Extensive Implementation of Highly Active Antiretroviral Therapy Shows Great Effect on Survival and Surrogate Markers in Vertically HIV–Infected Children

Salvador Resino,[1] José Mª Bellón,[1] Rosa Resino,[1] Mª Luisa Navarro,[2] José Tomás Ramos,[3] Mª Isabel de José,[4] Mª José Mellado,[5] and Mª Ángeles Muñoz-Fernández[1]

[1]Laboratorio de Inmunobiología of Hospital "Gregorio Marañón," [2]Servicio de Inmuno-Pediatría of Hospital "Gregorio Marañón," [3]Servicio de Inmuno-Pediatría of Hospital "12 de Octubre," [4]Servicio de Pediatría of Hospital "La Paz," and [5]Servicio de Pediatría-Infecciosas, Hospital "Carlos III," Madrid, Spain

We performed a retrospective observational study of 253 children vertically infected with human immunodeficiency virus (1994–2001) to assess the effectiveness of antiretroviral therapies (ARTs) on survival and surrogate markers at the population level. Children were divided into 3 groups according to the ART protocols used during the follow-up period: calendar period (CP) 1 (1994–1996) received combined therapy with 2 nucleoside reverse transcriptase inhibitors (NRTIs); CP2 (1997–1998) received implementation of highly active ART (HAART) with 3 drugs (NRTIs, protease inhibitors, and non-NRTIs); and CP3 (1999–2001) received extensive HAART. The children in the CP3 group had statistically significant longer survival periods, lower virus load (VL), highest undetectable VL proportion, and highest $CD4^+$ T cell counts. HAART is effective at the population level at decreasing VL, increasing $CD4^+$ T cells, and increasing the survival in a higher percentage of HIV-infected children.

The introduction of HAART has decreased mortality rates in HIV-infected adults and children [1–3] and has proven to be effective in suppressing virus load (VL) and increasing $CD4^+$T cells counts in children [4–6]. However, our current knowledge faces 2 major limitations. First, earlier therapeutic regimens were not too suitable for children and thus compromised their administration and/or adherence, or pharmacokinetics were inadequate [7]. In fact, several past treatment regimens failed to suppress HIV-1 replication in children because the required optimal drug concentrations were never reached [8]. Second, our current strategies are mostly based on the results of prospective clinical trials in which both children and their adherence to therapy were firmly controlled, whereas lack of compliance is usually one of the main causes of poor response rates in children [9, 10]. By contrast, observational and cohort studies allow the analysis of real-life effectiveness of different therapies [2, 11].

Studies of the behavior of the main markers of progression to AIDS in perinatally HIV-infected children receiving HAART outside the controlled setting of clinical trials are scarce. In this regard, we lack enough data

Received 28 August 2003; accepted 14 January 2004; electronically published 5 May 2004.

Financial support: Fundación para la Investigación y la Prevención del SIDA en España, FIPSE (grants 36365/02 and 12456/03), Comunidad de Madrid (grant 08.5/0034/2001), the Red Temática Cooperativa de investigación en SIDA (grant RIS G03/173) of FIS, the Red Temática Cooperativa de Investigación en Genética Clínica y Molecular (grant RIG C03/07) of FIS, Bristol-Myers Squibb, and Plan Nacional de Salud (grant SAF 2003-09209). S.R. is supported by a grant of FIS (grant CM0300038).

Reprints or correspondence: Dr. Mª Angeles Muñoz-Fernández, Laboratorio de Inmunobiología Molecular, Hospital General Universitario "Gregorio Marañón," C/ Doctor Esquerdo 46; 28007 Madrid, Spain (Mmunoz@cbm.uam.es).

Clinical Infectious Diseases 2004; 38:1605–12

1058-4838/2004/3811-0017$15.00

regarding the evolution of plasma VL and $CD4^+$ T cell percentage to monitor the efficacy of therapy in these children. Our objective was to compare the effectiveness of several therapeutic strategies throughout the years by monitoring the 2 most important markers of progression to AIDS and the mortality rates in HIV-infected children at the population level.

MATERIALS AND METHODS

Population and study design. This is a retrospective study of 253 vertically HIV-1–infected children observed during 1994–2001 who received care in the pediatric departments of 4 large Spanish pediatric referral hospitals (Hospitals "Gregorio Marañón," "12 de Octubre," "La Paz," and "Carlos III" in Madrid, Spain). Inclusion criteria involved having $CD4^+$ and VL quantification and having ⩾6 months of follow-up. From an initial cohort of 287 HIV-infected children, 15 children were excluded because we lacked data regarding their follow-up. A further 22 children were excluded for having <6 months of follow-up.

