STRONG SEX BIAS IN ELITE CONTROL OF PAEDIATRIC HIV INFECTION

Short title: Elite control in HIV infected children

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ABSTRACT

Background: Reports of post-treatment control following antiretroviral therapy (ART) have prompted the question of how common immune control of HIV infection is in the absence of ART. In contrast to adult infection, where elite controllers (EC) have been very well characterized and comprise approximately 0.5% of infections, very few data exist to address this question in paediatric infection.

Methods: We describe 11 ART-naïve EC from 10 cohorts of HIV-infected children being followed in South Africa, Brazil, Thailand, and Europe.

Results: All but one EC (91%) are female. The median age at which control of viraemia was achieved was 6.5yrs. Five of these 11 (46%) children lost control of viraemia at a median age 12.9yrs. Children who maintained control of viraemia had significantly higher absolute CD4 counts in the period of EC than those who lost viraemic control. Based on the data available from these cohorts, the prevalence of EC in paediatric infection is estimated to be 5-10-fold lower than in adults.

Conclusion: Although conclusions are limited by the study design, these data suggest that, whilst paediatric elite control can be achieved, compared to adult EC this occurs rarely, and takes some years after infection to achieve. Also, loss of immune control arises in a high proportion of children and often relatively rapidly. These findings are consistent with the more potent antiviral immune responses observed in adults and in females.

INTRODUCTION

The large majority of HIV-infected children rapidly develop AIDS in the absence of antiretroviral therapy (ART)(1). In comparison with adults, the children progress faster without ART, 50% developing AIDS within 1 year and 60% have died by 2.5 years(1, 2). Surprisingly, ART-naïve HIV-infected individuals with the spontaneous capacity to maintain normal CD4+ T cells counts, known as non-progressors, are more common in children than adults and comprise 5-10% of ART-naïve HIV-infected children(3-5). The features of paediatric non-progressors (PNP) are high viraemia in the presence of normal CD4+ T cell counts, reduced CCR5 expression in the central memory CD4 T-cell subset, low immune activation, and no correlation with protective major histocompatibility complex (MHC) class I molecules (HLA-B*27, HLA-B*57, HLA-B*58:01 and HLA-B*81:01)(6, 7). Many of these features are similar to those described in natural hosts of SIV infection such as the sooty mangabey(8, 9). Adult viremic non-progressors (AVNP) appear to be broadly similar(10, 11) to PNP but AVNP are exceptionally rare. Adult non-progressors typically have normal CD4 counts but low or undetectable viral loads and most express the protective HLA alleles described above(12, 13).

Although the non-progressor status is more frequently seen in the paediatric population, children with spontaneous control of viremia - often termed ‘elite controllers’ (EC) - have been scarcely described(7). Definitions of EC in HIV-infected individuals have varied but one that reached acceptance is: three or more consecutive viral loads spanning at least one year below 50 HIV RNA copies/mL, in the absence of ART (14-16). Estimates of the frequency of EC in adult infection range between 0.18-0.56%(15-19). The immune features responsible for elite control of viremia remain incompletely

123 understood, although contributing factors include the spectrum of MHC class I and killer
124 cell immunoglobulin-like receptors (KIR) molecules expressed, as well as specificity
125 and functionality of the HIV-specific CD8+ T-cell response (12, 13, 20-22).
126
127 Here we describe a group of 11 ART-naïve, vertically HIV-infected children who
128 fulfil the criteria of EC. We also describe a larger number of ART-naïve HIV-infected
129 children who achieved transient aviraemia (TA) on one or more occasions but who did
130 not meet the EC criteria. Identifying these paediatric EC provides an approximate
131 estimate of the frequency of natural immune control of HIV infection in children and
132 provides a context for anecdotal paediatric cases of post-treatment control(23-25).
133
134 **MATERIAL AND METHODS**
135 **Study participants**
136 We defined paediatric EC as vertically HIV-infected, ART-naive children with
137 three or more consecutive viral load measurements over a year or more, that were
138 below the limit of detection. The limit of detection varied according to center and the
139 historical period when the assays were done, and ranged between <150, <100, <50,
140 and <20 copies/mL. “Blips” higher than 1,000 copies/mL were not allowed(15, 16, 18).
141 The TA group was defined as ART-naïve, vertically infected children in whom one or
142 more HIV RNA measurements were below the limit of detection, but without fulfilling the
143 EC criteria. The CD4+ T cell count was not considered in the definition. Medical records
144 were reviewed for clinical data, viral loads and CD4+ and CD8+ T cells counts. All
145 patients were diagnosed before 10 years of age and/or were born to a mother with
146 confirmed HIV infection, supporting the notion that infection had occurred perinatally via
147 mother-to-child transmission.

