ORIGINAL ARTICLE

Impact of antiretroviral protocols on dynamics of AIDS progression markers

S Resino, J M Bellón, S Sánchez-Ramón, D Gurbindo, J Ruiz-Contreras, J A León, J T Ramos, M Á Muñóz-Fernández

Arch Dis Child 2002;**86**:119–124

See end of article for authors' affiliations

Correspondence to: Dr M Á Muñoz-Fernández, Departamento de Inmunología, Hospital General Universitario "Gregorio Marañón", C/ Doctor Esquerdo 46, 28007 Madrid, Spain; Mmunoz@cbm.uam.es

Accepted for publication 15 October 2001

Aims: To assess the "real life" effectiveness of different antiretroviral therapies (ART).
Methods: A retrospective multicentre observational study in 150 HIV-1 vertically infected children on the progression to AIDS (study A), and in 61 HIV-1 infected children on the evolution of the most relevant markers of progression (study B). All children were categorised into four groups: untreated (NT); on monotherapy (MT); on combination therapy (dual-ART); and on potent ART (HAART).
Results: No child in the HAART group progressed to AIDS, whereas 14 children in the NT and seven in the MT groups progressed to AIDS, respectively, the differences being statistically significant. There was a mean increase of 8 units of %CD4+ per year; this was greater in the HAART group than in the other groups. The mean decrease in viral load was 0.65 $\log_{10}$ copies/ml per year; this was greater in the HAART group than in the NT and MT groups. The HAART group had the lowest probability of returning to baseline %CD4+ and viral load.
Conclusion: Potent ART had the greatest protective effect against progression to AIDS in this observational study.

Paediatric HIV-1 infection is different from the adult disease[1 2]; this has important implications for antiretroviral treatment (ART) of children. Broadly, treatment of HIV-1 infected children has followed the patterns of adult treatment. However, in clinical practice established adult doses of several antiviral drugs have been found to be suboptimal for children, probably because of age dependent changes in pharmacokinetics and pharmacodynamics.[3] Indeed, several treatments have failed to suppress HIV-1 replication, because the required optimal concentrations of the drugs were not reached.[4 5] With a more rapid clinical evolution and with higher plasma viral load (VL), the available therapeutic arsenal to combat HIV-1 infection in children has been smaller than in adults. An additional problem of ART for HIV-1 infected children is adherence to treatment. Lack of compliance is one of the main causes of poorer response rates to treatment in children than adults.

However, some authors have shown that once the chronic infection is established (6–9 months after primary infection), a direct relation exists between VL and disease progression, regardless of others factors.[6 7] Reduction of VL with treatment is associated with improved prognosis.[6 8] Potent antiretroviral therapy is more effective in maintaining low VL and in providing clinical benefits to patients,[5 9] although unfortunately, not in all cases. Until recently, there were few alternatives to zidovudine for management of HIV-1 infection in children. Since the first study in children in 1988, which confirmed the efficacy of this type of monotherapy,[10] a broad range of different single and two drug regimens, and more recently combinations of three and four drugs, have been used.[11–13] New combination therapies have been shown to be effective in suppressing VL and increasing CD4+ T lymphocyte counts in children.[14 15] However, such data are derived from clinical trials, and not from observational studies in cohorts of children. Observational and cohort studies allow analysis of the "real life" effectiveness of different therapies, in contrast to clinical trials,[16] whose main goal is to determine the efficacy of the new therapeutic protocols, under trial conditions. Once individual protocols have been shown to be efficacious in clinical trials and are introduced into clinical practice, observational analysis should be performed. Accordingly, we carried out an observational study in a paediatric population to define the impact of the new therapies. As far as we know, few data exist concerning "real life" effectiveness of ART in the paediatric population as assessed by monitoring the two most important markers of progression to AIDS in children (plasma VL and CD4+ T cell percentage). We also evaluated longitudinally the effectiveness of different ART regimens for the control of immunological and virological markers of progression of disease in vertically HIV-1 infected children, and clinical progression to AIDS.

