Long-Term Effects of Highly Active Antiretroviral Therapy in Pretreated, Vertically HIV Type 1–Infected Children: 6 Years of Follow-Up

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Background. Several studies of children with human immunodeficiency virus (HIV) type 1 infection have demonstrated sustained increases in $CD4^+$ cell count, even when virological failure has occurred after receipt of highly active antiretroviral therapy (HAART), but these studies were of limited duration. Moreover, the $CD4^+$ cell count threshold at which antiretroviral treatment should be initiated is still unsettled. The aim of this study was to define the long-term impact of HAART on $CD4^+$ cell percentage and viral load according to $CD4^+$ cell percentages before HAART was initiated.

Methods. We conducted a retrospective study of 113 pretreated HIV-1–infected children stratified by pre-HAART $CD4^+$ cell percentage (<5%, 5%–15%, 15%–25%, and >25%). The inclusion criteria were as follows: initiating HAART with a protease inhibitor, having 6 years of follow-up after starting HAART, having a $CD4^+$ cell count or viral load recorded before initiation of HAART, and having received mono- or dual-nucleoside therapy before starting HAART.

Results. During the first 2 years of HAART, HIV-1–infected children experienced a significant increase in $CD4^+$ cell percentage and a decrease in viral load ($P < .05$). During their last 4 years of receiving HAART, we found a significant decrease in viral load but not an increase in $CD4^+$ cell percentage, because the $CD4^+$ cell percentage reached a plateau after the second year of HAART. Moreover, children with $CD4^+$ cell percentages of <5% at baseline did not achieve $CD4^+$ cell percentages of >25% after 6 years of HAART. Children with $CD4^+$ cell percentages of 5%–25% at baseline had a strong negative association with achieving $CD4^+$ cell percentages of >30% for at least 6 and 12 months but not with achieving $CD4^+$ cell percentages of >30% for at least 24 months.

Conclusions. Long-term HAART allowed for restoration of $CD4^+$ cell counts and control of viral loads in HIV-1–infected children. However, initiating HAART after severe immunosuppression has occurred is detrimental for the restoration of the $CD4^+$ cell count.

The efficacy of HAART is demonstrated by the fact that many patients achieve suppression of viral load below the detectable limit (≤400 copies/mL), allowing for an increase in the number of $CD4^+$ cells [1, 2], with a good clinical outcome [3, 4]. Previous receipt of antiretroviral therapy affects the overall response to subsequent receipt of HAART [2]. Moreover, other factors, such as age, quantity of $CD4^+$ cells, and viral load before beginning HAART, may alter responses to HAART [2, 5, 6]. In addition, the effects of HAART may differ between children and adults, because children have developing immune systems [7]. In this regard, HIV-1–infected children have higher viral loads and lower rates of virological response than adults [2, 8]. Several studies of HIV-1–infected children have shown sustained increases in $CD4^+$ cell count, even when virological failure has occurred after receipt of HAART [2, 5, 6], but these studies were of limited duration, and the long-term immunologic and virological evolution after prolonged

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HAART are not completely understood. Moreover, the threshold $CD4^+$ cell count at which antiretroviral treatment should be initiated is still unsettled [5, 6, 9], and there is a need to decide the optimal time to initiate HAART. With respect to this, Luzuriaga et al. [10] reported that an age of ⩽3 months at the initiation of HAART was associated with improved long-term virus suppression.

To address those issues, we conducted a retrospective study to define the long-term impact of HAART on $CD4^+$ cell percentage and viral load according to $CD4^+$ cell percentage (<5%, 5%–15%, 15%–25%, and >25%) before beginning HAART in pretreated, vertically HIV-1–infected children who were followed up for 6 years.

MATERIALS AND METHODS

Population and study design. A retrospective study of 113 vertically HIV-1–infected children recruited from October 1996 to November 1998 and followed up until November 2004 was conducted. These children are part of the cohort that we have been studying in 6 large pediatric referral hospitals in Madrid and Seville (Spain). All infants were identified as having HIV-1 infection on the basis of positive results of both DNA PCR and virus culture assays, as described elsewhere [11]. The only entry criterion for this cohort was having a vertical HIV-1 infection. Thus, 278 children were included in this cohort from 1996 to 1998: 113 pretreated children and 40 treatment-naive children who started HAART, 90 children who did not receive HAART (during 1996–1998), and 35 children who were lost to follow-up or who died after 1996. Of these 35 children, 25 did not receive HAART, and only 10 children died among those who started HAART between 1996 and 1998. The 10 children who died were not included in this study, because they underwent 6 years of follow-up after starting HAART.