Our study involved 10 clinics caring for HIV-infected children around the world: Kimberley Hospital (Kimberley, South Africa), Ithembalabantu Clinic (Durban, South Africa), the Family Clinical Research Unit in Tygerberg Academic Hospital (Cape Town, South Africa), Instituto Emílio Ribas (Sao Paulo, Brazil), Universidade Federal de Minas Gerais (Belo Horizonte, Brazil), Great Ormond Street Hospital (London, United Kingdom), St Mary's Hospital (London UK), Karolinska University Hospital (Stockholm, Sweden), Sant Joan de Déu Children's Hospital (Barcelona, Spain), and The HIV Netherlands Australia Thailand Research Collaboration, Thai Red Cross AIDS Research Centre (Bangkok, Thailand). The cohorts selected were designed to enable us to identify any children meeting the criteria for paediatric elite controllers. We therefore sought large cohorts of HIV-infected children in South Africa, the country with the largest number of paediatric HIV infections worldwide, as well as in Brazil and Thailand, countries also with substantial paediatric HIV epidemics, in order to sample study populations inside and outside of Africa, respectively. Finally we sought paediatric elite controllers among some of the smaller paediatric HIV cohorts being followed in Europe, which nonetheless are largely comprised of HIV-infected African children. Informed consent was obtained from all study participants, and for underage children, from their caregivers.

Statistical Methods

Clinical and laboratory results were described using absolute numbers, percentages, medians, and interquartile ranges (IQR). Comparisons were performed using Wilcoxon rank-sum test for continuous variables and Chi-square or Fisher's exact test for categorical variables as appropriate. Age to achieving paediatric EC status was

compared among those who maintained EC and those who rebounded via Kaplan Meier survival analysis using the log-rank test. We assumed a two-sided alpha error of 0.05 and used the statistical software StataSE 15.0 (StataCorp. College Station, TX: StataCorp LP), and GraphPad Prism Version 7 (GraphPad Software, CA). To test whether absolute or relative CD4 counts are different between the EC who maintained viraemia control and those who lost, the R package lmer4 was used to produce linear mixed-effects models. Age was modeled as a fixed effect and CD4 count (or percentage) as a random effect of each individual. P-values were calculated using the ANOVA function in R to compare two models. The null model states that CD4 count (or percentage) is proportional to age and the alternative model states that CD4 count (or percentage) is proportional to age and viraemic control status.

RESULTS

We identified 11 vertically infected paediatric EC according to the criteria described above (Table 1, Figure 1). Ten (91%) were female. The median age at enrolment was 6.7 years (IQR 2.9 - 8.1yrs). The group included 8 (73%) patients born in African countries, 2 (18%), in Latin America, and 1 (9%) in Asia.

Among the 11 study subjects, 7 were viraemic when enrolled but became aviraemic subsequent to enrolment, whereas 4 subjects were already aviraemic EC when enrolled (Table 1). The median age at which viraemic control was first achieved was 6.5 years (IQR 5.2 – 10.0) (Figure 2A). Only 3 patients, EC-3, EC-4 and EC-9 achieved viraemic control before 5 years of infection. Five of the 11 children (46%) became viraemic during follow up. The viral rebound arose at a median age of 12.6

years (IQR 7.9 – 13.1). The age at which EC was achieved did not predict whether EC would be maintained subsequently (Log-rank p=0.43; Figure 2B).

The median absolute CD4 T cell count and percentage in the 11 subjects at enrolment were 1,170 cells/μL (IQR 726 – 1,808) and 34% (IQR 31 – 38), respectively. All patients had an absolute and relative CD4 count within the normal range for age during the period of EC apart from EC-11 (Figure 1). In a linear mixed model analysis, absolute CD4 count was significantly higher in ECs who maintained control of viraemia than those who subsequently lost it (Figure 2C, p=0.03). CD4% was also higher among those maintaining viraemic control, although this did not reach statistical significance (p=0.72, Fig 2D).

