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The impact of viraemia on inflammatory biomarkers and CD4$^+$ cell subpopulations in HIV-infected children in sub-Saharan Africa

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Objective: To determine the impact of virological control on inflammation and cluster of differentiation 4 depletion among HIV-infected children initiating antiretroviral therapy (ART) in sub-Saharan Africa.

Design: Longitudinal cohort study.

Methods: In a sub-study of the ARROW trial (ISRCTN24791884), we measured longitudinal HIV viral loads, inflammatory biomarkers (C-reactive protein, tumour necrosis factor alpha, interleukin 6 (IL-6), soluble CD14) and (Uganda only) whole blood immunophenotype by flow cytometry in 311 Zimbabwean and Ugandan children followed for median 3.5 years on first-line ART. We classified each viral load measurement as consistent suppression, blip/post-blip, persistent low-level viral load or rebound. We used multi-level models to estimate rates of increase or decrease in laboratory markers, and Poisson regression to estimate the incidence of clinical events.

Results: Overall, 42% children experienced viral blips, but these had no significant impact on immune reconstitution or inflammation. Persistent detectable viraemia occurred in one-third of children and prevented further immune reconstitution, but had little impact on inflammatory biomarkers. Virological rebound to $\geq$5000 copies/ml was associated with arrested immune reconstitution, rising IL-6 and increased risk of clinical disease progression.

Conclusions: As viral load testing becomes more available in sub-Saharan Africa, repeat testing algorithms will be required to identify those with virological rebound, who need switching to prevent disease progression, whilst preventing unnecessary second-line regimen initiation in the majority of children with detectable viraemia who remain at low risk of disease progression.

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Introduction

Of the 1.8 million children living with HIV globally, over 80% live in sub-Saharan Africa. Although antiretroviral therapy (ART) has transformed clinical outcomes in HIV-infected children [1], only two-thirds were receiving treatment in 2019 [2]. World Health Organization (WHO) recommendations [3] for universal treatment mean that large numbers of HIV-infected children still need to start ART in sub-Saharan Africa.

HIV infection is characterized by inflammation and cluster of differentiation 4 ($CD4^+$) depletion, which underlie mortality in both children [4] and adults [5–8]. Virological suppression following ART leads to increases in $CD4^+$ cell count and reductions in morbidity and mortality [1]. Although there has been considerable research in adults [9–16], much less is known about the relationships between $CD4^+$ reconstitution, inflammation and viral load (VL) dynamics following ART initiation in children, particularly in sub-Saharan Africa.

We therefore characterized the interplay between VL suppression and loss of suppression, immune reconstitution, inflammation and clinical disease progression in a large cohort of HIV-infected children starting ART in Uganda and Zimbabwe.

Methods

This was a sub-study of the ARROW trial (ISRCTN24791884), in which 1206 children (age 3 months–17 years), eligible for first-line ART using WHO 2006 criteria [17], were recruited in Uganda and Zimbabwe [18]. The trial tested four HIV management strategies [18–20]. Children initiated ART with a regimen containing lamivudine + abacavir plus a non-nucleoside reverse transcriptase inhibitor (NNRTI), and had $CD4^+$ cell counts assayed 12-weekly. Long-term, two-thirds received 2NRTI + NNRTI maintenance, and one-third 3NRTIs.

Immunology sub-study

From June 2008, 316 children (97%) were enrolled in this sub-study (Figure, Supplemental Digital Content 1, http://links.lww.com/QAD/C105). HIV VL was assayed retrospectively on cryopreserved plasma taken pre-ART, at weeks 4, 24, 36 and 48 post-ART initiation, then every 24 weeks; additional VLs were assayed in children randomized to once- vs. twice-daily lamivudine + abacavir [20]. The lower limit of detection was 80 copies/ml because many low-volume samples were diluted 1:2. No VLs were used in real-time to make treatment decisions [18]. Children fulfilling WHO clinical or immunological criteria for failure switched to boosted protease inhibitor-containing second-line ART.

Inflammatory biomarkers [C-reactive protein (CRP), tumour necrosis factor alph (TNF-α), interleukin 6 (IL-6), soluble $CD14^+$] and IL-7 were assayed by ELISA on cryopreserved plasma taken pre-ART and then every 24 weeks. In Uganda, whole blood immunophenotyping by flow cytometry was done pre-ART and at weeks 4, 12, 24, 36 and 48 post-ART initiation, then 24-weekly. Assay details are in Text, Supplemental Digital Content 2, http://links.lww.com/QAD/C106.

Analysis

Analysis considered all measurements on first-line ART. To estimate the impact of long-term VL dynamics on immune responses, we first classified each individual VL measurement as consistent suppression, blip/post-blip, persistent low-level VL (pLLVL) or rebound [21]. We predefined consistent suppression as <10 000 copies/ml or >1$\log_{10}$ drop at week 4, <5000 copies/ml at week 24, declining or <400 copies/ml at week 36 and subsequently <80 copies/ml at all measurements. VL measurements ≥80 copies/ml were classified as a blip if the child had a previous VL <80 copies/ml and either subsequently re-suppressed <80 copies/ml (one or two VL≥80 copies/ml allowed; $n = 150$) or their last measurement was a single value ≥80 copies/ml ($n = 31$; Supplementary Methods). Those not re-suppressing <80 copies/ml but remaining <5000 copies/ml were defined as persistent low-level VL (pLLVL), whereas rebound was defined as confirmed VL ≥5000 copies/ml (following WHO 2010 guidelines [22]). We recorded the greatest degree of viral replication observed to have occurred, meaning children could move from response to blip, pLLVL and rebound states but not backwards through these states, i.e. if a child had ever experienced a VL blip they were subsequently classified as having previously 'blipped'.

