HIV-1 viral load and CD4 cell count in untreated children with vertically acquired asymptomatic or mild disease

Paediatric European Network for Treatment of AIDS (PENTA)*

Background: Plasma HIV-1 RNA levels are high in vertically infected infants. Information in older children is limited, particularly in those who have not received antiretroviral therapy.

Objectives: To describe the relationships between HIV-1 RNA, age and CD4 cell count in untreated vertically infected children.

Design: HIV-1 RNA was measured in 70 children [median age, 3.5 years (range, 0.4–11.9 years); median CD4 cell count, 881 $\times 10^6$/l (interquartile range, 576–1347 $\times 10^6$ cells/l)] enrolled in a randomized placebo-controlled trial comparing immediate with deferred zidovudine in asymptomatic or mildly symptomatic vertically infected children (PENTA-1 trial). Short-term variability was assessed by comparing HIV-1 RNA at –2 and 0 weeks (prior to randomization). The relationship between age and HIV-1 RNA, and CD4 cell count was analysed using data from all children prior to randomization and sequential samples from 35 remaining on placebo for up to 105 weeks, by fitting mixed linear models.

Results: The within-individual SD in viral load was 0.26 $\log_{10}$ copies/ml. The median plasma HIV-1 RNA at enrolment was 4.61 $\log_{10}$ (range, 2.3–6.56 $\log_{10}$ copies/ml), significantly higher in children aged ≤ 2 years (median, 5.23 $\log_{10}$ copies/ml) than in those aged > 2 years (4.51 $\log_{10}$ copies/ml; $P < 0.0001$). Mean HIV-1 RNA fell by 0.38 $\log_{10}$ copies/ml per year up to 2 years of age, by 0.21 $\log_{10}$ copies/ml per year from 2 to 4 years of age, and by 0.03 $\log_{10}$ copies/ml per year from 4 to 6 years of age reaching a nadir of 4.25 $\log_{10}$ copies/ml at 6 years. Mean $\log_{10}$ CD4 cell count declined steadily with age and was not significantly correlated with HIV-1 RNA, although there was some evidence that the rate of $\log_{10}$ CD4 cell decline was negatively correlated with the initial rate of HIV-1 RNA decline. No mutations associated with resistance to zidovudine were observed.

Conclusions: Age is a key factor in the interpretation of both viral load and CD4 cell count in vertically infected children.

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Introduction

Viral load, as measured by plasma HIV-1 RNA, has been shown to predict clinical outcome in HIV-1-infected adults [1], and is increasingly being used to make decisions about starting and changing antiretroviral therapy [2,3]. Most studies have reported on viral load in infancy [4–6] and have included mixed populations of infants with both rapidly and slowly progressive disease; a proportion of these infants and their mothers during pregnancy also received antiretroviral therapy. Fewer data have been published on viral load in older vertically infected children, particularly untreated children who have asymptomatic or mild disease. Such data could help to provide further guidance on the timing of initiation of antiretroviral therapy in children, particularly because, in line with treatment of adults, paediatricians are commencing therapy earlier in the course of disease. CD4 cell counts fall with age in HIV-uninfected children [7] but even after adjusting for age, CD4 cell counts appear to be less useful for predicting disease progression in infected children than in adults [8].

We describe the short-term variability in HIV-1 RNA, and the relationship between HIV-1 RNA, age and CD4 cell count in a substudy of untreated children who had asymptomatic or mild disease enrolled in the Paediatric European Network for Treatment of AIDS (PENTA)-1 trial.