An additional group of ART-naïve children with spontaneous TA was identified within the same cohorts. Twenty one children had at least one timepoint with viremia below the limit of detection and 4 had transiently suppressed the virus for more than one timepoint, however, without fulfilling the criteria for EC (Supplemental Table 1, Figure 3). Of note, TA(2)-1 was aviraemic on 3 occasions, but these spanned less than 1 year. In this child, the VL was always below 1,000 copies/mL over a follow-up period of 9 years.

DISCUSSION

To identify paediatric elite controllers (EC), we applied the definition described for EC adults by *Olson et al (16)* and *Yang et al* (15), being three consecutive undetectable plasma viral load measurements spanning at least one-year in ART-naïve subjects, without viraemic spikes higher than 1,000 HIV RNA c/ml. Ten cohorts of HIV-

infected children were studied, from South America (Brazil), Europe (UK, Spain and Sweden), Africa (South Africa) and Asia (Thailand). Within these cohorts we identified 11 children qualifying as EC. Although 4 of the paediatric EC were identified within European cohorts, all originated from sub-Saharan Africa. This group thus is likely represents a broad range of populations and subclades of HIV according to the global distribution: subtype B (Brazil), C (Southern Africa and Ethiopia), D (Uganda) and CRF01_AE (Thailand)(26).

Several features of paediatric EC are apparent even from this small group. The high proportion of females (91%) is consistent with the observation that adults females are 5-fold more likely than males to be EC(15). Although there appears to be an increased susceptibility among females specifically to in utero MTCT(27-31), this does not appear to apply to intra partum or post partum infection(28, 29), so overall the numbers of females infected via MTCT do not exceed those of males by more than approximately 10%(28). As in adult infection, where viral loads are typically lower in females than males(32), in some studies female children also have been shown to have lower viral loads(31, 33), although this sex difference was not observed in one large study of >2000 ART-naïve children(34). Among 282 paediatric slow progressors (PSP), defined as ART-naïve children maintaining absolute CD4 counts above 350 cells/mm3 and not meeting clinical criteria (WHO clinical disease stage 3 or 4) to initiate ART until at least 5 years of age, 59% were female(34), indicating that in female children absolute CD4 count declines more slowly than males. Nonetheless, the numbers of female EC in the group of children identified here are significantly higher than that of this large PSP cohort ($p=0.05$), suggesting that female children control viraemia better than males, as well as maintaining higher absolute CD4 counts. This is

consistent with adult studies demonstrating that the initial viral setpoint in females is lower than in males(35-37), in association with a more vigorous type I interferon response in acute infection in females(32, 38).

Despite the limitations inherent in the fact that these are not birth cohorts that were studied, many of the other features of paediatric EC appear to contrast with observations in adult EC. First, the time to achieve control of viraemia in this group of 11 children is a median of 6.5 years, whereas in adults the time to viral setpoint is approximately 6 weeks (39). Again, it should be noted that this estimate of the time to achieve EC among children does not take into account the possibility that some EC children who achieved EC at an earlier age were not recognized as EC as a result of having lost EC status by the time they presented; indeed, EC-1 and EC-6 experienced periods of undetectable viral load prior to fulfil the criteria of EC (Figure 1), and some TAs experienced more than one period of aviraemia (Figure 3). Equally, other children who would achieve EC at a future date were not included as EC because they had not yet achieved EC status at the time of the study. Second, EC status appears to be more short-lived in children than in adults, in whom the median time to viral rebound among adult women is >30 years(15). The relatively common occurrence of children identified as achieving undetectable viral loads only transiently (Figure 3, Supplemental Table 1) prompts the hypothesis that the developing immune response over childhood helps to bring viral load down over a period of years, but once sufficient selection pressure is brought to bear on the virus, in most cases it can escape. By contrast, in adults the critical battles between the immune system and the virus are waged chiefly, although not exclusively, in the first weeks of infection (40-42). Although lack of sample availability prevents precise measurement of the viral load among the EC children here,

certainly the number and frequency of blips of <1000 c/ml among the paediatric EC suggests that control of viral replication is more profound among adult EC. The average viral load among adult EC is reportedly 1-5 RNA c/ml(43-46) . All these above-mentioned differences between paediatric and adult EC are consistent with the aggressive antiviral immune response observed in adults compared with the relative tolerogenic immunity in childhood. In particular, HIV-specific CD8+ T-cell activity, representing the most potent arm of the antiviral immune response, does not reach adult levels until well into adolescence(47). This explains the strong association between adult EC and expression of certain 'protective' HLA, such as HLA-B*57 and HLA-B*27, that mediate an effective cellular immune response against HIV(12, 13, 20). Although lack of sample availability here prevents an assessment of the HLA type among the 11 paediatric EC identified, HLA that are protective in adult infection have less impact in PSP, consistent with a lesser role played by virus-specific CD8+ T-cells in paediatric infection(7).