We selected children for our study according to the following criteria: children who started receiving HAART with a protease inhibitor; children who completed 6 years of follow-up after starting HAART; children with a $CD4^+$ cell count or a viral load recorded before initiation of HAART; and children who received mono- or dual-nucleoside therapy before starting HAART. We included in the analysis only children who had received prior antiretroviral therapy at the time they began HAART so that the study group was more homogeneous with respect to previous exposure to antiretroviral drugs and accumulation of HIV resistance.

The study was approved by the ethical committees of all hospitals involved. Clinical classification was based on the 1994 revised guidelines of the Centers for Disease Control and Prevention (CDC) [12]. Children were monitored at least every 3 months with repeated interviews; physical examinations were performed in accordance with published guidelines [13]; and collection of blood samples for measurement of serial $CD4^+$ cell percentage, $CD8^+$ cell percentage, and viral load were performed, as described elsewhere [2]. There was not a uniform approach with respect to antiretroviral therapy. Instead, each pediatrician administered the appropriate antiretroviral therapy regimen and changed the drugs according to his or her interpretation of each patient's data and in accordance with international CDC [13] and European [14, 15] guidelines. The clinicians did not have access to resistance testing to guide treatment decisions. Adherence to antiretroviral drug therapy was measured by each pediatrician by examination of the number of doses taken by each child and by means of interviews with their parents or tutors. Fat distribution was assessed by clinical criteria, anthropometric measurements, and dual-energy x-ray absorptiometry. Lipodystrophy was defined on clinical grounds at last observation. HIV resistance testing was not available for the entire study period.

Statistical analysis. All statistical analyses were done with SPSS software, version 12.0 (SAS). All P values are 2-tailed. Statistical significance was defined as $P < .05$. Differences in mean values for different groups of children were analyzed using an independent-samples t test. Fisher's exact test was applied for categorical variables.

Initiation of HAART was defined as the first time a child took ⩾3 antiretroviral drugs that included a protease inhibitor. In terms of statistical analysis, subsequent changes in HAART were ignored.

First, to establish each patient's representative measures, we determined the mean $CD4^+$ cell percentage and $\log_{10}$ viral load each year before and after HAART initiation. $CD4^+$ cell percentage and $\log_{10}$ viral load were plotted by year of follow-up and stratified by $CD4^+$ cell percentage (<5%; 5%–15%; 15%–25%; >25%) before beginning HAART. Moreover, we analyzed the normalization of immunologic markers. We calculated the percentage of children with a mean $CD4^+$ cell percentage each year of >30% and the percentage of children with a mean viral load each year of <400 copies/mL.

Second, we performed a multivariate analysis to determine long-term response to HAART. Dependent variables were the mean differences in $CD4^+$ cell percentage and $\log_{10}$ viral load recorded each year from the year before HAART was begun (-1 year) to the second year of HAART and from the second to the sixth years of HAART. After we observed the evolution in $CD4^+$ cell percentage, we performed the data analysis a posteriori for years 2 and 6. We selected these points for analysis of a first stage of increase in $CD4^+$ cell percentage and a second stage of stabilization of $CD4^+$ cell percentage. Independent variables were strata of $CD4^+$ cell percentages. This analysis was adjusted for baseline patient characteristics (i.e., previous diagnosis of AIDS; duration of antiretroviral therapy and number of changes in antiretroviral therapy before beginning HAART; number of new drugs [nucleoside analogues] included in

HAART; $CD4^+$ cell percentage, viral load, and age at baseline; and adherence to HAART during follow-up).