Participants and methods

Participants

The PENTA-1 trial is a double-blind placebo-controlled trial comparing immediate with deferred zidovudine (ZDV) therapy. Children were randomly assigned to receive ZDV (immediate) or matching placebo (deferred) and followed every 3 months. Any child experiencing clinical progression or rapidly falling CD4 cell counts could be switched to open-label ZDV with the paediatrician and parents remaining blind to the original randomization. During 1992–1995, 197 vertically HIV-1-infected children with asymptomatic or mild disease who had not had prior antiretroviral therapy were enrolled. A subset of 70 children (35 randomized to ZDV and 35 to placebo) who had completed 2 years follow-up by December 1995 and had at least one baseline (weeks −2 or 0) sample available for analysis were included. The children were from France (n = 1), Germany (n = 4), Italy (n = 29), Spain (n = 9), United Kingdom (n = 12), and Brazil (n = 15). Samples were to be stored at weeks −2, 0, 2, 4, 8, 12, 24, 48, 72, and 96. Twenty-six (74%) of the children allocated to deferred treatment remained on placebo throughout the study; nine (26%) transferred to open-label ZDV as a result of clinical disease progression (no child developed AIDS) or falling CD4 cell counts after a median of 31 weeks (range, 9–99 weeks). Only data based on samples collected prior to the start of ZDV therapy were included from these nine children. Altogether, 246 samples (mean, 7.5 per child; range, 3–10 per child) taken over a period of 90 weeks (range, 11–105 weeks) in the placebo group and 59 samples taken before treatment in the ZDV group were included in the analysis.

Laboratory methods

Quantitative HIV-1 RNA assay

In 13 children, serum samples were collected throughout for each child and stored at −70°C. For the remaining 57 children, all samples were collected into EDTA tubes, separated within 6 h and plasma stored at −70°C. HIV-1 RNA was measured in 200 μl plasma or serum using a quantitative reverse transcriptase polymerase chain reaction (PCR) assay (Amplicor Monitor, Roche Diagnostic Systems, Branchburg, New Jersey, USA) from a single batch of kits in five laboratories. The lower limit of detection was 2.6 $\log_{10}$ copies/ml (400 copies/ml). Positive and negative assay controls from the kit were replaced on each plate by three standards [4.18 $\log_{10}$, 5.18 $\log_{10}$ copies/ml HIV-1 (clade B) and negative plasma] quantified by electron microscopy and kindly provided by the AIDS Clinical Trials Group Virology Quality Assurance Laboratories.

Quality assurance of HIV-1 RNA

Differences in the laboratory performance of the assay were assessed by comparison of the three control standards. A high level of agreement of results between laboratories was evident. The maximum difference between the five laboratories was 0.23 $\log_{10}$ copies/ml for the high positive control, 0.12 $\log_{10}$ copies/ml for the low control, and all negative controls were in agreement. The ratio of between to within laboratory variance was 4.18 for the high control and 2.71 for the low control.

Point mutation assay for genotypic resistance

ZDV resistance-associated point mutations at codons 41, 70 and 215 ($M_{41}L$, $K_{70}R$, $T_{215}Y/F$) of the reverse transcriptase gene were assayed in serum or plasma HIV-1 RNA as previously described [9].

CD4+ cell counts

CD4 cell percentage and absolute counts were measured at the same timepoints as HIV-1 RNA. Flow cytometry was performed locally in each of the clinical centres following the children.

Statistical analyses

HIV-1 RNA levels in plasma or serum were transformed prior to analysis and the results expressed as $\log_{10}$ copies/ml. Data from 39 children with two base-

line plasma HIV-1 RNA values (weeks −2 and 0) provided an estimate of the within-child variation. HIV-1 RNA levels prior to start of treatment (week 0 or nearest to the start of treatment and before day 1) on all 70 children and longitudinal data from the 35 children in the placebo group (but excluding results after start of ZDV in nine children) were used to explore the effect of age on HIV-1 RNA. The trajectory of $\log_{10}$ HIV-1 RNA over age was modelled using a smooth curve (allowed to vary randomly between different children) by fitting a mixed linear model using the statistical package MLn [10]. Age was divided into five intervals using four age-points (1, 2, 6, and 8 years) and a separate curve was fitted for each age interval and joined smoothly using natural cubic splines.

