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Interruption of antiretroviral treatment in HIV-infected children

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Dedicated to my parents and my grandfather.

INTERRUPTION OF ANTI-RETROVIRAL TREATMENT IN HIV-INFECTED CHILDREN

I. INTRODUCTION

Highly active antiretroviral therapy (HAART) has significantly reduced mortality of human immunodeficiency virus (HIV) disease [10]. However, it is now evident, that prolonged therapy, maintaining suppression of plasma viremia is not likely to eradicate HIV infection [34,82], which is why life-long therapy is necessary for most HIV-infected patients. A life-long treatment with HAART may lead to a broad spectrum of significant toxicities [4,32,43]. In addition, as a consequence of toxicity and the requirement for long-term daily medications, adherence to drug regimens is difficult, which can lead to the selection of resistant viral strains [96,18]. In an attempt to reduce the requirement for life-long therapy, several approaches to treatment interruption have been studied with patients who have maintained suppression of plasma viremia and relatively high CD4 cell counts while receiving HAART [25].

Structured treatment interruption (STI) is one option that has been studied thus far. STI is investigated in clinical trials for several reasons: to test whether protective immune responses can be induced, to limit drug exposure in order to minimize toxicity and to test whether response to salvage regimens in patients with multiple treatment failure can be increased [29]. Our approach has been to evaluate the possibility if treatment interruption could be a strategy for HIV-infected paediatric patients, whose life-long treatment is of great difficulty.

1. General information about HIV-Treatment

1.1 Historical notes

Zidovudine (azidothymidine, AZT) was the first antiretroviral agent licensed for AIDS in 1987 [51]. With the advent of AZT monotherapy the frequency of opportunistic infection was reduced and survival increased. The immune function in terms of the number of CD4-T-helper cells increased [75]. However, the drug does not prevent the progression from HIV seropositivity to AIDS or the neurological disease, when used in symptom-free HIV-infected people [13,5] and the beneficial effects in patients with AIDS wanes after about one year.

In 1992 and 1994 didanosine (ddI) and zalcitabine (ddC), two new nucleoside analogues, were licensed [15,14]. At the end of 1994 it became evident, that by using a combination of two nucleoside analogues applied at a low symptomatic or even symptom-free state, a better long-term effect can be achieved [21,40]. At the same time it became possible to quantify the antiviral effect of antiretroviral therapy and to compare the clinical effect with the intensity of the biologic effect [72]. Since the first treatment combinations that were used showed a reduction of the viral load (VL) of approximately 0.8 to 1 $\log_{10}$ copies/ml (compared to 0.3 to 0.5 $\log_{10}$ copies/ml for the first nucleoside monotherapies), there

were new hopes of stronger antiviral effects of other nucleoside combinations (a reduction of 1 to 1.5 $\log_{10}$ copies/ml) [54,92]. The introduction of protease inhibitors in combination with one or two nucleoside analogues led to permanent reductions of the viral load of 2 to 3 $\log_{10}$ copies/ml [41]. Since the beginning of 1996 the clinical benefit of some combination therapies became evident with a spectacular reduction of the risk of AIDS and mortality [64]. However, antiretroviral multitherapy is limited by the facts that antiretroviral agents do not eliminate the virus, that there is an important risk of development of drug resistance and that the long-term use of antiretroviral drugs still causes relevant toxicity [95].

1.2 Drug classes

Until today there are three groups of different drugs, 17 substances altogether [15]. Two classes of drugs work by interacting with the reverse transcriptase before the integration of the viral genes into the cellular DNA. The third group of drugs are protease inhibitors, interacting at the last stage of the virus reproduction cycle before the formation of viral particles [15,104]. Combination of drugs of these classes resulted in marked reductions of viral load and increase of CD4 cells.

1.2.1 Nucleoside Analogues (NRTI)

After an intracellular triple phosphorylation the nucleoside analogues inhibit the reverse transcriptase (RT) by competing with natural nucleosides. They then inhibit the prolongation of proviral DNA by interrupting to link with the next nucleoside through a 3'5'-phosphodiester. At the moment only six NRTIs are available for paediatric use: zidovudine, lamivudine, didanosine, stavudine, zalcitabine and abacavir. Pharmaceutical profiles and tolerances for children are very close to those observed in adults. Only the pharmacokinetics of zidovudine and lamivudine have been studied closely for new born children [5,15,104].

1.2.2 Non-Nucleoside Analogues (NNRTI)

Non-Nucleoside Analogues also inhibit the reverse transcriptase but without the necessity of being phosphorylated. Three molecules are licensed for adults but only efavirenz and nevirapine have been licensed and explored for children yet. They work selectively for HIV-1 but not for HIV-2 and an extremely quick appearance of resistant mutations during a monotherapy is known. Their strongest adverse effects are skin rash or even a Stevens-Johnson Syndrome. The incidence of hepatitis has increased with the use of nevirapine. Efavirenz may have adverse effects on the central nervous system [5,15 ,104].

1.2.3 Protease inhibitors (PI)

The viral protease splits the precursor polyproteins of the structure proteins and the viral enzymes. It interferes at a late stage of the viral cycle, when the virus is being assembled. Protease inhibitors therefore

act on chronically infected cells. As long as the protease inhibitor is effective, the infected cell produces a non infectious virus. Seven protease inhibitors are already commercialized or soon to be. There are important acute and long-term adverse effects and interactions of protease inhibitors with other drugs (gastrointestinal effects, lipodystrophy syndrome, insulin resistance) and the taste and form of application is very inconvenient, especially for children [5,15 ,104].

1.3 Treatment guidelines

The major goal of HIV therapy is to maintain the long-term health of the patient while avoiding drug-related toxicity and preserving viable future treatment options [46]. With the advent of combination therapy a profound and durable reduction of viral replication became achievable [22]. According to the guidelines for the use of antiretrovirals treatment should be offered to all patients with the acute HIV syndrome, those within 6 months of HIV seroconversion, and all patients with symptoms related to HIV infection. Biological and immunological factors hugely influence the recommendations for offering antiretroviral therapy in asymptomatic patients. In that case, antiretroviral therapy should be prescribed to individuals with fewer than 15 % CD4 T-cells or plasma HIV RNA levels exceeding 100,000 copies/ml [104].

Due to a better function of the thymus, the extent of immunological response of children to antiretroviral treatment is supposedly better than that of adults, but there is no comparison yet in studies using the same methods [67]. All children of HIV-infected mothers usually receive a zidovudine prophylaxis for six weeks with 2 mg/kg every six hours starting 12 hours after birth. In addition, a trimethoprim/sulfamethoxazol prophylaxis (TMP/SMX) is given to all HIV-infected children during the first year of life to prevent the occurrence of a pneumocystis carinii pneumonia. In some countries (for example USA), a TMP/SMX prophylaxis is systematically given to all infants of HIV-infected mothers during the first four months of life, until a HIV infection can definitely be excluded [20].

Table 1: Indications for initiation of antiretroviral therapy in children < 12 months of age with HIV infection [104]:

clinical category		**CD4 cell count**	**plasma HIV RNA**	**recommendation**
symptomatic (clinical category A, B or C)*	or	< 25 % (immune category 2 or 3)	any value	treat
asymptomatic (clinical category N)*	and	≥ 25 % (immune category 1)	any value	consider treatment

*according to the CDC Atlanta Paediatric Classification System

Concerning the benefits and risks of the use of antiretroviral agents in infants under 12 months, there are great therapeutical difficulties, due to a shortage of information on pharmacokinetic effects and tolerance of antiretroviral agents, especially of PIs over short- or long-term [20]. Since HIV infection progresses more rapidly in infants than in older children or adults, some experts would treat all HIV-infected infants under six months or under 12 months of age, regardless of clinical, immunological or virological parameters [104]. Early administration of antiretroviral treatment in the acute phase of HIV-infection leads to a reduction of viral load in the newborn before the irreversible damage of the immune system and therefore ideally leads to a maintenance of the CD4 cell reservoir. Apart from that, an early treatment keeps the virus from its dissemination. Because of the importance of the initial viral load for the disease progression, it is one of the aims of early treatment to achieve a better long-term prognosis by initially lowering the viral load [17,20].

