.7 years. In a substudy, naive and memory CD4 counts were recorded. Age-standardized measurements were analyzed using monophasic, asymptotic nonlinear mixed-effects models.

Results. One hundred twenty-seven children were studied. Older children had lower age-adjusted CD4 counts in the long term and at treatment initiation ($P < .001$). At all ages, lower counts before treatment were associated with impaired recovery ($P < .001$). Age-adjusted naive CD4 counts increased on a timescale comparable to overall CD4 T-cell reconstitution, whereas age-adjusted memory CD4 counts increased less, albeit on a faster timescale.

Conclusions. It appears the immature immune system can recover well from HIV infection via the naive pool. However, this potential is progressively damaged with age and/or duration of infection. Current guidelines may therefore not optimize long-term immunological health.

Antiretroviral therapy (ART), as the standard treatment for adults [1] and children [2] infected with human immunodeficiency virus (HIV), has significantly reduced mortality and morbidity in those to whom treatment is available [3]. HIV predominantly infects CD4+ T-lymphocytes, and untreated HIV infection is usually characterized by a steady decline over several years in the concentration of these cells in the blood (CD4 count). As CD4 count falls, patients become progressively less able to fight opportunistic infections, eventually leading to an AIDS diagnosis. Antiretroviral therapy acts by suppressing

HIV replication, often reducing viral load to concentrations undetectable using most current assays. Patients' own homeostatic mechanisms can then restore their CD4 counts to healthier levels.

The decision to initiate ART in HIV-infected children is usually based on guidelines such as those issued by the World Health Organization (WHO) [2] or the Paediatric European Network for the Treatment of AIDS (PENTA) [4]. These guidelines give thresholds for discrete age groups, indicating CD4 count and CD4 percentage below which ART is recommended based on observed, short-term risk of disease progression and mortality. Using these guidelines, ART may be delayed in some children with counts above the thresholds, with the aim of reducing the risks of long-term drug toxicity and viral resistance and avoiding some of the practical and social challenges of administering ART to children [5]. Current guidelines are thus predicated on the assumption that it is acceptable to delay treatment for social or economic

reasons while immediate short-term clinical risk can be avoided. This assumes that such delay is without any other long-term harm. In particular, the current treatment guidelines do not directly address long-term immune competence once children leave pediatric care and join adult clinics. As antiretroviral drugs improve in efficacy, acceptability, and diversity, survival of HIV-infected infants into adulthood is increasingly common, and thus the consequences of surviving childhood with HIV require consideration.

A number of lines of evidence suggest differences between children and adults in the level of T-cell repopulation, its speed and kinetics, and the relative proportions of naive and memory T cells that return to circulation. Children are known to have a more active thymus than adults [6, 7], and the rates at which cells enter and leave the naive T-cell pool are faster during development than in adulthood [8]. Consistent with this, younger age has been associated with greater increases in CD4 count following ART [9]. T-cell recovery in adults is biphasic, with a fast initial return of memory cells to circulation followed by a slower repopulation of the naive pool [10]. This biphasic character is less pronounced in children, [11] and their T-cell reconstitution is mainly through growth of the naive subpopulation [12, 13]—a contrast which may be a further result of age-related changes in thymic activity.

A number of pediatric studies have already shown poorer recovery of CD4 count on ART to be associated both with older age [14–17] and lower pre-ART count [15, 16, 18]. Here, we aim to assess more explicitly the long-term consequences in adulthood of delaying ART in children with CD4 counts above the guideline thresholds. We use longitudinal data from children starting ART as part of the PENTA 5 [19, 20] clinical trial, who had a range of pre-ART ages and CD4 counts. Using nonlinear mixed-effects models, we investigate the relationships between age, CD4 count at treatment initiation, and CD4 repopulation, and we illustrate the importance of the naive subpopulation for this recovery. Finally, we explore the consequences of our findings for HIV-infected, ART-naive children of different ages and with different CD4 counts.

MATERIALS AND METHODS

Data

PENTA 5 was a randomized, partly blinded clinical trial designed to compare the safety, tolerability, and effectiveness of 3 dual antiretroviral drug combinations, with or without a protease inhibitor, for the treatment of HIV in children. The trial's methods, primary findings, and long-term follow-up are described in detail elsewhere [19, 20]. One hundred thirty perinatally HIV-infected, treatment-naive children aged 3 months to 16 years were randomized to receive 2 of the 3 nucleoside reverse-transcriptase inhibitor (NRTI) antiretrovirals zidovudine, lamivudine, and abacavir (3 groups, open-label). Children with more advanced

disease also received the open-label protease inhibitor nelfinavir, whereas symptom-free children were randomly assigned either nelfinavir or a placebo. Outcome was assessed at clinic visits by recording the levels of markers including CD4 count and viral load.

