

# Transient viral load increases in HIV-infected children in the UK and Ireland: what do they mean?

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**Objectives:** To investigate transient increases in viral load during sustained suppression in children in the UK and Ireland Collaborative HIV Paediatric Study (CHIPS).

**Design:** Cohort of HIV-infected children from 39 centres.

**Methods:** Transient viraemia was defined as  $\geq 1$  detectable viral loads ( $\geq 50$  copies/ml) between two undetectable values ( $< 50$  copies/ml)  $< 280$  days apart, during a period of sustained viral suppression (from a confirmed level of  $< 50$  copies/ml until the last undetectable measurement before antiretroviral therapy change or until a confirmed level of  $> 50$  copies/ml).

**Results:** Of 595 children initiating HAART without previous treatment, 347 (58%) achieved sustained suppression. Of these, 78 (23%) experienced 109 episodes of transient viraemia (median 134 copies/ml); 92 (84%) had levels of  $< 1,000$  copies/ml (maximum 39,839). Transient viraemia was more common during second-line therapy (25/100 child-years [CY]) and

following a previous episode (19/100 CY) compared with first-line therapy without a previous episode (11/100 CY). Rates decreased with age at HAART initiation (incidence rate ratio [IRR] 0.95 per year older;  $P=0.05$ ), but were higher in those suppressed for longer (IRR 1.63 in those suppressed for  $\geq 1$  year versus  $< 1$  year;  $P=0.03$ ). CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts were similar before and after transient viraemia. Of detectable viral loads during periods of suppression 44% were transient increases rather than virological failure: experiencing transient viraemia did not increase subsequent virological failure ( $P=0.20$ ).

**Conclusions:** Transient viraemia is relatively common among children on HAART, occurring more frequently in those starting HAART at younger ages, on second-line therapy and after longer suppression. It does not appear to affect CD4<sup>+</sup> or CD8<sup>+</sup> T-cell counts or the risk of subsequent virological failure. Natural variation, assay effects and adherence might all have a role.

## Introduction

During periods of viral suppression on antiretroviral therapy (ART) many HIV-infected individuals have transient periods of viraemia, during which HIV RNA viral load temporarily becomes detectable. It is generally thought that these transient increases in viral load represent random biological and statistical variation rather than clinically significant increases in viraemia [1] and that they are not a predictor for longer-term virological or immunological failure in adults [2–4]. The occurrence of transient viraemia has also been linked to the emergence of drug-resistant HIV variants [5,6]. By contrast, two studies have found associations between intermittent viraemia and

long-term virological failure [7,8]. One complication in interpreting the research evidence in adults is that definitions of transient viraemia vary between studies, making the comparison of results difficult [4]. For example, Greub *et al.* [8] define a 'blip' as a single HIV RNA test  $> 50$  copies/ml and  $< 500$  copies/ml and a 'bump', which was a stronger predictor of virological failure, as multiple HIV RNA  $> 50$  and  $< 500$  copies/ml between two undetectable measures, regardless of the duration of such low-level viraemia. By contrast, Easterbrook *et al.* [7] defined intermittent viraemia as any number of HIV RNA levels  $> 400$  copies/ml followed by a measurement

<400 copies/ml. Transient increases in viral load might be genuine 'blips' in viral load or might indicate virological failure: predictors and concurrent changes in other parameters might be very different in these two situations and different definitions of transient viraemia might obscure genuine effects.

Although there are a number of papers describing transient increases in viral load in adults on ART, there are no reports of transient viraemia in children. As children generally have lower virological suppression rates than adults [9], and higher viral load at ART initiation [10,11], patterns of transient viraemia might be different in children. To address this issue we explore the incidence, characteristics, predictors and subsequent consequences of transient increases in viral load in children with virological suppression (<50 copies/ml) in the Collaborative HIV Paediatric Study (CHIPS) cohort of HIV-infected children in the UK and Ireland.

## Materials and methods

CHIPS is a multicentre cohort of HIV-infected children under care in the UK and Ireland since 1996 [12]. Analyses are based on data reported to CHIPS up to December 2005, from 39 hospitals in the UK and Ireland, and account for approximately 90% of all children reported to the National Study of HIV in Pregnancy and Childhood (NSHPC) and alive in 2005. Data for 2005 could be incomplete and subject to reporting delay. Ethics approval for CHIPS was obtained from the London Multicentre Research Ethics Committee (MREC), UK.