The apparent temporal association between loss of EC status and puberty in some cases (loss of viraemic control arising at 12-14 years of age in EC7, EC8 and EC-11) might seem surprising if greater potency of the HIV-specific CD8+ T-cell response develops through adolescence. However, in many paediatric slow progressors, the higher frequency and broader HIV-specific CD8+ T-cell responses observed with increasing age are accompanied by increased immune activation, CD4 decline and, ultimately, increased viraemia(6).

The question of the prevalence of EC among infected children versus adults is difficult to address when analyzing this study's data, given that the paediatric cohorts

297 from which these 11 children have been identified have not been followed from birth. In
298 particular the European cohorts, that are largely comprised of children who were born
299 in sub-Saharan Africa, are clearly selected for those who survived long enough to have
300 emigrated. The fact that the average age at enrolment of these children is 6.7 years
301 illustrates the point that many healthy children are likely to remain undiagnosed,
302 especially in less well-resourced countries with high adult seroprevalence where the
303 priority is to treat adults and children with known infection and prevent disease
304 progression. The most accurate estimates of EC prevalence in paediatric HIV are from
305 Kimberley, Northern Cape and Stellenbosch, Western Cape, South Africa, in which the
306 structure of paediatric care is such that a high proportion of HIV-infected children have
307 been followed. In these two provinces, of 11,539 children followed, there were 3
308 paediatric EC. These data would yield an estimated prevalence of 0.026% (95% CI 0 -
309 0.06%). This may represent an underestimate since healthy EC children are less likely
310 to be identified in such settings. Furthermore, since it appears that maintenance of EC
311 status can be quite short-lived in paediatric infection, it is likely that some paediatric EC
312 have lost control of viraemia by the time that they present to clinicians. Conversely,
313 other children who are future EC have not achieved control of viremia when first
314 presenting.
315
316 The other 8 cohorts studied, as described above, are relatively selected and
317 therefore would represent an overestimate of prevalence. Overall, the 10 cohorts
318 provide a paediatric EC prevalence estimate of 0.08% (95% CI 0.04 - 0.14%). Given
319 the prevalence of EC among adult cohorts of ~0.5%, and even after considering the
320 imperfections of prevalence estimate here of paediatric EC, these data suggest that EC
321 prevalence is 5-10-fold lower among infected children than adults.

The observation of TA in a number of ART-naïve children, as noted above, is not described in adult infection nor has it been noted in paediatric infection. Possible reasons why TA have not been described in paediatric infection previously, even though within the Kimberley cohort alone, some 20 paediatric TA were identified (Supplemental Table 1), include the fact that the HIV-infected children whose viral loads are most frequently monitored are the relatively rare ones being cared for in well-resourced settings in Europe or North America. By contrast, in the more poorly resourced sub-Saharan African countries where >90% HIV-infected children live, dedicated outpatient follow up facilities, let alone frequent and regular monitoring of viral loads, are only encountered exceptionally. The mechanisms underlying TA remain unknown. It is possible that a proportion of these would eventually become EC, but in the majority, an undetectable viral load is followed by a viral load 2 $\log_{10}$ higher. One might speculate that, as the paediatric immune response becomes more effective at controlling viraemia with increasing age, so the strength of selection pressure for escape mutants intensifies. Further studies to define the mechanisms of immune control and loss are relevant to designing of strategies to achieve post-treatment control in children. However, it is of interest that among these paediatric TAs, 8 of 21 (38%) are male, a proportion similar to that among PSPs (41%) (34) and higher than among EC. Numbers are too small to be definitive but these suggest the possibility that male children might be more susceptible to viral rebound once aviraemia has been achieved compared to female children.

The present study has several limitations resulting from the unstructured availability of paediatric cohorts and the absence of longitudinal data from birth. The

rarity of paediatric EC limits the analyses that are possible in order to describe this unique group more fully. Furthermore, paucity of samples from the majority of these subjects, many of whom are no longer being followed up, prevents a more comprehensive analysis of the potential immune mechanisms that may be operating in the children to achieve control of viraemia. However, this study does provide the first initial description of a group of longitudinally tracked paediatric EC, and the striking differences that exist between these and adult EC. Finally, the prevalence estimates of paediatric EC is relevant in future studies of post-treatment control in children where the impact of ART or another intervention is being evaluated.