Three scales of CD4 cell counts were explored (untransformed, square-root and $\log_{10}$-transformed). The $\log_{10}$ scale was found to be the best in reducing skewness, stabilizing the variance and achieving linearity with age. CD4 cell counts were also adjusted for age by calculating CD4 cell z-scores, based on data from uninfected children born to HIV-infected mothers in the European Collaborative Study [11]. Using data at enrolment from all children and those at subsequent timepoints in the placebo group, the relationship between CD4 cell count and HIV-1 RNA was investigated by jointly fitting $\log_{10}$ CD4 cell count linearly on age and $\log_{10}$ HIV-1 RNA piecewise linearly with a change point at 4.5 years (chosen to give maximum reduction in deviance relative to no change point) in a mixed linear model [10].

Table 1. Clinical and immunological characteristics of 70 children enrolled into the PENTA-1 virology substudy.

	n (%)
Age (years)	
≤ 2	18 (26)
2–4	23 (33)
4–6	15 (21)
> 6	14 (20)
Sex	
Male	30
Female	40
Ethnicity	
White	51
Black African	11
Other	8
CDC clinical stage	
N	27 (39)
A	26 (37)
B	17 (24)
CD4 cell count ($\times 10^6$/l)	
≤ 200	4 (6)
200–499	12 (17)
500–999	22 (31)
≥ 1000	32 (46)
CD4 cell percentage (%)	
< 5	1 (1)
5–15	7 (10)
15–24	21 (30)
≥ 25	41 (59)
CD4 cell z-score	
< −2	27 (39)
−2 to −1	22 (31)
−1 to 0	13 (19)
> 0	8 (11)
Total	70 (100)

CDC, Centers for Disease Control and Prevention paediatric disease criteria.

Results

Baseline characteristics

The characteristics of the 70 children included in the analysis are shown in Table 1. The median age was 3.5 years (range, 0.4–11.9 years). Median values and interquartile ranges (IQR) for absolute CD4 cell count, CD4 cell percentage and CD4 cell z-scores were 881×10^6 cells/l (IQR, $576–1347 \times 10^6$/l), 26% (IQR, 19–31%), and −1.58 (IQR, −2.81 to −0.8), respectively. The majority of children (76%) were classified as having no symptoms (stage N) or mild symptoms (stage A), according to the revised 1994 Centers for Disease Control and Prevention (CDC) classification of HIV infection in children [12]. Of the 17 (24%) children classified as stage B, six had asymptomatic lymphocytic interstitial pneumonitis (LIP), five had a previous single bacterial chest infection, and three had both (Table 1). The remaining three children had oral herpes (n = 1), or presumptive non-respiratory bacterial infections (n = 2). Eleven children were presumed to have non-B clade viruses because their mothers were born in Africa; of these, six had LIP and two had a single chest infection.

HIV-1 RNA

To assess the short-term variability in $\log_{10}$ HIV-1 RNA, Fig. 1 shows the difference between weeks −2 and 0 plotted against the mean of the values. The within-child SD was 0.26 $\log_{10}$ (coefficient of variation, 5.5%). In four children the difference between the two values was greater than 0.5 $\log_{10}$; however, three of these had low viral load (< 3.3 $\log_{10}$ copies/ml).

All 70 children had detectable HIV-1 RNA at baseline. The median and geometric mean were 4.61 and 4.51 $\log_{10}$ copies/ml, respectively, but the range was very wide (2.3–6.6 $\log_{10}$; Fig. 2). Fifty-four per cent of children had levels below 4.7 $\log_{10}$ copies/ml and 27% had levels below 4.0 $\log_{10}$ copies/ml. The distribution among the 11 African children (median, 4.53 $\log_{10}$ copies/ml; range, 3.43–5.54 $\log_{10}$ copies/ml) did not appear to be significantly different from the remaining children (median, 4.61 $\log_{10}$; range, 2.3–6.56 $\log_{10}$ copies/ml; Wilcoxon rank-sum test, $P = 0.3$). The distribution of age and CD4 cell z-scores was also similar in both groups (data not shown). At baseline, the median HIV-1 RNA copy number was significantly