Third, we performed a logistic regression analysis to determine the OR necessary to reach a normal $CD4^+$ cell percentage (>30%). The dependent variable was achieving and maintaining a $CD4^+$ cell percentage of >30% for at least 6, 12, and 24 months of the follow-up period. Independent variables were $CD4^+$ cell percentage, viral load, and age. This analysis was adjusted for baseline patient characteristics (previous diagnosis of AIDS; duration of antiretroviral therapy and number of changes in antiretroviral therapy before beginning HAART; number of new drugs [nucleoside analogues] included in HAART; $CD4^+$ cell percentage, viral load and age at baseline; and adherence to HAART during follow-up).

RESULTS

Characteristics of HIV-1–infected children according to $CD4^+$ cell strata. Table 1 shows characteristics at baseline of pretreated, vertically HIV-1–infected children. The mean age at which the children first entered the cohort was ~7 years, with the follow-up period lasting until early teen years. During the follow-up period, 2 children progressed to AIDS, and 1 child died. Twenty-seven children (23.8%) had a $CD4^+$ cell percentage of <5%, and none had a viral load below the limit of detection at baseline. Only 15 children (13.2%) had received monotherapy before initiating HAART, and the other children had received combined therapy before initiating HAART. Moreover, 41 children (36.2%) received only 1 nucleoside analogue HIV reverse-transcriptase inhibitor as part of HAART, 36 (31.8%) received 2 nucleoside reverse-transcriptase inhibitors, and 7 (6.1%) received 1 nonnucleoside analogue HIV reverse-transcriptase inhibitor.

The most common antiretroviral drugs included in first-line HAART were stavudine (76%) and lamivudine (67%) as nucleoside reverse-transcriptase inhibitors, nelfinavir (34%) and ritonavir (25%) as protease inhibitors, and efavirenz (10%) as a nonnucleoside reverse-transcriptase inhibitor. Two nucleoside reverse-transcriptase inhibitors plus 1 protease inhibitor was the most common HAART regimen (73.6%). Only 57 children (50.4%) retained their initial HAART regimen after 2 years of follow-up, and 19 children (16.8%) received >2 HAART regimens.

Evolution of $CD4^+$ cell percentage and viral load according to $CD4^+$ cell strata. We observed that children with $CD4^+$ cell percentages of <5% at baseline did not achieve $CD4^+$ cell percentages of >25% after 6 years of HAART (figure 1*A*). Moreover, children who had $CD4^+$ cell percentages of 5%–25% at baseline achieved $CD4^+$ cell percentages of >25% but did not reach the percentage of children with $CD4^+$ cell percentages of >25% at baseline. When we analyzed achievement of $CD4^+$ cell percentages of >30%, we observed similar trends (figure 1*B*). However, evolution of viral load was not affected by baseline $CD4^+$ cell percentage (figures 1*C* and 1*D*).

Table 2 shows the mean changes in $CD4^+$ cell percentage and

Table 1. Demographic and clinical characteristics of vertically HIV-1–infected children at baseline, before beginning HAART.

Characteristic	$CD4^+$ cell percentage stratum at baseline				
	<5% (*n* = 27)	5%–15% (*n* = 34)	15%–25% (*n* = 29)	>25% (*n* = 23)	*P*
Age, mean years ± SD	7.4 ± 0.5	7.7 ± 0.6	6.9 ± 0.8	4.9 ± 0.7	.049
Male sex	18 (66.7)	15 (44.1)	10 (34.5)	7 (30.4)	.038
Diagnosis of AIDS[a]	18 (66.7)	18 (52.9)	13 (44.8)	8 (34.8)	.136
Receipt of antiretroviral treatment before beginning HAART					
Monotherapy	2 (7.4)	7 (20.6)	2 (6.9)	4 (17.4)	
Combined therapy	5 (18.5)	7 (20.6)	10 (34.5)	12 (52.2)	.033
Monotherapy plus combined therapy	20 (74.1)	20 (58.8)	17 (58.6)	7 (30.4)	
Duration, mean months ± SD	36.6 ± 4.2	40.4 ± 4.9	39.7 ± 6.2	15.9 ± 1.9	.003
Number of changes in antiretroviral protocol before beginning HAART	2.5 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	1.7 ± 0.14	.036
No. of new drugs included in HAART regimen initiated at baseline ± SD[b]	1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	.650
1 NRTI	7 (25.9)	15 (44.1)	11 (37.9)	8 (34.8)	.798
2 NRTIs	10 (37.0)	9 (26.5)	8 (27.6)	9 (39.1)	
1 NNRTI	2 (7.4)	1 (2.9)	3 (10.3)	1 (4.3)	.641

NOTE. Data are no. (%) of children, unless otherwise indicated.