MATERIALS AND METHODS

Patients

Between August 1988 and February 1999, a retrospective follow up study of a cohort of 240 vertically HIV-1 infected children from the Departments of Paediatrics of the General University Hospitals "Gregorio Marañón" and "12 de Octubre" Madrid, and "Virgen del Rocío" Hospital Sevilla, Spain, was performed. Of 240 HIV-1 infected children who had at least two different immunological and virological evaluations, 150 children who had adequate data on AIDS stage at entry and subsequent progression were selected for analysis of their progression to AIDS, while receiving various ART regimens given for up to 30 months (study A). Those children who were already in clinical category C, and those for whom we did not have enough evaluations were excluded from study A.

The children were divided into four groups according to the ART protocol given during the follow-up: (1) NT group (not treated): 58 ART naïve children; (2) MT group (on monotherapy): 36 children treated with a nucleoside analogue HIV-1 reverse transcriptase inhibitor (NRTI) alone, and who

Abbreviations: ART, antiretroviral therapy; CDCP, Centres for Disease Control and Prevention; dual-ART, combination therapy; HAART, potent antiretroviral therapy; MT, monotherapy; NRTI, nucleoside analogue HIV-1 reverse transcriptase inhibitor; NT, not treated; PI, HIV protease inhibitor; RR, relative risk; VL, viral load

Table 1 Characteristics of a cohort of 150 HIV-1 infected children at entry to the study

	NT	MT	dual-ART	HAART
	(n=58)	(n=36)	(n=31)	(n=25)
Clinical category				
A	46 (43.0%)	22 (20.6%)	20 (18.7%)	19 (17.8%)
B	12 (27.9%)	14 (32.6%)	11 (25.6%)	6 (14.0%)
Age	1.4 (0.2; 11.0)	4.3 (0.6; 15.1)	5.4 (0.3; 15.0)	6.1 (1.0; 13.5)
Immunological category				
>25% CD4+	38 (49.4%)	13 (16.9%)	15 (19.5%)	11 (14.3%)
15–25% CD4+	10 (34.5%)	9 (37.9%)	4 (17.2%)	3 (10.3%)
<15% CD4+	7 (18.9%)	11 (24.3%)	12 (29.7%)	10 (27.0%)
Lymphocyte subsets				
%CD4+	32.8 ± 3.76 (8; 66)	24.8 ± 5.12 (1; 62)	26.0 ± 6.24 (1; 55)	22.1 ± 5.38 (1; 44)
%CD8+	32.2 ± 3.82 (5; 71)	39.5 ± 5.66 (7; 69)	39.4 ± 8.10 (7; 60)	48.5 ± 5.70 (21; 82)
Viral load				
Log_{10} VL	4.30 ± 0.28 (2.30; 6.37)	4.69 ± 0.36 (2.30; 6.08)	4.55 ± 0.44 (2.30; 6.41)	4.14 ± 0.42 (2.48; 6.06)

In each group, number of children are classified according to clinical and immunological categories (%within rows). Immunological and virological characteristics: results expressed as mean ± 2 SE (min; max). Age expressed as median (min; max).

had previously been ART naive; (3) CT group (on combination therapy): 31 children treated with two NRTIs; and (4) HAART group (on potent antiretroviral therapy): 25 children treated with two NRTIs and at least one HIV protease inhibitor (PI) (table 1). No child in the dual ART or HAART groups was included in the NT or MT groups. Progression to AIDS was defined as progression to clinical category C. Children who entered the study earlier tended to have no therapy or monotherapy, compared with those who entered the study later.

Sixty one of the 240 children were enrolled into a longitudinal study to assess the evolution of the most relevant immunological and virological markers of progression to AIDS (study B). For inclusion in this second study group, patients had to meet the following selection criteria: (a) duration of follow up 12–24 months; (b) aged above 2 years when they entered the study; and (c) to have had at least four determinations of log_{10} plasma VL and %CD4+ and %CD8+ lymphocytes, in order to estimate the slope as well as the overall trend of these variables. Of the 61 children, 13 were in the NT, 16 the MT, 15 the dual-ART, and 17 the HAART groups (table 2).