Because the concentration of $CD4^+$ T cells in the blood of healthy individuals varies considerably during childhood [21], measured CD4 counts were converted to z scores standardized for age (CD4 z scores) [22]. The z scores indicate the rank of a recorded CD4 count within the expected distribution for HIV-negative children of the same age born to HIV-positive mothers, expressed in terms of the standard normal distribution. Thus a z score of 0 indicates that the child has the expected CD4 count for their age, and a z score of ± 1.96 indicates that their CD4 count is at the 2.5th or the 97.5th percentile of expected CD4 counts for their age, respectively. Scores less than -3 are below the 1 in 1000th percentile. Although some authors have used CD4 percentage, which is more stable with age, in preference to CD4 count, standardized CD4 count has been shown to have greater prognostic value and so was used here [23].

Although most children entered the trial with CD4 z scores clustered round the median, at $z = -2.3$ (approximately the first percentile in an HIV-uninfected population), there were a number of lower, outlying scores. Four children had a pre-ART z score less than -12, and 21 scores less than -12 were observed subsequently, in these children and 4 others. The formulas used to calculate z scores do not allow the youngest children to have extremely low scores, and z scores are unstable with respect to CD4 count at low values. A very low z score thus indicates a CD4 count very low for age but does not give reliable information about exactly how low. For this reason, and based on preliminary fits and sensitivity analyses, all z scores less than -12 were truncated at -12.

Cell counts for the naive ($CD4^+CD45RA^+$) and memory ($CD4^+CD45RO^+$) subpopulations of the $CD4^+$ T-cell pool were available in a 26-patient subset of the PENTA 5 participants (recruited in specific centers where 4-color flow cytometry was available) during the early part of the trial [12, 13]. Because z score conversion formulas are not available for these subpopulations, we corrected by dividing by the count expected in a healthy child of the same age [24].

Modeling CD4 z Scores

We used a nonlinear mixed-effects model [25] to fit the evolution of children's CD4 z scores with time on ART. The nonlinear curve used was an asymptotic function (Figure 1) such that z_{ij}, the z score for patient i at time t_{ij} weeks after treatment initiation, is modeled as:

$$z_{ij} = asy_i - (asy_i - int_i)e^{-ct_{ij}} + \epsilon_{ij} \qquad (1)$$

The function's parameters have clear biological and clinical relevance: the patient-specific parameters int_i and asy_i
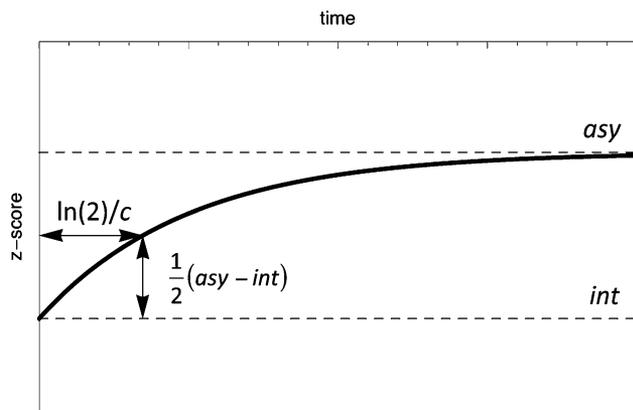
Figure 1. Schematic representation of the curve used as the basis of the nonlinear, mixed-effects models. Labels int, asy and c refer to Equation (1), and represent pre-ART CD4 z score, long-term CD4 z score and characteristic recovery time, respectively.

represent, respectively, the y intercept, or z score at treatment initiation, and the asymptotic or long-term z score achieved on ART. The constant c characterizes the rate of increase in z score, with $\ln(2)/c$ being the time taken for half the ultimate increase in z score on ART from int to asy to have occurred. Each patient-specific parameter is the sum of a universal fixed effect describing the mean response of the whole population and a patient-level random effect describing the deviation of an individual from this mean. Association between pre-ART and long-term CD4 z scores is included through correlation between these child-level random effects. The term ϵ_{ij} represents noise and measurement error. The inclusion of the exponential function hints at an underlying model for the mechanism of T-cell reconstitution, which could be described using ordinary differential equations.