Table 2: Indications for initiation of antiretroviral therapy in children ≥ 1 year of age infected with HIV [104]:

clinical category		**CD4 cell count**		**plasma HIV RNA**	**recommendation**
AIDS **(clinical category C)***	or	< 15 % (immune category 3)		any value	treat
mild-moderate symptoms **(clinical category A or B)***	or	15 - 25 % (immune category 2)	or	≥ 100,000 copies/ml	consider treatment
asymptomatic **(clinical category N)***	and	> 25 % (immune category 1)	and	< 100,000 copies/ml	Many experts would defer therapy and closely monitor clinical, immune and viral parameters.

*according to the CDC Atlanta Paediatric Classification System

The first treatment prescribed after the prophylaxis is often the classic combination of two NRTIs + one PI or two NRTIs + one NNRTI. Combinations including NNRTIs should only be given to children who are considered to have a good compliance because of a rapid development of resistances against that group of antiretrovirals. Furthermore, it has to be determined which situations allow a treatment interruption for a short while without risking serious morbidity, and which limit of immunodeficiency can be tolerated without risking the quality and duration of immunological reconstitution. All recommendations given are therefore limited and open to further improvement [7,11,38].

1.4 Toxicity

Side-effects that appear in children taking antiretroviral drugs are: allergic reactions (nevirapine, efavirenz), gastro-intestinal effects (didanosine), nephrolithiasis (indinavir) and neurological effects (efavirenz). Hepatocellular insufficiency that can appear after taking nevirapine has not yet been reported in children, whereas mitochondrial toxicity has been seen in children as well as lipodystrophy. Neither the incidence of lipodystrophy in children nor its long-term effect on the cardiovascular system have been fully explored yet [50].

1.4.1 Lipodystrophy syndrome

This syndrome is characterized by body fat redistribution leading to peripheral fat wasting and central obesity with accumulation of visceral fat and enlargement of the breast and neck ("buffalo neck") [9]. Metabolic abnormalities have also been reported, such as elevated triglyceride and cholesterol levels, and insulin resistance or type 2 diabetes mellitus [80]. Lipodystrophy and specifically the loss of subcutaneous fat (lipoatrophy) is a significant and distressing side-effect of antiretroviral therapy [8]. Multiple surveys have shown that clinical factors such as age, the CD4 cell count, and others are important predisposing factors. Some observational studies have found an association between lipodystrophy and either complete viral suppression, a history of a low CD4 cell count or, in particular, the change in CD4 cell count during therapy [79]. The role of protease inhibitors and nucleoside reverse transcriptase inhibitors (NRTI) in the occurrence of lipodystrophy has been investigated in different studies [61,44,94,70]. The exact mechanisms with which PIs [102,81,55] and NRTIs [12,98,59,52,78] contribute to this syndrome has yet to be evaluated. Although the initial identification of this syndrome was coincident with the widespread use of PI-containing regimens, more recent reports have concluded that it may also occur in the absence of PIs [68,93].

There are several possible sequelae of lipodystrophy syndrome. Firstly, adherence to antiretroviral therapy could be compromised because of cosmetic effects, leading to virological and even clinical failure [60,65]. Secondly, the metabolic effects can lead to an increase in cardiovascular disease [23], and several case reports have described premature coronary-artery disease in patients with few or no risk factors that were receiving protease inhibitor therapy [45,31]. Increased exercise can decrease central fat accumulation but at the expense of increased peripheral fat wasting [77].

1.4.2 Mitochondrial toxicity

One of the major toxicities of NRTI therapy, particularly over medium-term to long-term, is thought to be secondary to inhibition of mitochondrial DNA polymerase γ, resulting in impaired synthesis of mitochondrial enzymes that generate ATP by oxidative phosphorylation. These include myopathy (zidovudine), neuropathy (stavudine, didanosine, zalcitabine), hepatic steatosis and lactic acidaemia (didanosine,

stavudine, zidovudine) and possibly also peripheral lipoatrophy (possibly all NRTIs, although predominantly with stavudine) and pancreatitis (didanosine) [9,6]. The most serious mitochondrial toxicities are lactic acidosis and pancreatitis.

The management of mitochondrial toxicity is generally limited to cessation of the causative drug and sometimes of other drugs that might exacerbate the condition. Some toxic effects, such as peripheral neuropathy, may worsen for several weeks after drug cessation ("coasting phenomenon"). Several agents have been used in the treatment of congenital mitochondrial diseases with limited success [9,89]. These agents include essential vitamin coenzymes (thiamine and riboflavin), electron acceptors (vitamin C), antioxidants (compound Q), and L-carnitine. Patients with zidovudine-induced myopathy and NRTI-induced peripheral neuropathy have been shown to have reduced concentrations of L-carnitine [9].

1.4.3 Allergic reaction

Allergic reactions in HIV-1-infected patients are about 100 times more common than in the general population. An allergic reaction typically manifests as an erythematous, maculopapular, pruritic and confluent rash with or without fever beginning after one to three weeks of therapy. All licensed NNRTIs (nevirapine, delavirdine, efavirenz), the NRTI abacavir (hypersensitivity) and the PI amprenavir are common antiretroviral drugs that cause allergic reactions, which is rare in other NRTIs or PIs [9].

The pathogenesis of such allergic reactions is unknown. Suggested causes and mechanisms include the degree of immunodeficiency or immune activation, the longer duration and higher doses of therapy, altered drug metabolism associated with gluthation deficiency or slow acetylator phenotype and co-infections with cytomegalovirus and Epstein-Barr virus [9]. Recent studies have shown a potential genetic predisposition for a hypersensitivity reaction to abacavir [47]. Two different teams of investigators have identified a strong association between a rare HLA type and the risk of developing abacavir hypersensitivity [30,69]. About 50 % of antiretroviral allergic reactions resolve spontaneously despite continuation of therapy. Therapy should be stopped if there is mucosal involvement, blistering, exfoliation, clinically significant hepatic dysfunction, fever greater than 39°C or pruritus [9]. Desensitisation is unstudied for NRTIs and may be inappropriate for antiretroviral allergic reactions and abacavir hypersensitivity, since it would necessitate a period of subtherapeutical drug concentration and so favour development of drug resistance [9].

1.5 Resistance to antiretroviral therapy

Viral resistance is an important problem for the treatment of HIV-infected patients and probably the strongest limit. Even though the mortality rate in HIV-infected children has decreased to almost zero in the last two or three years in industrialised countries, the majority of European and American studies note an insufficient suppression of viral replication until today, which is stronger in children than in adults

[17,20,101]. A large number of children stay clinically asymptomatic without any sign of immunodeficiency, but have a persisting viral replication, which, even if existing at a low level, can easily lead to the emergence of resistant viral strains. One of the main reasons for that is probably the difficulty of adherence to a combination therapy [101,88,3], which is mainly due to adverse effects, toxicity or the fact that sometimes numerous pills have to be taken a day, which can be disturbing in everyday life [104,39].

The selection of resistant viral strains, due to growing selection of mutations under subtherapeutical levels, can have a durable influence on the efficiency of a treatment, also because cross-resistance between substances of the same drug family is widespread. Especially resistance against NNRTIs appears rapidly and is difficult to burn out, so that it is strongly advised to carry out resistance-testing before every start of a new treatment [37].

However, resistance and insufficiency may not only be caused by a subtherapeutical dosage of antiretroviral treatment but also by a potentially different replication profile of the virus in children. Even though the development of resistant virus strains has not yet been fully explored in children, it is probably the major reason for a remaining viral replication during antiretroviral treatment [104,101].

1.6 Structured treatment interruption (STI)

A few years ago, treatment interruption was not considered as treatment option, and patients who interrupted therapy generally did so of their own will, which was described as non-adherence. “Drug holidays” were occasionally recommended though, for example to allow resolution of drug-toxicity, but it was assumed that once started, antiretroviral treatment was a life-long commitment [35]. Today antiretroviral treatment may be interrupted for the following reasons:

- Request for treatment interruption by the patient,
- carelessness/non-observance,
- adverse effects/toxicity,
- change of treatment guidelines [57].

For four or five years now, the strategy of structured treatment interruption (STI) in HIV infected patients has been discussed mainly for three therapeutical reasons: (1) to stimulate the anti-HIV immune response after viremia has been suppressed by treatment, (2) to raise compliance by increasing time off therapy, improving quality of life and diminishing toxicity, and (3) in the case of the occurrence of multiresistant virus strains. In that case therapy is interrupted before starting a salvage therapy in the hope of achieving a resusceptibility of the virus to different available antiretroviral agents [103,63,100]. By interrupting HAART and later restarting all antiretroviral agents simultaneously, the development of drug resistance can be reduced to a minimum.