All infants were diagnosed as HIV-1 infected on the basis of positive results in both DNA polymerase chain reaction and virus culture assays, as described previously.[17] Clinical classification of the children was based on the 1994 revised guidelines of the Centres for Disease Control and Prevention

Table 2 Treatment of children with HIV-1 at entry to the study

	Study A (n)	Study B (n)
Not treated	58	13
Monotherapy	**36**	16
AZT	20	6
ddI	15	9
d4T	1	1
Combination therapy	31	15
AZT+ddI	10	5
3TC+ddI	10	4
AZT+3TC	6	1
D4T+ddI	3	4
AZT+d4T	1	0
3TC+d4T	1	1
HAART	25	17
3TC+d4T+saquinavir	4	5
D4T+nelfinavir+nevirapine	4	0
AZT+3TC+saquinavir	3	2
3TC+d4T+ritonavir	2	3
3TC+d4T+indinavir	2	2
3TC+d4T+amprenavir	2	0
AZT+3TC+ritonavir	1	2
3TC+d4T+indinavir+efavirenz	1	0
3TC+d4T+nelfinavir+efavirenz	1	0
3TC+ddI+ritonavir	1	2
3TC+ddI+nelfinavir	1	0
D4T+ddI+indinavir	1	0
D4T+ddI+nelfinavir+efavirez	1	0
D4T+ddI+efavirenz	1	0
3TC+d4T+ddI+ritonavir	0	1

AZT, zidovudine.

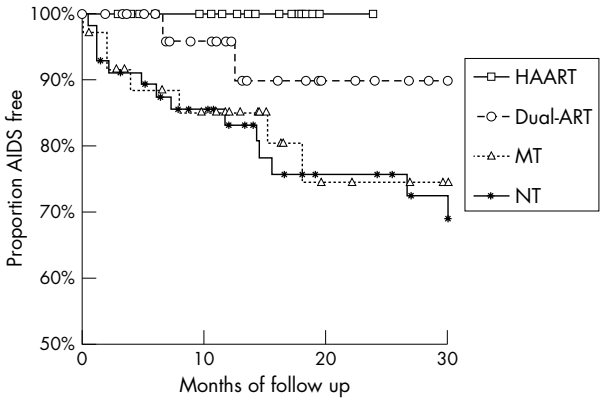

Figure 1 Kaplan–Meier curve of progression to AIDS, classified according to antiretroviral treatment regime.

(CDCP).[18] The study was conducted according to the declaration of Helsinki and approved by the ethical committee for all hospitals involved. Drugs were prescribed by the treating physician according to CDCP guidelines[3] after obtaining written informed consent from legal guardians. Response to therapy was controlled at 0, 3, 6, 9, 12, 15, and 18 months by serial measurements of %CD4+ and %CD8+, VL, and by collecting clinical data, according to published guidelines.[3]

Quantification of T cell subsets in peripheral blood

T lymphocyte subsets in peripheral blood were quantified by direct immunofluorescence using monoclonal antibodies of the T series and multiparameter flow cytometry (FACScan, Becton-Dickinson, Heidelberg, Germany), as described previously.[17]

Quantitative HIV-1 RNA assay

Blood samples were collected in EDTA tubes, separated within four hours, and plasma was stored at −70°C. The VL was measured in 200 µl of plasma by reverse transcriptase polymerase chain reaction (Amplicor monitor kit, Roche Diagnostic Systems, Brandenburg, New Jersey, USA). The lower limit of detection was 200 HIV-1 RNA copies/ml.[19 20]