We compared multivariate models for the fixed-effects parts of int and asy using backward selection with an exit P value of .01 (as our goal was to identify a parsimonious model for predicting CD4 response in general), considering possible effects of sex, NRTI randomization group, and pre-ART age, viral load, Centers for Disease Control and Prevention disease stage, and weight- and height-for-age z scores, and using P values from likelihood ratio tests. A more formal, mathematical description of the final model is given in the Supplementary Appendix.

In fitting the models we assumed an autocorrelation of 0.2 between any 2 contiguous observations of z score on the basis of preliminary work that suggested that, although the inclusion, or otherwise, of an autocorrelation influences parameter values slightly, its magnitude has very little effect.

Modeling Changes in the Naive and Memory Subpopulations
We used the same asymptotic curve (Equation 1) to fit a nonlinear, mixed-effects model to the natural logarithm of the age-corrected naive and memory cell counts (ratio of observed counts to those expected in an uninfected child of

the same age). The logarithm converts the positive ratio scale to one that can take any value, positive or negative, meaning that the assumption of a single term noise and measurement error ϵ_{ij} is plausible. The log-ratio is also more comparable to z score in that a value of zero indicates an average cell count at this age, whereas negative log-ratios correspond to below-average numbers. Because this data set is more limited, we used a somewhat simpler model incorporating fixed and random effects for int and asy but not taking any covariates into account. This model is described in full in the Supplementary Appendix.

All fitting was carried out in the statistical environment R [26], using the nlme package [27]. The relationships between fixed and random effects at different ages, and trajectories of CD4 count predicted by the model, were plotted using Mathematica [28].

RESULTS

Patients
Of 130 children enrolled in PENTA 5, 127 started ART [19]. The median age at ART initiation was 5.3 (interquartile range [IQR], 2.4–8.6) years; median pre-ART CD4 count was 620 (IQR, 343–912) cells/μL^{-1}; median pre-ART z score was −2.3 (IQR, −4.1 to −1.3) (corresponding to approximately the 1st [2 in 10 000th, 10th] percentile of the counts expected for an uninfected child of the same age), and median follow-up time was 5.7 (IQR, 5.1–6.5) years (Figure 2). (See [19] for more details of pre-ART characteristics and follow-up.) One child was excluded from the analysis because no pre-ART viral load measurement was available.

At treatment initiation the median age of the 26 children with immunophenotyping was 5.4 (IQR, 2.9–8.5) years; median pre-ART CD4$^+$CD45RA$^+$ count was 348 (IQR, 122–546) cells/μL^{-1}, and median pre-ART CD4$^+$CD45RO$^+$ count was 194 (IQR, 105–231) cells/μL^{-1}. The median ratios to expected healthy cell numbers were 0.25 (IQR, 0.12–0.44) (naive) and 0.51 (IQR, 0.28–0.65) (memory). Median follow-up to last immunophenotypes was 2.6 (IQR, 2.3–2.9) years.

Figure 2 gives an overview of the changes in CD4 z score and normalized naive and memory cell count that followed ART initiation, showing mean values across the whole trial. There is an initially steep increase in CD4 z score and normalized naive count, which becomes slower as the populations appear to approach homeostatic set points. The normalized memory cell count, meanwhile, shows no clear trend with time.