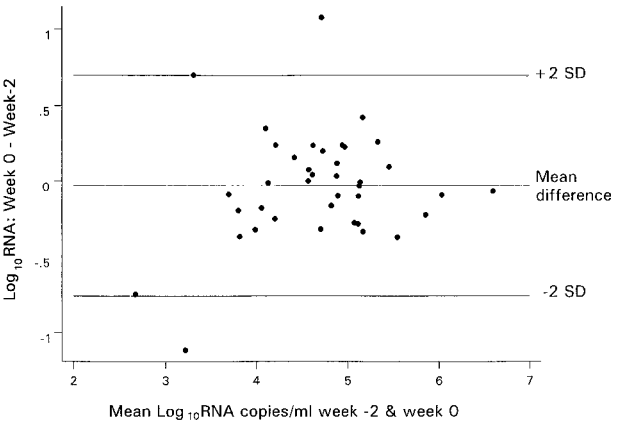

Fig. 1. Within-individual variability of $\log_{10}$ HIV-1 RNA expressed as the mean difference (± 2 SD) between HIV-1 RNA at −2 and 0 weeks in 39 children prior to randomization, plotted against the mean of the two values.

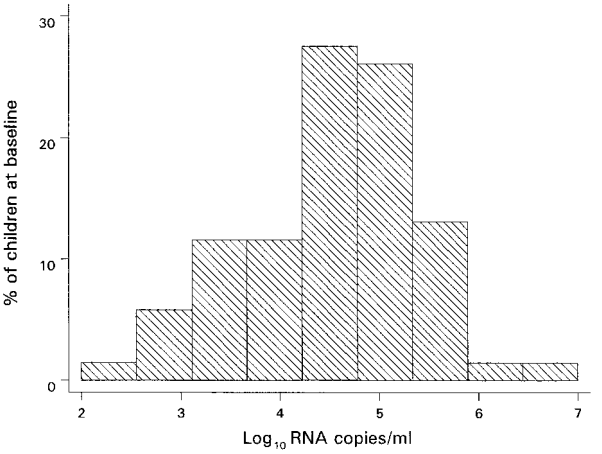

Fig. 2. Frequency distribution of plasma (n = 57) or serum (n = 13) $\log_{10}$ HIV-1 RNA in 70 children at enrolment.

higher in children aged ≤ 2 years (median, 5.23 $\log_{10}$ copies/ml; IQR, 4.74–5.56 $\log_{10}$ copies/ml) compared with those aged > 2 years (median, 4.51 $\log_{10}$ copies/ml; IQR, 3.67–4.89 $\log_{10}$ copies/ml; Wilcoxon rank-sum test, $P < 0.0001$). The median HIV-1 RNA (4.51 $\log_{10}$ copies/ml) in children with CDC clinical stage B disease was not significantly different from that in children in stage N (4.57 $\log_{10}$ copies/ml) and stage A disease (4.91 $\log_{10}$ copies/ml; $P = 0.16$).

Changes in HIV-1 RNA with increasing age for individual children randomized to placebo are shown grouped by age at trial entry in Fig. 3. The baseline values were higher in the younger children and fell more steeply over the subsequent 2 years than in the older children. Fig. 4 shows the scatterplot of these data combined with baseline HIV-1 RNA levels for the 35 children randomized to ZDV, against age and the fitted mean level as a function of age together with pointwise 95% confidence intervals. On average, HIV-1 RNA fell significantly by 0.38 $\log_{10}$ copies/ml (SE, 0.18) per year over the first 2 years and by 0.21 $\log_{10}$ copies/ml (SE, 0.08) per year from 2 to 4 years. The estimated mean decline between 4 and 6 years was 0.034 $\log_{10}$ copies/ml (SE, 0.09) per year and the mean minimum level was 4.25 $\log_{10}$ copies/ml. After age 6 years, there were too few data to give any reliable indication of trend. Results were very similar if only those children in the placebo group were included (data not shown). If region of origin (Europe, 44 children; Brazil and Africa, 26 children) was included as a covariate in the model, viral load was slightly lower in the African and Brazilian children compared with the European children ($P = 0.08$). However, the shape of the trajectory of mean HIV-1 RNA with age was very similar ($P = 0.41$).

Antiretroviral resistance

No child had ZDV-associated genotypic mutations at baseline, and none in the placebo group developed mutations while remaining on placebo.