Statistical analysis

CD4+ and CD8+ counts are expressed as percentages. In all analyses, HIV-1 RNA concentrations were transformed to $\log_{10}$ scale in order to normalise their distribution. Slopes of %CD4+ and %CD8+ and $\log_{10}$ VL within individuals were calculated, expressing them as %CD4+ and %CD8+ per year and $\log_{10}$ VL per year. Slope calculations were then analysed by multiple linear regression analysis to assess the effects of antiretroviral combination therapy on the trends (slopes) of peripheral blood CD4+ T lymphocytes and plasma VL during the course of follow up. This multiple linear regression analysis was performed for all children collectively and adjusted for baseline %CD4+, %CD8+, $\log_{10}$ VL, and age, to compensate for differences among groups at entry to the study. Estimated marginal means of the slopes of each group according to the ART protocol were obtained. Progression to AIDS was determined by the Greenwood method using Kaplan–Meier curves. Statistical significance was set at $p < 0.05$; probabilities were compared by the log rank test (Mantel–Haenzel). This analysis was performed for all children according to ART groups. The time to reach baseline and its relative risk (RR) were estimated by the proportional hazard Cox regression equation for VL and %CD4+. RR <1 indicates a protective effect.

RESULTS

Influence of ART protocol on the clinical evolution of HIV-1 disease

In study A, 150 HIV-1 infected children were followed for up to 30 months. Table 1 shows baseline immunological and virological characteristics. Table 2 shows drugs used in both groups.

Figure 1 shows progression to AIDS. No child in the HAART group progressed to AIDS and only two of the 31 children in the dual-ART group progressed to AIDS. The NT and MT groups progressed to AIDS faster (14 of 58 and seven of 36 children, respectively; $p = 0.03$ and $p = 0.04$ compared with the HAART group).

Longitudinal study of immunological and virological progression

In study B, two of 17 children in the HAART group were treated with an NRTI, and another two with two nucleoside analogues prior to enrolment; the remaining 13 children were ART naïve. Table 3 shows baseline clinical, virological, and immunological characteristics of the 61 children.[18] When the baseline %CD4+ and %CD8+ were analysed, no statistically

Table 3 Baseline clinical, immunological, and virological characteristics of a cohort of 61 HIV-1 infected children

	NT	MT	dual-ART	HAART
	(n=13)	(n=16)	(n=15)	(n=17)
Clinical category				
A	8 (53.3%)	3 (20.0%)	0	4 (26.7%)
B	3 (15.8%)	6 (31.6%)	5 (26.3%)	5 (26.3%)
C	2 (7.4%)	7 (25.9%)	10 (37.0%)	8 (29.6%)
Immunological category				
CD4 >25%	7 (35.0%)	6 (30.0%)	4 (20.0%)	3 (15.0%)
CD4 15–25%	1 (8.3%)	4 (33.3%)	2 (16.7%)	5 (41.7%)
CD4 <15%	5 (17.2%)	6 (20.7%)	9 (31.0%)	9 (31.0%)
Follow up (months)	21.60 ± 0.96	16.94 ± 2.52	16.13 ± 2.84	15.46 ± 1.06
Age (years)	6.8 (2.4; 10.8)	4.8 (1.3; 13.7)	6.4 (3.0; 11.8)	5.6 (2.2; 15.5)
Lymphocyte subsets				
%CD4	26.08 ± 9.82	22.75 ± 8.80	15.37 ± 8.72	14.49 ± 5.54
%CD8	33.08 ± 11.1	43.94 ± 7.66	39.27 ± 9.44	43.86 ± 8.46
Viral load				
$\log_{10}$ VL	3.97 ± 0.36	4.48 ± 0.44	4.55 ± 0.60	4.84 ± 0.28

In each group, number of children are classified according to clinical and immunological categories (%within rows). Immunological and virological characteristics: results expressed as mean ± 2 SE (min; max). Age expressed as median (min; max).