STI is especially considered for those patients for whom other treatment options are no longer successful or even harmful and can only be carried out on the strict condition that both viral load and CD4 cells

are regularly checked. Immunological and clinical consequences of treatment interruption vary in every patient [57]. Each HIV-infected person has to be regarded individually and their medical history must be closely examined (CD4 cell counts should not have decreased below 300-350/mm^3) before deciding together with the patient and care-giver whether a treatment interruption is useful [74]. Unfortunately, with the exception of some reports of sustained suppression, fairly prompt virological relapse and the occurrence of AIDS events after discontinuation of antiretroviral treatment has been the predominant pattern observed thus far in clinical trials [36,62,84,42]. Along with a rapid increase of viral load a brisk decrease of CD4 cells can be observed [16,105,49] and a lot of patients run the risk of manifesting a state that resembles a primary infection so that a restart of antiretroviral treatment becomes necessary [20].

One rationale for interrupting therapy, and one of the earliest to be studied, argues that periodic exposure of the immune system to low-level viremia might stimulate HIV-specific cellular immunity. This appears to be retained in patients, whose disease course progresses only slowly over a long period of time, but is lost in the majority of chronically infected patients [53]. It was hoped that the HIV-specific immune response stimulated by intermittent therapy might ultimately allow the immune system to control HIV without the need of drug therapy. Interest in this approach was stimulated in part by reports of the famous "Berlin patient", who initiated antiretroviral therapy shortly after infection and who was able to maintain an undetectable viral load and strong HIV-specific CD4 and cytotoxic T-lymphocyte (CTL) responses without antiretroviral therapy after a number of unplanned treatment interruptions [62]. Enthusiasm for this approach has waned, at least for chronically infected patients, due to discouraging data from clinical trials.

In the ongoing Staccato study, 600 patients on successful antiretroviral treatment were to be randomised to either continued therapy, CD4-guided therapy, or one-week-on/one-week-off therapy. A scheduled preliminary analysis evaluated effectiveness in the 1-week-on-1-week-off arm. This schedule showed an unacceptably high failure rate and was therefore terminated. Of 36 evaluable patients, 19 (53%) had two successive HIV RNA concentrations > 500 copies/ml at the end of the week off therapy, and were classified as virological failure. Most of those who failed (eleven patients) took didanosine, stavudine, saquinavir, and ritonavir [2].

The Swiss and Spanish Intermittent Treatment Trial (SSITT) used an eight-weeks-on/two-weeks-off schedule in 133 chronically infected patients who had been successfully treated with antiretroviral therapy. After 52 weeks, 18 % of the patients showed their immune systems responding against HIV off therapy (viral load < 5000 copies/ml). After 96 weeks, 11 % were still showing response off therapy. It was noted, for example, that the best results were observed in patients who had low baseline viral loads to begin with [48].

The STI strategy may especially be viable in patients treated during primary infection. A study group at the Massachusetts General Hospital has reported data from a select group of HIV-infected patients who

were diagnosed and treated before Western blot seroconversion [99]. Of 14 such patients, eight maintained viral loads < 5000 copies/ml after at least one STI. The results have been very promising but are possibly due to early initiation of treatment since they were not seen in other studies [42,83,76]. Controlled trials are being planned to assess the effectiveness of STI after early antiretroviral therapy. Efforts to study STIs in this patient population are limited by the fact that only few patients are diagnosed during acute infection.

Another rationale for interruption of therapy is to allow re-emergence of wild-type virus and thereby improve response to salvage therapy in heavily experienced patients with extensive drug resistance (Structured Intermittent Therapy = SIT). It has been clearly demonstrated that discontinuation of antiretroviral therapy leads to replacement of resistant virus by wild-type virus. One study group has demonstrated that in patients who discontinue a failing HAART regimen, drug susceptibility increases at a median of six weeks after discontinuation and is associated with increases in plasma HIV-1 RNA, decreases in CD4 cell count, and increases in viral replicative capacity, sometimes referred to as "fitness" [19]. This is believed to reflect the re-emergence of the more virulent wild-type virus, replacing the mutant virus maintained by the selective pressure of the failing regimen [35]. Hypothetically multidrug resistant virus might be so unfit, that it would not persist indefinitely in the absence of therapy and would thus give patients a renewed opportunity for durable viral suppression [19].

However, in highly experienced patients failing antiretroviral therapy, discontinuation of HAART prior to initiation of salvage therapy is a promising but potentially hazardous approach. While it may allow restoration of drug-susceptible virus, there is also a significant decline in CD4 cell count and the potential for opportunistic infections or other complications of untreated HIV infection. Deeks and colleagues concluded from their study, that despite the presence of reduced drug susceptibility, antiretroviral-drug therapy can provide immunologic and virologic benefit. This benefit reflects continued antiviral-drug activity and the maintenance of viral population with a reduced replicative capacity [19]. Furthermore, the virological benefit after re-initiation of therapy is expected either to be transient or to require a complex multidrug salvage regimen in order to maintain suppression [35].

The remaining treatment interruption approaches seek to decrease total time on therapy, either through time-based treatment cycles (SIT) or CD4 cell count based cycles ("pulse therapy"). The idea of pulse therapy is to keep the CD4 cell count above a predetermined threshold using cycles of therapy followed by prolonged interruptions. There are two studies supporting an approach for pulse therapy: a study from Argentina [58] and an observational cohort study at Johns Hopkins University [87]. There may be a subset of patients, presumably those with relatively low viral load set points and good CD4 cell count responses to HAART, who will be able to discontinue therapy safely for prolonged periods of time [35].

Regarding antiretroviral therapy and its consequences in adults, including new strategies to enforce the fight against HIV, it is important to find out, whether children can profit from similar strategies. Although

viral resistance, adverse-effects and risks of treatment interruption are already documented in different studies for adults, no data address them in children yet. With this pilot study we retrospectively evaluated the effects of treatment withdrawal in HIV-infected children in order to prepare a prospective study.

II. PATIENTS AND METHODS

1. Study population

We studied all HIV-1 infected children that attended the Paediatric Clinic of Haematology and Immunology at the Hôpital Necker-Enfants Malades in Paris, France, who had received antiretroviral treatment for at least ten months, before stopping therapy completely for an individual length of time between 1996 and 2000. Altogether, 300 children are treated for their HIV infection at Necker-Enfants Malades, 250 of which receive antiretroviral treatment. We had a study population of 35 eligible paediatric patients who were under medical treatment in that hospital. In a retrospective study we observed this group concerning their discontinuation of antiretroviral therapy after months or years of receiving treatment.

2. Classification of the group

The CDC Atlanta Paediatric Classification System was used to define infection status and clinical and immunological conditions before treatment interruption.

Table 3: CDC Atlanta Paediatric Classification System [104]

	non symptomatic	**mildly symptomatic**	**moderately symptomatic**	**severely symptomatic**
no suppression CD4 > 25 %	N1	A1	B1	C1
moderate suppression CD4 =15–24 %	N2	A2	B2	C2
severe suppression CD4 < 15 %	N3	A3	B3	C3

Table 4: Clinical categories of the CDC Atlanta Paediatric Classification System [104]

category	symptoms
A	lymphadenopathy hepatomegaly splenomegaly parotitis dermatitis
B	anaemia candidiasis cardiomyopathy CMV hepatitis LIP (lymphoid interstitial pneumonia) nephropathy
C	Children, who have any condition listed in the 1987 surveillance case definition for acquired immunodeficiency syndrome, with the exception of LIP (which is a category B condition).

Immunological parameter (HIV RNA viral load and CD4 T-lymphocyte count) were collected every three months and recorded in addition to other information about every child in special forms, including:

- name,
- age,
- type of contamination,
- date and reason for treatment interruption,
- medical history of viral load and CD4 T-cell count (noted were the highest plasma HIV RNA count and the lowest CD4 T-lymphocyte count ever detected during antiretroviral therapy).

3. Treatment

Antiretroviral therapy was prescribed individually for each child on the basis of guidelines for the use of antiretroviral agents in HIV-infected children and adolescents and was adopted to the child's weight, age and unique therapeutical and management considerations. Most of the children received a zidovudine prophylaxis for six weeks after birth. Few children had not been followed up from birth and therefore had not received any prophylaxis. As soon as their seropositivity was discovered, they received either zido-

vudine or lamivudine monotherapy adopted to their current weight. If clinical symptoms had already appeared, the children initially received combination therapy.