CD4 cell count

Mean $\log_{10}$ CD4 cell count fell linearly by 0.084 $\log_{10}$ cells/μl (SE, 0.012) per year. In terms of absolute CD4 cell counts, this gives mean cell counts at 1, 2, 4, 6 and 8 years of 1413, 1175, 794, 501 and 355×10^6 cells/l, respectively. The CD4 cell z-score declined by approximately 0.46 (SE, 0.08) per year and the CD4 cell percentage by 1.4% (SE, 0.47%) per year.

HIV-1 RNA and CD4 cell count

The estimated correlation between $\log_{10}$ CD4 cell count and $\log_{10}$ HIV-1 RNA during the first 2 years of life was very weak [r = +0.15; 95% confidence interval (CI), −0.65 to 0.65]. Thereafter the correlation estimate inverted and appeared to increase with age to r = −0.5 (95% CI, −0.7 to 0.7) by 5 years. However, at all ages the 95% CI was wide and included zero. There was marginal evidence ($P = 0.08$) that the rate of decline in $\log_{10}$ CD4 cell count was greater in children with slow initial decline in $\log_{10}$ HIV-1 RNA in the first 5 years of life.

Discussion

This study provides information on HIV-1 RNA in a cohort of vertically infected children who had not received antiretroviral therapy and did not develop AIDS. The short-term variability of HIV-1 RNA was similar to that reported in adults showing that HIV-1 RNA values can vary by approximately 0.5 $\log_{10}$ (threefold) on repeated measurements within days of each other [13,14]. The greatest variability occurred

