Combined antiretroviral therapy reduces hyperimmunoglobulinemia in HIV-1 infected children

The Italian Register for HIV Infection in Children* (IRHIC)

Objective: To evaluate the effect of combined antiretroviral therapy on serum immunoglobulin (Ig) levels in HIV-1 perinatally infected children.

Methods: Data from 1250 children recorded by the Italian Register for HIV Infection in Children from 1985 to 2002 were analysed. Since Ig levels physiologically vary with age, differences at different age periods were evaluated as differences in *z*-scores calculated using means and standard deviations of normal population for each age period. Combined antiretroviral therapy has become widespread in Italy since 1996, thus differences in Ig *z*-scores between the periods 1985–1995 and 1996–2002 were analysed. Data according to type of therapeutic regimen were also analysed.

Results: Between the two periods 1985–1995 and 1996–2002, significant ($P < 0.0001$) decreases in IgG (6.29 ± 4.72 versus 4.44 ± 4.33), IgM (9.25 ± 13.32 versus 5.61 ± 7.93), and IgA (10.25 ± 15.68 versus 6.48 ± 11.56) *z*-scores, together with a parallel significant ($P < 0.0001$) increase in CD4 T-lymphocyte percentages, were found. These decreases were confirmed regardless of whether the children were receiving intravenous Ig or not. Ig *z*-scores were significantly higher in children receiving mono-therapy than in those receiving double-combined therapy (IgG, $P < 0.0001$; IgM, $P = 0.003$; IgA, $P = 0.031$) and in the latter children than in those receiving three or more drugs ($P < 0.0001$ for all *z*-scores). Ig *z*-scores correlated inversely with CD4 T-lymphocyte percentages and, directly, with viral loads.

Conclusions: Our data show that in HIV-1 infected children combined antiretroviral therapy leads to reduction of hyperimmunoglobulinemia which parallels restoration of CD4 T-lymphocyte percentage and viral load decrease, which it turn probably reflects improved B-lymphocyte functions.

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Keywords: children, HIV-1 infection, combined antiretroviral therapy, immunoglobulins

Introduction

High levels of serum immmunoglobulins (Ig)G , M and A have been reported in adults [1] and children [2,3] infected with HIV-1. Most Ig produced in excess reflect a generalized HIV-1 driven polyclonal B-cell activation and are not antigen specific. They are ineffective in protecting against infections [1] and high

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Ig levels correlate with increased risk of bacterial infections [4] and poor prognosis [5].

Combined antiretroviral therapy (ART) effectively suppresses HIV-1 replication in most patients, restores defective T-lymphocyte mediated immunity [6] and reduces morbidity and mortality rates [7–9]. Nevertheless little is known on its effects on B-lymphocyte functions and Ig production. To our knowledge, no study has evaluated the effect of combined-ART on serum Ig levels in children. We have previously demonstrated the effect of such therapy on the survival rate of HIV-1 infected children in a large observational population-based study [9]. In this study we report data on Ig levels in a similar large, unselected population followed-up over a 17-year period.

Methods

Data collection

Data were collected by the Italian Register for HIV Infection in Children, which is a nationwide multicentre study of children perinatally exposed to HIV-1 instituted in 1985 by the Italian Association of Paediatrics [9]. The data source is a network of 106 paediatric clinics distributed throughout Italy. The data are transmitted to the two coordinating centres at Department of Paediatrics of the Universities of Florence and Turin. Data collection started on 1 June 1985. Information concerning data collection has been described in detail elsewhere [2,9,10]. Data are prospectively collected every 6–12 months. In the present study data collected up to 31 December 2002 were analysed.

Clinical HIV-1 stage was classified according to the recommendations laid down by the Centers for Disease Control and Prevention (CDC) [11]. Ig levels were measured by laser nephelometry. Viral loads were evaluated quantitatively by Amplicor HIV Monitor test and results are expressed as Log_{10} HIV-1 RNA copies/ml. CD4 T-lymphocyte counts were measured by standardized fluorescent-activated cell sorting technique. According to the USA guidelines for the use of ART in paediatric HIV infection [12], CD4 T-lymphocyte percentages, rather than their absolute counts, were taken into account as these percentages reflect the immune status of HIV-1 infected children more accurately.