[a] According to Centers for Disease Control and Prevention criteria.

[b] Protease inhibitor was not included.

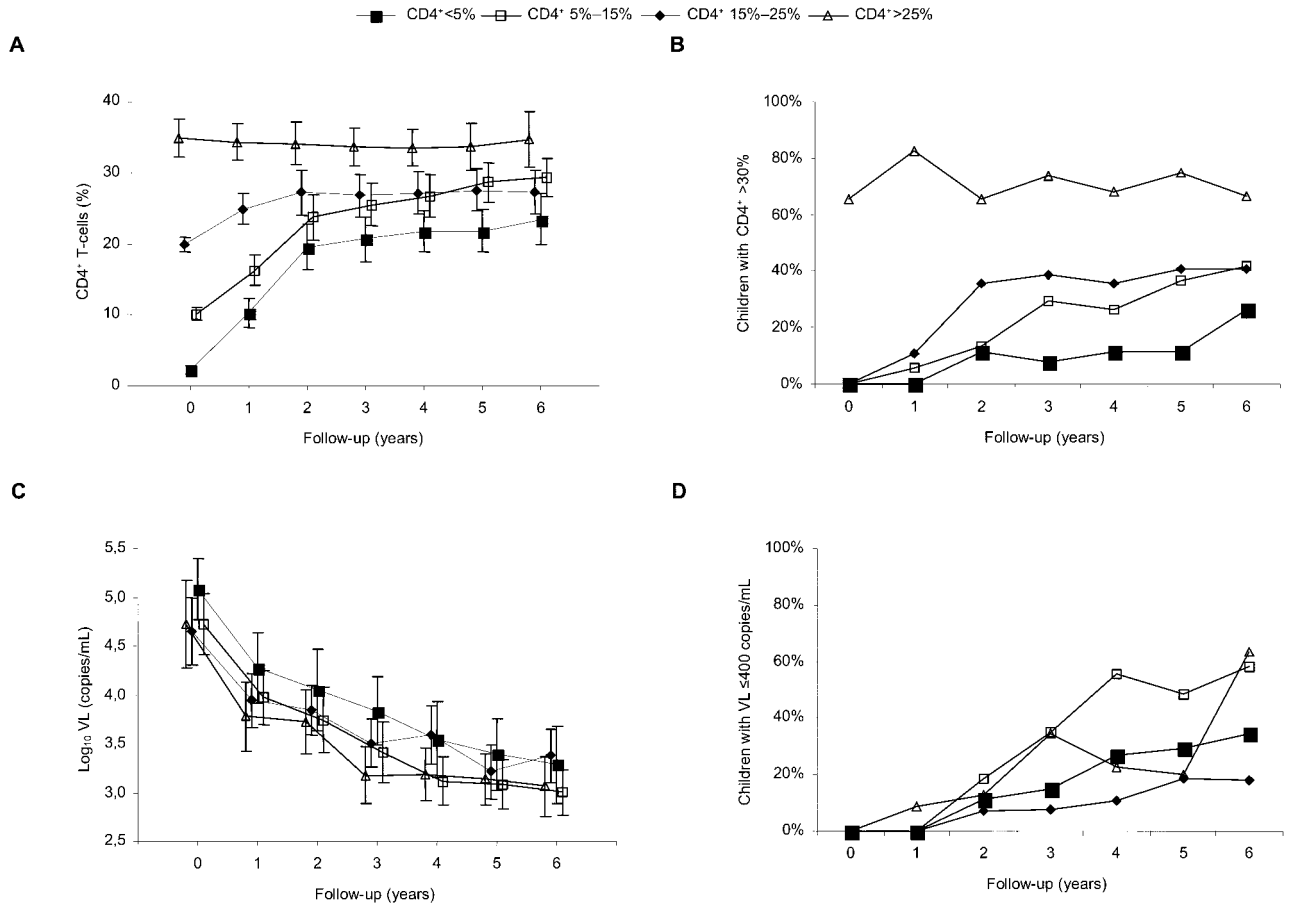

Figure 1. Evolution of $CD4^+$ cell percentages and $\log_{10}$ viral loads (VLs) during follow-up of pretreated, vertically HIV-1–infected children receiving HAART, according to baseline $CD4^+$ cell percentages. *A* and *C* show $CD4^+$ cell percentages and $\log_{10}$ VLs as mean values ± 2SE. *B* and *D* show percentages of HIV-1–infected children with a mean $CD4^+$ cell percentage of ⩾30% and a mean VL of ⩽400 copies/mL for each year.

$\log_{10}$ viral load (copies/mL) from the time before HAART was begun (−1 year) to the second and sixth years of HAART, according to $CD4^+$ cell percentage strata. During the first 2 years of HAART, we found a significant increase in $CD4^+$ cell percentage and a decrease in viral load ($P < .05$). When we analyzed the data from the last 4 years of follow-up (from the second to sixth years), we did not find a significant increase in $CD4^+$ cell percentage. After the second year of HAART, $CD4^+$ cell percentage reached a plateau and remained stable until the sixth year of follow-up. Moreover, we found a decrease in viral load after the second year of HAART.

We also analyzed the long-term effect of HAART on $CD4^+$ cell percentage (table 3). Children with $CD4^+$ cell percentages of <5% at baseline had a strong negative association with achieving a $CD4^+$ cell percentage of >30% for at least 6, 12, and 24 months. Children with $CD4^+$ cell percentages of 5%–25% had only negative associations with achieving a $CD4^+$ cell percentage of >30% for at least 6 months and 12 months. However, we did not find an association between $CD4^+$ cell percentage strata and long-term control of viral load. Seventy-seven children (68.1%) had a viral load below the limit of detection for at least 6 months, 69 children (61.0%) had a viral load below the limit of detection for at least 12 months, 57 children (50.4%) had a viral load below the limit of detection for at least 18 months, 55 