

AIDS

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## **Early Antiretroviral Therapy Reduces HIV DNA Following Perinatal HIV Infection**

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## ABSTRACT

The impact of antiretroviral therapy (ART) on the size of the HIV reservoir has implications for virological remission in adults, but is not well characterised in perinatally acquired infection (PaHIV). In a prospective observational study of 20 children with PaHIV and sustained viral suppression on ART for >5 years, proviral DNA was significantly higher in deferred (>4 years) versus early (first year of life) ART recipients ( $p=0.0062$ ), and correlated with age of initiation ( $p=0.13$ ;  $r=0.57$ ). No difference was seen in cell-associated viral RNA ( $p=0.36$ ). Identifying paediatric populations with smaller reservoirs may inform strategies with potential to induce ART-free remission.

Key words: Perinatally infected children, early antiretroviral therapy, HIV reservoir

## BACKGROUND

Despite the undeniable gains made in prevention of mother-to-child transmission more than 200,000 children were born with HIV in 2014 and, if they have access, face a lifetime on antiretroviral therapy (ART). Lessons learnt from the 'Mississippi baby' and adult seroconverter studies including the VISCONTI and SPARTAC cohorts suggest that early ART reduces the size of the viral reservoir and can induce remission<sup>1-6</sup>.

Infants acquire HIV on an immature immune system. They have an active thymus with capacity for immune regeneration, with an increased proportion of naive T cells with lower CCR5 expression and reduced susceptibility for HIV infection, and a smaller proportion of long lived resting memory CD4<sup>+</sup> T cells that harbour the majority of the HIV reservoir<sup>7</sup>. Infants have reduced HIV-specific cytotoxic T cell (CTL) responses and, if treated within the first months of life, are frequently seronegative<sup>8</sup>.

Increasing evidence demonstrates the association of early treatment in infancy with reduced proviral DNA reservoir size and restricted HIV-specific immune responses<sup>9</sup>. Given the approximate known timing of transmission in perinatal infection and the relatively preserved immune system, very early treated children may offer an optimal cohort in which to consider immunotherapeutic strategies towards ART free remission<sup>10</sup>. This study analysed the size of the HIV reservoir in blood in relation to age at starting suppressive ART in perinatally infected children on therapy for more than 5 years.

## **METHODS**

### **Participants**

A prospective observational cohort study of 20 children with perinatally acquired HIV infection with sustained viral suppression ( $<50$  copies HIV RNA/ml (c/ml)) on ART for  $> 5$  years were recruited to the CHERUB-yc (Collaborative HIV Eradication of Viral Reservoirs: UK BRC; Young people and Children) cohort.

Two groups of perinatally infected children were compared: those commencing ART in the first year of life (early; EG) or after four years of age (deferred; DG). HIV DNA, cell associated HIV RNA (CA-RNA), and ultra low plasma HIV viral load (lower limit of detection 5 HIV RNA c/ml) were compared between groups. Serological status was assessed by plasma venous sampling using the 4<sup>th</sup> generation Abbott Architect HIV Ag/Ab Combo assay, performed at entry into the CHERUB-yc cohort.

For each child, baseline data collection included gender, ethnicity, age at starting ART and ART drug regimens received. CD4<sup>+</sup> T cell count, percentage and CD4/CD8 ratios were collected at ART initiation and at time of reservoir analysis. Plasma viral load (pVL) data were collected at ART initiation and included time to viral suppression, defined as a plasma HIV VL  $<50$  c/ml. Routinely collected viral load data was reviewed to ensure continuous viral suppression on ART for more than 5 years.

### *Measurement of HIV-1 DNA*

CD4<sup>+</sup> T cells were enriched from frozen PBMC samples by negative selection (Dynabeads) to a purity of >97%. CD4<sup>+</sup> T cell DNA was extracted (Qiagen) and used as input DNA for PCR. Cell copy number and Total HIV DNA levels were quantified in triplicate using previously published assays.

### *Unspliced cell-associated HIV-1 RNA Transcript quantitation*

RNA was isolated and subjected to two rounds of PCR using semi-nested primers. HIV measurements were normalized to input cellular RNA using the 18S gene with the Amplifluor Human/Mouse 18S rRNA Primer Set (FAM labeled) (Millipore).

### *Ultra Low Plasma HIV Viral Load*

RNA was isolated from 3ml EDTA plasma by the method of Boom and amplified in a semi-nested PCR with limited cycles of first round amplification and a real-time TaqMan second round. Each run contained a negative (normal human plasma - NHP) and positive (high RNA copy plasma diluted in NHP to 29 copies/ml) control. The detection limit of the method was 4 HIV RNA copies/ml plasma.