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Authors contribution:

VAV designed research, analysed the data, and wrote the paper. PZ, JR, JM, MC, AVZ, DS EA RA, CB, CFG, LN, TP, WNS, JA, DP, BT, JP, PJ, GTW, and MC did the recruitment at their sites and helped with data management. NG and MAA analysed the data. PG designed the study, analysed the data and wrote the paper. All authors critically reviewed the manuscript.

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Table 1

PID	Cohort	Country of Origin	Number in cohort*	Sex	Evidence of MTCT†	Year of birth	Age at enrollment (yrs)	Age at EC (yrs)	Age at loss of viral control (yrs)
EC-1	London, UK§	Ghana	400	Female	a,b	2004	3	6	-
EC-2	Durban, RSA§	South Africa	200	Female	a,b	2002	8	11	-
EC-3	Belo Horizonte, Brazil§	Brazil	644	Female	a,b	1993	4	<4	-
EC-4	Kimberley, RSA	South Africa	2,501	Male	a,b	2007	3	6	10.4 (started ART)
EC-5	Stellenbosch, RSA	South Africa	9,038	Female	a,b	1999	10	<10	-
EC-6	Barcelona, Spain§	Ethiopia	100	Female	a	2002	8	9	-
EC-7	London, UK§	Uganda	278	Female	a,b	2001	12	<12	13
EC-8	London, UK§	Zimbabwe	278	Female	a,b	2000	7	10	12.6
EC-9	Stellenbosch, RSA	South Africa	9,038	Female	a,b	2010	2	2	3.9
EC-10	Sao Paolo, Brazil§	Brazil	500	Female	a,b	1999	3	4	8
EC-11	Bangkok, Thailand§	Thailand	382	Female	a,b	1996	6	<6	13.7

* Number of patients in the study cohort who did not meet the criteria for being an EC.

† a: diagnosis pre 10yrs; b: mother with known HIV infection

§: local clinics – the EC number and cohort size can not represent the HIV-infected children population in that region.
MTCT: mother-to-child-transmission; EC: elite controller; ART: antiretroviral therapy

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FIGURE LEGENDS

Figure 1. Longitudinal data of viral load (red), absolute CD4 (blue), relative CD4 (green), and CD4:CD8 (pink). A. Paediatric elite controllers (EC) maintaining control of viraemia after achieving elite control. B. Paediatric EC who become viraemic after a period of elite control. Hashed-line represents the RNA HIV limit of detection of 50 copies/ml. The empty triangles represent viral load assays with the limit of detection above 50 copies/mL. The 10^{th}, 50^{th} and 90^{th} percentiles are represented by the three gray lines for absolute CD4 count and CD4 percentage.

Figure 2. A. Kaplan-Meier curve for age to achieve paediatric EC status among the 11 subjects. B. Kaplan-Meier curves for age to achieve paediatric EC status among those who maintained EC and those who rebounded; log-rank test was used to compare the curves. C and D: Mixed linear model of absolute and relative CD4 count, respectively, for those with persistent EC status (blue) and transient EC status (red).

Figure 3. Longitudinal data of viral load (red), absolute CD4 (blue), relative CD4 (green), and CD4:CD8 (pink). A. Transient aviraemia (TA) with 2 or more viral loads below the limit of detection. B. TA with 1 viral load below the limit of detection. Hashed-line represents the RNA HIV limit of detection of 50 copies/ml. The empty triangles represent viral load assays with the limit of detection above 50 copies/mL. The 10^{th}, 50^{th} and 90^{th} percentiles are represented by the three gray lines for absolute CD4 count and percentage CD4.

Figure 1.