We recorded all regimens received until the day of treatment interruption including:

- dosage,
- reasons for changing dosage,
- route of administration,
- adverse effects,
- start and type of the first antiretroviral therapy ever received,
- reason for changing therapy, date of onset and combination of new treatment,
- clinical effects appearing after discontinuing therapy,
- reason for restarting therapy, date of onset and combination of new treatment.

We subdivided the range of treatment regimens into three different groups. (1) children that received a bitherapy, consisting of two NRTIs, (2) those who took therapies including PIs, no matter how many drugs were used altogether, (3) those who took a therapy other than those two listed above. No child received a monotherapy by the time antiretroviral therapy was interrupted.

4. Treatment interruption

Antiretroviral combination therapy was stopped either by the consulting doctor, the child's care-givers or the child itself in the time between January 1996 and December 2000. We classified all children into three groups according to the following reasons of their treatment interruption:

- toxicity (n=11),
- inefficiency (n=17),
- inconvenience (n=7).

4.1 Adverse effects and toxicity

For eleven children (31.4 %) treatment interruption was decided for reasons of severe adverse-effects and toxicity. Common intolerances to drugs were caused by indinavir, ritonavir, nelfinavir, and efavirenz. Side-effects that often occurred were hepatic toxicity such as cholestase (n=3) and hepatitis, especially when taking PIs and repetitive skin rash appeared after intake of NNRTIs (n=4). Gastro-intestinal effects, such as nausea (n=5), intractable vomiting (n=2) and chronic diarrhoea (n=3) were usually caused by NRTIs but also after taking nelfinavir. Nephrolithiasis caused by an incompatibility to indinavir was also a common adverse-effect (n=5). One child though had to stop therapy because of a severe lipodystrophy syndrome she developed after taking a combination therapy that included nelfinavir for eleven months.

4.2 Inefficiency

17 children (48.6 %) stopped antiretroviral therapy for reasons of virological failure. They were in a good clinical state and remained asymptomatic without any sign of immunodeficiency. However, viral replication persisted on a high level and could not be successfully suppressed mostly because of the occurrence of multiresistance but also because of non-adherence to the treatment. In such cases it was decided to change antiretroviral therapy to a more successful combination therapy. However, rapid changes of combination therapy risk a limitation to future treatment options, especially in long-term HIV therapy. Consequently, treatment was interrupted to burn out resistance and to possibly achieve a change back to the "wild type" virus. Three asymptomatic children, whose viral load and CD4 cells stayed in a steady state stopped treatment since they did not need further therapy for the moment and were also grouped into the category "inefficiency".

4.3 Inconvenience

The third reason for treatment interruption was inconvenience and applied to seven children (20 %). This category includes lack of adherence and the child's or care-givers' decision to stop therapy for reasons of incompatibility, inability to swallow or simply carelessness. This was often the case, when parents suffered from severe HIV infections themselves and were hospitalised, so that an orderly care for the child, including regular treatment administration was not evident. Older children who had reached the age of puberty and took antiretroviral therapy independently, stopped medication themselves. Often they found an antiretroviral treatment too uncomfortable or embarrassing even in terms of outing themselves in public.

5. Immunological and virological tests

Plasma HIV RNA was tested approximately every three months. The average time between the last plasma HIV RNA test during antiretroviral treatment and the first during treatment interruption was 118 ± 65 days. Amplicor HIV-1 Monitor RNA polymerase chain reaction (PCR) test (Roche Diagnostics, Branchburg, NJ) was used to quantify plasma HIV RNA concentrations. PCR is a very sensitive method that measures the presence or amount of RNA or DNA of a specific organism or virus (for example: HIV) in the blood or tissue [71]. The Amplicor RNA PCR test is a highly sensitive test, proven to detect viral loads as low as 50 RNA copies per ml [90].

The CD4 cell count was regularly checked approximately at the same time as the viral load (every three months). The average time between the last CD4 cell count before treatment interruption and the first CD4 cell count during treatment interruption was 121 ± 66 days. The CD4 and CD8 T-lymphocyte count was measured by flow cytometry and noted in both absolute numbers and percentages. Flow cy-

tometry uses fluorescent antibodies to detect IgG or IgM antibodies bound to autologous or allogenic cells. It is used to measure cell size, number, viability, and nucleic acid content [71].

6. Resistance testing

To estimate the presence of major mutations in the HIV reverse transcriptase and protease genes, genotypic resistance was tested before treatment interruption at a time when the child was still under antiretroviral therapy. In order to detect new resistance development or the decline of former gene mutations and drug resistance, tests were carried out regularly in the absence of antiretroviral treatment. Finally, another test was run in the case of a restart of therapy, when a new combination of efficient agents had to be defined for the child.

7. Restart of therapy

Antiretroviral therapy was resumed whenever CD4 cell counts declined to less than 15 % or viral load was continuously detected above 5 $\log_{10}$ copies/ml. As soon as severe clinical symptoms appeared, therapy was restarted, even if immunological parameters had not yet reached such dangerous levels. In two cases the child itself or the child's care-givers elected to resume drug treatment independent of these thresholds.

8. Analysis

In order to evaluate the data for CD4 cell changes after treatment interruption, the analysis is based on the following equations. CD4 X0 is defined as the baseline. The baseline is the last CD4 cell count before treatment interruption, measured at day t0. CD4 X1 was the first CD4 cell count measured after treatment interruption at day t1 (CD4 cells were noted in % of white blood cells).

Therefore, ΔCD4 is:

$$\Delta CD4 = \frac{CD4X1 - CD4X0}{(t1 - t0)}$$

This equation shows the percentage of lost or gained CD4 cells per day of treatment interruption.

This way, we calculated the average CD4 cell loss during treatment interruption. The average CD4 cell loss can be more or less severe, depending on how high or low the patient's CD4 cell baseline was. Therefore, the baseline was also included into the equation.

Subsequently, Δ'CD4 describes the loss or gain of CD4 cells per day of treatment interruption according to the individual baseline. The result is therefore noted in % of the individual baseline of each child. Δ'CD4 is:

$$\Delta' CD4 = \frac{\left(CD4X1 - CD4X0\right) \times 100}{CD4X0} / (t1 - t0)$$

Similar equations were used to analyse the data for viral loads. VL X0 is defined as the last viral load before treatment interruption at day t0, setting the baseline. If VL X1 is the first viral load detected after treatment interruption on day t1, ΔVL is:

$$\Delta VL = \frac{VLX1 - VLX0}{(t1 - t0)}$$

showing the average viral load gained or lost per day of treatment interruption. Therefore Δ'VL is:

$$\Delta' VL = \frac{\left(VLX1 - VLX0\right) \times 100}{VLX0} / (t1 - t0)$$

and stands for the average gain or loss of plasma HIV RNA according to the baseline per day of treatment interruption.

Differences between categorical variables were tested in order to interpret the course of CD4 cell counts after treatment interruption regarding:

- age (older or younger than five years),
- sex,
- reasons of treatment interruption (inefficiency versus toxicity/inconvenience),
- medical history of viral load (more or less than 5 $\log_{10}$ copies/ml of HIV RNA),
- medical history of CD4 T-cell count (more or less than 5 %).

We did the same analysis for the results of viral load gain or loss, concerning the same variables like:

- age (older or younger than five years),
- sex,
- reason of treatment interruption,
- medical history of viral load,
- medical history of CD4 T-cell count.

In all 35 HIV-1 infected children the empirical equations described above were used to interpret the dynamics of HIV-1 RNA and CD4 cells in the time after treatment interruption.

III. RESULTS

1. Features of the study group

Overall 35 children born to HIV-infected mothers between 1981 and 1997 were studied; 27 of these children (77.1 %) were prospectively followed up from birth. All children acquired HIV-infection through vertical transmission. 20 children are of European, 14 of African and one child is of Caribbean origin. All children received antiretroviral therapy for at least ten months before interrupting treatment at some point between 1996 and 2000. Sixteen children were born to mothers who were injecting drug users, 12 were born to mothers, who came from continents with a high HIV infection rate such as Africa and seven children were born to mothers, who had acquired HIV infection through transfusions or infected sexual partners. The group consisted of 16 girls and 19 boys, with the youngest patient being three years and eight months old and the oldest child 19 years and seven months old at study-end in December 2000.