Age at ART Initiation Affects Both Pre-ART and Long-term CD4 z Scores
The rate of z score increase in our fitted model (Equation 1) was $c = (0.034 \pm 0.003)$ week^{-1} (estimated value $\pm$ standard error; this notation is used throughout), implying a time for half-recovery of $\ln(2)/c \approx 20$ weeks. According to the model parameters (fixed effects) estimated from our data, an average
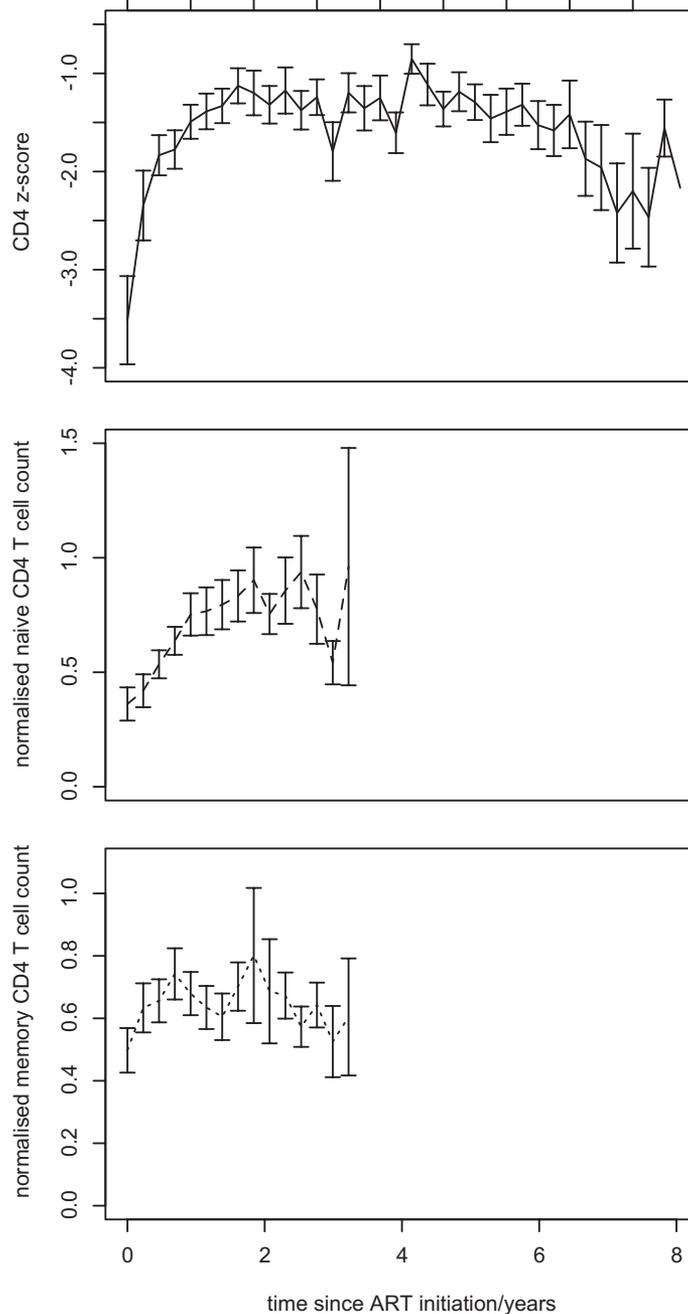
Figure 2. Mean *z* score (solid line) and age-standardized naïve (dashed line) and memory (dotted line) cell counts at 12-week time points during the Paediatric European Network for the Treatment of AIDS 5 trial (error bars: standard error). Within each 12-week window centered at weeks 0, 12, 24, and so on, the mean was calculated using the score closest to the center of the window for each child. The table above the graphs gives the numbers of children with measurements available in the 48 weeks following the time points indicated. Slightly lower means at the latest time points are due to a cohort effect with the earliest participants in the trial (with longest follow-up) having lower trajectories throughout. ART, antiretroviral therapy.

child—of age 5.3 years, viral load 120 000 RNA copies/mL^{-1}, WHO disease stage N/A/B, and weight-for-age *z* score −0.6 (approximately the median values for this population)—would

be expected to have a pre-ART CD4 *z* score of −2.6 ± 0.2 and a long-term score of −1.1 ± 0.1. Children's expected pre-ART score was −0.41 ± 0.07 lower for each year older ($P < 10^{-4}$),

-1.1 ± 0.3 lower per $\log_{10}$ copies/mL^{-1} higher viral load ($P = .001$), and -3.0 ± 0.8 lower if they had pre-ART disease stage C ($P < 10^{-3}$). Their long-term CD4 z score was -0.15 ± 0.03 lower for each year older at ART initiation ($P < 10^{-4}$), -1.1 ± 0.4 lower if they had pre-ART disease stage C ($P = .01$), and -0.31 ± 0.08 lower per unit higher pre-ART weight-for-age z score ($P < 10^{-3}$). These results are summarized in Table 1, together with univariate models of the effects of these and other factors on pre-ART and long-term z score. Figure 3 illustrates how the fixed-effects or average z score trajectory is affected by age at ART initiation.

There was a strong positive correlation ($r = 0.54$; $P \sim 10^{-11}$) between the child-level random effects for pre-ART and long-term CD4 z score (Supplementary Figure 1).