Here we define HAART as regimens containing  $\geq 3$  drugs from  $\geq 2$  classes, or triple-NRTI regimens including tenofovir or abacavir, and consider children who initiated HAART without previous ART apart from having received drugs to prevent mother-to-child transmission. Because single-drug substitutions for toxicity or poor formulation tolerability are relatively common, we define switch to second-line (or third-line) as any switch of  $\geq 3$  drugs (regardless of reported reason) or a switch of two drugs with reported reasons being for 'failure' (CD4<sup>+</sup> T-cell count, viral load or clinical failure or resistance) with HIV-1 RNA >50 copies/ml. Substitutions of one drug (within or across class) or two drugs for reasons other than failure (including toxicity, personal request and simplification), as well as switches with viral load <50 copies/ml, are not counted as changes to second-line therapy.

During first- and second-line therapy, we define periods of sustained virological suppression as the time from the first of two consecutive undetectable viral loads (<50 copies/ml) to the last viral load <50 copies/ml before confirmed viral load failure (persistent viral load >50 copies/ml) or switch to

second- or third-line treatment (minimum observed duration of sustained suppression 3 months, 84% >6 months). Undetectable viral loads with a detection limit >50 copies/ml (for example <400 copies/ml) were assumed to be <50 copies/ml if they were within 4 months of a viral load <50 copies/ml. In order to allow for the fact that some children have more frequent viral load measurements, owing to the observational nature of the data, transient increases in viral load within periods of sustained virological suppression were defined as one or more detectable viral loads  $\geq 50$  copies/ml between two undetectable viral loads (<50 copies/ml) <280 days apart without a change in treatment (ignoring single drug substitutions for simplification/personal reasons and the addition of a single drug to the regimen). We did not put a limit on the viral load at the time of the transient viraemia, choosing to define this by resuppression without major change of regimen within a set period of time in order to contrast with incipient failure, as paediatricians would not consider a single raised value even of >1,000 copies/ml as failure in clinical practice. The time period of 280 days was chosen to capture a common pattern of a single detectable viral load between two undetectable viral loads on a 3-monthly visit schedule allowing for a single missed visit. Conversely, confirmed viral load failure was defined as the first of two or more consecutive viral load measurements >50 copies/ml spanning more than 280 days. Where the transient viraemia consisted of multiple detectable viral loads (19% of episodes), we defined the viral load at the time of the transient increase as the geometric mean of the measured values. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts at each transient increase in viral load were the closest values within  $\pm 3$  days of the first detectable viral load measurement with values available.

We then evaluated predictors of the rate of transient viraemia during periods of sustained virological suppression using univariable and multivariable Poisson regression considering the following: gender; age, CD4<sup>+</sup> T-cell count, viral load and prior Centers for Disease Control and Prevention (CDC) B or C events at HAART initiation; current therapy (first- or second-line, including protease inhibitor [PI] or non-nucleoside reverse transcriptase inhibitor [NNRTI]); time-dependent effects of having experienced transient viraemia previously and of current calendar year, age and duration of virological suppression. As there were a small number of missing values of CD4<sup>+</sup> T-cell count and viral load at HAART initiation, 10 multiple imputations (MICE) were used in multivariable models [13,14], to avoid excluding different children from different analyses. Multivariable models were based on backwards elimination using the Akaike Information Criteria. Finally we considered

predictors of the first detectable viral load during the period of suppression being either transient viraemia or confirmed viral load failure using cause-specific hazards within a competing risks framework [15].

## Results

Of the 1,065 children enrolled in CHIPS up to December 2005, 595 started HAART without previous treatment and were eligible for this analysis, of whom 347 (58%) went on to achieve sustained virological suppression <50 copies/ml. Seventy-eight children (13% of total, 22% of those with sustained suppression) experienced a total of 109 transient increases in viral load, the majority during first-line therapy, reflecting duration of follow up (Table 1).