The average age at the time antiretroviral therapy was started was 3.5 years ± 3.8 and the median age 2.0 years [0;15.3]. At the time, antiretroviral therapy was stopped, the average age was 10.4 years ± 4.9 years and the median age 11.1 years [1.3;18.3]. Altogether, 29 children (82.9 %) were over five years old and only six (17.1 %) were under five years when antiretroviral therapy was interrupted. The average time, for which treatment was taken was 80.8 months ± 48.6 before withdrawal of antiretroviral therapy, the median time was 79 months [10;160]. The time of treatment administration mainly depended on the child's age, since older children generally received antiretroviral therapy for a longer period of time.

Regarding the patients' clinical and immunological state before treatment interruption, a CD4 cell count below 5 % (at a time while the patient was receiving HAART) was defined as "a medical history of severe immunodeficiency". A viral load (VL) above 5 $\log_{10}$ copies/ml (at a time while the patient was receiving HAART) was defined as "a medical history of severe increase of viral load".

Table 5: Medical history of immunodeficiency and severe increase of viral load

medical history	number of children
viral load > 5 $\log_{10}$ copies/ml *	10
viral load < 5 $\log_{10}$ copies/ml	25
CD4 cell count < 5 % **	13
CD4 cell count > 5 %	22
viral load > 5 $\log_{10}$ and CD4 cell count < 5 %	5

* A VL > 5 $\log_{10}$ copies/ml while receiving HAART was defined as "a medical history of a severe increase of viral load".

** A CD4 cell count < 5 % while receiving HAART was defined as "a medical history of severe immunodeficiency".

All children were classified according to the CDC Atlanta Paediatric Classification System:

Table 6: Immunological and clinical state of the patients

CDC Atlanta Classification System	number of children
N3	1
A1	3
A2	4
A3	4
B1	2
B2	1
B3	18
C3	2

Apart from one child staged N3, all children developed mild symptoms, such as lymphadenopathy, hepatomegaly, splenomegaly or parotitis which are classified A. Common symptoms in the group of children classified B were candidiasis, HSV or a herpes zoster, three children developed a lymphoid interstitial pneumonia (LIP). The two children categorized C had developed a malignant Non Hodgkin Lymphoma.

2. Treatment

21 children (60 %) in our cohort took an antiretroviral regimen that included a PI, 16 of which took a treatment that consisted of one PI and two NRTIs (mostly AZT + 3TC or 3TC + d4T). Four of the children receiving PIs used a quadritherapy.

Six children (17.1 %) had a dual combination regimen consisting of only two NRTIs in different combinations (such as AZT + 3TC ; AZT + ddC ; d4T + ddI ; d4T + 3TC).

Eight children (22.9 %) took a therapy, which did not include a PI. Those were combinations of either two NRTIs + one NNRTI or three NRTIs. None of the children received a monotherapy by the time treatment was stopped.

Table 7: Treatment combinations

number of agents	combination	number of children
bitherapy	2 NRTI	6
triple therapy	2 NRTI + 1 PI	16
	2 NRTI + 1 NNRTI	6
	1 NRTI + 2 PI	1
	3 NRTI	2
quadritherapy	2 NRTI + 2 PI	1
	2 NRTI + 1 NNRTI + 1 PI	2
	1 NRTI + 1 NNRTI + 2 PI	1

Table 8: PIs used in treatment combinations

PI	number of children
nelfinavir	11
amprenavir	3
indinavir	3
saquinavir	2
ritonavir	1
lopinavir	1

3. Treatment interruption

3.1 Clinical course

The average time of observation after treatment interruption was 325 ± 294 days, with a median time of 258 days [11;1536]. In the absence of antiretroviral treatment, three children developed slight symptoms like skin reactions (erythema or prurigo) or candidiasis and one child developed a herpes zoster infection in addition to a trichophyton infection. Another child suffered from thrombopenia and epistaxis and one child developed a LIP during treatment interruption, so that these two children had to restart therapy. They were the only two children, who restarted therapy due to clinical events. 21 children were in a good state of health and still without antiretroviral therapy by the time we finished the study at the end of December 2000.

3.2 Virological and immunological course

Viral loads increased in all children after treatment interruption, and CD4 cell counts decreased in the same way. However, in the course of treatment interruption, we noted three different types of virological and immunological reaction:

- 14 children immediately developed a brisk increase of viral replication (above 5 $\log_{10}$ copies/ml) and a sharp decrease of CD4 cells (below 15 %). Ten of these children remained on such levels, so that a restart of therapy became necessary. The other four children had high viral loads but not constantly above 5 $\log_{10}$ copies/ml and their CD4 cell counts did not constantly decrease to under 15 % so that a restart of therapy was not decided.
- Eleven children stayed in a steady state. They had constantly low viral loads (between 3 and 4 $\log_{10}$ copies/ml) or even undetectable levels, and their CD4 cell counts were always above 15 %. Nine of these children were still without therapy two years after treatment interruption, in two cases the child or the parents wished to resume therapy.
- The last group consists of ten children, who alternated between these two variations. They were dealing well with the cessation of their therapy, but in contrast to the second group, they reached higher levels of viral load and lower CD4 cell counts from time to time, which kept them from maintaining a steady state. Two of these children restarted therapy because of clinical events (lymphoid interstitial pneumonia and thrombopenia with frequent episodes of epistaxis). The other eight children were still without therapy by the end of the study.

3.2.1 Virological course

The average time between the last plasma HIV RNA test during antiretroviral treatment and the first test after treatment interruption was 118 ± 65 days. During that time, the viral load increased by 0.944 ± 1.416 $\log_{10}$ copies/ml, which are 0.008 ± 0.012 $\log_{10}$ copies/ml per day. Therefore, according to the individual baseline, a child gained 0.2 ± 0.6 % of the baseline viral load per day of treatment interruption. Consequently, a child with a baseline of 10,000 copies (4 $\log_{10}$ copies/ml) showed an increase of viral load of approximately 20 copies per day of treatment interruption.

3.2.2 Immunological course

The average time between the last CD4 cell count directly before treatment interruption and the first CD4 cell count during treatment interruption was 121 ± 66 days. The average loss of CD4 cells during that time was 0.06 ± 0.12 % CD4 cells per day, which is 0.3 ± 0.5 % of the individual baseline. Subsequently, a child had lost approximately 30% of the baseline CD4 cell count by the time CD4 cells were first measured in the absence of therapy (after 121 ± 66 days).

4. Factors associated with immunological decline

Assuming that the course of HIV RNA viral load and CD4 cell count depended on certain factors, distinctions were made between the medical history of viral load and CD4 cell count, age, sex and reason for treatment interruption. A 'p' lower than 0.05 was defined as being statistically significant.

4.1 Associations with the decline of CD4 cell count

In children under five years of age (n=6) a loss of 3.0 ± 3.4 % CD4 cells was observed at the first measurement after treatment interruption (after 121 ± 66 days), which is 0.025 ± 0.028 % per day. Children older than five years (n=29) lost 6.9 ± 13.2 % CD4 cells (0.057 ± 0.109 % per day). Male patients (n=19) showed a loss of 5.1 ± 7.2 % (0.042 ± 0.059 % per day), while female children (n=16) lost 7.6 ± 16.4 % CD4 cells during treatment interruption (0.063 ± 0.135 % per day).

According to the different reasons for treatment interruptions, we made a distinction between those patients, who stopped therapy for reasons of inefficiency and those who discontinued for other reasons such as toxicity or inconvenience. Those of the first group (n=17), who interrupted therapy for reasons of low efficacy lost 8.7 ± 15.2 % CD4 cells (0.072 ± 0.126 % per day), while a loss of only 3.8 ± 8.2 % CD4 cells was observed for the remaining children (n=18) that interrupted treatment for reasons of toxicity or inconvenience (0.031 ± 0.068 % per day).

In addition, the association between the medical history of viral load before treatment interruption and the course of CD4 cells after treatment interruption was examined. The 10 children who had a medical history of a severe increase of viral load (> 5 $\log_{10}$ copies/ml) had a decline of 7.6 ± 13.9 % CD4 cells (0.063 ± 0.115 % per day). The 25 children who had a medical history of viral load below 5 $\log_{10}$ copies/ml lost 2.8 ± 4.9 % CD4 cells (0.023 ± 0.04 % per day). We also studied the association between the medical history of CD4 cells before treatment interruption and the course of CD4 cells after treatment interruption. The 13 children with a medical history of severe immunodeficiency (CD4 cell count < 5 %), lost 6.7 ± 13.7 % (0.055 ± 0.113 % per day) in contrast to the 22 children with a stronger immune system (CD4 cell count > 5 %) who lost 5.3 ± 9.6 % CD4 cells (0.044 ± 0.079 % per day).