The Naive Subpopulation Is the Main Source of CD4$^+$ T-Cell Reconstitution in Children

We found a rate of population growth in the naive subpopulation, $c = 0.051 \pm 0.006$ weeks^{-1}, corresponding to a time to half the eventual naive cell recovery of $1n(2)/c \approx 14$ weeks. The size

of the naive subpopulation increased but remained below healthy levels even in the long term; the log ratio of actual to expected cell numbers rose from -1.70 ± 0.23 to -0.33 ± 0.09, corresponding to an increase from an average of 18% to 72% of the expected naive subpopulation based on age. The normalized memory cell count, which was higher than normalized naive count before ART at -1.24 ± 0.27 (29%), was lower than normalized naive count in the long term, approaching -0.50 ± 0.08 (61%). This comparatively small increase in memory cell count was on a much faster timescale: $c = 0.254 \pm 0.042$ weeks^{-1} (ie, taking only $1n(2)/c \approx 3$ weeks for half-recovery). Thus, on the evidence both of timescale and magnitude of recovery, T-cell reconstitution in these children appears to arise mainly from the naive compartment.

Predictions

Figure 4A shows the average pre-ART and long-term CD4 z scores across different ages in PENTA 5 based on our fitted model (solid line, all random effects zero, corresponding to a child of given age who is average with respect to other characteristics, as

Table 1. Summary of Univariate and Multivariate Covariate Models of Pre–Antiretroviral Therapy (Intercept) and Long-term (Asymptote) CD4 z Score

Factor	Median (IQR) or No. (%)		Univariate Models (Estimate ± SE; P Value)	Multivariate Model (Estimate ± SE; P Value)
Pre-ART age (y)	5.3 (2.3–5.8)	Intercept	-0.28 ± 0.07; $P < 10^{-3}$	-0.41 ± 0.07; $P < 10^{-3}$
		Asymptote	-0.16 ± 0.03; $P < 10^{-3}$	-0.15 ± 0.03; $P < 10^{-3}$
Pre-ART viral load ($\log_{10}$copies/mL^{-1})	5.1 (4.5–5.5)	Intercept	-0.39 ± 0.37; $P = .29$	-1.12 ± 0.34; $P = .001$
		Asymptote	0.23 ± 0.18; $P = .22$	…
Pre-ART WHO disease stage (C vs not C)	12 (9.4%)	Intercept	-3.4 ± 0.9; $P < 10^{-3}$	-2.97 ± 0.85; $P < 10^{-3}$
		Asymptote	-0.60 ± 0.48; $P = .21$	-1.11 ± 0.44; $P = .01$
Pre-ART height-for-age z score	-0.88 (-1.6 to -0.2)	Intercept	0.0010 ± 0.2149; $P = .96$	…
		Asymptote	-0.26 ± 0.10; $P = .01$	…
Pre-ART weight-for-age z score	-0.62 (-1.3 to 0.2)	Intercept	0.16 ± 0.20; $P = .41$	…
		Asymptote	-0.27 ± 0.10; $P = .005$	-0.31 ± 0.08; $P < 10^{-3}$
Sex (male vs female)	72 (57 %)	Intercept	-0.88 ± 0.55; $P = .11$	…
		Asymptote	-0.51 ± 0.28; $P = .60$	…
NRTI group				
(Zidovudine/lamivudine vs lamivudine/abacavir)	36 (28%)	Asymptote	0 ± 0.3; $P = 1.00$	…
(Zidovudine/abacavir vs lamivudine/abacavir)	44 (35%)	Asymptote	-0.4 ± 0.3; $P = .16$	…

The second column describes the distribution of each covariate within the population. Entries in the format "X (Y to Z)" are median (interquartile range) and entries in the format "X (Y%)" are number (%). Each univariate model (apart from NRTI group) looked for the effect of a single covariate on both intercept and asymptote simultaneously. Estimates are per year older (age), per $\log_{10}$ copies/mL^{-1} higher (viral load), or per unit z score higher at ART initiation (height/weight-for-age). Including further factors in the multivariate model did not significantly improve the fit. For pre-ART CD4 z score (intercept) there was no additional effect of sex (-0.94 ± 0.43 in boys vs girls; $P = .03$), pre-ART weight-for-age ($+0.022 \pm 0.177$ per 1 unit higher; $P = .90$), or pre-ART height-for-age (-0.060 ± 0.177 per 1 unit higher; $P = .73$). Similarly, for long-term CD4 z score there was no effect of sex ($+0.16 \pm 0.23$ in boys vs girls; $P = .47$), pre-ART viral load (-0.17 ± 0.20 per $\log_{10}$ copies/mL^{-1} higher; $P = .36$), height-for-age (-0.013 ± 0.131 per 1 unit higher; $P = .94$), or randomized NTRI group (-0.038 ± 0.275 and -0.26 ± 0.26 for zidovudine/lamivudine and zidovudine/abacavir, respectively, vs lamivudine/abacavir; $P = .56$).