The median viral load during episodes of transient viraemia were 118 copies/ml (interquartile range [IQR] 72–344) during first-line therapy and 167 copies/ml (IQR 80–519) during second-line therapy (rank sum  $P=0.68$ ), with 86 (79%), 92 (84%) and 104 (95%) being <500, <1,000 and <10,000 copies/ml respectively and all being <40,000 copies/ml (Table 1). The time from the first detectable viral load to the following undetectable viral load reflected the most common pattern of 3-monthly clinical visits with a single detectable viral load between two undetectable values (median 91 days, range 14–216). Transient increases in viral load occurred in children of all ages from 1 to 15, and after a median of 1.4 years of sustained virological suppression, with 63% occurring after >1 year of viral loads <50 copies/ml. The median CD4<sup>+</sup> (%) and CD8<sup>+</sup>

**Table 1.** Characteristics of transient viraemic episodes and children who experience transient viraemia

Parameter	First-line	Second-line	Overall
Number of children, <i>n</i> (%)	595 (100)	132* (100)	595 (100)
Children reaching sustained virological suppression, <i>n</i> (%)	300 (50)	60 (45)	347† (58)
Child years with sustained suppression			
Total	684.6	98.8	783.4
Median (IQR) per child‡	1.8 (0.9–3.8)	1.3 (0.4–2.7)	1.8 (0.9–3.7)
Children experiencing transient viraemia, <i>n</i> (% of those reaching sustained suppression)	59 (20)	19 (32)	78§ (22)
1 episode	43 (14)	12 (20)	55 (16)
2 episodes	12 (4)	5 (8)	17 (4)
3 episodes	2 (1)	2 (3)	4 (1)
4 episodes	2 (1)	0 (0)	2 (1)
Total number of episodes, <i>n</i>	81	28	109
Transient viraemia rate, per 100 CY (95% CI)	11 (8–14)	25 (17–39)	12 (10–15)
Rate <1,000 copies/ml, <i>n</i>	9	21	10
Rate ≥1,000 copies/ml, <i>n</i>	2	5	2
Subsequent episodes after first episode of transient viraemia, <i>n</i>	22	9	31
Child years at risk after first episode of transient viraemia, <i>n</i>	95.2	17.6	112.8
Rate of subsequent episodes, per 100 CY (95% CI)	19 (13–29)	15 (5–43)	18 (12–27)
Age at ART initiation in children who experienced transient viraemia¶, years	3.5 (0.6–8.3)	3.3 (1.8–7.4)	3.3 (0.8–7.9)
By age group, <i>n</i> (% of children starting ART at this age and achieving viral suppression)			
<1	15 (29)	3 (43)	18 (32)
1–3	13 (20)	8 (50)	21 (26)
4–8	20 (18)	6 (29)	26 (21)
9–12	8 (15)	2 (17)	10 (16)
≥13	3 (16)	0 (0)	3 (14)
Viral load during episode of transient viraemia, median copies/ml (range)	118 (51–39,839)	167 (51–36,442)	134 (51–39,839)
Age at episode¶, years	7.2 (4.7–11.0)	8.7 (6.0–12.3)	7.7 (4.9–11.3)
Time since HAART initiation at time of episode¶, years	2.8 (1.4–4.2)	4.6 (3.7–5.3)	3.2 (1.8–4.9)
Duration of sustained suppression at time of episode¶, years	1.4 (0.7–2.9)	1.3 (0.7–2.2)	1.4 (0.7–2.4)
Duration of suppression after the first episode¶, years	1.8 (0.8–1.7)	0.9 (0.6–1.7)	1.4 (0.7–2.3)
Any change in ART during episode¶, <i>n</i> (% of episodes)	5 (6)	4 (14)	9 (8)
Multiple viral loads >50 cells/ml during episode, <i>n</i> (% of episodes)	14 (17)	7 (25)	21 (19)

\*The 132 children with data on second-line therapy are a subset of the 595 children with data on first-line therapy. †A total of 347 children reached sustained virological suppression at some point during either first or second-line therapy (13 in both first- and second-line therapy). ‡For children reaching suppression. §No child experienced transient viraemia during both first- and second-line therapy. ¶Values given as median (interquartile range). #During six episodes of transient viraemia a single drug was substituted within class, one substituted a single drug cross-class, one dropped a single drug from a four-drug regimen and in one episode in a child aged 5 zidovudine was added to lamivudine+abacavir+nevirapine. All nine changes were for personal reasons or simplification and the median viral load during the episode of transient viraemia was 120 copies/ml (range 67–3,000), similarly to overall. ART, antiretroviral therapy; CI, confidence interval; HAART, highly active ART.