Compared to those on 2NRTI + NNRTI long-term, children on 3NRTI maintenance were more likely to experience pLLVL and rebound [21]; however, as our goal was to estimate the impact of VL dynamics on biomarkers regardless of cause, we did not adjust for first-line regimen or other randomizations. We used multilevel models to estimate rates of increase or decrease for each of the following outcomes: inflammatory biomarkers; $CD4^+$%; $CD4^+$-for-age (the ratio of observed:expected count for the child's age [23]); $CD8^+$-for-age; $CD4^+/CD8^+$ ratio; $CD4^+$ sub-populations; weight-for-age and height-for-age (details in Text, Supplemental Digital Content 2, http://links.lww.com/QAD/C106).

Incidence of clinical events (WHO stage 3/4 events and deaths) were estimated using Poisson regression, and the impact of time-dependent biomarker values on clinical outcomes estimated using time-updated Cox regression.

All analyses used Stata 15.1 (StataCorp, College Station, Texas, USA). All *P*-values are two-sided.

Ethical approvals

Caregivers gave written informed consent for all children; if older children (8–17 years) were aware of their HIV status, they gave additional assent or consent following national guidelines. ARROW was approved by Research Ethics Committees in Uganda, Zimbabwe and the UK.

Results

Of 316 children in the immunology sub-study, three (0.9%) died before week 4. Of the remaining 313 children, 311 (99.4%) achieved an initial VL response <5000 copies/ml at week 24 and were included in analyses; two children never achieved VL response. Median (interquartile range, IQR) age at ART initiation was 5.4 years (2.2, 9.4) and median $CD4^+$% 13% (8%, 19%) (Table 1). Median follow-up on first-line ART was 3.6 years (IQR 3.5, 3.7). Only four (1.3%) children switched to second-line regimens during follow-up. Cytokine measurements were available for 99.4% ($n = 309$) children pre-ART and for 83.6% of subsequent 24-weekly measurements [median (IQR) 7 (7, 8) time-points per child].

Overall changes in $CD4^+$ populations and inflammatory biomarkers on first-line antiretroviral therapy

Children showed rapid $CD4^+$ reconstitution on ART (Fig. 1a). $CD4^+$% increased by 10% [95% confidence interval (CI) 9–10%] over the first 12 weeks on ART, 7% (6–8%) through week 72, then 0.2% (0.2–0.3%) every 12 weeks throughout follow-up (all $P < 0.001$). $CD4^+$-for-age and $CD4^+/CD8^+$ ratio increased similarly, with the mean $CD4^+/CD8^+$ ratio stabilizing at just over 1. $CD8^+$-for-age declined more slowly, stabilizing at around 1.6 by week 96.

$CD4^+$ reconstitution was driven by increases in the proportion of naive ($CA45RA^+$ $CD4^+$ cells over the first 24 weeks ($P < 0.001$), most of which were $CD31^+$, with slower rises subsequently ($P < 0.001$) (Fig. 1b). Conversely, the proportion of $CD45RA^-CD31^-$ $CD4^+$ cell counts decreased over the first 24 weeks ($P = 0.001$), then more slowly throughout follow-up ($P < 0.001$).

IL-7 decreased substantially over the first 24 weeks ($P < 0.001$), then more slowly throughout follow-up ($P < 0.001$) (Fig. 1c). The proportion of proliferating ($Ki67^+$) $CD4^+$ cells dropped strikingly by 8% (7–9%) over the first 48 weeks ($P < 0.001$) (Fig. 1c), then rose by 1% (1–2%) to week 72 ($P < 0.001$), driven by increases in memory ($CD45RA^-$) $CD4^+$ cells, and did not change significantly thereafter ($P = 0.58$).

The proportion of activated ($HLA\text{-}DR^+$) $CD4^+$ cells decreased more slowly and over a longer period, by 5% (4–6%) over the first 72 weeks on ART ($P < 0.001$), and then by 0.4% (0.3–0.5%) every 24 weeks thereafter ($P < 0.001$) (Fig. 1c).

Different trajectories on first-line ART were observed for each of the inflammatory markers (Fig. 1d). TNF-α declined over the first 48 weeks then rose again to week 72, before subsequently declining more slowly (all $P < 0.001$). CRP also dropped substantially over the first 48 weeks, but then rose through week 96 and subsequently declined ($P \leq 0.001$). IL-6 dropped by 28% (24–32%) over the first 24 weeks ($P < 0.001$), but did not change significantly thereafter ($P = 0.69$). sCD14 declined much more slowly throughout follow-up ($P = 0.001$).

At ART initiation, $CD4^+$% was strongly associated with cell populations and biomarkers [4]. Despite this, initial responses to ART were broadly similar, and normalization of each pathway occurred regardless of $CD4^+$ cell count (Figure, Supplemental Digital Content 3, http://links.lww.com/QAD/C107) or age at ART initiation (data not shown).

Taken together, the post-ART dynamics showed a sharp rise in $CD4^+$ cells, particularly over the first 12 weeks, driven predominantly by recent thymic emigrants, and an initial decline in activated and proliferating $CD4^+$ cells over 24–48 weeks, accompanied by substantial decreases in IL-7 and inflammatory biomarkers (except sCD14). After 48 weeks of ART, the proportion of proliferating $CD4^+$ cells, and concentrations of TNF-α and CRP, began to rise.

Despite good immunological responses, a proportion of children experienced VL blips and loss of VL control, to either pLLVL (<5000 copies/ml) or rebound (confirmed ≥5000 copies/ml) (Fig. 1e). Fastest transitions occurred between consistent VL suppression and blip, and between pLLVL and rebound (Figure, Supplemental Digital Content 4, http://links.lww.com/QAD/C108). We therefore investigated how changes in $CD4^+$ subpopulations and biomarkers related to consistent VL suppression or VL non-suppression after initial virologic response.

Consistent viral load suppression

Of 311 children achieving an initial VL response, 103 (33.1%) consistently sustained VL <80 throughout follow-up, 93 (29.9%) experienced pLLVL or rebound, and 115 (37.0%) had one or more VL blips (Fig. 1E). Whilst VL was consistently maintained <80 copies/ml, increases in $CD4^+$%, $CD4^+$-for-age and $CD4^+/CD8^+$ ratio on ART (Figure, Supplemental Digital Content 5, http://links.lww.com/QAD/C109) were only slightly greater than the whole cohort, which included children with blips, pLLVL and rebound (Fig. 1).