All infants received a diagnosis of HIV-1 infection on the basis of positive results of both DNA PCR and viral culture assays [12]. Clinical classification was based on the 1994 revised guidelines of the Centers for Disease Control and Prevention (CDC) [13]. This study was conducted according to the declaration of Helsinki and approved by the ethical committees of all hospitals involved. The children had follow-up visits at least every 3 months with repeated interviews, physical examinations, and blood sample collection, in accordance with published guidelines [7]. T lymphocyte subsets in peripheral blood were quantified by flow cytometry (FACScan, Becton-Dickinson Immunocytometry Systems) [12], and HIV-1 RNA levels were measured in plasma with a quantitative RT-PCR assay (Amplicor Monitor; Roche Diagnostic Systems) [14].

There was no strict approach regarding antiretroviral treatment (ART). Instead, each pediatrician administered the appropriate ART regimen and changes the drugs according to his or her interpretation of the patient's data and following international CDC [7, 15] and European [16] guidelines. The adherence of antiretroviral drugs was measured by pediatricians by examination of the dose token and through interviews with parents or tutors. Response to therapy was evaluated every 3 months by serial assessment of percentage of $CD4^+$cells, percentage of $CD8^+$ T cells, and VL and clinical data.

Exposure variables. Descriptive analyses were performed to establish what ART protocols and protease inhibitor (PI) and nonnucleoside analogue HIV-1 reverse transcriptase inhibitor (NNRTI) drugs were administered and the proportion of children treated over time (figure 1). When a child in a calendar year switched his or her ART protocols or antiretroviral drugs (PI or NNRTI), we counted them as many times as they had changed. On this basis, the children were divided into 3 groups according to the ART protocols used during the period of follow-up, according to calendar period (CP) [2, 17]. CP1 (1994–1996) comprised 161 children who received combination therapy with 2 nucleoside reverse-transcriptase inhibitors (NRTIs). More than 40% of the children were not receiving ART, and >35% were receiving monotherapy. CP2 (1997–1998) comprised 153 children receiving HAART with 3 drugs (NRTIs, PIs, and an NNRTI). Less than 50% of the children were receiving HAART. CP3 (1999–2001) comprised 205 children receiving HAART with a broader use of nelfinavir and efavirenz. More than 60% of the children were receiving HAART and <10% were not receiving ART.

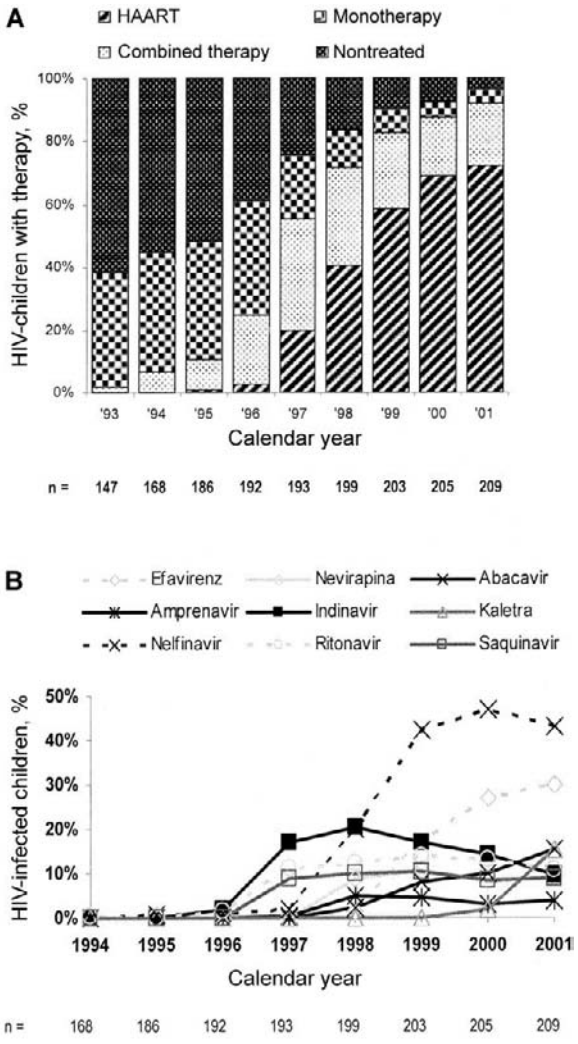


Figure 1. Use of antiretroviral protocols *(A)* and antiretroviral drugs *(B)* by HIV-1–infected children, according to calendar year.