**Table 2.** Univariable and multivariable predictors of transient viraemia: Poisson regression

	Univariable model			Multivariable model		
	IRR	95% CI	P-value	IRR	95% CI	P-value
Sex, female versus male	0.73	0.47–1.12	0.15	–	–	–
Age at HAART initiation, per year	0.95	0.90–1.01	0.08	0.95	0.90–1.00	0.05
CD4% at HAART initiation, per 5% increase	1.05	0.96–1.15	0.29	–	–	–
Viral load at HAART initiation, per log <sub>10</sub> copies/ml	1.18	0.89–1.58	0.25	–	–	–
CDC B/C event before HAART initiation	1.21	0.79–1.84	0.38	–	–	–
Current age						
0–3 years	1.00	–	0.91	–	–	–
4–12 years	0.90	0.56–1.44	–	–	–	–
13 years or older	0.93	0.48–1.80	–	–	–	–
Current calendar year						
1997–99	1.00	–	0.19	–	–	–
2000–02	1.09	0.43–2.75	–	–	–	–
2003–05	1.49	0.59–3.78	–	–	–	–
Current duration of suppression						
<1 year	1.00	–	0.05	*	–	–
1–2 years	1.71	1.14–2.56	–	–	–	–
2–3 years	1.60	0.98–2.63	–	–	–	–
3–4 years	1.99	1.11–3.56	–	–	–	–
≥4 years	1.58	0.78–3.22	–	–	–	–
Therapy						
First-line	1.00	–	–	1.00	–	–
Second-line	2.43	1.59–3.71	<0.001	2.25	1.46–3.48	<0.001
Previous episodes of transient viraemia						
None	1.00	–	–	1.00	–	–
≥1	1.87	1.24–2.82	0.003	1.66	1.07–2.56	0.02
Current regimen contains NNRTI	0.62	0.42–0.92	0.02	0.67	0.46–0.98	0.03
Current regimen contains PI	1.70	1.15–2.53	0.006	–	–	–

Incidence rate ratio (IRR) is univariable and multivariable (backwards elimination based on Akaike Information Criteria) Poisson regression of transient viraemia on period of sustained suppression. \*Adjusted IRR for children suppressed for >1 year compared with children suppressed for <1 year is 1.63 (95% confidence interval [CI] 1.05–2.53),  $P=0.03$ , with other effects unchanged. CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

T-cell counts during transient viraemia were 858 cells/mm<sup>3</sup> (IQR 634–1,314; 32%, IQR 26–36) and 970 cells/mm<sup>3</sup> (IQR 720–1,190) respectively. Changes in CD4<sup>+</sup> or CD8<sup>+</sup> T-cell counts were not associated with transient viraemia, with median differences of -20 (IQR -127–140) and +4 (-178–128) respectively from the preceding counts (sign rank  $P=0.85$  and  $0.78$ , respectively) and median differences of +17 (IQR -117–130) and +35 (-110–190), respectively, to the next counts (sign rank  $P=0.44$  and  $0.07$ , respectively).

Overall transient increases in viral load occurred at a rate of 12 per 100 child-years (CY; 95% confidence interval [CI] 10–15) of suppression (Table 1), although the rate was higher in second-line than in first-line therapy (25 versus 11 per 100 CY respectively; unadjusted IRR 2.4 [95% CI 1.6–3.7];  $P<0.001$ ; Table 2), with similar rates for transient viraemia <1,000 and ≥1,000 copies/ml (Table 1). Most children (71%) had just one transient increase in viral load, with only six (8%) of those with transient viraemia having three or

four distinct episodes. However, the rate was slightly higher after the first transient increase (increasing from 12 [95% CI 10–15] per 100 CY before to 18 [95% CI 12–27] after the first episode;  $P=0.003$ ; Table 1) suggesting that some children were at greater risk.