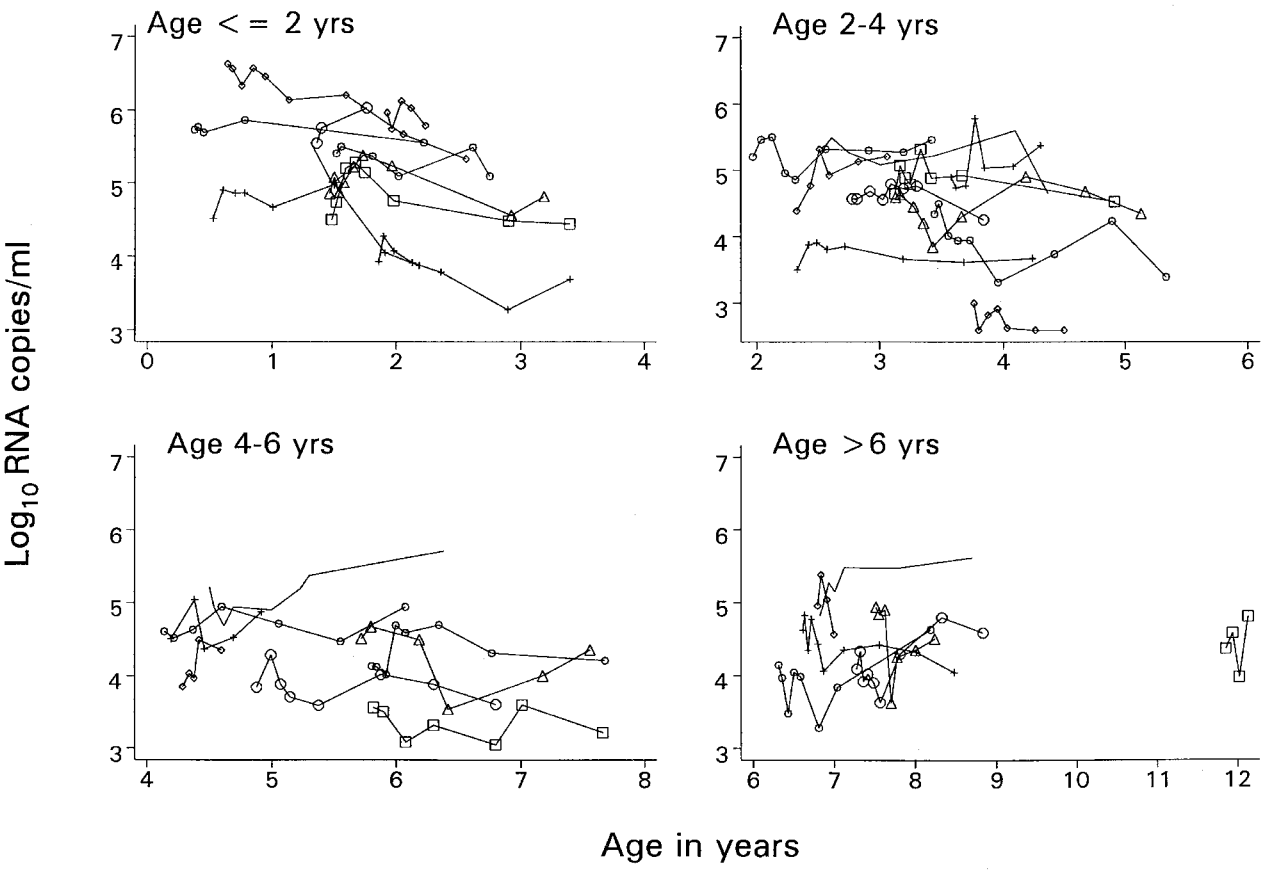

Fig. 3. Profiles of $\log_{10}$ HIV-1 RNA in 35 children in the placebo group plotted against age at enrolment.

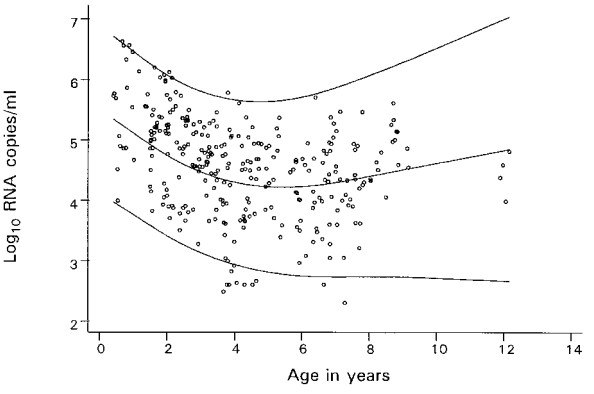

Fig. 4. Log_{10} HIV-1 RNA in 305 samples from 35 children on placebo for up to 105 weeks (296 samples), and from 35 children in the zidovudine group prior to treatment (59 samples), plotted against age. The fitted line and 95% confidence interval around it were derived from the fitted mixed linear model.

towards the lower limits of the sensitivity of the assay.

The median viral load at baseline in children in our study (4.61 $\log_{10}$ copies/ml) was similar to that in adults in the Delta trial (4.71 $\log_{10}$ copies/ml) [15]. However, in the Delta trial, 47% of adults had AIDS-related complex or AIDS at enrolment, and about 10% per year progressed to AIDS or death during the 30 months of follow-up. In contrast, 76% of children in our study were classified as asymptomatic (stage N or A disease) [12] at randomization and no child developed stage C (AIDS-defining) disease during follow-up. Among the 24% with stage B disease, the majority had asymptomatic LIP or a single bacterial infection, neither of which have been shown to be associated with poor survival [16].

We observed no significant differences between the distribution of baseline HIV-1 RNA in black African children and the rest of the children. However, the numbers are small and other data have suggested that PCR-based assays may underestimate HIV-1 RNA by up to 1 $\log_{10}$ in individuals infected with non-B clade viruses [17–19]. Because a proportion of children from Brazil may also have had non-B clade viruses, we undertook further analyses including region of origin (Europe or Africa and Brazil) as a covariate in the model. The results showed that although inclusion of these children slightly decreased HIV-1 RNA overall, there was no evidence that subtype-dependent skewing was contributing to the effect of age on HIV-1 RNA.