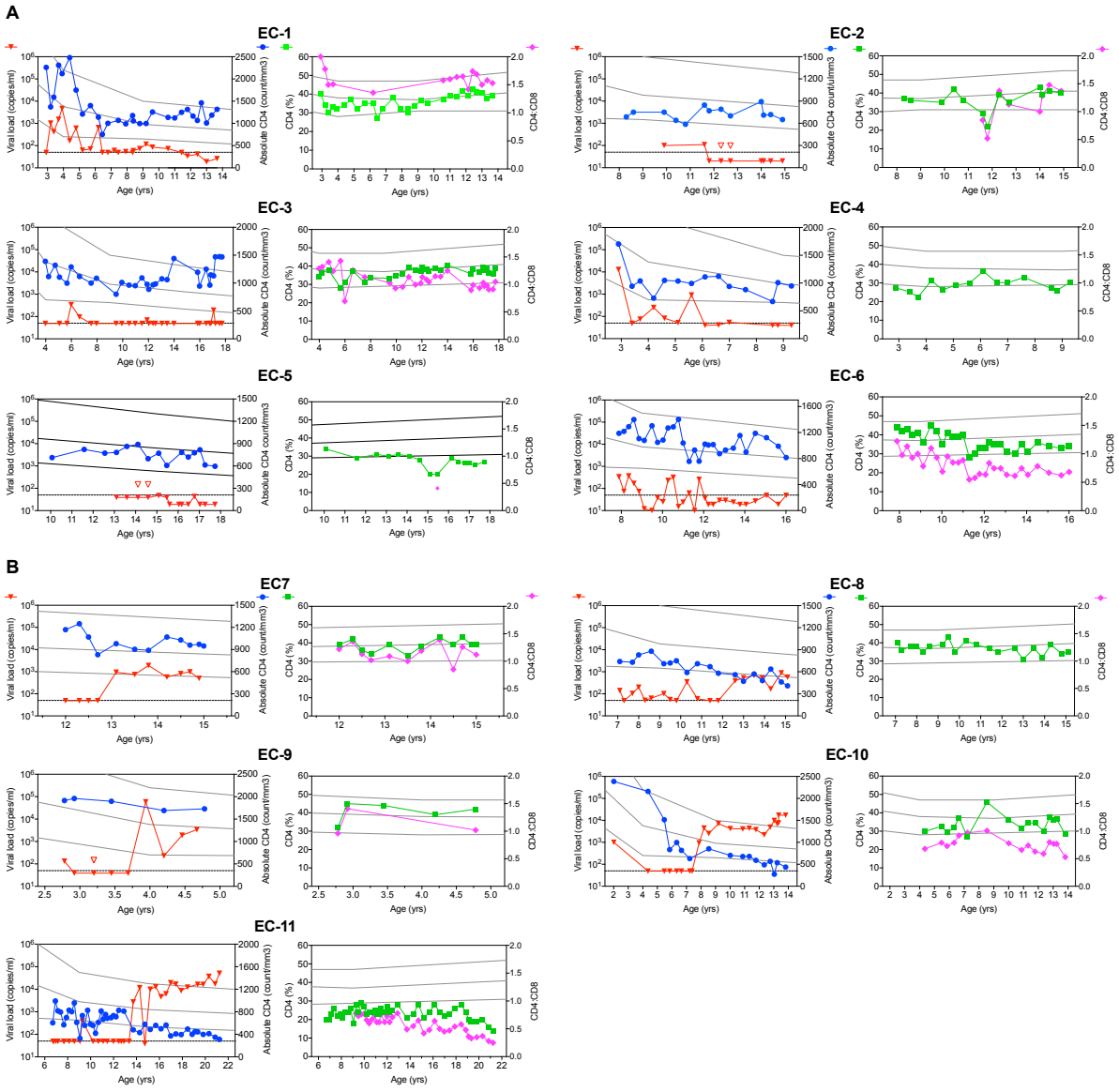

A
EC-1
EC-2
EC-3
EC-4
EC-5
EC-6
B
EC7
EC-8
EC-9
EC-10
EC-11
Viral load (copies/ml)
Absolute CD4 (count/mm3)
CD4 (%)
CD4:CD8
Age (yrs)

Figure 2.