Outcomes. We assessed the time to death calculated for each CP and grouped by the distribution of the main type of ART administrated over time. Also, we calculated the mean $CD4^+$ T cell count and $\log_{10}$ VL per year, to be considered as each patient's representative measure per year and CP. Per-

Table 1. Clinical, immunologic, and virologic parameters of HIV-1–infected children at entry in each calendar period.

Parameter	CP1 (1994–1996)	CP2 (1997–1998)	CP3 (1999–2001)
No. of HIV-infected children	161	153	205
Age, mean years ± SEM	4.1 ± 0.26	6.3 ± 0.31	7.7 ± 0.27
Time of birth, no. of children			
Before 1994	123	107	135
1994–1996	38	36	45
1997–1998	…	10	17
1999–2001	…	…	8
CDC clinical category, no. of children			
A	58	55	81
B	37	25	37
C	66	73	87
Immunologic characteristics			
$CD4^+$ T cells, mean % ± SEM	21.8 ± 1.06	23.6 ± 1.14	28.3 ± 0.88
$CD8^+$ T cells, mean % ± SEM	39.4 ± 1.31	42.7 ± 1.21	39.4 ± 0.96
$CD4^+$ T cell percentage of >25%, percentage of children	36.9	44	59.4
$CD4^+$ T cell percentage of 15%–25%, percentage of children	24.7	22.7	25.7
$CD4^+$ T cell percentage of <15%, percentage of children	35.7	33.3	11.4
Virologic characteristic			
Log_{10} VL, mean copies/mL ± SEM	4.61 ± 0.08	4.20 ± 0.07	3.80 ± 0.07
VL of ⩽500 copies/mL, percentage of children	3.1	5.3	17.6
VL of 500–5000 copies/mL, percentage of children	8.2	22.5	31.3
VL of 5000–30,000 copies/mL, percentage of children	32	35.1	26.4
VL of >30,000 copies/mL, percentage of children	56.7	37.1	24.7

NOTE. CDC, Centers for Disease Control and Prevention; CP, calendar period; CT, combination therapy; MT, monotherapy; VL, virus load.

centages of $CD4^+$ T cells and VL were calculated only within groups integrated by >6 children. We calculated the percentage of children with $CD4^+$ T cell percentages of <15%, 15%–25%, and >25%; and the percentage of children with VL values of <500, 500–5000, 5000–30,000, >30,000 copies/mL per year and CP. These $CD4^+$ T cell counts and VLs were then used to measure the changes during each CP and were plotted by CP and age. The age appears on the x-axis, expressed in mean values of the interval it represents (e.g., the interval between 4 and 5 years of age is reflected as "age, 4.5 years").

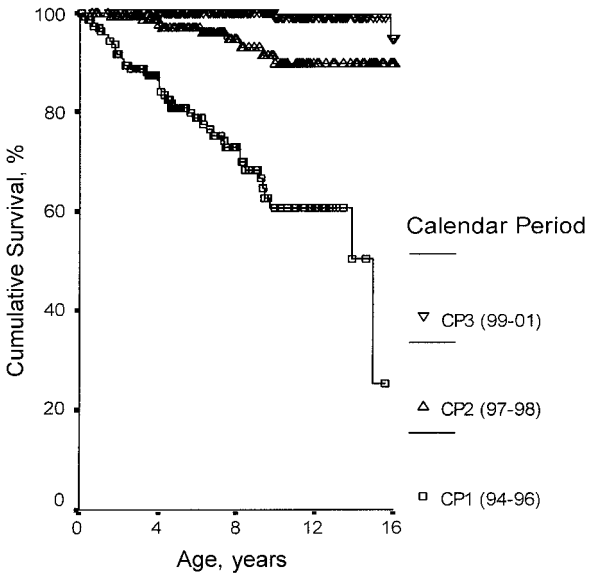
Statistical analysis. We determined the progression to death (cumulative incidence of survival) by the Greenwood method by Kaplan-Meier curves. Groups were compared by the log-rank test (Mantel-Haenszel). We estimated the relative proportion (RP) of survival by proportional-hazard Cox regression according to the effect of CP, and adjusted it by other potential determinants, such as $CD4^+$ and $CD8^+$ T cell percentage, VL, age, and diagnosis of AIDS at the entry of each CP. All listed variables were used together to calculate the adjusted RP of survival. We prefer to express the RP of survival because we want to highlight the benefits of HAART, such as survival markers. The CP was introduced in the model as a time-dependent variable.