Table 1. Characteristics at ART initiation of all children with initial VL response, and those subsequently experiencing viral load blips, persistent low level viral load and/or rebound.

Factor (at ART initiation)	Total with initial VL response, $N=311$, median (IQR) or n (%) [number missing]	Ever had VL blip, $N=130$, median (IQR) or n (%) [number missing]	P (ever vs. never had VL blip)	Ever had persistent low level viral load, $N=59$, median (IQR) or n (%)	P (ever vs. never had pLLVL)	Ever had rebound, $N=59$, median (IQR) or n (%)	P (ever vs. never had rebound)
Male	146 (46.9%)	62 (47.7%)	0.82	25 (42.4%)	0.43	29 (49.2%)	0.71
Country/centre			<0.0001		0.75		0.15
Uganda/A	50 (16.1%)	14 (10.8%)		8 (13.6%)		13 (22.0%)	
Uganda/B	79 (25.4%)	26 (20.0%)		18 (30.5%)		15 (25.4%)	
Uganda/C	78 (25.1%)	26 (20.0%)		15 (25.4%)		18 (30.5%)	
Zimbabwe/D	104 (33.4%)	64 (49.2%)		18 (30.5%)		13 (22.0%)	
Age (years)	5.4 (2.2, 9.4)	5.3 (2.3, 8.4)	0.17	6.5 (2.7, 10.2)	0.34	7.8 (1.7, 12.3)	0.14
WHO stage			0.49		0.54		0.54
1/2	100 (32.2%)	39 (30.0%)		17 (28.8%)		17 (28.8%)	
3/4	211 (67.8%)	91 (70.0%)		42 (71.2%)		42 (71.2%)	
Real-time monitoring			0.93		0.82		0.82
$CD4^+$ monitoring	154 (49.5%)	64 (49.2%)		30 (50.8%)		30 (50.8%)	
No $CD4^+$ monitoring	157 (50.5%)	66 (50.8%)		29 (49.2%)		29 (49.2%)	
Long-term ART			0.06		<0.0001		0.001
2NRTI + NNRTI	204 (65.6%)	93 (71.5%)		20 (33.9%)		28 (47.5%)	
3NRTI	107 (34.4%)	37 (28.5%)		39 (66.1%)		31 (52.5%)	
Viral load (copies/ml)	216800 (67000, 641200)	273400 (94300, 704300)	0.10	249100 (92200, 726200)	0.30	275100 (94800, 1039200)	0.22
TNF-α (pg/ml)	23.9 (19.3, 29.7) [3]	25.4 (21.0, 30.1) [2]	0.02	25.5 (19.8, 30.5)	0.29	24.8 (18.7, 32.9)	0.60
IL-6 (pg/ml)	5.8 (4.5, 8.2) [5]	6.3 (4.9, 9.0) [3]	0.007	5.5 (4.6, 7.2)	0.51	6.3 (4.7, 10.6) [1]	0.20
CRP (mg/l)	4.0 (1.4, 13.5) [2]	4.3 (1.5, 13.2) [2]	0.63	4.4 (1.6, 14.4)	0.24	6.3 (1.6, 29.4)	0.03
$sCD14^+$ (mg/l)	2.1 (1.6, 2.5) [2]	2.1 (1.6, 2.5) [2]	0.38	2.1 (1.6, 2.4)	0.81	2.3 (1.8, 2.8)	0.03
IL-7 (pg/ml)	9.8 (3.4, 17.5) [14]	10.6 (4.1, 18.7) [3]	0.09	9.8 (5.1, 18.5)	0.51	8.7 (4.4, 16.1) [4]	0.89
$CD4^+$%	13 (8, 19)	13 (10, 18)	0.78	13 (9, 16)	0.73	10 (5, 15)	0.003
$CD4^+$-for-age	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)	0.36	0.3 (0.2, 0.4)	0.37	0.2 (0.1, 0.3)	0.0005
$CD8^+$-for-age	2.1 (1.4, 3.0)	2.0 (1.3, 2.9)	0.65	2.0 (1.4, 2.8)	0.40	2.0 (1.4, 2.9)	0.94
$CD4^+$:$CD8^+$	0.3 (0.1, 0.4)	0.3 (0.2, 0.5)	0.37	0.2 (0.2, 0.4)	0.49	0.2 (0.1, 0.3)	0.0005
% CD4 $CD45RA^{+a}$	48.4 (31.2, 62.2) [14]	53.3 (35.0, 64.0) [5]	0.20	46.5 (27.2, 57.1) [3]	0.15	42.6 (23.0, 54.2) [4]	0.009
% $CD4^+$ $CD45RA^+CD31^{+a}$	36.9 (22.4, 49.7) [14]	40.9 (28.3, 50.8) [5]	0.22	33.6 (20.0, 45.7) [3]	0.22	30.6 (18.0, 42.5) [4]	0.02
% $CD4^+$ $CD45RA^+CD31^{-a}$	7.5 (4.2, 14.1) [14]	9.5 (4.4, 15.0) [5]	0.19	6.6 (3.9, 13.8) [3]	0.39	5.7 (3.0, 9.0) [4]	0.02
% $CD4^+$ $CD45RA^-CD31^{-a}$	34.5 (24.6, 45.7) [14]	32.5 (24.8, 41.8) [5]	0.21	38.8 (25.9, 44.8) [3]	0.31	40.9 (27.7, 47.2) [4]	0.0504
% $CD4^+$ $Ki67^{+a}$	8.7 (4.8, 14.3) [12]	9.7 (5.6, 14.1) [4]	0.44	8.0 (4.5, 16.7) [4]	0.84	13.2 (5.7, 23.5) [4]	0.004
% $CD4^+$ HLA-DR+[a]	8.7 (5.1, 12.9) [16]	8.7 (4.8, 12.5) [5]	0.58	8.5 (4.8, 12.5) [4]	0.60	9.4 (6.1, 15.3) [6]	0.20
Weight-for-age	−2.2 (−3.3, −1.3)	−2.2 (−3.3, −1.5)	0.84	−2.1 (−3.9, −1.5)	0.25	−2.2 (−3.3, −1.5)	0.45
Height-for-age	−2.5 (−3.4, −1.6)	−2.8 (−3.4, −1.9)	0.04	−2.5 (−3.4, −1.8)	0.83	−2.1 (−3.3, −1.3)	0.10

Numbers in square brackets indicate missing data (Uganda only for immunophenotyping). Children experiencing more than one of viral load blips, persistent low-level viral load and rebound included in all relevant columns, see Methods for definitions. Chi-squared or rank-sum tests used to compare categorical and continuous factors at ART initiation, respectively. Some of these data have previously been shown in [21]. ART, antiretroviral therapy; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; pLLVL, persistent low-level viral load; VL, viral load.