4.2 Associations with the increase of viral load

For the six children under five years of age, we observed a gain of 0.354 ± 0.59 copies/ml HIV RNA at the first measurement after treatment interruption (0.003 ± 0.005 $\log_{10}$ copies/ml per day). Those above the age of five years showed a gain of viral load of 1.18 ± 1.416 $\log_{10}$ copies/ml during that time (0.010 ± 0.012 $\log_{10}$ copies/ml per day). This result is statistically significant with p=0.05.

For the 19 male children of our cohort the viral load increased by 1.18 ± 1.298 $\log_{10}$ copies/ml (0.010 ± 0.011 $\log_{10}$ copies/ml per day). Female children showed an increase of 0.826 ± 1.416 $\log_{10}$ copies/ml (0.007 ± 0.012 $\log_{10}$ copies/ml per day).

In terms of cessation of antiretroviral therapy, we distinguished between inefficiency of therapy and other reasons such as toxicity or inconvenience. Children, who interrupted therapy because of inefficiency of their present drug regimen, gained 0.944 ± 1.534 $\log_{10}$ copies/ml after treatment interruption (0.008 ± 0.013 $\log_{10}$ copies/ml per day). The 18 children who discontinued therapy for other reasons showed a HIV RNA gain of 1.062 ± 1.18 $\log_{10}$ copies/ml (0.009 ± 0.010 $\log_{10}$ copies/ml per day).

Concerning the medical history of CD4 cell count, the 13 children with a medical history of severe immunodeficiency (CD4 cell count < 5 %) showed an increase of HIV RNA of 0.944 ± 1.062 $\log_{10}$ copies/ml (0.008 ± 0.009 $\log_{10}$ copies/ml per day) while those patients with a CD4 cell count above 5 % gained 1.18 ± 1.18 $\log_{10}$ copies/ml (0.010 ± 0.010 $\log_{10}$ copies/ml per day). We also observed an association between the medical history of viral load before treatment cessation and the course of viral load afterwards. This led to the result that the 10 children with a medical history of a severe increase of viral load gained 1.18 ± 2.36 $\log_{10}$ copies/ml (0.010 ± 0.02 $\log_{10}$ copies/ml per day). The remaining 25 children, who had a HIV RNA of less than 5 $\log_{10}$ copies/ml, gained 0.59 ± 0.708 $\log_{10}$ copies/ml by the time the viral load was first measured after treatment interruption (0.005 ± 0.006 $\log_{10}$ copies/ml per day).

Table 9: Associations with the decline of CD4 cell count and the increase of viral load (VL) (at the time of the first measurement after treatment interruption)

	decrease of CD4 cells in % (after 121 ± 66 days)	p	increase of VL in log_{10} copies/ml (after 118 ± 65 days)	p
medical history of viral load*				
< 5 log (copies)	-2.8 ± 4.9 (n=25)	n.s.	0.59 ± 0.708 (n=25)	n.s.
> 5 log (copies)	-7.6 ± 13.9 (n=10)		1.18 ± 2.36 (n=10)	
medical history of CD4 cells**				
< 5 %	-6.7 ± 13.7 (n=13)	n.s.	0.944 ± 1.062 (n=13)	n.s.
> 5 %	-5.3 ± 9.6 (n=22)		1.18 ± 1.18 (n=22)	
sex				
male	-5.1 ± 7.2 (n=19)	n.s.	1.18 ± 1.298 (n=19)	n.s.
female	-7.6 ± 16.4 (n=16)		0.826 ± 1.416 (n=16)	
age				
< 5 years	-3.0 ± 3.4 (n= 6)	n.s.	0.354 ± 0.59 (n=6)	0.05
> 5 years	-6.9 ± 13.2 (n=29)		1.18 ± 1.416 (n=19)	
reason for interruption				
inefficiency	-8.7 ± 15.2 (n=17)	n.s.	0.944 ± 1.534 (n=17)	n.s.
others	-3.8 ± 8.2 (n=18)		1.062 ± 1.18 (n=18)	

n.s.= not significant ($p > 0.05$)

* A viral load > 5 log_{10} copies/ml while receiving HAART was defined as "a medical history of a severe increase of viral load".

** A CD4 cell count < 5 % while receiving HAART was defined as "a medical history of severe immunodeficiency".

5. Genotypic resistance during treatment interruption

In addition to plasma HIV RNA and CD4 cell count, the changes in genotypic drug resistance patterns were investigated. Patients were tested for HIV resistance before and after treatment interruption by standard methods. Those patients to whom therapy was reintroduced, were tested again before they started a new drug regimen, to analyse, which molecules were still active and could therefore be used for further treatment. The impact of treatment interruption on the resistance profile was assessed in all cases in which a second genotypic analysis was available during or at the end of the interruption period before antiretroviral treatment was reintroduced. In the first plasma sample of all patients before treatment interruption, 27 of 35 children showed major mutations in the reverse transcriptase gene (RT gene). In 30 cases out of 35 children major mutations of the protease gene were found in the first test. A second test was done for ten children, nine of whom were in the middle of a treatment interruption, whereas one child restarted therapy before the second test took place. Among these ten children however, a general reversion of major mutations in the RT gene occurred in seven patients while the other three showed no change in the

mutation pattern at all. By regarding the seven children who experienced a reversion of resistant virus mutants, we noticed a complete reversion of all mutations in the RT gene in three children. In one child three of the original seven mutations were detected, in another child one of the three that were detected in the first test. The remaining two children showed only one major mutation of the RT gene less compared to the first test of genotypic resistance.

Concerning the protease gene, three of the ten children in question showed at least one or two major mutations less throughout the period of treatment interruption. In one child, however, only one of originally seven major mutations was detected after five months of treatment interruption. It is interesting to see, that the same child has had nine major mutations of the RT gene in the first test, which were no longer detectable in the second test. Five children showed no reversion at all in the protease gene and in one child an additional major mutation was observed in the second test, which had not been detected before treatment interruption. Twelve out of 35 children had stopped antiretroviral therapy because of multiresistance development, four of them are documented to have less major mutations, one of them had completely changed back to the wild type virus. The results show, that a shift to drug-sensitive virus may also occur in HIV-infected children in the absence of antiretroviral drugs.

6. Restarting therapy

In 14 children (40 %) treatment was reintroduced. Reinstitution of antiretroviral therapy was associated with a prompt reduction in plasma viral load and an increase of CD4 cells within one month in all patients who resumed treatment. The 14 children who restarted therapy were seven boys and seven girls with an average age of 11.8 ± 4.8 years and a median age of 12 years [4.3;17.9].

In ten of the 14 cases therapy was restarted because of an important increase of viral load after treatment interruption (above 5 $\log_{10}$ copies/ml) or a severe decrease of CD4 cells (below 15 %). In two cases therapy was restarted because of clinical events such as a LIP (n=1) and a thrombopenia followed by frequent episodes of epistaxis (n=1). Less severe clinical symptoms such as skin allergies (n=3) were hardly decisive for treatment reintroduction, except where they appeared in line with immunological events. In two cases the child or the child's care giver elected to resume therapy. When a restart of antiretroviral therapy was decided, the genotype of resistance was tested for all molecules the child had been taking so far.

7. Treatment combinations used for reintroduction of therapy

The most common therapy for those restarting antiretroviral treatment was a combination of two NRTIs and one NNRTI, which was prescribed to five of the 14 children. Three children restarted with a treatment consisting of two NRTIs and nelfinavir (PI), while a regimen including two PIs was prescribed three times. The other three children took various combinations, all consisting of one NNRTI in addition to PIs or NRTIs.

8. Development of immunological effects after reintroduction of therapy

After reintroduction of antiretroviral therapy, plasma HIV RNA decreased to levels of 2 or 3 $\log_{10}$ copies/ml within one month and undetectable levels of viral replication were finally reached in all children after approximately six months. An increase of CD4 cells of at least 2 % were noted in all children in the first month after restarting therapy. After six months most children had reached a level of at least 15 % of CD4 cells. The clinical symptoms, which had appeared in some children in the absence of antiretroviral therapy regressed quickly within the first two months and no sequels were noted.

9. Case reports

In order to describe the three different types of response to the cessation of antiretroviral treatment, we present three case reports.