Further details of assays and ethical approvals are available in on-line supplementary material , <http://links.lww.com/QAD/B118>

## RESULTS

### Participant characteristics

In the 'early treatment' group (EG; n=10), 6 were female and 7 were of black African ethnicity with a median age at start of ART of 18 weeks (range 4-35 weeks) (Table 1). Median baseline viral load was >500,000 c/ml, the upper limit of routine pVL quantification. Eight infants presented with CDC C diagnoses. Two were followed from birth, one of whom received 4 weeks of post exposure prophylaxis (PEP) triple therapy with nevirapine (NVP), lamivudine and zidovudine started within 4 hours of birth in 2003. Following cessation of PEP the infant rapidly became viraemic (pVL >500,000 c/ml) and commenced NVP with triple nucleosides at treatment doses. Nine infants commenced NVP with triple nucleoside backbone and one infant received NVP with a dual NRTI backbone. Viral suppression to <50 c/ml was achieved by a median of 24 weeks (range 11-47 weeks) on ART.

For the 'deferred treatment' group (DG; n=10) 6 were female and 7 were of black African ethnicity with a median age initiating ART of 8.0 years (range 6.5-10.6 years). The median CD4+ T cell count at ART initiation was 250 cells/ $\mu$ L (range 0-1050 c/ml) with a median pVL of 197,980 c/ml (range 39,600- >500,000 c/ml), six children having presented with a CDC C category diagnosis. Eight children commenced non-nucleoside reverse transcriptase inhibitor based ART with dual (4) and triple (4) nucleoside backbone and two children commenced a boosted protease inhibitor (lopinavir/ritonavir) based regimen with a dual nucleoside backbone. Viral suppression to <50 c/ml was achieved in a median of 19 weeks (range 10-28).

### **Deferred ART was associated with lower CD4/CD8 ratio**

At time of recruitment into the CHERUB-yc cohort, the median duration on suppressive ART was 9.9 years (range 7.6-12.6) in the EG versus 7.3 years (range 5-10.2 years) in the DG group (Table 1). Median CD4+ T cell counts were 968 cells/ul (range 761-2192 cells/ul) and 739 cells/ul (range 457-1310 cells/ul) with median CD4/CD8 ratios of 1.4 (range 0.9-2.4) and 1.2 (range 0.6-2.2), respectively. After more than 5 years of suppressive ART, three children in the deferred treatment group had persistently abnormal CD4/CD8 ratios ( $<0.9$ ), with normalised CD4/CD8 ratios in all early treated children. Four early treated children were HIV Ab/ag negative by 4th generation assay, with all children in the deferred treatment group being HIV Ab/ag seropositive.

### **No difference in persistent viraemia according to age of ART initiation.**

In assessing low level persistent viraemia, ultra sensitive plasma HIV viral load was  $<5$  HIV RNA copies/ml in 10/10 in the DG and detectable in two children in the EG (pVL of 6 and 57 copies/ml )(Table 1).

### **Greater HIV reservoir associated with delay in ART initiation.**

In assessing the size of the latent reservoir Total HIV DNA was significantly higher in those starting ART after 5 years of age compared to those starting ART in the first year of life ( $p=0.0062$ ; Mann Whitney) (Figure 1a). There was no statistical difference in levels of CA-RNA levels in the two groups ( $p=0.11$ ; Mann Whitney) (Figure 1b). Age of ART initiation when analysed as a continuous variable correlated with proviral DNA ( $p=0.013$ ;  $r=0.57$ )(Spearman's Rank Correlation)(Figure 1c).

### **DISCUSSION**

In this small cohort of children with perinatally acquired HIV, early ART significantly reduced HIV DNA in CD4+ T cells when compared to ART commenced in chronic PaHIV. Studies of adult seroconverters identify HIV DNA as a key predictor of post-treatment viral control and progression<sup>6</sup>. As identifying paediatric populations with lower viral reservoirs may allow optimal selection of individuals likely to achieve ART-free remission, these data provide further evidence for early, sustained ART in PaHIV. It is worth noting that even though the majority of participants were already CDC Stage C at diagnosis (14/20), many of these children were able to achieve low reservoir sizes in the peripheral blood with long term sustained VL suppression on ART.

Early treatment for all infants diagnosed before their first birthday, irrespective of CD4+ T cell count, has been global standard of care since 2008. This was after a randomised controlled trial of early versus deferred ART (the Children with HIV Early Antiretroviral



Therapy (CHER) study) showed a reduction in mortality of 76% compared with initiating ART based on a CD4+ T cell count threshold<sup>11-12</sup>. Consistent with our findings, subsequent analysis of this cohort showed that children who started ART within 12 weeks of life had lower levels of HIV DNA than those in the deferred ART group: median 27 vs 100 copies per 100,000 PBMCs after 96 weeks versus 81 weeks of ART respectively<sup>13</sup>.