[a]Uganda only.

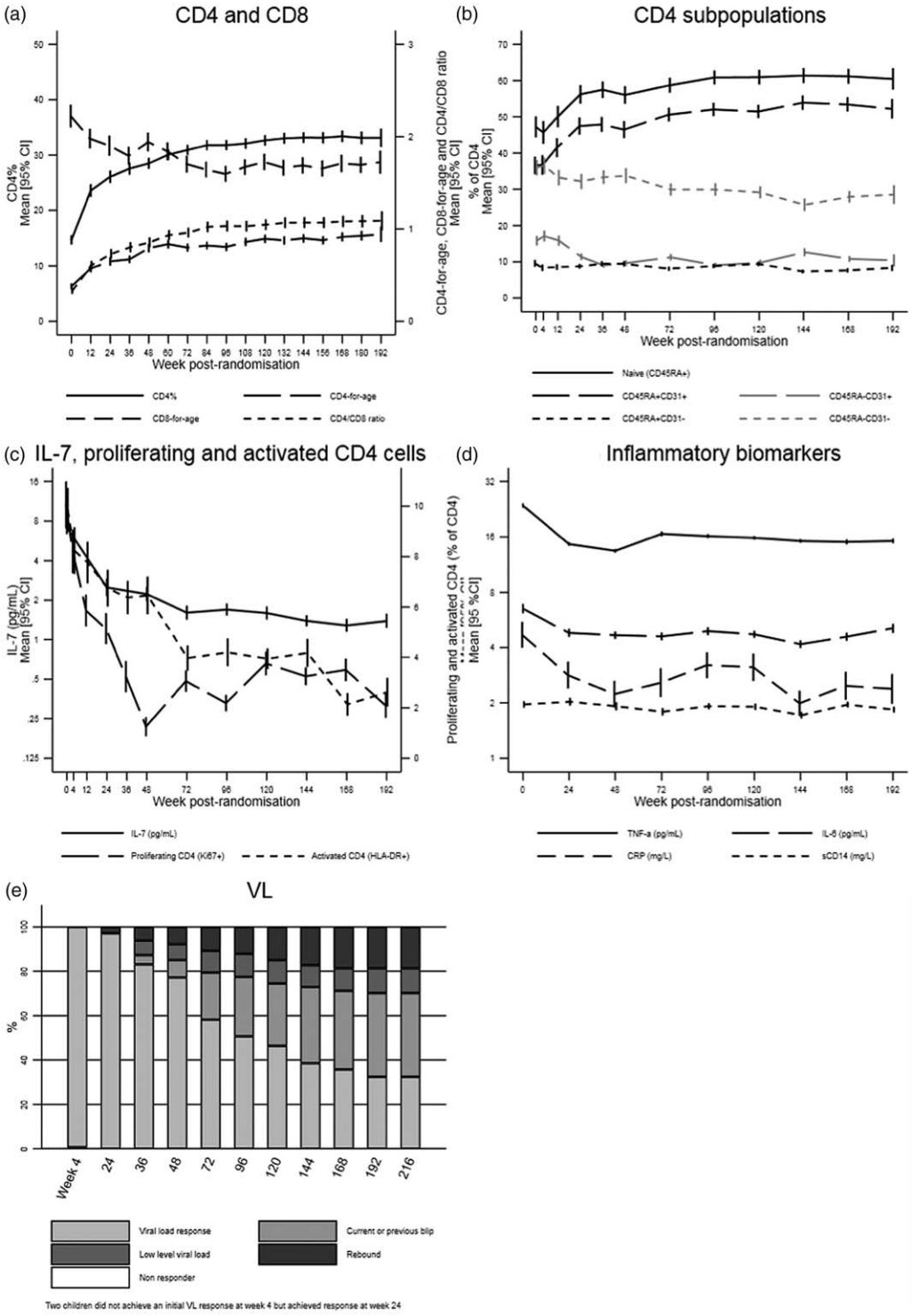


Fig. 1. Overall changes in biomarkers and VL on first-line ART. (a) $CD4^+$ and $CD8^+$; (b) $CD4^+$ subpopulation; (c) IL-7, proliferating and activated $CD4^+$ cells; (d) inflammatory biomarkers; and (e) VL. VL, viral load.

After children were stable on ART from 48 weeks, those with consistent VL suppression had ongoing increases in $CD4^+$%, $CD4^+$-for-age and $CD4^+/CD8^+$ ratio (Fig. 2), while their $CD8^+$-for-age, IL-7 and TNF-α continued to decrease (Figs. 2 and 3, and Figure, Supplemental Digital Content 6, http://links.lww.com/QAD/C110). There was no evidence of changes in other inflammatory biomarkers (IL-6, CRP, sCD14) once children were stable with consistent VL suppression ($P > 0.3$). The proportion of naive ($CD45RA^+$) and recent thymic emigrant ($CD45RA^+CD31^+$) $CD4^+$ cells continued to increase significantly, while the proportion of $CD45RA^-CD31^-$