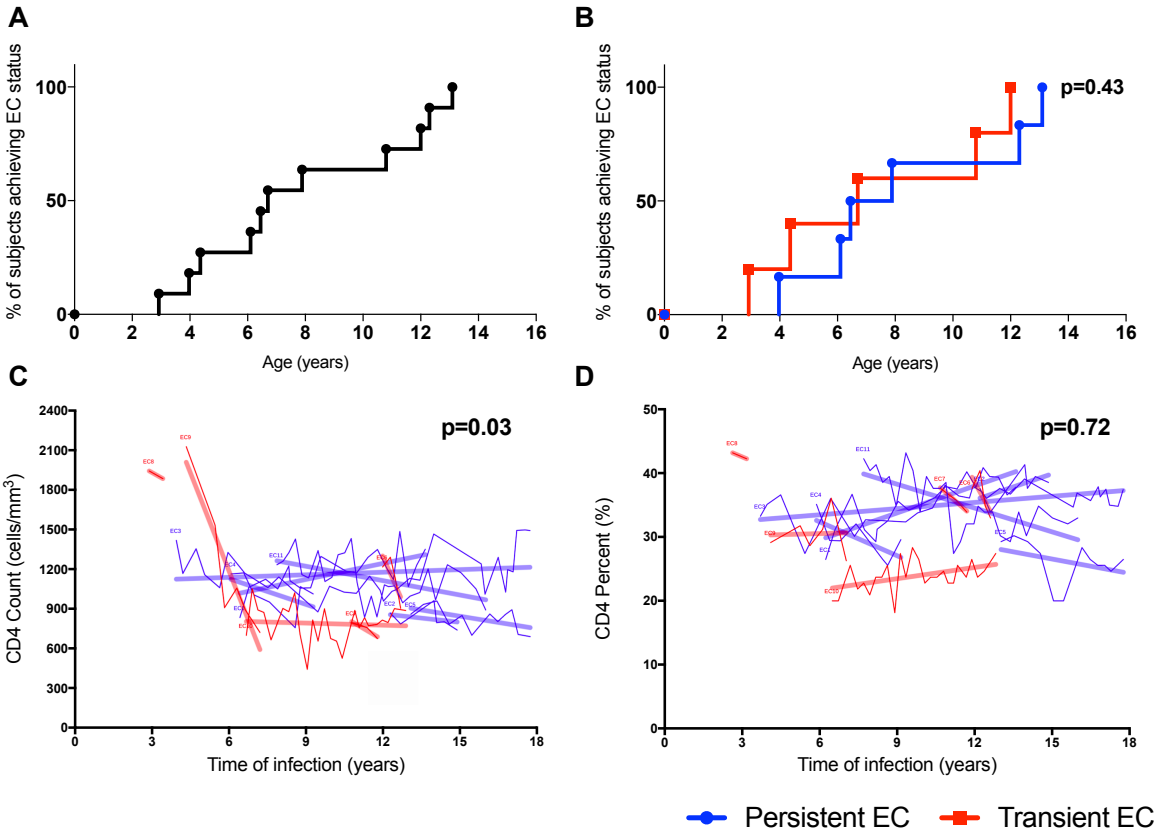
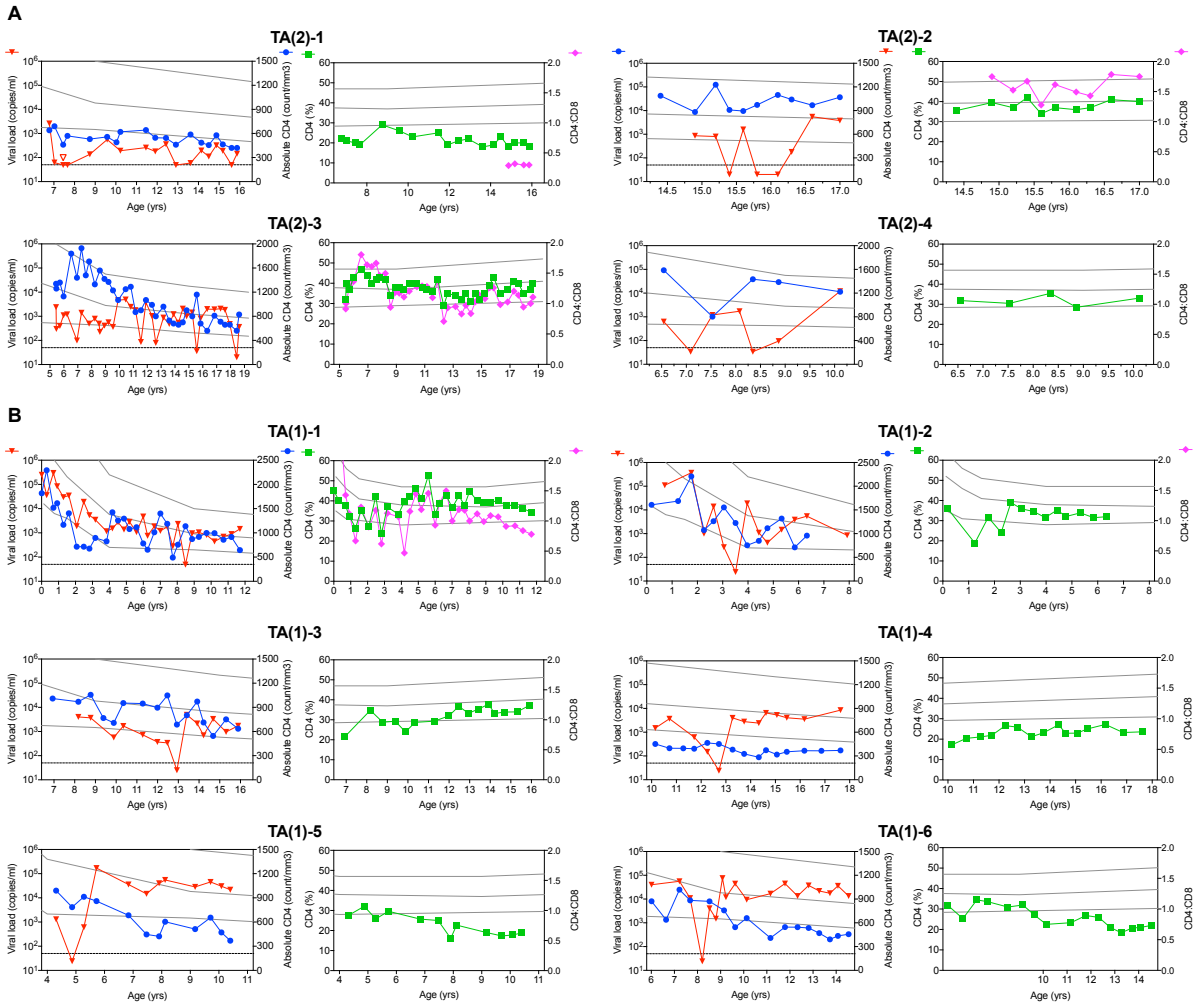