children (44.6%) had a viral load below the limit of detection for at least 24 months, and 36 children (31.8%) had a viral load below the limit of detection for at least 36 months. We also analyzed viral load and age at baseline as independent variables, and we found that only viral load had an OR of 2.08 (95% CI, 1.06–4.08) to achieve a $CD4^+$ cell percentage of >30% during at least 6 months of follow-up.

Furthermore, 15 (55.5%) of 27 children with a $CD4^+$ cell percentage of <5% and 11 (32.3%) of 34 children with a $CD4^+$ cell percentage of 5%–15% at baseline did not achieve a $CD4^+$ cell percentage of >25% after 4 years of follow-up. However, $CD4^+$ cell percentages of most of the other children were restored to >25% (without immunodeficiency, according to the CDC guidelines), although most of these children did not achieve undetectable viral loads (<50%). When we performed a multivariate logistic regression analysis for discordant re-

Table 2. Summary of estimated mean changes in CD4⁺ cell percentage and log_{10} viral load from baseline to the second and sixth year of HAART among HIV-1–infected children.

Baseline CD4⁺ cell percentage stratum	From baseline to second year of HAART		From second year to sixth year of HAART	
	CD4⁺ cell percentage (95% CI)	Log_{10} viral load, copies/mL (95% CI)	CD4⁺ cell percentage (95% CI)	Log_{10} viral load, copies/mL (95% CI)
<5%	16.4 (12.1–21.5)[a]	−0.7 (−1.1 to −0.3)[a]	3 (−1.7 to 7.7)	−0.6 (−1 to −0.2)[a]
5%–15%	13.1 (8.05–18.1)[a]	−1.1 (−1.4 to −0.6)[a]	3.5 (−0.5 to 7.5)	−0.7 (−1.1 to −0.3)[a]
15%–25%	8.3 (4.6–12.1)[a]	−0.8 (−1.1 to −0.5)[a]	−3.1 (−8 to 1.6)	−0.5 (−0.9 to −0.2)[a]
>25%	−2.7 (−6.7 to 1.29)	−1 (−1.3 to −0.6)[a]	−1.1 (−5.2 to 4.9)	−0.6 (−1 to −0.2)[a]

NOTE. Multivariate analysis was adjusted for previous diagnosis of AIDS, duration of previous antiretroviral therapy (nucleoside analogue), number of changes in antiretroviral therapy before initiation of HAART, number of new drugs (nucleoside analogues) included in HAART, viral load and age at baseline, and adherence to HAART during the follow-up period.