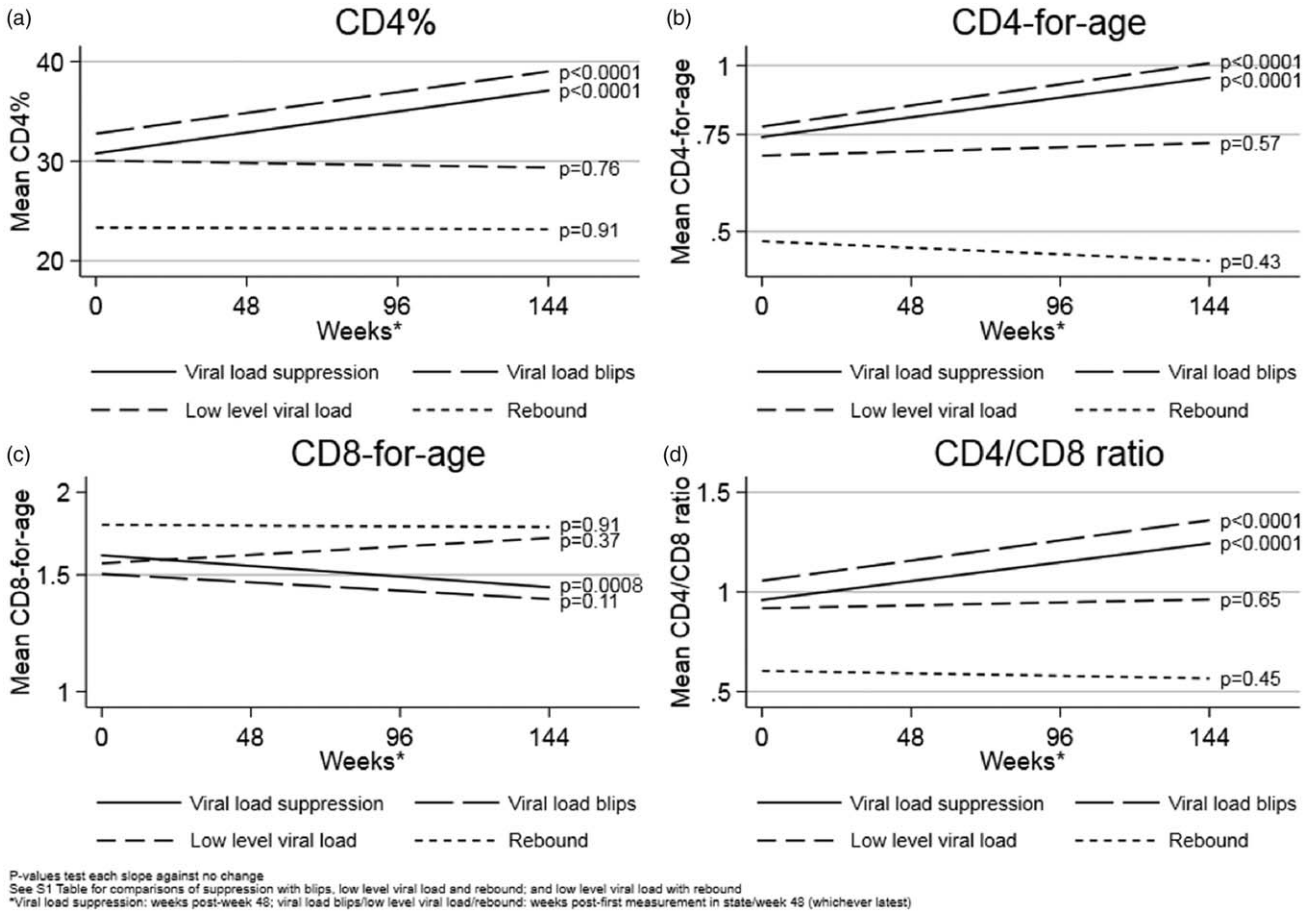


Fig. 2. $CD4^+$ and $CD8^+$ over time with consistent VL suppression, previous VL blips, pLLVL and rebound. pLLVL, persistent low-level viral load; VL, viral load.

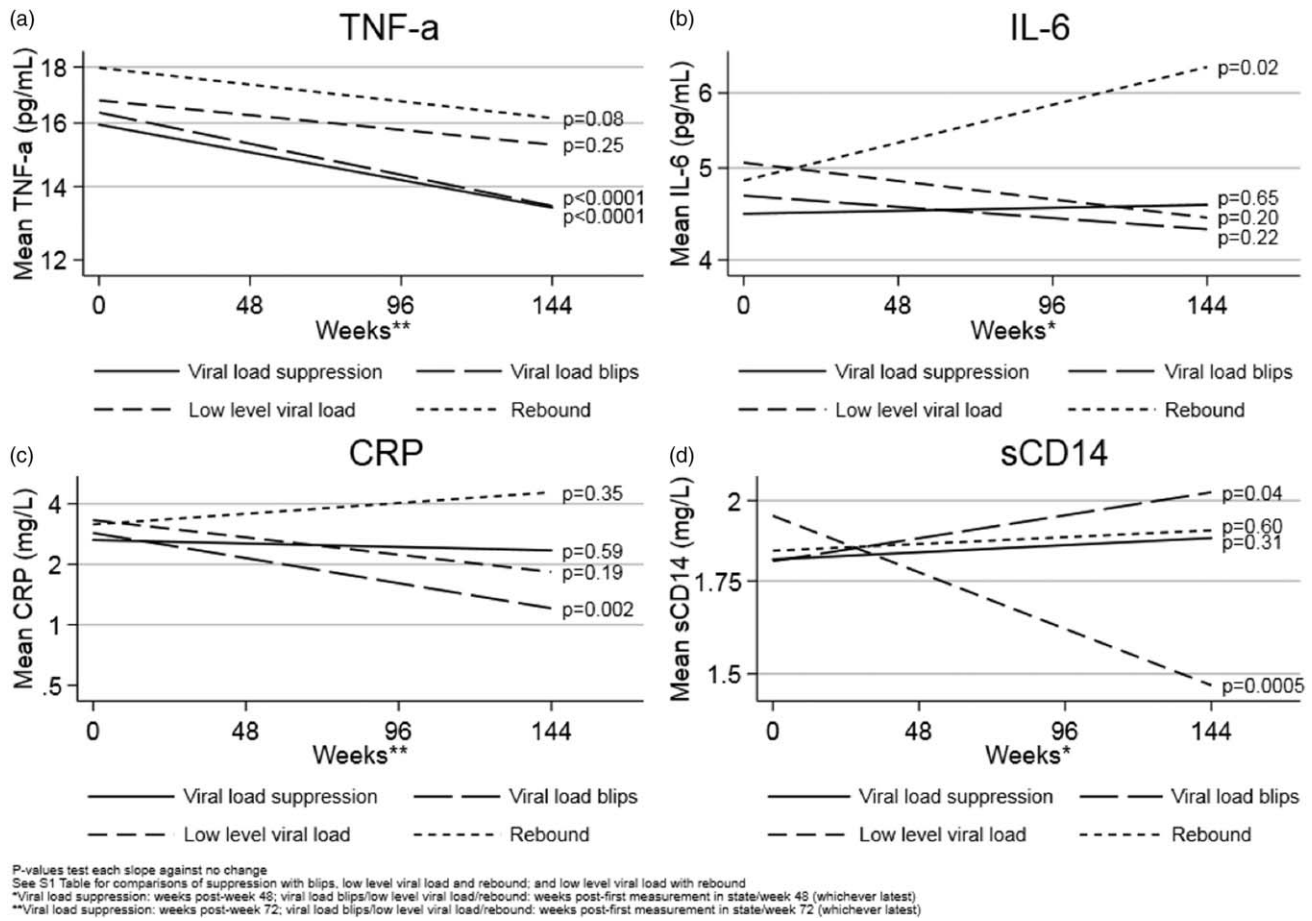
and $CD45RA^+CD31^-CD4^+$ and HLA-DR^+ cells decreased (Figure, Supplemental Digital Content 7, http://links.lww.com/QAD/C111).