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; NRTI, nucleoside reverse-transcriptase inhibitor; SE, standard error; WHO, World Health Organization.
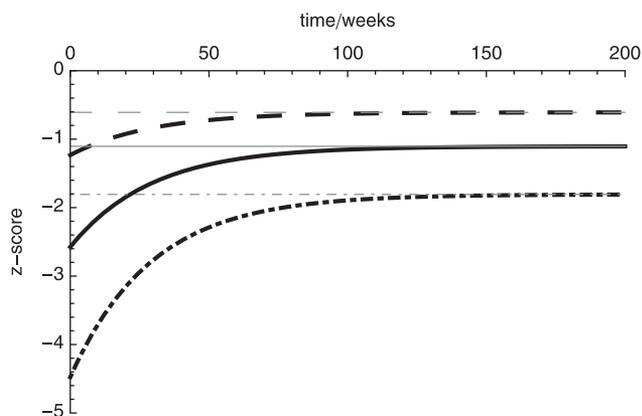
Figure 3. *z* Score trajectories for an average child (see text) starting antiretroviral therapy (ART) at the median age, 5.3 years (solid line) and at ages 2 years (dashed line) and 10 years (dashed-and-dotted line) (from fixed-effects model). Gray horizontal lines show the positions of the asymptotes. A *z* score of −2 corresponds to the 2.5th percentile for CD4 count in a healthy population.

described above). The covariance matrix of the random effects (Supplementary Table 1) can be used to predict the effect of pre-ART on long-term CD4 *z* score for children whose pre-ART scores are above or below the average for HIV-infected children their age (see Supplementary Appendix). In Figure 4, these predictions are shown for children aged 2, 6, and 10 years. At every age, a higher pre-ART score corresponds to a higher score in the long term, but in general younger children have higher scores both pre-ART and in the long term. Figure 4B shows some trajectories of CD4 count predicted by the model, based on initiating ART having reached WHO thresholds at 2, 6, or 10 years of age.
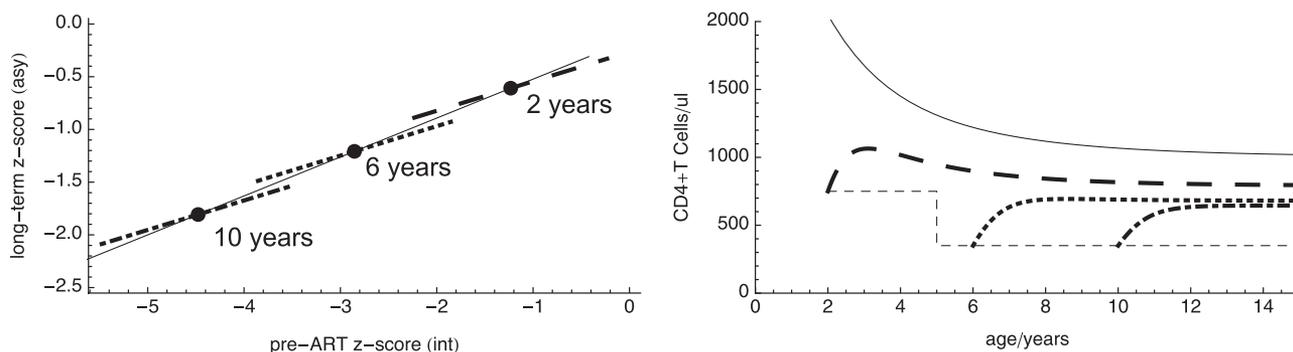
They illustrate that the threshold currently specified for the treatment of younger children results in a higher count in the long term than the threshold recommended for older children. Two effects are combined in this observation. First, pre-ART CD4 counts correspond to different *z* scores depending on age: the thresholds correspond to *z* scores from −2.3 to −1.6 for ages 2–5 and from −3.9 to −4.5 for ages 5–15. Second, the relationship between pre-ART and long-term CD4 *z* score is also age-dependent, as shown for 3 examples in Figure 4A.