[a] $P < .05$.

sponse to HAART, we found a significant association between immunodeficiency (CD4⁺ cell percentage, <25%) during follow-up and duration of antiretroviral therapy before HAART (OR, 1.04; 95% CI, 1–1.07; $P = .012$), adherence to therapy of >90% (OR, 0.66; 95% CI, 0.06–0.01; $P = .002$), and CD4⁺ cell percentage at baseline (OR, 0.75; 95% CI, 0.62–0.921; $P = .006$).

Lipodystrophy and hyperlipidemia. At the end of the study, we found that 57 children (50.4%) had lipodystrophy, 16 (14.1%) of whom had serious lipodystrophy (severe lipoatrophy and lipohypertrophy). We did not find statistical differences among CD4⁺ cell percentage strata. Moreover, children had smooth plasma cholesterol and triglyceride levels, and they did not have significant increases during follow-up ($P > .05$). Hyperlipidemia was defined as plasma cholesterol and triglyceride concentrations of >200 mg/dL and >170 mg/dL, respectively. Twenty-nine percent of children at study entry, 54% at the third year of HAART, and 34% at the end of the study had cholesterol levels >200 mg/dL. Thirty-four percent of children at study entry, 35% at the third year of HAART, and 37% at the end of the study had triglyceride levels of >170 mg/dL.

DISCUSSION

The overall effectiveness of long-term HAART in HIV-1–infected children has rarely been studied, and few studies reflect the evolution of CD4⁺ cell percentage and viral load in a large cohort of HIV-1–infected children throughout long-term HAART [5, 6]. We report a study of the effect of HAART in a large group of pretreated, HIV-1–infected children beginning HAART who were followed up for 6 years outside the controlled setting of a clinical trial. We stratified HIV-1–infected children by strata of CD4⁺ cell percentage at baseline.

The capacity of CD4⁺ cell percentage regeneration during long-term HAART has not been well defined. Although long-term HAART allowed restoration of CD4⁺ cell percentage, initiation of HAART before severe immunosuppression may be more effective for restoration or maintenance of a normal CD4⁺ cell percentage [5]. Addressing this issue is particularly important, because it may provide an answer regarding whether HAART should be initiated for patients with a CD4⁺ cell percentage of <15% or <20% [15] or for patients with a percentage that indicates normal immune function (≥25%) [16] to achieve the best clinical outcome. Previous studies reported that increases in CD4⁺ cell counts among HIV-1–infected children undergoing HAART depend on CD4⁺ cell counts at baseline [5, 17]. In our study, HIV-1–infected children with CD4⁺ cell percentages of <5% at baseline experienced a slower restoration of CD4⁺ cell percentage than did HIV-1–infected children with baseline CD4⁺ cell percentages of 5%–15%, and restoration of CD4⁺ cell percentage to a normal level could not be achieved during long-term HAART. HIV-1–infected children who had a low CD4⁺ cell percentage when HAART was initiated were less likely to attain percentages approaching the normal range by the sixth year of HAART. This may reflect the inhibitory effect of HIV on thymic function [7]. Moreover, CD4⁺ cells are productively infected by HIV [18] and undergo apoptosis induced by an abnormal cellular activation [19] when viral load is not controlled. In addition, high viral load is associated with activation of the immune system [20], and it is used as a predictive marker of virological failure [21–23] and increases in CD4⁺ cell percentage [24–26]. However, in our study, increases in CD4⁺ cell percentage after HAART initiation were unrelated to pre-HAART viral load. Moreover, we observed HIV-1–infected children with CD4⁺ cell percentages of 5%–25% at baseline who achieved CD4⁺ cell percentages near 25% in the third year of HAART, and we observed plateaus in their CD4⁺ cell percentages, just as has been reported elsewhere regarding adults [5, 27, 28]. This may represent a strong argument for deferring initiation of HAART, at least until a patient achieves a CD4⁺ cell percentage of 15%–20%, at which time the immediate risk of clinical disease progression remains small [29]. A limitation of these data could be that CD4⁺ cell percentage

Table 3. Results of logistic regression analysis of achieving CD4⁺ cell percentages of >30% for at least 6, 12, and 24 months.