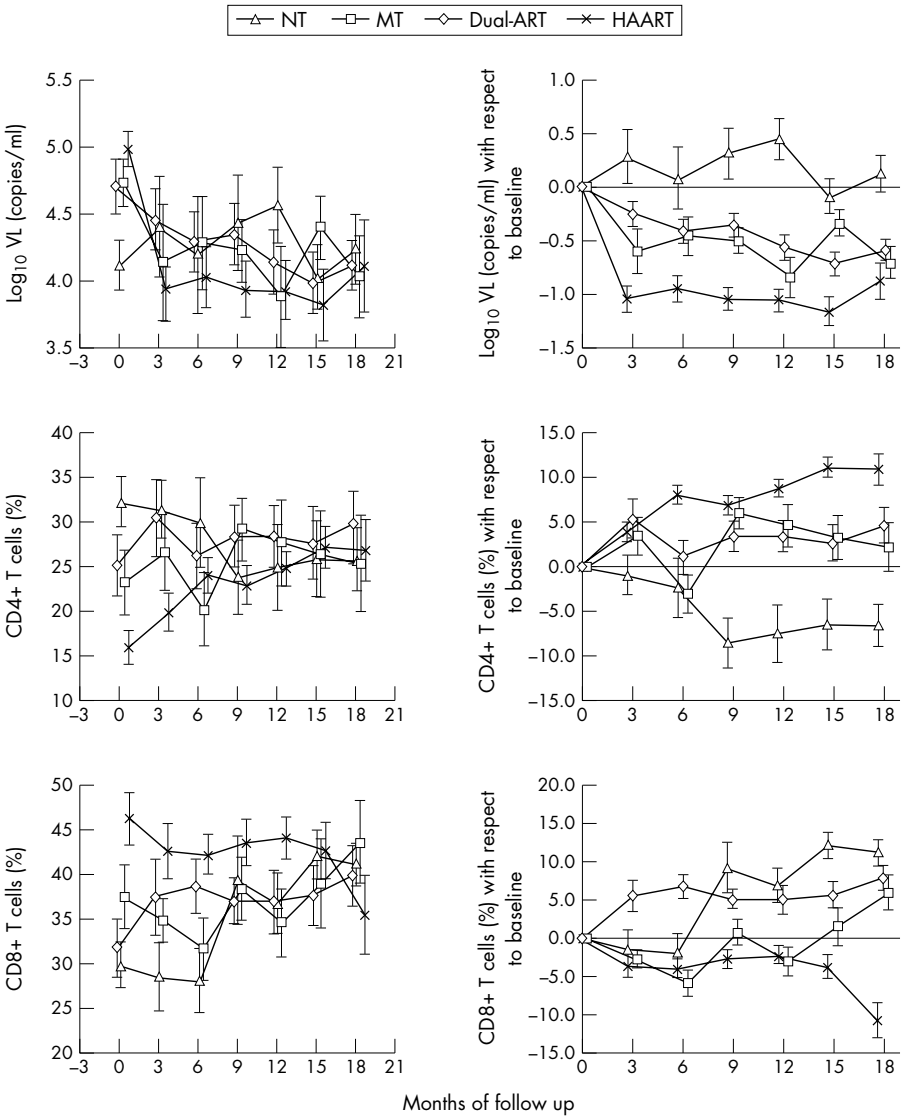

Figure 2 Mean CD4+ and CD8+ T lymphocyte percentages in peripheral blood, and plasma $\log_{10}$ VL (copies/ml) in the different groups of HIV-1 infected children. Error bars represent 1 SD.

significant differences between treatment groups were observed, although there was a trend towards lowest %CD4+ in children receiving more complete treatment. However, significant differences were found when analysing normalised VL values ($\log_{10}$) between the NT and HAART groups ($p = 0.01$).

The mean values of %CD4+ and %CD8+ in peripheral blood, and $\log_{10}$ VL in all groups were analysed at 0, 3, 6, 9, 12, 15, and 18 months of follow up. In the NT group there was no reduction of mean VL, which remained high throughout the follow up period (fig 2). In contrast, %CD4+ decreased, %CD8+ increased, with inversion of the CD4:CD8 ratio over time, as expected from the known natural history of infection. In the MT group, %CD4+ and %CD8+ remained constant during the first 12 months, but after 15 months of therapy a clear fall in VL and a concomitant increase in %CD8+ were observed (fig 2). When analysing the HAART group, a completely different pattern of response to treatment from the other groups was observed: there was a dramatic decrease in VL after the third month of treatment, which remained low during the remaining follow up period, and an increase in %CD4+, with stable %CD8+ (fig 2).