For the purpose of this study, the date of birth was assumed to be the time of infection [3]. For each CP, the time from birth to death was subdivided into several periods. The children entered the risk set at the date of birth, the date of entry in the cohort group, or the date at the beginning of each CP [3]. In CP3, for instance, if an individual child was included in the study period at age 1 year, he was assessed at age 1, 2, or 3 years, corresponding to each year of the study period.

Differences in proportion of HIV-infected children by $CD4^+$ counts and VL were analyzed by Fisher's exact test. For the purpose of this analysis, the children were distributed by age in such a way that when we analyzed one period, no child was integrated in >1 age group. For example, when a child was 2.5 years old, he or she was included only in a single CP.

RESULTS

Demographic, clinical, immunological, and virological characteristics of HIV-infected children at the entry in each CP are



	Total	Number Events	Number Censored	Percent Censored
CP1 (94-96)	161	42	119	73.91
CP2 (97-98)	153	9	144	94.12
CP3 (99-01)	205	2	203	99.02
Overall	519	53	466	89.79

Log Rank Statistic and (*P*)

Factor	CP1 (94-96)	CP2 (97-98)
CP2 (97-98)	30.48 (<.0001)	
CP3 (99-01)	84.71 (<.0001)	11.64 (.0006)

Figure 2. Kaplan-Meier curve of survival, classified according to calendar period (CP).

illustrated in table 1. During the follow-up period, 83 children developed AIDS [13].

We assessed the survival in relation to CP and age, as shown in the Kaplan-Meier curve in figure 2. Longer survival rates were evident for CP3 than for CP1 or CP2, with these differences being statistically significant in all cases ($P < .001$). The crude and adjusted relative proportions (RPs) of survival by CP were also calculated (table 2). For crude survival RP, CP2 and CP3 presented statistical differences with CP1 ($P < .0001$). Differences between CP2 and CP3 were also statistically significant (RP, 9.7; 95% CI, 2.03–46.5; $P = .004$). When RP values were adjusted for each CP, those differences were also statistically significant (table 2). The statistically significant survival RP values of the variables of the adjusted model were as follows: 1.06 for $CD4^+$ T cells (95% CI, 1.02–1.10), 1.03 for $CD8^+$ T cells (95% CI, 1.0–1.07), 0.38 for $\log_{10}$ VL (95% CI, 0.19–0.78), 3.8 for age (95% CI, 2.34–6.25), and 0.42 for AIDS (95% CI, 0.16–1.07). However, differences between CP2 and CP3 were not statistically significant.

The proportion of children stratified by VL levels was assessed (figure 3). The percentage of children with VL <500 copies/mL was longer in CP3 than in the other 2 periods. We also found a lesser percentage of children with higher VL levels (>30,000 copies/mL) in CP3 than in CP1 or CP2. The lowest values of VL levels were found during CP3. The largest differences in VL were found when comparing children from CP3 to those of the same age from CP1 and CP2. Differences between VL in children in CP1 and CP2 were very small.

In CP3, >50% of the children had $CD4^+$ T cell percentages of >25% (i.e., a preserved immune system) (figure 4). The difference between this and the other CPs was statistically significant. A very small proportion of the children had $CD4^+$ T cell percentages <15% (i.e., severe immunodeficiency). The highest differences were seen between CP1 and CP3. CP3 had greater values than CP2, particularly from 5 years of age onward.