Group	No. of children	At least 6 months: Percent of children with CD4⁺ cell percentage of >30	At least 6 months: OR (95% CI)	At least 6 months: *P*	At least 12 months: Percent of children with CD4⁺ cell percentage of >30	At least 12 months: OR (95% CI)	At least 12 months: *P*	At least 24 months: Percent of children with CD4⁺ cell percentage of >30	At least 24 months: OR (95% CI)	At least 24 months: *P*
All children	113	49.6	1.08 (1.04–1.14)	<.001	40.7	1.12 (1.06–1.17)	<.001	26.8.	1.08 (1.03–1.14)	.001
Baseline CD4⁺ cell percentage stratum										
<5%	27	25.9	0.06 (0.01–0.29)	<.001	7.4	0.02 (0.01–0.12)	<.001	7.4	0.07 (0.01–0.46)	.005
5%–15%	34	47.1	0.16 (0.04–0.68)	.013	41.2	0.13 (0.03–0.55)	.006	26.5	0.26 (0.07–1.06)	.062
15%–25%	29	51.7	0.24 (0.06–0.99)	.049	46.4	0.23 (0.06–0.80)	.023	25	0.27 (0.07–1.06)	.061
>25%	23	78.3	…	…	73.9	…	…	52.2	…	…

NOTE. *P* < .05 indicates statistical significance. Logistic regression analysis was adjusted for previous diagnosis of AIDS, duration of previous antiretroviral therapy (nucleoside analogue), number of changes in antiretroviral therapy before initiation of HAART, number of new drugs (nucleoside analogues) included in HAART regimen, viral load and age at baseline, and adherence to HAART during the follow-up period.

at initiation of HAART depended on age at the time of availability of protease inhibitors. First, very few young children were likely to have a very low CD4⁺ cell percentage; second, such children may have been at higher risk of immunologic failure than older children with similar CD4⁺ cell percentages at initiation of HAART, because the latter children had been selected because they survived the previous period. The role of age in the restoration of CD4⁺ cell percentage is difficult to evaluate, because our study was not a randomized clinical trial, and the independent effect of initial CD4⁺ cell percentage may not be totally controlled for by adjustment for age in logistic regression.

Moreover, the 10 children who died were not included in this study. They had CD4⁺ cell percentages of <15% and viral loads of >4 $\log_{10}$ at baseline, and, later, they experienced increases in CD4⁺ cell percentage and decreases in viral load. Evidently, a slight overestimation of immunologic restoration may have occurred, because the level of CD4⁺ cell restoration was lower among the children who died than among children with a CD4⁺ cell percentage of <15% who were included in the study.