In a Spanish cohort of very early treated children, those who initiated ART under 12 weeks had a 6-fold reduction in HIV DNA measured in CD4+ T cells, when compared to those commencing ART between 12 weeks and 1 year of age<sup>14</sup>. Although the deferred treatment group was earlier than in our study, these data are again consistent with our own results. A similar association with age of virological control was seen in a large cohort of adolescents, median age 14.3 years. After a decade on ART those who had suppressed within the first year of life had a lower median proviral load compared to those who suppressed aged 1-5 years and after age 5 years (4.2 copies/million PBMCs versus 19.4 and 70.7 copies/million PBMCs respectively  $p < 0.001$ )<sup>15</sup>. In partial contrast to our findings, a study of 12 children who initiated ART within 1.2 months of birth and sustained viral suppression for an average of 5 years demonstrated that ART initiated from early infancy does not prevent the establishment of a reservoir of latent provirus, but does significantly limit the evolution of HIV in viral reservoirs<sup>9</sup>.

Four of the ten early treated children in this study remained HIV seronegative by 4<sup>th</sup> generation assay after almost a decade of suppressive ART, comparable to 43% of the

CHER cohort starting therapy by 12 weeks of age<sup>8</sup>. All those commencing ART in childhood in our cohort, or after 24 weeks of age in the CHER cohort were seropositive. Whilst such infants may be optimal candidates for interventions leading to ART free remission, antibody seronegativity potentially raises confirmatory diagnostic issues, particularly for carers questioning the original diagnosis<sup>8</sup>.

In assessing evidence of low level viral turnover, HIV pVL was <5 c/ml in all patients in the deferred ART group but detectable in two children in the early treated group. One child had a plasma viral load around the limit of detection (6 copies/ml) however a second child, on nevirapine, abacavir and lamivudine had a pVL 57 c/ml on the low copy assay with a paired routine clinical pVL of less than 20 c/ml. Subsequent routine clinical follow up 3 months later confirmed virological failure with a pVL of 2500 c/ml, prompting an ART switch.

This is a small single centre cohort analysis of the latent HIV reservoir in perinatally infected children, the majority of whom started ART in the early 2000s with symptomatic disease. Whilst the cohort lacks the homogeneity of other studies, even in this setting, early, continuous suppressive therapy limits the size of the latent reservoir. In the prospective Collaborative HIV Paediatric Study (CHIPS), covering more than 95% of children diagnosed in the UK and Ireland, only 2% of children commenced ART before 6 months of age and remain suppressed at current follow up. Accordingly, multicentre international collaborations, such as those outlined in the EPIICAL project are required to

establish a co-ordinated approach to achieving early ART free remission in children living with HIV<sup>10</sup>.

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The authors report no conflicts of interest

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**Table 1. Demographics, Presenting Characteristics and ART initiation**

	Early Treated Group (n=10)  ART < 1 year	Deferred Group (n=10)  ART > 5 years
Female sex (%)	6 (60)	6 (60)
Black African ethnicity (%)	7 (70)	7 (70)
Median current age years (range)*	9.9 (8.8-14.0)	15.6 (12.4-16.6)
Parameters at ART Initiation:		
Median age commenced ART (range)*	18 weeks (4-35)	8 years (6.5-10.6)
CDC category C	8 (80)	6 (60)
Median pre-ART CD4+ T cell count cells/uL (range)	820 (140-3720)	250 (0-1050)
Median pre-ART CD4+ T cell % (range)	28 (6-51)	14 (0-32)
Median VL (c/ml)	>500,000	197,980

	(2514 - >500,000)	(39,600->500,000)
Initial ART Regimen NNRTI based (%)	10 (100)	8 (80)
Median time to viral suppression (VL<50 c/ml)	24 weeks (range 11-47)	19 weeks (range 10-28)
Median duration of suppressive ART years (range)	9.9 (7.6-12.6)	7.3 (5.0-10.2)
Median CD4+ T cell count cells/uL (range)	1124 (813-1413)	717 (546-994)
Median CD4+ T cell % (range)	43% (29-55%)	38% (28-55%)
Median CD4/CD8 (range) [n with CD4/CD8, <0.9]	1.4 (0.9-2.4) [0]	1.0 (0.6-2.2) [3]
Seronegative on HIV 4 <sup>th</sup> generation Ab/Ag Test	4 (40%)	0
Ultra low plasma VL <5 copies/ml	8 (80)	10 (100)

Details of the participant cohort including those who initiated ART before 1 year and after 5 years. ART antiretroviral therapy; CDC Centre for Disease Classification.

\*P <0.001; Mann-Whitney.



## Figure 1. Association of the HIV Reservoir with Timing of ART Commencement

Total HIV DNA (A) and unspliced intracellular HIV RNA (B) measured in purified blood CD4+ T cells were compared in participants who commenced ART before or after the age of 1 year (Mann-Whitney Test). Total HIV DNA was correlated with age of ART initiation as a continuous variable (Spearman's Rank Correlation)(C).