Treatment

The specific therapy offered was based upon Italian and USA guidelines [13,14]. Only therapeutic courses administered for at least 30 days were considered in the study [15]. Therapy was classified as one of the following: (i) mono-therapy, i.e., one nucleoside reverse transcriptase inhibitor (NRTI); (ii) double-combined therapy, i.e., two NRTI or one NRTI and one non-nucleoside reverse transcriptase inhibitor; (iii) combined therapy with three or more drugs.

Statistical analysis

Age and duration of treatment were expressed as median and range. Viral loads and CD4 T-lymphocyte percentages were expressed as mean and standard deviation. Since Ig levels vary with age, differences at different age periods were evaluated through differences in *z*-scores. Means and standard deviations for the normal population for each age period were calculated using values from a previous study by de Martino and colleagues [2].

In this study 2 statistical analyses were performed: (i) Analysis of Ig *z*-score differences between the periods 1985–1995 and 1996–2002. Since 1996 the use of combined-ART has become widespread in Italy and ART with three or more drugs was introduced into the treatment of HIV-1 infected children [9]. Therefore, it was decided to analyse differences between the periods 1985–1995 and 1996–2002. Student's t test was used to analyse changes in Ig *z*-scores and CD4 T-lymphocyte percentages between the two periods. (ii) Analysis of Ig *z*-score differences according to type of therapeutic regimen. One-way analysis of variance, with Bonferroni *post hoc* test, was used to evaluate changes in Ig *z*-scores, CD4 T-lymphocyte percentages and viral loads in children receiving mono-, double-combined therapy, or three of more drugs. Each therapeutic course was considered separately. Data from children who were not receiving ART at the time of Ig determinations were also analysed. Linear regression analysis was used to investigate the relationship of Ig *z*-score along with CD4 T-lymphocyte percentages and viral loads.

Statistical analyses were performed using the SPSS software package (SPSS 11.5; Chicago, IL). $P < 0.05$ was considered statistically significant.

Intravenous Ig (IVIG) courses

During the study period (1985–2002) a varying proportion of children received IVIG (from 0% in 1985, up to 62.7% in 1992 and down again to 0.22% in 2002). To avoid the potential bias on IgG *z*-scores due to the administration of IVIG, data from children receiving IVIG and those who were not were analysed together and separately.

Results

Ig *z*-scores in the periods 1985–1995 and 1996–2002

From 1985 to 2002 data from 1250 HIV-1 perinatally infected children (5061 determinations) were collected.

The median follow-up time was 10.7 years (range, 0.1–17.3 years). Significant ($P < 0.0001$) decreases in IgG, IgM and IgA *z*-scores were evident between the two study periods and paralleled by a significant ($P < 0.0001$) increase in CD4 T-lymphocyte percentages (Table 1).

Of 1250 children, 518 (41.4%) had received IVIG. The treatment had been administered for a median of 35 months (range, 1–120 months). IgG *z*-scores were significantly higher in children receiving IVIG than in those who were not (6.09 ± 4.50 versus 6.70 ± 4.60 in the period 1985–1995, $P = 0.02$; and 5.06 ± 4.55 versus 4.30 ± 4.27 in the period 1996–2002, $P < 0.0001$). No differences in IgM or IgA *z*-scores were present.

Excluding data from children receiving IVIG, significant ($P < 0.0001$) decreases in Ig *z*-scores were confirmed between the periods 1985–1995 and 1996–2002 (IgG *z*-score, 6.09 ± 4.50 versus 5.06 ± 4.55; IgM *z*-score, 9.67 ± 14.67 versus 5.48 ± 7.79; IgA *z*-score, 10.17 ± 17.06 versus 6.39 ± 11.97). Furthermore, even in the subgroup of children receiving IVIG significant ($P < 0.0001$) decreases in Ig *z*-scores were present (IgG *z*-score, 6.70 ± 4.60 versus 4.30 ± 4.27; IgM *z*-score, 8.45 ± 10.24 versus 6.16 ± 8.36; IgA *z*-score, 10.38 ± 12.67 versus 6.95 ± 9.30).