In addition to differences between rates of transient viraemia on first- versus second-line therapy and before and after the first episode, we found that higher rates of transient viraemia were associated with younger ages at HAART initiation (adjusted IRR 0.95 [95% CI 0.90–1.00] per year older;  $P=0.05$ ; Table 2). For example, 29% of children initiating HAART aged ≤3 years and achieving sustained virological suppression subsequently experienced a transient increase in viral load compared with only 15% of children aged ≥9 years at HAART initiation (Table 1). However current age was not a predictor either univariably or multivariably. Rates of transient viraemia were also lower in children currently receiving NNRTIs (adjusted IRR 0.67 [95% CI 0.46–0.98];  $P=0.03$ ) and conversely

**Table 3.** Predictors of transient viraemia versus failing during periods of sustained virological suppression: cause-specific hazards

	Transient viraemia: multivariable model			Failure: multivariable model			Het <i>P</i> -value*
	CHR	95% CI	<i>P</i> -value	CHR	95% CI	<i>P</i> -value	
Sex, female versus male	0.71	0.44–1.15	0.16	1.24	0.86–1.78	0.25	0.05
Age at HAART initiation, per year	0.93	0.87–1.01	0.07	1.04	0.98–1.10	0.16	0.01
CD4 <sup>+</sup> T-cell % at HAART initiation, per 5% increase	1.02	0.92–1.13	0.71	0.99	0.91–1.08	0.84	0.69
Viral load at HAART initiation, per log <sub>10</sub> copies/ml	1.08	0.81–1.42	0.61	0.92	0.70–1.21	0.56	0.41
CDC B/C event before HAART initiation	1.17	0.73–1.88	0.52	0.83	0.57–1.20	0.31	0.23
Current calendar year							
1997–1999	1.00	–	0.72	1.00	–	0.48	0.48
2000–2002	0.68	0.25–1.85	–	1.63	0.51–5.16	–	–
2003–2005	0.66	0.25–1.79	–	1.27	0.40–4.07	–	–
Therapy							
First-line	1.00	–	–	1.00	–	–	0.33
Second-line	3.32	2.03–5.42	<0.001	2.45	1.55–3.88	<0.001	–
Regimen contained NNRTI	0.56	0.17–1.83	0.34	0.64	0.25–1.46	0.26	0.91
Regimen contained PI	0.78	0.25–2.45	0.67	0.54	0.23–1.29	0.17	0.61

\**P*-value for heterogeneity in the effect of each factor on the risk of transient viraemia versus failing from a cause-specific hazard (CHR) model. CDC, Centers for Disease Control and Prevention; CI, confidence interval; HAART, highly active antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

were higher in children currently receiving PIs. Approximately 65% of NNRTIs received during periods of virological suppression were nevirapine, with the remaining 35% being efavirenz, and the predominant PI was nelfinavir (approximately 75%). Although duration of suppression was not a significant predictor when categorized by year (Table 2), children suppressed for  $\geq 1$  year had significantly higher rates of transient viraemia than those suppressed for  $< 1$  year (adjusted IRR 1.63 [95% CI 1.05–2.53];  $P=0.03$ ; effect of other predictors unchanged). Similar results were obtained in a cause-specific analysis of transient viraemia for  $< 1,000$  copies/ml only.

During periods of virological suppression we observed a total of 109 episodes of transient viraemia and 141 virological failures (as defined in *Materials and methods*) so that 44% of detectable viral loads following a period of suppression were transient increases rather than failures. For a child with virological suppression, the probability of the next viral load being a transient increase, failure or remaining  $< 50$  copies/ml was 0.05, 0.06 and 0.89 respectively. Using time-dependent models for the outcome virological failure, we found no evidence for a higher rate of virological failure in children who had experienced transient viraemia (HR 0.74 [95% CI 0.46–1.17] compared with those who had never experienced transient viraemia;  $P=0.20$  [adjusted for pre-HAART variables: sex, age, CD4<sup>+</sup> T-cell count, viral load and CDC B/C events, line of ART and current calendar year]). Similarly, children who had a transient increase in the last year (adjusted HR 0.93 [95% CI 0.59–1.47];  $P=0.77$ ), or a transient increase  $> 1,000$  copies/ml

(adjusted HR 0.84 [95% CI 0.35–2.06];  $P=0.71$ ) were not at higher risk of subsequent virological failure.