DISCUSSION

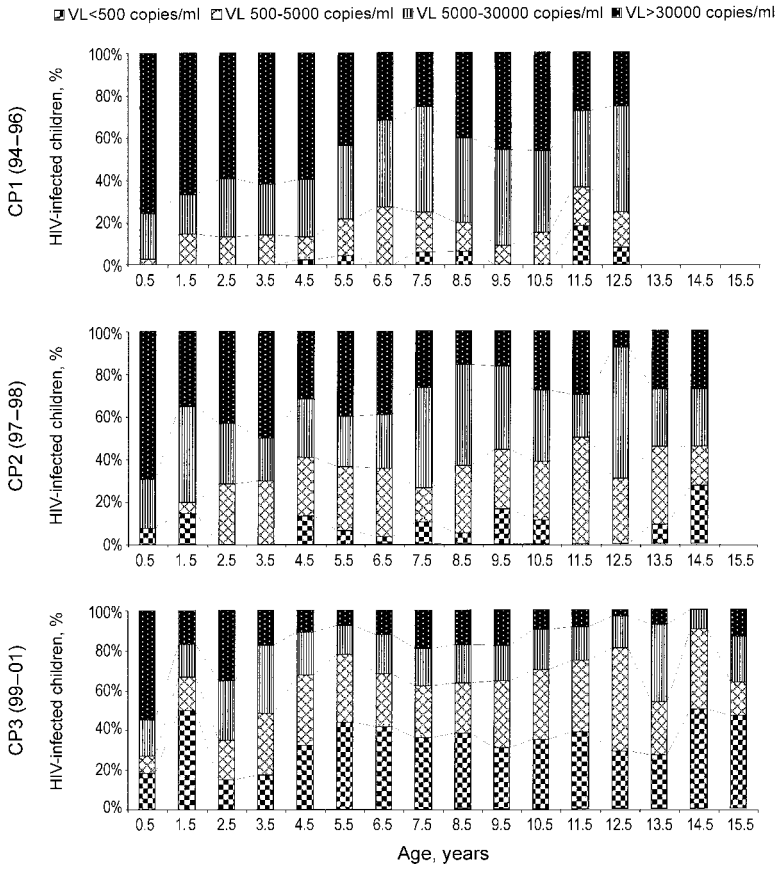
The introduction of HAART around the mid-1990s was a major breakthrough in treating HIV-infected patients. The overall effectiveness of ART on HIV-infected patients has been studied at the population level [2, 3, 11, 18, 19]. To date, however, few studies reflect the evolution of a large cohort of HIV-infected children throughout the different stages of the different HAART regimens used. We recruited a large group of children whose ART evolved in such a manner that >60%–70% were receiving HAART at the end of the study. These percentages are higher than in other studies [2, 3] and thus may more faithfully represent what is actually happening at the population level in Spain.

We divided the studied time lapse between 1997 and 2001 into 2 subperiods according to the percentage of children receiving HAART: CP2 (1997–1998) and CP3 (1999–2001). The earlier years of ART in children faced important limitations because approved pediatric formulations of PI were few, with poor administration and adherence. The introduction of new formulations of nelfinavir and efavirenz in 1999 [20, 21] extended the use of HAART to a larger number of HIV-infected children. This is reflected in our present data, which indicates a clear improvement in survival, a decrease of VL levels, and an increase of percentage of $CD4^+$ T cells throughout CP3.

One of the key benefits of HAART is the control of viral replication. Plasma VL is known to have prognostic value [14].

Table 2. Crude and adjusted relative proportions (RPs) of survival, by calendar period (CP), in HIV-infected children.

	Crude analysis of survival		Adjusted analysis of survival	
CP	RP (95% CI)	*P*	RP (95% CI)	*P*
CP1 (1994–1996)	1 (…)	…	1 (…)	…
CP2 (1997–1998)	9.40 (1.96–44.9)	.005	5.8 (0.99–34.3)	.050
CP3 (1999–2001)	59.19 (13.3–263.3)	<.001	6.5 (1.12–37.6)	.036