DISCUSSION

In adults starting ART, an initial reconstitution of T cells through redistribution is followed by a second, slower phase of repopulation starting at around 3 weeks [10, 29]. Our work in children is more consistent with a uniform rate of repopulation throughout recovery, similar to that shown by van Rossum et al [11]. When we applied the piecewise linear mixed models used successfully to model adults' biphasic recovery [29], we found no significant improvement in fit over our asymptotic model. Moreover, attempts to fit a nonlinear, mixed-effects model incorporating 2 rates of recovery resulted in a model that was effectively monophasic. Interestingly, similar studies to ours have also arrived at models that utilize a single rate of recovery in children [14] but a biphasic one in adults [30]. Modeling naive and memory subpopulation reconstitution also supports a possible explanation—namely, reconstitution in children, in contrast to adults, is mainly via the naive T-cell compartment [12]. This agrees with current understanding that de novo production of naive CD4$^+$ T cells by the thymus falls with age and predicts immunological recovery on ART [31]. It is further corroborated

Figure 4. *A*, Relationship between pre–antiretroviral therapy (ART) and long-term CD4 *z* score predicted for average children starting ART at ages 0.25–16 y (random effects of zero; solid line). Filled circles indicate average CD4 *z* score pre-ART and long-term for children aged 2, 6 and 10 y at ART initiation. For children whose pre-ART scores differ from the average for age, the dashed and dotted lines show the predicted long-term CD4 *z* scores in children aged 2 (dashed line), 6 (dotted line) and 10 (dashed-and-dotted line) y. The labels int and asy refer to Equation (1), and represent pre-ART and long-term CD4 *z* score, respectively. *B*, *z* = 0 (solid line), the average CD4 count in an uninfected child; World Health Organization guidelines for ART initiation, based on absolute CD4 count (light dashed line), and 3 predicted CD4 count trajectories initiating ART at ages 2 (dashed line), 6 (dotted line), and 10 years (dashed-and-dotted line) according to these guidelines.
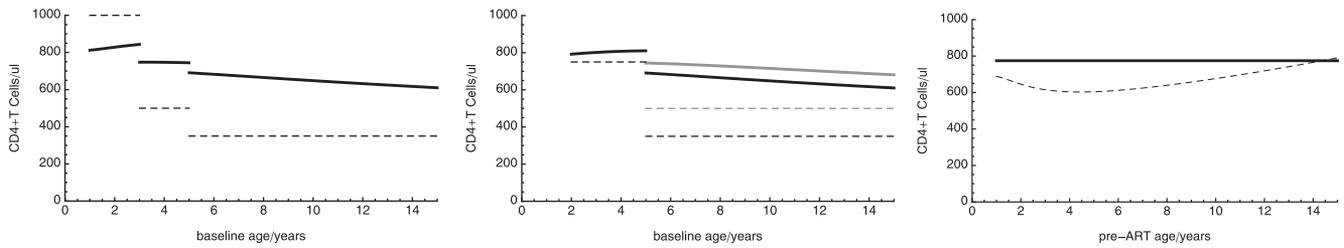
Figure 5. Comparison of sets of CD4 count thresholds for treatment initiation: Paediatric European Network for the Treatment of AIDS 2009 [4] (*A*) (similar to US guidelines) and World Health Organization 2010 [2] (*B*, black line). Dashed lines give the thresholds for CD4 count. Solid lines give CD4 count predicted by the model at age 20 years, for children starting antiretroviral therapy (ART) at pre-ART ages indicated and according to the thresholds. In (B), the gray line shows the expected consequences of setting a threshold of 500 cells/μL^{-1} for CD4 count in children >5 years. If a z score of −1 at age 20 years (CD4 count of 774 cells/μL^{-1}, corresponding to the 16th percentile of expected CD4 counts in uninfected persons; solid line) represents an acceptable long-term immunological outcome, then the dashed line in (*C*) shows the age-dependent CD4 count threshold at which our model indicates therapy should be started in order to achieve this goal.

by our observation that, as a proportion of healthy values, naive CD4 count recovers on a similar timescale to total CD4 reconstitution.

As expected, we found that pre-ART z scores were lower in older children. Although the gap narrowed with therapy, older children also had lower long-term z scores. A number of studies measuring CD4 percentage in children starting ART are consistent with ours [14–17], and there are a variety of potential explanations. Less complete recovery may be connected to an age-related decrease in thymic activity. More advanced disease in older children, who have been infected for longer, may also have caused permanent changes to the immune system and lymphoid tissue that contribute to poorer long-term status [32]. Finally, older children may be beginning to exhibit the slower recovery of the naive pool seen in adults, evidenced in our fixed c model as a lower asymptote. Not all studies concur with our findings, however. Hainaut et al [33], for example, found no significant association between age and the increase in CD4 count achieved after 12 months of ART, but their study was limited by small population size (19 patients). Age also had no effect on z score 6 months after ART initiation in data from the European Collaborative Study [18]. Their 5-month to 5-year age category, however, included children <2 years of age, who are known to be at significantly increased risk of disease progression and in whom treatment is now universally recommended [2]. This increased risk may have masked the benefit of younger age that we identified.