Sustained suppression of viral load can be achieved in HIV-1–infected children receiving HAART [8]. However, it is difficult to achieve a sustained decrease in viral load below the detection limits of the assays [2]. Our study provides evidence from current clinical practice that demonstrates that prolonged HAART can achieve a viral load below the limit of detection for a long-term period (36 months) only in a small proportion of children, as has been reported elsewhere regarding adults [30]. Moreover, in the management of HIV-1–infected children beginning HAART, it is crucial to detect signs associated with incomplete suppression of viral load so more effective therapies can be implemented. Our data indicate that HIV-1–infected children with low viral loads at baseline and high adherence to antiretroviral therapy during the follow-up period achieved and maintained low viral loads during the follow-up period, as has been reported elsewhere regarding adults [31, 32]. Other authors have found that HIV-1–infected children with virological failure had desirable immunologic and clinical outcomes [33, 34]. Our results also suggest that HIV-1–infected children can achieve a restored CD4⁺ cell percentage without complete suppression of viral load. A percentage of children had immunologic failure, and they could not achieve restored CD4⁺ cell percentages. These children with immunologic failure could have had resistance to a high number of drugs, because they had received antiretroviral therapy for a long time before beginning HAART and had lower adherence to HAART during the follow-up period. These factors are associated with resistance to drugs [31, 35]. Moreover, a lower CD4⁺ cell percentage at initiation of HAART may be associated with very severe damage in the immune system that hinders the recovery of CD4⁺ cell percentages to >25%. On the other hand, table 1 suggests that two-thirds of the children with a CD4⁺ cell percentage of <5% were male, whereas only approximately one-third of the children with a CD4⁺ cell percentage of >25% were male. However, we believe that this difference was coincidental and was not caused by a less desirable evolution of CD4⁺ cells in boys. Furthermore, immune restoration was similar in boys and girls with CD4⁺ cell percentages of <5% at baseline. Several studies have reported associations between age and restoration of CD4⁺ cell counts [5, 6, 9]. We found that children ⩾5 years old at baseline had lower baseline CD4⁺ cell percentages and slower increases in CD4⁺ cell percentages than did children aged <5 years at baseline (data not shown). HIV-1–infected children ⩾5 years old at baseline had greater increases in CD4⁺ cell percentage and achieved a mean CD4⁺ cell percentage of ⩾25% after the second year of HAART. This could be because there was a functional thymus. In contrast to immunologic responses, we found an association between evolution of viral load and age.

We retrospectively evaluated HIV-1–infected children receiving HAART after receiving prior treatment and evaluated the impact of protease inhibitor–based HAART on $CD4^+$ cell percentage and HIV RNA detection (viral load, >400 copies/mL) after at least 6 years of HAART. However, the major limitation of the study is the lack of a uniform approach to treatment decisions, as well as changes in treatment guidelines over time. Also, all of the children in the study received prior antiretroviral therapy with nucleoside analogues before they received HAART. Receipt of prior therapy and receipt of new drugs included in the HAART regimen may also play an important role in response to HAART, but we did not find an association of these variables with long-term response to HAART. Another limitation of our study is previous drug resistance. The presence of resistant HIV in this cohort obviously influences the outcome of therapy, but antiretroviral therapy (monotherapy and combined therapy), time receiving antiretroviral therapy, switches in antiretroviral therapy protocols, and numbers of new drugs in HAART regimens were similar among children with $CD4^+$ cell percentages of <25%.

The continued and prolonged use of HAART has resulted in the development of complications, including changes in body fat distribution and metabolic disturbances, such as insulin resistance and atherogenic dyslipidemia [36]. However, a mild but not significant increase in plasma cholesterol and triglyceride levels was observed during the follow-up period. These protease inhibitor–experienced, HIV-1–infected children may also have had increased lipid and cholesterol levels at entry into the study. The possibility of hypercholesterolemia in HIV-1–infected children with other risk factors for cardiovascular diseases may be of concern and requires routine monitoring during HAART.

Puberty is a time of somatic growth and hormone-mediated changes, and, theoretically, these physiological changes can affect drug pharmacology and cause adverse effects. Beregszaszi et al. [37] reported that puberty seems to be the time when children are most likely to develop lipodystrophy and metabolic complications, although the findings of this study provide no evidence in bivariate or multivariate analyses demonstrating that stage of puberty affects the risk of lipodystrophy. During the first years of the study, children did not receive any treatment for lipid disorders. However, when plasma lipid levels became elevated after several years of HAART, children were treated in accordance with international guidelines, which recommend improvements in diet and exercise as the first intervention. Pharmacological agents (statins) are not recommended for routine use in children and adolescents, except in consultation with both a lipid specialist and a pediatric HIV specialist [38]. Unfortunately, we did not have precise data about these treatments in this retrospective study. We will conduct studies specifically designed to evaluate lipid disorders in HIV-1–infected children in the future.

In conclusion, although long-term HAART allowed restoration of $CD4^+$ cell percentages and control of viral loads in HIV-1–infected children, HAART initiation after severe immunosuppression may be less effective for restoration or maintenance of a normal $CD4^+$ cell percentage. These data argue in favor of not delaying initiation of HAART in young children.

SPANISH GROUP OF PAEDIATRIC HIV INFECTION

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