Impact of viral load blips

Overall, 130 (41.8%) children experienced blips to a median (IQR) 200 (110, 750) copies/ml; 19 (14.6%) had already experienced a blip by week 48 and 43 (33.1%) had more than one blip on first-line ART. After their first blip, children still had significant increases in $CD4^+$%, $CD4^+$-for-age and $CD4^+/CD8^+$ ratio, and non-significant decreases in $CD8^+$-for-age, which did not differ from those during consistent suppression ($P > 0.7$, Fig. 2, Table 2). There was also no evidence that changes in IL-6, sCD14, TNF-α and IL-7 differed between children with blips and children with consistent suppression ($P > 0.2$; Table 2). If anything, CRP actually decreased faster in those with blips compared to consistent VL suppression ($P = 0.03$). After their first blip, children still had non-significant increases in $CD45RA^+CD31^+$ and naive $CD4^+$ cells, and decreases in $CD45R^-CD31^-$ and HLA-DR^+ $CD4^+$ cells, which did not differ from those during consistent suppression ($P > 0.3$). $Ki67^+$ $CD4^+$ cells decreased ($P = 0.02$), whereas they did not change during suppression (suppression vs. blips $P = 0.01$).

Impact of persistent low-level viral load

Overall, 59 (19.0%) children experienced pLLVL after median 72 weeks on ART (IQR 36, 120), with median (IQR) VL 720 copies/ml (IQR 150, 2430) and $CD4^+$% 31% (24%,37%) during pLLVL. Over half (53.6%) of time in pLLVL was spent with the most recent VL measurement <1000 copies/ml. VL did not increase over time during pLLVL ($P = 0.32$). Although pLLVL did not lead to $CD4^+$ depletion, it arrested further immune reconstitution (Table 2): none of $CD4^+$%, $CD4^+$-for-age, $CD4^+/CD8^+$ ratio or $CD8^+$-for-age changed significantly over time during pLLVL (Fig. 2), although the proportion of memory ($CD45RA^-CD31^-$) and activated (HLA-DR^+) $CD4^+$ cells continued to decrease (Figure, Supplemental Digital Content 6, http://links.lww.com/QAD/C110; and Figure, Supplemental Digital Content 7, http://links.lww.com/QAD/C111). Similar to findings after a first blip, $Ki67^+$ cells decreased ($P = 0.04$), whereas they did not change during suppression (suppression vs. pLLVL $P = 0.02$); there



P-values test each slope against no change
See S1 Table for comparisons of suppression with blips, low level viral load and rebound; and low level viral load with rebound
*Viral load suppression: weeks post-week 48; viral load blips/low level viral load/rebound: weeks post-first measurement in state/week 48 (whichever latest)
**Viral load suppression: weeks post-week 72; viral load blips/low level viral load/rebound: weeks post-first measurement in state/week 72 (whichever latest)

Fig. 3. Inflammatory cytokines over time with consistent VL suppression, previous VL blips, pLLVL and rebound. pLLVL, persistent low-level viral load; VL, viral load.

Table 2. Comparisons of trajectories in $CD4^+$ subpopulations, inflammatory biomarkers, weight-for-age and height-for-age according to VL dynamics.

	Consistent suppression vs. blips vs. pLLVL vs. rebound	Consistent suppression vs. blips	Consistent suppression vs. pLLVL	Consistent suppression vs. rebound	Rebound vs. pLLVL	Consistent suppression + blips vs. pLLVL + rebound
$CD4^+$%	$P < 0.0001$	0.94	0.003	<0.0001	0.85	<0.0001
$CD4^+$-for-age	0.004	0.75	0.046	0.003	0.34	0.0001
$CD8^+$-for-age	0.17	0.71	0.053	0.17	0.42	0.01
$CD4^+/CD8^+$ ratio	<0.0001	0.72	0.02	<0.0001	0.45	<0.0001
% $CD4^+$ $CD45RA^+$	0.83	0.61	0.64	0.54	0.43	0.33
% $CD4^+$ $CD45RA^+CD31^+$	0.89	0.64	0.96	0.50	0.62	0.32
% $CD4^+$ $CD45RA^+CD31^-$	0.41	0.61	0.59	0.15	0.14	0.32
% $CD4^+$ $CD45RA^+CD31^-$	0.31	0.41	0.12	0.83	0.16	0.84
TNF-α	0.49	0.68	0.35	0.30	0.90	0.07
IL-6	0.03	0.20	0.17	0.04	0.008	0.17
CRP	0.04	0.03	0.34	0.28	0.11	0.18
$sCD14^+$	0.0006	0.23	0.0003	0.98	0.002	0.02
IL-7	0.21	0.94	0.26	0.07	0.73	0.01
% $CD4^+$ $Ki67^+$	0.02	0.01	0.02	0.29	0.68	0.34
% $CD4^+$ $HLA\text{-}DR^+$	0.53	0.34	0.42	0.93	0.66	0.98
Height-for-age *Z*-score	0.03	0.049	0.85	0.01	0.102	0.13
Weight-for-age *Z*-score	0.37	0.92	0.98	0.09	0.15	0.38