In the HAART group there was a trend towards an increase in CD4+ T lymphocytes (9.89 (1.78) increase in % CD4 per year). Similar differences, although of the opposite sign, were observed with respect to $\log_{10}$ VL in the HAART group. In this group of children, a clear downward trend in VL during the entire study period was observed (−0.66 (0.18) $\log_{10}$ VL per year). There were significant differences between the HAART group and the other groups for %CD4+ trends and $\log_{10}$ VL trends ($p < 0.01$). The mean increase of 8 units of %CD4+/year was greater in the HAART group than in the other groups. Likewise, when VL was analysed, highly significant differences were found between the HAART group and the NT and MT groups, with an estimated mean difference of −0.65 $\log_{10}$ per year. The negative sign indicates that in the one year period, the decrease in VL was 0.65 $\log_{10}$ VL, greater in the HAART group than in the NT and MT groups.

Kaplan–Meier and Cox regression analyses to define success versus failure of therapy

Time to return to baseline VL and %CD4+ (considered to be two end points representing failure of therapy), were assessed by the Kaplan–Meier method and Cox regression analysis, as well as the relative risk (RR) of reaching both parameters. As table 4 shows, virological differences were noted between the three groups of treated children in time and RR of reaching baseline VL. Similarly, with regard to time in reaching baseline %CD4+ T cells, immunologically important differences were

Table 4 Time to reach baseline viral load and CD4+ T lymphocyte percentage compared to NT group (Kaplan–Meier and Cox regression analysis)

		VL ⩾ baseline			%CD4+ ⩽ baseline		
	n	%	Months	RR	%	Months	RR
MT	16	61.5	8.2 (5.7; 10.6)	0.62 (0.25; 1.53)	66.5	8.0 (4.7; 11.3)	0.46 (0.19; 1.14)
dual-ART	15	58.5	7.7 (0; 15.85)	0.67 (0.26; 1.71)	40	14.8 (8.2; 21.4)	0.34* (0.12; 0.91)
HAART	17	29.5	—	0.27** (0.09; 0.78)	23.5	—	0.18** (0.05; 0.55)

%, percentage of children who reached nadir values; *p<0.05; **p<0.01.
Values are expressed as median (95% confidence interval); RR determined with respect to NT group.

noted between groups (table 4). A total of 42.8% of children in the MT group, 47.6% in the dual-ART group, and 50% in the HAART group achieved undetectable VL. Highly significant differences between the three groups of treated children compared with the NT group ($p < 0.05$) were observed. When analysing RR for reaching baseline %CD4+ values, children from the dual-ART ($p = 0.025$) and HAART groups ($p = 0.002$) showed highly significant differences compared with the NT group, indicating a greater protective effect.

DISCUSSION

We report an observational study to assess the progression to AIDS and the evolution of markers of HIV-1 infection progression (VL and %CD4+) in HIV-1 infected children receiving different ART regimens. It is important to consider that essential aspects of treatment such as nutritional management and prophylaxis against *Pneumocystis carinii* pneumonia, changed substantially over the course of the 11 year period of the study. Prophylaxis is probably at least as important as ART in preventing the onset of AIDS in children. As expected from the natural history of HIV-1 infection, in the NT group of children a decrease in CD4+ T cells was observed, not just as a consequence of infection, but also of increasing age.[1 21] In addition, an increase of the %CD8+ and VL in parallel with the decrease of %CD4+ was observed, in accordance with data published elsewhere.[1 22] Our results indicate that the HAART group had a significant difference in the rate of progression to AIDS compared to the MT group, but not to the dual-ART group, in agreement with previous results.[23] However, a better trend of evolution of both %CD4+ and VL is clearly seen in children on HAART, as has also been reported in the literature.[5 24]

Monotherapy regimens for the management paediatric infection have been shown to be of benefit in symptomatic children, resulting in a clear improvement in quality of life, growth, and neurological development, as well as immunological and virological parameters,[25–28] as confirmed in this study. However, our data indicate that this beneficial effect is transitory and disappears with time, as is illustrated in the trends in VL and %CD4+ (fig 2), probably as a consequence of the development of drug resistance.