Longitudinal data from cohorts of perinatally infected children followed from birth, including mixed populations of slow and fast progressor children, have reported that HIV-1 RNA peaks at median values of 5.0–6.0 $\log_{10}$ copies/ml at 2 months of age and falls to around 4.0–5.0 $\log_{10}$ copies/ml after 2 years [4–6]. Studies of cohorts including older children [20,21] reported falls not dissimilar to those seen in our study (0.29 and 0.21 $\log_{10}$ copies/ml per year, respectively), but included children on treatment (mainly ZDV monotherapy) and a proportion of these children had AIDS. In our study of untreated children, none of whom had AIDS, mean HIV-1 RNA fell by over 1.0 $\log_{10}$ copies/ml over the first 5 years of life, the fall being greatest in the first 2 years. HIV-1 RNA in adults appears to fall to a 'setpoint' within 6–9 months of acquisition of HIV infection [22]. This may reflect an immaturity of the immune system in young HIV-infected children, which fails to contain viral replication to the same extent as in infected adults.

It is well established that absolute CD4 cell count, and to a lesser extent CD4 cells as a percentage of total lymphocytes, fall with age in uninfected children [7]. High CD4 cell counts are a feature in infants and younger children with HIV infection, irrespective of the course of the disease [8]. In our study, the mean absolute CD4 cell count fall was linear on a $\log_{10}$ scale and therefore decreased with increasing age, with the greatest decline (250×10^6 cells/l) in the first year, and the smallest (73×10^6 cells/l) in 6–8 year-olds. Thus, only after the age of about 6 years is the rate of decline similar to that observed in asymptomatic infected adults (60×10^6 cells/l per year) [23]. The greater decline in the youngest children is possibly due to the combined effects of disease progression and the natural fall with age.

We estimated a change in the correlation between HIV-1 RNA and CD4 cells with age from weakly positive in the first 2 years to inverse correlation by 4–5 years. However, the 95% CI around the estimates were very wide at all ages and were consistent with no correlation. There was also marginal evidence suggesting that CD4 cell count declines faster in children with an initial slow decline in HIV-1 RNA. Further data are required to substantiate both these findings. Mofensen *et al.* [20] previously reported a weak inverse correlation ($r = -0.12$) between baseline CD4 cell percentage and HIV-1 RNA in 254 children with mild-to-moderate disease and a similar median age (3.5 years) distribution to those in our study. However, no differences in the nature of the correlation were reported with age. Inverse weak correlations have been observed in other paediatric studies including older children with AIDS [21]. The complex nature of the relationship between these two parameters in vertically infected children is not surprising in view of the dynamics of CD4 cell count changes with age in young uninfected children and the continuing viral load decline over the first 4–5 years of life in HIV-infected children. The relationship between these two parameters may become more similar to that observed in adults [1] only after about 5 years of age.

Viral load is an important predictor of disease progression in HIV-infected adults. There are fewer data in children, but in the trial of immunoglobulin therapy in children, HIV-1 RNA load in infancy was predictive of rapid progression to AIDS or death [20]. We have not reported any analyses of HIV-1 RNA as a predictor of clinical progression to AIDS in this study because only a subset of children from the PENTA-1 trial were included. However, this will be reported when follow-up in the trial is completed.

In conclusion, in this study of asymptomatic or mildly symptomatic vertically HIV-infected children, very high viral loads were seen in the younger children, which declined non-linearly up to 5 years of age in the absence of antiretroviral therapy. The relationship between HIV-1 RNA and CD4 cell count appeared to change with age, becoming inverted only after 2 years. More data are required to explore this and the suggestion that CD4 cell declines are greater in children with slow declines in viral load. It has been shown that HIV-1 RNA can fall to undetectable levels in vertically infected children when treatment is commenced with three-drug antiretroviral regimens soon after the diagnosis of HIV has been confirmed [24]. However, the availability of appropriate formulations of most of the protease inhibitor drugs for young children lags behind that for adults, and data on the long-term tolerability and compliance with triple therapy regimens is lacking. In the absence of data from trials to provide guidance on the timing of commencement of antiretroviral therapy in children with slow disease progression, paediatricians need to use clinical judgement and serial measurements of viral load and CD4 cell counts, bearing in mind that age is a key factor in the interpretation of both these parameters.

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Appendix

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