Ig *z*-scores according to type of antiretroviral therapy.

Data from 633 children who underwent 1758 therapeutic courses (611 courses of mono-therapy; 465 courses of double-therapy; 682 courses of therapy with three or more drugs) were available (Table 2). Median duration of therapeutic courses was 17.4 months (range, 1.0–34.2 months).

Ig *z*-scores were significantly higher in children receiving mono-therapy than in those receiving double-combined therapy and in the latter children than in those receiving three or more drugs (Table 2). Data from 1051 children (median age, 4.3 years; range, 0.1–19.2 years) who were not receiving ART at the time of Ig determinations were also available. Within this population IgG, IgM, and IgA *z*-scores were 5.22 ± 4.09,

Table 1. Features and immunoglobulin (Ig) *z*-scores in HIV-1 infected children in the periods 1985–1995 and 1996–2002.

	1985–1995	1996–2002	*P*
Children (n)	927	799	
Age (years) [median (range)]	3.2 (0.2–13.0)	7.5 (0.3–19.3)	
CD4 T lymphocytes percentage (mean ± SD)	22.8 ± 13.3	24.8 ± 12.0	< 0.0001
Viral load (log_{10} RNA copies/ml) (mean ± SD)	Not available[a]	3.67 ± 1.47	
z-score (mean ± SD)			
IgG	6.29 ± 4.72	4.44 ± 4.33	< 0.0001
IgM	9.25 ± 13.32	5.61 ± 7.93	< 0.0001
IgA	10.25 ± 15.68	6.48 ± 11.56	< 0.0001

[a]Information on viral load has been collected since 1997.

Table 2. Features and immunoglobulin (Ig) *z*-scores in children receiving mono-therapy, double-combined therapy or therapy with three or more drugs.

	Mono-therapy[a]	Double-combined therapy[b]	Combined therapy with ⩾ 3 drugs[c]	*P*
Children (n)	486	333	347	
Therapeutic courses (n)	611	465	682	
Age (years) [median (range)]	4.3 (0.2–14.7)	7.9 (0.7–17.30)	10.7 (0.6–19.30)	
CD4 T lymphocytes (%) (mean ± SD)	16.69 ± 11.36	18.23 ± 11.88	22.41 ± 10.29	< 0.0001 for a vs. c; < 0.0001 for b vs. c
Viral load (log_{10} RNA copies/ml) (mean ± SD)	4.49 ± 1.03	4.15 ± 0.93	3.72 ± 1.49	< 0.0001 for a vs. c; < 0.0001 for b vs. c
Ig z-score (mean ± SD)				
IgG	6.56 ± 4.10	5.20 ± 3.52	3.44 ± 3.23	< 0.0001 for a vs. b < 0.0001 for b vs. c
IgM	8.17 ± 9.70	6.57 ± 6.42	4.55 ± 5.58	0.003 for a vs. b; < 0.0001 for b vs. c
IgA	9.37 ± 9.50	7.92 ± 9.64	5.25 ± 7.37	0.031 for a vs. b; < 0.0001 for b vs. c

7.27 ± 12.54, and 8.21 ± 15.74, respectively ($P < 0.0001$ versus children receiving three or more drugs).

Ig z-scores correlated inversely with CD4 T-lymphocyte percentages (IgG, $r = -0.16$; $P = 0.0001$; IgM, $r = -0.17$; $P < 0.0001$; IgA, $r = -0.21$; $P < 0.0001$) and, directly, with viral loads (IgG, $r = 0.19$; $P < 0.0001$; IgM, $r = 0.18$; $P < 0.001$; IgA, $r = 0.21$; $P < 0.0001$).