A
% of subjects achieving EC status
100
50
0
0 2 4 6 8 10 12 14 16
Age (years)
B
% of subjects achieving EC status
p=0.43
Age (years)
C
CD4 Count (cells/mm3)
p=0.03
Time of infection (years)
D
CD4 Percent (%)
p=0.72
Time of infection (years)
Persistent EC
Transient EC

Figure 3.


A
TA(2)-1
TA(2)-2
TA(2)-3
TA(2)-4
B
TA(1)-1
TA(1)-2
TA(1)-3
TA(1)-4
TA(1)-5
TA(1)-6
Viral load (copies/ml)
Absolute CD4 (count/mm3)
CD4 (%)
CD4:CD8
Age (yrs)

Supplemental Table 1

PID	Cohort	Country of Origin	Sex	Evidence of MTCT†
TA(2)-1	GOS, London, UK	Zimbabwe	Female	a,b
TA(2)-2	Durban, RSA	South Africa	Male	a,b
TA(2)-3	Stockholm, Sweden	Somalia	Female	a,b
TA(2)-4	Kimberley, RSA	South Africa	Male	a,b
TA(1)-1	Sao Paolo, Brazil	Brazil	Male	a,b
TA(1)-2	Kimberley, RSA	South Africa	Female	a,b
TA(1)-3	Kimberley, RSA	South Africa	Female	a,b
TA(1)-4	Kimberley, RSA	South Africa	Female	a,b
TA(1)-5	Kimberley, RSA	South Africa	Male	a,b
TA(1)-6	Kimberley, RSA	South Africa	Female	a,b
TA(1)-7	Kimberley, RSA	South Africa	Male	a,b
TA(1)-8	Kimberley, RSA	South Africa	Male	a,b
TA(1)-9	Kimberley, RSA	South Africa	Male	a,b
TA(1)-10	Kimberley, RSA	South Africa	Female	a,b
TA(1)-11	Kimberley, RSA	South Africa	Female	a,b
TA(1)-12	Kimberley, RSA	South Africa	Female	a,b
TA(1)-13	Kimberley, RSA	South Africa	Female	a,b
TA(1)-14	Kimberley, RSA	South Africa	Female	a,b
TA(1)-15	Kimberley, RSA	South Africa	Male	a,b
TA(1)-16	Kimberley, RSA	South Africa	Female	a,b
TA(1)-17	Bangkok, Thailand	Thailand	Female	a,b

† a: diagnosis pre 10yrs; b: mother with known HIV infection

TA: transient aviraemic