Considering the relative risks of transient viraemia versus failing, we found similar predictors of transient viraemia as in the Poisson analysis (Table 3). However, whilst children who started HAART at younger ages and were currently sustaining virological suppression had greater risks of experiencing a transient increase than older children, their risk of confirmed virological failure was lower than that of older children (heterogeneity  $P=0.01$ ). This most probably reflects a stronger selection effect in the youngest children starting HAART in terms of achieving sustained virological suppression. Thus younger children with suppressed viral load were at higher risk of transient viraemia, whereas older children were at higher risk of failing. There was also a suggestion that whilst girls might be at lower risk of transient viraemia than boys, their risk of failure was no different (heterogeneity  $P=0.05$ ). Therefore, relatively, girls with sustained virological suppression might be more likely to fail than to have a transient increase in viral load whereas boys might be more likely to have a transient increase in viral load than fail. We found similar effects of sex and age on low-level blips  $< 1,000$  copies/ml and transient viraemia  $\geq 1,000$  copies/ml, both in contrast to the effects of virological failure.

Of the 347 previously untreated children starting HAART and achieving sustained virological suppression, we were only able to identify three resistance tests performed during a transient increase in viral load (two from the same child during a single period of suppression) and eight carried out within 6 months of the first confirmed viral load failure following a period of

suppression (two from the same child after two separate periods of suppression) from the UK National Resistance database [16]. None of these nine children with resistance tests during a transient increase in viral load or failure had any resistance test results available before the period of sustained virological suppression. Viral loads and other characteristics during transient viraemia/failure were broadly representative of the larger group. In one child with two resistance tests during separate transient viral load increases in separate periods of suppression (child A) it is possible that resistance was acquired (Table 4): the child had no resistance mutations in the first test but had acquired the 82A PI mutation [17] during their second period of transient viraemia a year later. Four of the remaining eight children with resistance data available during transient viraemia or failure showed RT mutations, but without baseline data it is impossible to determine the likelihood of resistance being acquired during transient viraemia or at failure or existing beforehand.

## Discussion

Here we have shown that transient increases in viral load are fairly common, arising in 22% of children with maximal suppression on HAART, similar proportions to the 27–41% reported in adults [2,4,18]. Although these transient increases occur as commonly

as virological failure during periods of virological suppression, they appear to have no significance in terms of concurrent changes in CD4<sup>+</sup> or CD8<sup>+</sup> T-cell counts, and do not appear to affect subsequent virological failure over a median follow up of 1.4 years after the first transient increase in viral load, again similarly to adults [2,3,19]. One possible explanation is that transient viraemia is merely a result of measurement error or natural variation of low-level viraemia, and is an artefact of the lower limit of detection of viral load assays. The lack of effect on CD4<sup>+</sup> or CD8<sup>+</sup> T-cell counts or on subsequent virological failure would support this random explanation, as does the fact that the majority (84%) of transient increases observed in our study had low viral loads <1,000 copies/ml. However, the fact that even with a relatively small number of children we were able to identify a number of statistically significant predictors of transient viraemia (and low-level blips) suggests that there might be causes beyond natural or assay variation [20], although this is in contrast to at least one large adult study which found no significant predictors of transient viraemia [4]. In particular, adherence can be more challenging in children, who depend on a caregiver for medication and are often unaware of their diagnosis. Poor adherence might increase the chance of both transient viraemia and failure, in addition to other factors which might influence these outcomes in different

**Table 4.** Mutations and prior NNRTI/PI exposure in 10 patients where resistance tests are available either during an episode of transient viraemia or during failure after a period of suppression containing an episode of transient viraemia

Child	Viral load at test*	Mutations present		Exposure to drug class at resistance test (duration)			Previous NNRTI/PI exposure (duration)
		RT	PI	NNRTI	PI	NRTI	
<b>During an episode of transient viraemia</b>							
A	36,442	None	None	–	LPV (2 years)	ddl+d4T	NVP (4 months)
A	4,322	None	82A	–	LPV (3 years)	ddl+d4T	NVP (4 months)
B	20,400	106A	None	NVP (1.5 years)	–	ZDV+3TC	–
<b>At failure after a period of suppression containing an episode of transient viraemia</b>							
C	787	None	None	NVP (4 years)	–	ddl+d4T	–
D	40,200	None	None	EFV (7 months)	–	3TC+d4T	NFV (3 years)
E	9,343	None	None	–	–	–	NVP (1.5 years)
F	40,796	None	None	NVP (3 years)	–	ZDV+3TC+ABC	–
G	12,383	103N, 181C, 190A	None	NVP (8 months)	–	ZDV+3TC	–
G	424,286	190A	None	–	LPV (7 months)	ddl+ABC	NVP (9 months)
H	198,013	181C	None	NVP (1.5 years)	–	ZDV+3TC+ABC	–
I	12,945	65R, 103N, 106M	None	EFV (3 years)	–	3TC+ABC	–