IVIG were administered during 915 out of 1758 therapeutic courses (52.0%). IgG z-scores were higher in children receiving IVIG compared to those who were not (5.51 ± 3.96 versus 4.16 ± 3.55; $P = 0.02$). Excluding data from children receiving IVIG, significant differences in IgG z-scores were confirmed between children receiving mono-therapy, double-therapy or three or more drugs (mono-therapy, 6.88 ± 4.59; double-therapy, 4.88 ± 3.46; three or more drugs, 3.11 ± 2.73; $P < 0.0001$ for mono- versus double-therapy; $P = 0.001$ for double-therapy versus three or more drugs). Moreover, even in the subgroup of children receiving IVIG IgG z-scores were significantly ($P < 0.0001$) higher in children receiving mono- and double-therapy compared to those receiving three or more drugs (mono-therapy, 6.52 ± 4.04; double-therapy, 5.31 ± 3.54; three or more drugs, 3.74 ± 3.59).

Discussion

In this observational study on a large data set we demonstrated that combined-ART is associated with significant reductions in Ig z-scores. Firstly, significant decreases in Ig z-scores between the periods 1985–1995 and 1996–2002 have been documented. The potential influence of IgG replacement therapy on IgG levels was avoided by analysing separately data from children receiving IVIG and those who were not. In both groups of children, significant decreases in Ig z-scores between the period 1985–1995 and the period 1996–2002 were confirmed. We previously demonstrated that the survival rate of Italian HIV-1 perinatally infected children remained stable up to the year 1995 and it significantly improved since 1996, when double combined-ART became widespread and potent ART was introduced into the care of HIV-1 infected children [9]. Similarly, in this study significant reductions in Ig z-scores between the periods 1985–1995 and 1996–2002 may be ascribed to the effectiveness of combined-ART.

Ig z-scores correlated directly with viral load and inversely with CD4 T-lymphocyte percentages and were significantly lower in children receiving three or more drugs than in those receiving other therapeutic regimens. A gradient effect was evident since Ig z-scores were lower in children treated with three or more drugs than in those receiving double-therapy, and were lower in the latter children than in those receiving mono-therapy. When analysing separately data from children who had received IVIG or had not received it, significant differences in IgG z-scores between children receiving mono-, double-therapy or three or more drugs were confirmed.

T-lymphocyte reconstitution with combined-ART has been extensively documented [16–19], but limited data are yet available on the effects on B-lymphocyte function and Ig concentrations. To our knowledge, this is the longest reported observation on the effects of combined-ART on HIV-1 induced hyperimmunoglobulinemia and the only study on children. Studies on limited subsets of adults [20–23] reported that combined-ART restores abnormal B-lymphocyte function and leads to a significant decrease in Ig levels. Nevertheless, in one study [20] abnormal Ig levels were still present in 45% of patients. This is consistent with our finding in children since decreased, but still abnormal Ig z-scores, were found. This could be explained by the fact that, in the most of children, combined-ART led to significantly decreased, but not to undetectable, viral loads and, parallel to this, significantly increased, but not normal, CD4 T-lymphocyte percentages.

Mechanisms responsible for hyperimmunoglobulinemia in HIV infection are still unclear [24]. HIV-1 infection is associated with polyclonal expansion of B-lymphocytes, as documented by the fact that hypergammaglobulinemia is usually polyclonal and involves several isotypes [22]. B-lymphocyte polyclonal activation may be driven by some HIV-1 proteins acting as superantigens, such as glycoproteins 120 and 160 [25]. Abnormal T-lymphocyte regulatory B-lymphocyte response is also likely to be involved. T-lymphocyte surface ligands that interact with B lymphocytes at several stages of their activation have been found to be abnormally expressed [26,27]. Finally, HIV-1 infection induces a dysregulation of cytokine production, including interleukin (IL)-10 [24] and IL-15 [28], which may promote polyclonal B-lymphocyte activation and increased Ig production. During effective combined-ART the T-lymphocyte function restoration may lead to the recovery of the T–B lymphocyte cooperation. Consistent with this, significant B-lymphocyte reconstitution has been described in HIV-1 infected children treated with regimens containing a protease inhibitor [29].

In conclusion, our data show that in HIV-1 infected children combined-ART leads to a reduction of Ig levels which parallels the restoration of CD4 T-lymphocyte percentage and reduction in viral load and probably reflects improved B-lymphocyte functions.

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APPENDIX

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