P values are shown for each comparison. Modelled trajectories are shown in Figs. 2 and 3, and in Supplementary Digital Content 6 and 7 (http://links.lww.com/QAD/C110 and http://links.lww.com/QAD/C111). pLLVL, persistent low-level viral load; VL, viral load.

was no evidence that changes in any other $CD4^+$ sub-population during pLLVL were different to those during consistent VL suppression ($P > 0.1$). IL-7, IL-6, CRP and TNF-α did not change significantly over time during pLLVL (Fig. 3). By contrast, during pLLVL sCD14 decreased significantly, albeit by a relatively small absolute amount. Although the absolute level of IL-6 was generally higher in pLLVL than in VL suppression (or after experiencing blips), there was no evidence of differences in how these biomarkers changed over time ($P > 0.1$, Table 2).

Impact of rebound

Overall, 59 (19.0%) children experienced rebound after median 72 weeks on ART (IQR 36, 120), with median (IQR) VL 31910 copies/ml (11 190, 86 430) and $CD4^+$% 23% (16%, 31%) during rebound (Table 1). Having experienced rebound led to a significant effect on the overall levels of $CD4^+$, $CD8^+$, $CD4^+$ sub-populations, and proportions of proliferating ($Ki67^+$) or activated ($HLA\text{-}DR^+$) cells, but only a modest impact on inflammatory cytokines (Figs. 2 and 3; Supplemental Digital Content 8, http://links.lww.com/QAD/C112). There was no evidence that VL changed over time in rebound ($P = 0.14$). Similar to pLLVL, there was no evidence of progressive declines in $CD4^+$% during rebound (0.1% decrease/year [95% CI 1.0% decrease−0.9% increase], $P = 0.91$), or in $CD4^+$-for-age and $CD4^+/CD8^+$ ratio, but changes were significantly smaller than the increases seen during suppression ($P < 0.001$, Fig. 2, Table 2). There was no significant change in $CD8^+$-for-age or $Ki67^+$ cells, with no evidence of any differences from changes seen in consistent suppression or pLLVL ($P > 0.1$). $HLA\text{-}DR^+$ and TNF-α continued to decrease despite VL rebound, with no evidence of a difference in trajectory compared with consistent VL suppression ($P > 0.3$). In contrast, IL-6 increased significantly faster during rebound than during pLLVL or suppression ($P < 0.05$); CRP changed similarly to IL-6 but differences were not significant. There was no evidence of a change in sCD14 or IL-7 during rebound.

Clinical events and growth by viral load dynamics

After week 48, clinical events (WHO stage 3/4 events and deaths) were relatively rare ($n = 10$) but occurred at a greater rate during rebound (53/1000 child-years [95% CI 20−116]) than during suppression (5/1000 child-years [95% CI 1−17]) and after blips (9/1000 child-years [95% CI 1−34]; rebound vs. suppression: $P = 0.003$); there were no events in children with LLVL (95% CI 0−46). Both deaths after week 48 (one pulmonary tuberculosis, one pneumonia) occurred in children with rebound. Combining LLVL (no events) and blips, the increased risk associated with rebound persisted after adjusting for time-updated $CD4^+$-for-age (rebound vs. suppression: hazard ratio (HR) = 5.94 (95% CI 0.91−38.64), $P = 0.06$). There was no evidence of additional effects of inflammatory cytokines ($P > 0.17$). In the subset of 205 children with immunophenotyping, there was some evidence that high $HLA\text{-}DR^+$ was associated with increased risk of clinical events (HR = 1.13 [0.98−1.30], $P = 0.09$, adjusting for VL group and $CD4^+$-for-age; $P = 0.04$ adjusting for VL group alone).

After week 48, height-for-age increased regardless of VL suppression (all $P < 0.0001$, Figure, Supplemental Digital Content 9, http://links.lww.com/QAD/C113), but increases were slower during rebound than suppression ($P = 0.01$) (Table 2). Weight-for-age changes were much smaller after week 48, with no evidence of changes in weight-for-age during rebound ($P = 0.39$).

Discussion

In this study of HIV-infected children in Uganda and Zimbabwe, most had excellent virological, immunological and clinical responses on first-line ART. Children showed rapid immune reconstitution with naive $CD4^+$ cells, and a rapid decline in activated and proliferating T-cells and most inflammatory biomarkers. Despite good initial virological suppression, only one-third remained consistently undetectable long-term; 42% had one or more blips, and almost one-third had persistent detectable viraemia. We found no evidence that blips were deleterious, whereas persistent detectable viraemia prevented further immune reconstitution, and virological rebound (≥5000 copies/ml) was associated with more inflammation and clinical disease progression.

$CD4^+$ counts rose rapidly following ART initiation, with concomitant declines in IL-7, peripheral T-cell proliferation and activation. Immune reconstitution was mainly driven by $CD45RA^+CD31^+$ $CD4^+$ cells, which are predominantly recent thymic emigrants [24,25], together with a decline in memory $CD4^+$ cells. Most inflammatory biomarkers (IL-6, CRP, TNF-α) also declined over the first 48 weeks on ART. Normalization of each pathway did not strongly depend on the baseline $CD4^+$ cell count or age, suggesting that in settings where children continue to present with advanced disease [26–29], excellent restoration of immune and inflammatory pathways is achievable.