We found a significant positive correlation between pre-ART and long-term CD4 z scores. Other studies in children [15, 16, 18] and adults [34] have agreed that better initial status is associated with improved recovery. Moreover, in the PENTA 11 trial of CD4-driven interruptions to children's ART, a higher nadir CD4 percentage (nadir here being the lowest CD4 percentage ever measured in an individual) independently predicted better recovery from treatment interruptions [35], and other long-term studies have also suggested that severe immunosuppression at

ART initiation in children damages the potential for recovery of the CD4 population [36, 37]. It seems likely that a lower pre-ART count is a sign of more advanced disease, which leads to impaired recovery.

There are a number of simplifications and limitations to our analysis. First, although we modeled c as constant with respect to age and as having no random effects, there is evidence that age and other factors may, in fact, affect the speed of T-cell repopulation [11, 16, 18, 33]. However, models allowing c to vary with age or including random effects did not converge consistently, probably because of the limited population size, and so we were not able to identify such an effect definitively in our data. Second, although the model and its parameterization are useful for predicting long-term CD4 count from a given pre-ART value, as well as illustrating the dominant contribution of the naive subpopulation to reconstitution, they shed little light on mechanisms of recovery. A mechanistic model described by differential equations would be more informative here and might be a productive avenue for further investigation [38]. Another simplification in our model is that, because our goal was to predict long-term CD4 count on the basis of different thresholds for starting ART, it does not take into account the roles of viral suppression on ART and treatment failure. These are clearly both important factors for long-term recovery, and in adults bivariate models for simultaneous longitudinal measurements of both CD4 count and viral load have been shown to be superior, in terms of likelihood, to univariate models, as presented here [39]. Finally, very long-term longitudinal studies will add to our understanding in the future by following patients' progress further into adulthood.

Despite some limitations, our model has demonstrated that the current approach to ART, which concentrates on short-term risks, may not optimize children's health prospects in the longer term in resource-rich settings where multiple effective and acceptable therapies are now available. As a general rule, we have shown that delay in starting ART during childhood will impair the expected

CD4 concentration on entering adulthood. For example, in a well patient whose CD4 count is just above the PENTA 2009 treatment threshold throughout childhood, the effect of delaying treatment from the first to the 15th birthday is to reduce long-term CD4 count in adulthood by 200 cells/μL^{-1}, from around 810 to 610 cells/μL^{-1} (Figure 5A). The model also highlights some interesting and potentially important observations. First, it would appear that in early life large changes in CD4 cell concentration thresholds for ART initiation may only influence long-term CD4 levels to a limited extent. For example, in a child aged 3 years, the 500 cells/μL^{-1} difference in pre-ART count between 1000 and 500 cells/μL^{-1} results in a change in long-term level of only around 90 cells/μL^{-1}: from 840 to 750 cells/μL^{-1}. However, after the age of 5, when the treatment threshold remains constant until adulthood, our model predicts a steady decline in the expected long-term CD4 count (Figure 5). This has implications for children aged >5 years who are clinically well and in whom the CD4 count is above the threshold. Our model would indicate that a substantial delay in ART initiation, although logistically desirable, may be detrimental in the long term, at least assuming reasonable treatment compliance. Figure 5C gives the CD4 counts in children of different ages, which are predicted to result in a CD4 count in adulthood of 774 cells/μL^{-1} (CD4 z score of -1; approximately the 16th percentile). These may be compared with the current WHO thresholds shown in Figure 5B. In children aged <5 years, the WHO thresholds are somewhat higher, indicating that current treatment guidelines designed to minimize disease progression are likely also to maintain a relatively good long-term CD4 z score. However, in children aged $\geq$5 years, the WHO thresholds are lower, suggesting that although their use reduces the likelihood of disease progression effectively, it may not be optimal from the point of view of maintaining CD4 count in adulthood.

Our model thus provides a first indication for the importance of considering long-term prospects when deciding when to initiate ART in children. It also serves to highlight the need to reevaluate treatment thresholds in the context of the increased likelihood of HIV-infected children surviving to adulthood.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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