9.1 Continual steady state for more than one year after treatment interruption

The patient was born in 1986, having acquired HIV perinatally. At the age of seven months antiretroviral therapy was initiated with a zidovudine monotherapy after a series of infections of the respiratory tract. Due to a haematological intolerance to zidovudine she received a dual therapy of stavudine and lamivudine. At the age of ten she suffered from a serious LIP, so that a combination triple therapy became necessary (lamivudine, stavudine, indinavir). Triple therapy was changed twice because of incompatibility before it was stopped completely for reasons of a still existing pruritus under stavudine, abacavir and nelfinavir. The patient was categorized B3, antiretroviral treatment had been given for 12 years and two months. The lowest CD4 T-cell count had been 4 % (under bitherapy) and the highest plasma HIV RNA detected was 3 $\log_{10}$ copies/ml (under triple therapy). On the day of treatment interruption she had a CD4 T-cell count of 17 % and a viral load of < 1.3 $\log_{10}$ copies/ml. There were no mutations on the RT gene nor on the protease gene.

Plasma HIV RNA increased immediately after treatment interruption to 3.1 $\log_{10}$ copies/ml, while the CD4 T-cell count fell to 15 %. Three months later, viral load had reached a steady level of about 3.7 $\log_{10}$ copies/ml, where it remained over the years with slight moderations, mainly ranging between 3.5 $\log_{10}$ and 4.1 $\log_{10}$ copies/ml. CD4 cells rose up to 21.5 % after five months of treatment interruption and remained at a steady level of about 19 %. In 15 months of treatment interruption, the patient developed no dangerous decrease of CD4 cells, but remained in a steady immunological state until the time of study end in December 2000 without further therapy.

Table 10: Continual steady state after treatment interruption

date	05.07. 1999	31.08. 1999	**10.11. 1999**	**29.12. 1999**	**08.03. 2000**	**07.06. 2000**	**01.08. 2000**	**29.08. 2000**	**31.10. 2000**	**03.01. 2001**
treatment	on	on	**off**	**off**	**off**	**off**	**off**	**off**	**off**	**off**
viral load in RNA copies/ml	150	< 20	**1400**	**5300**	**4800**	**3300**	**12000**	**5900**	**7200**	**5500**
viral load in log_{10} *	2.2	< 1.3	**3.1**	**3.7**	**3.7**	**3.5**	**4.1**	**3.8**	**3.9**	**3.7**
CD4 cells/µl in absolute numbers	516				**379**	**343**	**425**	**340**	**331**	**400**
CD4 cells in %	18.9	17	**15**	**15**	**21.5**	**18.6**	**19.4**	**19.9**	**19.9**	**17.5**

* method used: Amplicor HIV monitor (Roche)

9.2 Immunological effects alternate without reaching a steady state

The patient, born in 1994, interrupted therapy for reasons of inefficiency of therapy. Antiretroviral treatment had been started postnatally with a monotherapy of lamivudine to which zidovudine was added a month later. Finally she received a monotherapy of zidovudine, which was stopped completely after two years since a zidovudine monotherapy did not seem useful. For two years she stayed asymptomatic without therapy, with a CD4 cell count of 34 % and a largely ranging viral load. A combination therapy of two NRTIs and one PI was restarted because of a buccal mycosis. In 1999, the plasma HIV RNA rose to a peak level of > 4 log_{10} copies/ml, demonstrating the inefficiency of her current drug regimen.

Eventually, antiretroviral treatment was stopped two months later. At that time, the viral load was 3.1 log_{10} copies/ml and the CD4 cell count was 32.5 %. She was categorised A1. The lowest CD4 cell count ever detected was 30 % and the highest plasma viremia was > 4 log_{10} copies/ml. Immunological parameters tested after four months of treatment interruption showed a decrease of CD4 cell count to 28.6 % and an increased viral load of 5.7 log_{10} copies/ml.

The CD4 cell count sank to 28.6 % after treatment interruption and the viral load varied between 5.7 log_{10} and 3.7 log_{10} copies/ml. The final CD4 cell count at study end was 33.3 %, which was higher than during antiretroviral therapy. The plasma HIV RNA was 4.5 log_{10} copies/ml. The patient stayed asymptomatic until study end 15 months after treatment interruption.

Table 11: Immunological effects alternate without reaching a steady state

date	13.07.1999	22.09.1999	**25.01.2000**	**05.04.2000**	**15.11.2000**
treatment	on	on	**off**	**off**	**off**
viral load in RNA copies/ml	>10000	1200	**51730**	**4900**	**53000**
viral load in log_{10} *	> 4	3.1	**5.7**	**3.7**	**4.5**
CD4 cells/µl in absolute numbers	503	521	**353**	**330**	**427**
CD4 cells in %	33.1	32.5	**28.6**	**25**	**33.3**

* method used: Amplicor HIV monitor (Roche)

9.3 Prompt relapse of high HIV RNA levels requiring early restart of therapy

After a perinatal HIV infection in 1992, this patient developed a buccal mycosis and started a zidovudine monotherapy at the age of one year. Didanosine was added in 1996. After developing a severe neutropeny in 1997, therapy was changed to a triple combination therapy. But the neutropeny continued and the child showed great difficulty in tolerating nelfinavir, so that in regard of the low CD4 count and a high viral load, therapy was finally changed into another triple combination regimen (lamivudine, stavudine, amprenavir). The viral load continued rising to peak levels of more than 5 log_{10} copies/ml, while the CD4 cell count stayed under 10 %. High plasma HIV RNA had been common in the medical history of this patient, the highest being > 6 log_{10} copies/ml, the lowest CD4 T-cell count was 2 %.

Because of the many changes of antiretroviral treatment combinations and only minor success in the suppression of plasma HIV RNA, a treatment interruption was decided. On the day therapy was stopped the detected viral load was 5.1 log_{10} copies/ml, the CD4 T-cell count 11 %.

Two months after discontinuing antiretroviral treatment plasma HIV RNA had risen to 6 log_{10} copies/ml and CD4 cells decreased to 4 %, in addition, erythema and pruritus had developed. Subsequently, antiretroviral therapy was restarted three months after the initial treatment interruption. After restarting therapy, plasma viral loads went down to less than 2 log_{10} copies/ml within one year and CD4 cell levels rose to 19 %. The child stopped her treatment again 18 months after the first interruption, because of difficulties in taking amprenavir and immediately experienced a viral load of > 6 log_{10} copies/ml while the number of CD4 cells sank to 8 %. Treatment had been stopped for two different reasons: firstly as a therapeutical strategy due to an insufficient treatment, and secondly due to a personal dislike to the regimen after a long period of a steady and asymptomatic state of health. Both immunological reactions were

similar. No sequelae were detected after reinitiation of therapy, even though the patient had a history of severe plasma viremia and immunodeficiency. It is noteworthy, that plasma HIV RNA fell clearly below the level detected before therapy had been interrupted for the first time.

Table 12: Prompt relapse of high VL levels and low CD4 cell counts after treatment interruption

date	02.02.1999	09.03.1999	**11.05.1999**	**29.06.1999**	13.07.1999	11.08.1999	14.09.1999
treatment	on	on	**off**	**off**	on	on	on
viral load in RNA copies/ml	358000	130000	**858000**	**545800**	1032	731	< 350
viral load in log_{10} *	5.55	5.11	**5.93**	**5.74**	3.01	2.86	< 2.54
CD4 cells/µl in absolute numbers	not done	166	**16**	**39**	not done	not done	97
CD4 cells in %		11	**4.1**	**3**			4.3

* method used: Amplicor HIV monitor (Roche)

date	16.11.1999	18.01.2000	21.03.2000	13.06.2000	23.08.2000	24.10.2000	**28.12.2000**
treatment	on	on	on	on	on	on	**off**
viral load in RNA copies/ml	< 200	< 220	< 20	< 400	<400	<400	**>1000000**
viral load in log_{10} *	< 2.3	< 2.3	< 1.3	< 2	< 2	< 2	**> 6**
CD4 cells/µl in absolute numbers	181	256	not done	365	462	574	**not done**
CD4 cells in %	7.4	16.7		14.9	17.6	18.7	**8**

* method used: Amplicor HIV monitor (Roche)

IV. DISCUSSION

We present the results of a retrospective study dealing with the interruption of antiretroviral therapy in HIV-1 infected children. It is the first paediatric study about treatment interruption to date and was located in the Hôpital Necker-Enfants Malades in Paris, France.