The causes of inflammation in HIV-infected children are poorly understood, but may include co-infections and enteropathy as well as HIV itself [30]. There are complex inter-relationships between inflammatory biomarkers; for example, pre-ART levels of IL-6 or CRP predict mortality on ART, whilst levels of TNF-α and sCD14 do not [4]. In this analysis, the pattern of changes in inflammatory markers showed distinct differences. All biomarkers declined on ART over 48 weeks, although

reductions in soluble CD14 were very small, similar to other cohorts [31,32]. After 48 weeks, IL-6 remained stable, whilst the concentrations of TNF-α and CRP rose at the population level, before slowly declining again. Changes were similar in children who remained virologically suppressed, suggesting that these population-level effects could not be explained by the temporary or ongoing loss of virological control. It is striking that detectable viraemia only impacted inflammatory marker levels once VL was quite high (≥5000 copies/ml); at lower levels, loss of virological control had little effect on the overall level or subsequent evolution of inflammatory markers. This suggests that inflammatory pathways are controlled by a complex system of homeostatic regulation, which tends to maintain an inflammatory equilibrium, similar to the stability of the HIV viral load set-point; it is only once the perturbation is sufficiently great, due to high-level viral load rebound, that control of the system is disrupted. Once virological rebound occurred, and inflammatory markers stabilized at higher levels, WHO stage 3/4 events and deaths occurred at a greater rate, independently of $CD4^+$-for-age. In HIV-infected adults, higher levels of inflammation on ART are associated with elevated morbidity and mortality and there is considerable interest in evaluating adjunctive therapies to reduce inflammation [33,34]. In children, we did not find evidence that inflammation was associated with morbidity independently of VL, but few children had serious morbid events on ART.

Despite limited laboratory (and no VL) monitoring, children showed a good initial virological response to ART, as reported in other African cohorts [35], but frequently had episodes of detectable viraemia during median 3.5 years of follow-up. This was often due to blips – that is, isolated detectable HIV RNA followed by a return to virological suppression [36]. We found that blips were not deleterious: $CD4^+$ populations continued to normalize during and following blips and there was no rise in inflammatory biomarkers or increase in the rates of subsequent progression to pLLVL or rebound. To our knowledge, no previous studies have evaluated the impact of blips in HIV-infected children in sub-Saharan Africa. The mechanisms underlying blips may relate to assay variability at the limits of detection [15], statistical variation [11], the release of virus from long-lived reservoirs [14,16], intercurrent infections or vaccination [9,10] and incomplete ART adherence [12], although whether the drivers are the same in adults and children is unknown. It is interesting that whilst the overall increase in $CD4^+$ cells was comparable to that found in suppressed individuals, we saw a reduction in inflammation during blips, with declines in CRP and $Ki67^+$ $CD4^+$ cells that were greater than among those with virological suppression.

One-third of children had persistent detectable viraemia on ART; however, this was only deleterious for disease progression when ≥5000 copies/ml – a level higher than recent guidelines suggest for the switch to second-line therapy [37]. In cross-sectional and longitudinal cohort studies, detectable viraemia has been reported in 24–34% children on ART [38–41] and switch to second-line therapy for ART failure is often delayed [42]. We show here that while persistent viral replication (<5000 copies/ml) did not cause a decline in $CD4^+$ cells and had no substantial impact on inflammatory biomarkers or clinical disease progression, it prevented further immune reconstitution and rise in $CD4^+/CD8^+$ ratios. This indicates that persistent low-level virus prevents children from realizing their full potential to achieve normal or high levels of $CD4^+$ cell immune reconstitution. This may become particularly important when children enter adulthood.

Children with higher levels of viraemia (≥5000 copies/ml) had poorer immune reconstitution and increased levels of IL-6, a key inflammatory biomarker associated with mortality [4]. These children had a greater risk of clinical disease progression (WHO stage 3/4 events or death), and slower height gain than children with virological suppression. Where virological monitoring is available, it clearly has a role in identifying children with persistently detectable viraemia at a reasonably high level, in whom immunological and clinical disease progression and increased inflammation will develop over time. Our results suggest that switching treatment at lower VL thresholds may not necessarily provide long-term advantages given the limited treatment options for children in low-income countries, and the challenges of adherence [43].

The present study had strengths and weaknesses. ARROW is the largest trial of children initiating ART in sub-Saharan Africa. Because VL was not measured in real-time, our data provide a realistic impression of the long-term impact of blips and virological failure in a large cohort where adherence counselling was not undertaken following detectable viraemia. However, several different VL platforms were used across trial sites, which can affect the chance of detecting low-level blips [15,44]. We had good clinical data, including independently adjudicated WHO stage 3/4 disease and deaths, but event rates on ART were low, limiting our ability to establish clinical sequelae of each virological state. However, the low clinical event rates also support no major clinical harms being associated with persistent viraemia.

In summary, we show that most children starting ART in sub-Saharan Africa maintained consistent virological suppression or only experienced viral blips, which were not deleterious and do not warrant a switch to second-line ART. Ongoing recovery of $CD4^+$ and $CD8^+$ parameters occurred at the same rate in children with blips as those who were consistently suppressed, and both groups maintained stable levels of inflammatory markers.

In contrast, persistently detectable viraemia occurred in one-third of children. Persistent low-level viraemia limited further immune reconstitution, and once ≥5000 copies/ml, children had increased inflammatory biomarkers and clinical disease progression. This highlights the importance of strategies to enhance ART adherence, since even low-level viraemia hinders long-term immune recovery. Children with confirmed VL ≥5000 copies/ml should be prioritized for adherence support, due to the risk of disease progression, and switched to second-line regimens. Given the push to scale-up virological monitoring across Africa, this study shows the challenges that are likely to arise in interpreting VL results, especially if only conducted infrequently. Repeat testing algorithms are required to identify those with the virological rebound, who need switching to prevent disease progression, whilst preventing unnecessary second-line regimens in the majority of children with detectable viraemia who remain at low risk of disease progression.

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Conflicts of interest

There are no conflicts of interest.

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