\*Viral load on the day of the resistance test or else the closest viral load measurement within the 2 months before the test or up to 8 days after. 3TC, lamivudine; ABC, abacavir; d4T, stavudine; ddl, 2',3'-dideoxyinosine; EFV, efavirenz; LPV, lopinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; ZDV, zidovudine.

ways. In particular, transient increases in viral load of a higher magnitude might be more likely to be a consequence of adherence problems than low-level episodes, which might be more likely to be due to measurement error or natural variation. We found gender and time on therapy to be associated with higher rates of transient viraemia and low-level blipping, factors which have also been associated with poorer adherence. It is noteworthy that the risk of transient viraemia was >1.5 times higher after >1 year of sustained suppression, suggesting that ongoing efforts to promote adherence might be important, and was also significantly higher during second-line rather than first-line therapy.

Our observation that age at HAART initiation is a stronger determinant of transient viraemia than current age is intriguing. Young children have very high viral loads when starting HAART and it is possible that incomplete suppression early in the course of HAART initiated early in life increases the latent viral burden that could manifest later as low-level viral rebound, although in this dataset we only found a non-significant trend towards more transient viraemia in those with higher baseline HIV RNA after adjusting for age, possibly due to low power. Although slightly more younger children started therapy with a PI than older children (37%  $\leq 3$  years versus 27%  $\geq 9$  years), confounding of treatment and age is not likely to explain these findings, as adjusted and unadjusted effects of age were similar. We also found that NNRTI use was associated with lower rates of transient viraemia and PI use was associated with higher rates of transient viraemia, although the majority of children with sustained virological suppression on PIs were taking nelfinavir, which is now infrequently used in children. Numbers were too few to consider individual drugs separately, but the longer half-lives of NNRTIs might provide some protection against the effect of incomplete adherence on transient viraemia.

One limitation of our study is the observational nature of cohort data. Although viral load and CD4<sup>+</sup> T-cell counts are typically measured 3-monthly, in some cases there were much shorter intervals between measurements around a transiently raised viral load, whereas in other cases measurements continued 3-monthly. We defined transient viraemia on the basis of a restricted but consistent period of time and clearly distinguished a transient increase from incipient failure or an ongoing period of sustained low-level viraemia, which might have different consequences. We also allowed the viral load to reach any level during transient viraemia providing that the child resuppressed. An alternative would be to put a limit on the viral load. However in order to directly compare transient viraemia and incipient failure any transient increases over this threshold would then be defined as failure,

but few clinicians would classify a single viral load of 1,000–40,000 copies/ml followed by resuppression as viral load failure. Instead we focus on resuppression without major change in treatment within a consistent time period to avoid making arbitrary decisions about what to consider as a transient increase and how to classify transient increases with higher viral loads. Notably, very few transient increases according to our definition were >10,000 copies/ml. Although different definitions have been used in adult studies (any detectable viral loads after two undetectable measurements <50 copies/ml in those who again achieve suppression providing that there is no change in drugs in [4,19], compared with single detectable viral load between two undetectable measurements <50 copies/ml in [1,2]) we observed fairly similar rates of transient viraemia to these adult studies. However, data on viral or other intercurrent infections, which could induce a transient increase in viral load, are not collected in CHIPS, nor are data on adherence. Although we attempted to evaluate the effect of transient increases in viral load on resistance [5], these data are not formally collected within the cohort, and comparison with the UK National Resistance database identified only a few children with any resistance data, and even fewer baselines, thus limiting the conclusions that could be drawn from these data.

Overall, it appears that although transient increases in viral load are relatively common among children with sustained viral suppression on HAART, as in adults they can largely be ignored as they do not appear to affect CD4<sup>+</sup> or CD8 T-cell counts or risk of subsequent virological failure, at least over the medium term. Natural variation, assay effects and adherence might all have a role in transient viraemia, and it is certainly possible that improved attention to adequate dosing and adherence following an initial raised viral load contribute to their lack of overall effect. Nevertheless, even if an immediate effect of transient viral load increases cannot be detected, their importance for replenishing the viral reservoir should not be underestimated, as it has been suggested that it is particularly children who experience transient viraemia who have poorer decreases in HIV-1 DNA [21].

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