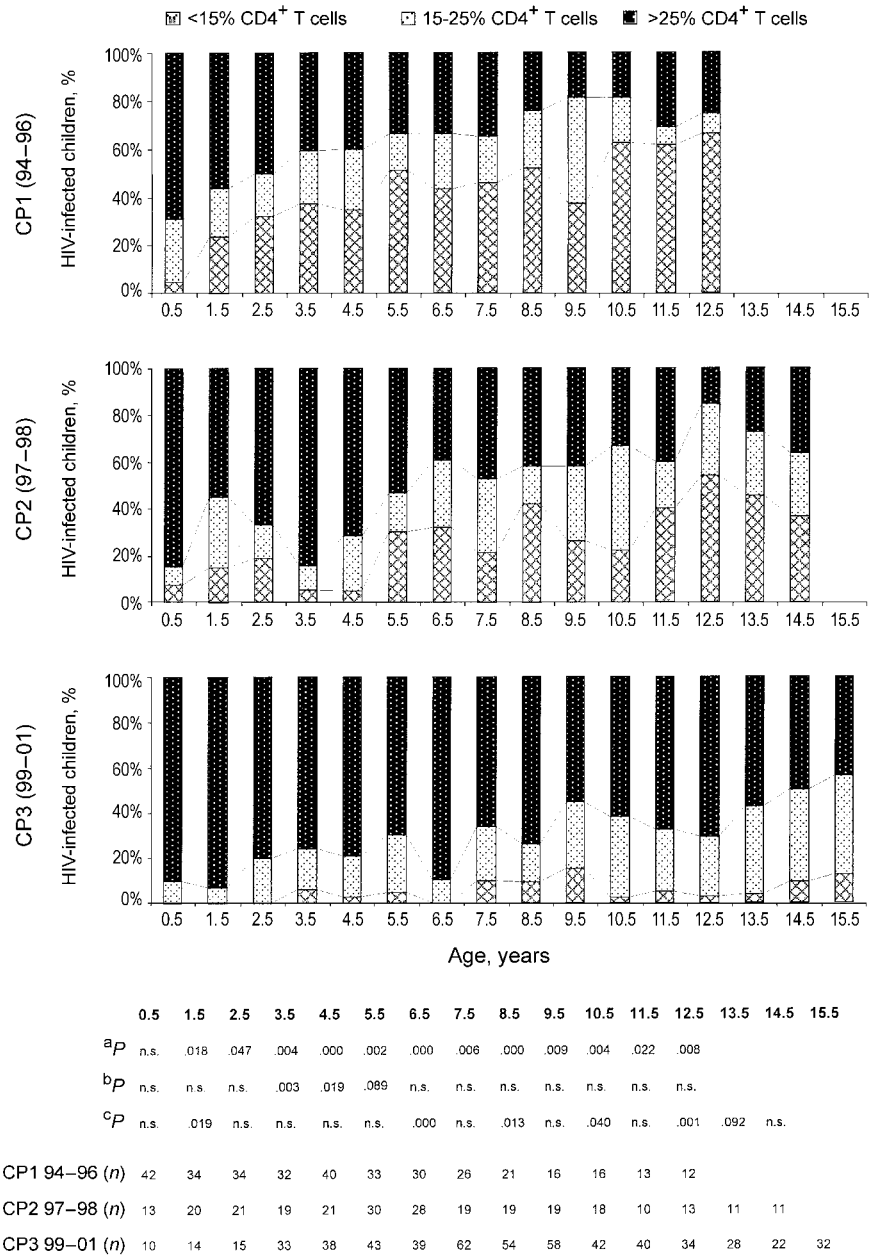


	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5
[a]*P*	n.s.	.005	.073	.001	.000	.001	.062	n.s.	.070	.055	.009	n.s.	.059			
[b]*P*	n.s.	.031	n.s.	n.s.	.037	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			
[c]*P*	n.s.	n.s.	n.s.	.012	.050	.001	.010	n.s.	n.s.	n.s.	.093	n.s.	n.s.	.037		
CP1 94–96 (*n*)	33	27	22	21	37	23	22	16	15	11	13	11	12			
CP2 97–98 (*n*)	13	20	21	20	22	30	28	19	19	18	18	10	13	11	11	
CP3 99–01 (*n*)	11	12	19	35	37	41	41	58	52	62	40	36	31	26	20	30

Figure 3. Virus loads (VLs; <500, 500–5000, 5000–30,000, and >30,000 copies/mL) in peripheral blood in the different groups of HIV-1–infected children, according to calendar period (CP) and age. The numbers under the figures represent the number of children in each age category during each time period. We compared the percentage of HIV-infected children with virus loads of >30,000 copies/mL. [a]CP1 (1994–1996) versus CP3 (1999–2001). [b]CP1 (94–96) versus CP2 (97–98). [c]CP2 (97–98) versus CP3 (99–01). n.s., Not significant.

Thus, the control of HIV replication while receiving HAART allows an increase of $CD4^+$T cell count [22] and function [23], leading to a decrease in opportunistic infections and tumors [11]. Although HAART rarely achieves its sustained suppression in children [24], the impact of new drugs and protocols of HAART on CP3 demonstrated the reduction of VL and the restoration of $CD4^+$T cell counts [8, 23]. This would account for the increase of the survival observed in our cohort of children, in agreement with previous reports [2, 3]. However, the RP of CP2 and CP3 decreased after adjusting the calculations for baseline $CD4^+$ T cell counts, $CD8^+$ T cell counts, $\log_{10}$ VL, age, and diagnosis of AIDS. This could be the consequence of the effect of these variables on the type of ART administered after each visit to the pediatrician. Our study demonstrates an overall recovery in children in worse clinical, immunological, and virological stages upon the implementation of novel, more effective antiretroviral drugs.