Dual therapy has been shown to be much more effective than monotherapy in adults; studies in ART naïve symptomatic children were therefore initiated, with similar conclusions.[13 29] Nevertheless, in our study combination therapy was not shown to be more effective than monotherapy: the decrease of VL and increase of %CD4+ were similar in both MT and dual-ART groups, and no significant differences in the longitudinal trends of both parameters were observed. These data contradict results obtained in clinical trials that included only naïve children.[12 13 27] However, in our study, among the group of children on combination therapy, 12 of 15 had previously received monotherapy and were changed to dual therapy after treatment had failed.

The benefits of HAART in infants and children previously treated with nucleoside analogues have also been shown previously.[14 30] In agreement with other studies, a dramatic decrease in VL after the third month of treatment was observed in children in the HAART group, with a concomitant increase of %CD4+ and %CD8+ that subsequently remained within the normal range for age in each child. These data support the greater effectiveness of HAART than other regimens, in accordance with clinical trials.[30] The increase in %CD4+ was significantly higher in the HAART group; inhibition of viral replication presumably allows recovery of CD4+ T cell numbers, in agreement with previous studies.[5 24] Undetectable VL was achieved in half of the children, and a dramatic reduction of VL occurred in the remainder. This incomplete reduction reflects differences between the evolution of infection in adults and children. Therefore, failure to reach undetectable VL in children should probably not be considered as treatment failure, at least with currently available drugs.[5 11 14] The time taken for %CD4+ and VL (two end points of therapy failure) to return to baseline, was longer in all three groups of children receiving ART than in untreated children. The time to reach undetectable VL was the same for all three groups of treated children, although a higher proportion in the HAART group reached it. Several factors may be operating; for example, some doses may not result in serum concentrations sufficient to inhibit viral replication completely, leading to the earlier occurrence of quasispecies resistant to administered drugs.

In this observational study, differences between ART treatment regimens appear less significant than in clinical trials. Adherence to treatment by the children was not monitored in this study, whereas in clinical trials measures are taken to ascertain adherence. However, the data presented here are derived from routine clinical practice, where children have a wide variety of social and other conditions (including adoption, being orphaned, living with grandparents or relatives, or in community centres). The higher the number of drugs administered, the more difficult it is to determine the efficacy of treatment.

Our study shows that in this area as in so many others, children are not simply small adults. HIV-1 infection in children is mostly acquired by vertical transmission, a special form of acute infection. Further studies are required to investigate more effective therapeutic strategies for suppressing VL to undetectable amounts and to increase %CD4+ rapidly and effectively.

ACKNOWLEDGEMENT

We thank Dolores García Alonso for excellent technical assistance and Melody Nicholls for revising the English. This work was supported by grants from the Foundation for Research and Prevention of AIDS in Spain (FIPSE 3008/99), from Fondo de Investigación Sanitaria (00/0207), from the Bristol-Myers Company, from the National Health Research Development Program (SAF 99-0022), and the Community of Madrid.

Authors' affiliations
S Resino, J M Bellón, S Sánchez-Ramón, M Á Muñóz-Fernández, Department of Immunology, General University Hospital "Gregorio Marañón", Madrid, Spain

D Gurbindo, Department of Paediatrics, General University Hospital "Gregorio Marañón"
J Ruiz-Contreras, J T Ramos, Department of Paediatrics, University Hospital "12 de Octubre", Madrid, Spain
J A León, Department of Paediatrics, Unit of Paediatric Infectious Diseases, University Hospital "Virgen del Rocío", Sevilla, Spain

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