In our multivariate model, $CD4^+$ T cell value, VL, and previous diagnosis of AIDS had statistical significance, as expected [25]. In addition, $CD8^+$ T cell counts could be directly associated with survival in adjusted Cox regression models. This is in agreement with our previous findings in HIV-infected children, in which a low $CD8^+$ T cell count is directly associated with clinical progression [14, 26] and response to HAART [27]. Moreover, age was inversely related to survival. This can be the result of the effect of natural selection in the study population.



	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5
[a]*P*	n.s.	.018	.047	.004	.000	.002	.000	.006	.000	.009	.004	.022	.008			
[b]*P*	n.s.	n.s.	n.s.	.003	.019	.089	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			
[c]*P*	n.s.	.019	n.s.	n.s.	n.s.	n.s.	.000	n.s.	.013	n.s.	.040	n.s.	.001	.092	n.s.	
CP1 94–96 (*n*)	42	34	34	32	40	33	30	26	21	16	16	13	12			
CP2 97–98 (*n*)	13	20	21	19	21	30	28	19	19	19	18	10	13	11	11	
CP3 99–01 (*n*)	10	14	15	33	38	43	39	62	54	58	42	40	34	28	22	32

Figure 4. Values of the percentage of CD4+ T cells (<15%, 15%–25%, and >25%) in peripheral blood in the different groups of HIV-1–infected children according to calendar period (CP) and age. The numbers under the figures represent the number of children in each age category during each time period. We compared the percentage of HIV-infected children with CD4+ T cell percentages of >25%. [a]CP1 (1994–1996) versus CP3 (1999–2001). [b]CP1 (1994–1996) versus CP2 (1997–1998). [c]CP2 (1997–1998) versus CP3 (1999–2001).

The present study began in 1994, when combination therapy was in use. At that time, some HIV-infected children were already receiving treatment, but not with HAART. Many of them were in advanced stages of infection, with many dying during CP1 (1994–1996) and, to a lesser degree, during CP2 (1997–1998). A high rate of HIV vertical transmission still persisted during the period of 1994–1997 [28, 29]. Many of these HIV-infected children died during the first years of life, negatively affecting the survival curve. After these first years, many of those who survived had a longer life expectancy [30].

As the years went by, the children who were better adapted to the infection survived and ended up added to the pool of HIV-infected children treated with combination therapy or HAART, thus increasing the global survival and mean age of that group. In our study, we found that VL levels were lowest in older HIV-infected children, possibly as a result of some

kind of natural selection process, perhaps because they are immunologically adapted (stronger antiviral response) or because they are infected with less pathogenic strains of HIV [31–33]. For this reason, in the adjusted Cox regression model, age was included as a covariate, and RP values were corrected by age.

Response to therapy has been shown to be highly dependent on the patient's adherence [9, 34]. Traditionally, children's adherence to ART has been poor [9, 10]. The adherence to ART was not strictly monitored in this study, as opposed to standard clinical trials. This may have an important, unaccounted effect on the interpretation of our results. However, the data presented here derive from routine clinical practice. Moreover, with the development of ART and prophylaxis treatments for opportunistic infections, for many children, HIV infection has become a chronic illness. Also, in the past few years, the precocious diagnosis of HIV infection has improved. Now it is faster because there is more information and the disease of HIV-infected mothers is more controlled, so ART for vertically HIV-infected children begins early. These factors could influence in the survival of HIV-infected children, and such factors were not analyzed in this study.

Our study shows the high effectiveness of HAART at the population level. HAART decreases VL, increases $CD4^+$ T cell values, and increases survival. However, the toxicities associated with its long-term use may significantly outweigh the early initiation of treatment in clinically stable children with a relatively preserved immune status [35, 36]. Recent guidelines for adults and children have outlined treatment recommendations in the light of long-term challenges of chronic ART [37]. Therefore, future studies to design more effective and reliable therapeutic strategies are required.

Acknowledgment

We thank Vanessa Campo-Ruiz for her assistance in translating and editing the article.

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