In this study we collected first information on treatment interruption in HIV-infected children to find out, whether it could be feasible. 60 % of the children in our study group (n=21) remained without therapy for a prolonged period of time, thus reducing the risk of toxicity and the development of resistant virus strains. Even though 14 children restarted antiretroviral treatment, a general reduction of time on antiretroviral therapy could be achieved in all children. The results show a viral rebound in all patients within the first few weeks of treatment interruption. Certain subgroups however showed tendencies towards a less significant loss of CD4 cells and a less severe increase of viremia during the time of treatment interruption. In general we found a trend, that male children lost less CD4 cells and had a slightly less increase of HIV RNA copies during treatment interruption than female patients. Children under five years of age lost less CD4 cells and gained less HIV RNA copies than children above that age. Patients with a medical history of good immune responses and low HIV plasma viremia lost less CD4 cells and gained less HIV RNA copies than children with a severe medical history. In contrast, children who stopped therapy due to an insufficient treatment were seen to loose more CD4 cells and gain more HIV RNA copies than those, who had stopped therapy for other reasons (toxicity/inconvenience). Due to a shortage of information on treatment interruption in HIV-infected children, the results we present can only be compared to those of adult studies.

According to the Swiss HIV Cohort Study in adults, occasional treatment interruptions of less than three months neither worsen nor improve disease outcome on an average term (three to four years). The results suggest that interruptions might be non-risky, particularly when viremia is low and CD4 cell count is high, but they still require confirmation [97]. Hirschel reported from the Swiss-Spanish Intermittent Treatment Trial (SSITT), that a substantial minority of patients taking combination therapy can be safely managed without drugs for at least several months. He also noted, that the best results came from the rare patients who started combination therapy during primary HIV infection [48]. In the meantime multiple studies investigated the different outcomes of treatment interruption in adults [100,99,19,73,24]. These trials were performed for different reasons (see I.1.6). A number of clinical trials using highly diverse protocols of STI have been conducted in patients who have initiated their first highly active antiretroviral treatment either during primary [91] or during chronic [36,85,86,33] HIV infection. Protocols used in STI studies usually vary widely with regard to the number and the duration of STI. However, their results are often similar to ours concerning the fact that viral rebound occurs in principally all patients shortly after

treatment interruption and that certain patients are more likely to profit from treatment interruptions than others.

1. Random treatment interruption

There are few reports on "random withdrawal" of antiretroviral therapy in patients who did not undergo a scheduled program for treatment interruption. One report from the 9th Conference on Retroviruses and Opportunistic Infections (CROI) in Seattle in February 2002 [56] points out, that interruption of antiretroviral therapy can only be done in a strategically scheduled interruption. A European study of more than 3,600 HIV-infected patients reports that, of the 16 % who interrupted their therapy for other reasons, 51 % suffered an AIDS-defining illness or death during the interruption. The remaining 49 % restarted therapy [56].

The EuroSIDA observational study by Lundgren et al. [66] reports data on the risk of developing a new AIDS-defining event (ADE) and/or death after interrupting or stopping antiretroviral therapy. Only patients who had been off therapy for at least three months were included in the analysis. After three months off therapy, the median reduction in CD4 cell count was 30 cells/mm^3 and the increase in plasma HIV-1 RNA level was 1.0 $\log_{10}$ copies/ml. Overall, the risk of development of an ADE or death increased by more than fivefold, compared with the risk while receiving antiretroviral therapy. The risk was directly correlated to the CD4 cell count at the time of therapy discontinuation. Lundgren conceded that this observational study is limited by not being designed to evaluate structured treatment interruptions, and therefore may be biased by analysing patients with many different reasons for stopping therapy, possibly including some who were in the terminal stages of disease. The strong correlation between lower CD4 cell count and much higher risk of developing an ADE/death is not surprising and has been noted in many other studies before; however, the magnitude of the increased risk (80 % among those who stopped therapy with a CD4 cell count < 50 cells/mm^3) is noteworthy. In any case, the risk of therapy interruption in patients with decreased CD4 cell counts and development of ADE/death in this study is even higher than in similar reports by Veronica Miller [73] and Steven Deeks [19] and their colleagues when evaluating whether a treatment interruption results in a beneficial reversion of viral phenotype from drug-resistant to wild-type, and provides reason for pause when considering this strategy.

Another European study came to a similar result concerning random interruption of antiretroviral therapy and also reports that the risk to develop serious complications seemed particularly strong for those whose CD4 cell counts were already fairly low around 200 cells/mm^3 [56].

In our study, none of the 35 children, who interrupted antiretroviral therapy randomly, suffered from AIDS-defining illness or death during the interruption. According to Hirschel [48], this could be due to the fact, that all children had been treated during primary infection and therefore had better chances to maintain a safe immunological state without therapy for months. Regarding our results, random interrup-

tion of antiretroviral treatment may not necessarily be dangerous for every patient, provided the patient is under regular medical observation. This would suggest, that there are possibly more options for treatment withdrawal, which need further investigation in children.

2. Medical history before treatment interruption

An observational cohort study at the Johns Hopkins University includes 101 patients who discontinued therapy with the intention of restarting based on laboratory or clinical parameters [87]. Using a baseline CD4 cell count of > 500 cells/mm^3 as a referent, those with a CD4 cell count < 200 cells/mm^3 were seven times more likely to resume therapy. Patients with high viral load set points and low CD4 cell count at the time of their original start of therapy were 2.9 times more likely to resume therapy [87]. The study refers to the pulse therapy strategy, in which the goal is to keep the CD4 cell count above a predetermined threshold using cycles of therapy followed by prolonged interruptions. Presumably those patients with relatively low viral load set points and good CD4 cell count responses to HAART will be able to discontinue therapy safely for a longer period of time. Apparently the original state of the immune system plays an important role for the outcome of treatment interruption not only in adults but also in children as we can conclude from our study. Similar to the adult studies named above, children with low CD4 cell counts (CD4 cells < 5 %) and high viral load set points (> 5 $\log_{10}$ copies/ml) lost more CD4 cells and gained more HIV RNA copies during treatment interruption than others. In addition, they were more likely to resume therapy (ten of the 14 children who restarted antiretroviral therapy were children with a medical history of low CD4 cells and high viral loads).

3. Early start versus late start of antiretroviral therapy

A debate continues over whether to start therapy as soon as possible after infection or at a later point. Arguments for very early intervention are, that therapy is able to stop the virus from destroying as much of the immune system as it might if left unchecked. Later intervention would delay the development of side effects and toxicities [56].

Most children of our study group received antiretroviral therapy shortly after birth, so that they are candidates for early intervention strategies. According to a study by Walker and colleagues, early intervention during seroconversion predisposes the patient to good results during and after treatment interruption [99]. Patients of Walker's study were diagnosed and treated before Western blot seroconversion and eight of 14 patients maintained viral loads < 5000 copies/ml after at least one STI. Similar successful results could hardly be seen in other studies, which is probably because treatment was not initiated as early as in Walker's study. It is possible however, that children who have been treated since birth could benefit even more from antiretroviral treatment interruption than adults with chronic HIV infections. On the other hand, the immune system is not yet fully developed at the time of birth, which is another factor

to influence the results of treatment and treatment interruption. Controlled trials are necessary, in order to assess the effectiveness of treatment interruption after early onset of antiretroviral therapy. Unfortunately, only few patients are diagnosed during acute infection and controlled trials are therefore difficult to carry out in adults.

Rosenberg and colleagues from the Massachusetts General Hospital have studied a group of acute infected patients who underwent STIs in order to augment HIV-specific immune responses [91]. HIV RNA levels increased in all patients during the first STI within 17 days, but soon dropped in some patients below 5,000 copies/ml. It is noteworthy that, compared to the first STI, the majority of patients experienced a much slower rebound in HIV RNA levels during the second STI that would require them to restart therapy. This would account for the thesis, that immune response is broadening and is becoming stronger during treatment interruptions, which may indicate why the patients are able to control their virus. The role of STI in patients with primary infection appears promising, but the potential in chronically infected patients (infected with HIV for more than six months) is not very clear yet. According to Dybul [27], some studies do suggest that autoimmunisation can occur in a very small percentage of effectively treated chronically infected patients, but the benefit of autoimmunisation in these patients over more than one year off therapy is almost zero.

4. Short and long cycles of STI

Another study by Dybul et al. [24] reports that in a small cohort of patients with undetectable plasma viral loads after HAART, repeated cycles of one-week-on followed by one-week-off HAART did not lead to rebound of viral load during the drug-free period in any of the eight subjects investigated. This schedule is thought to minimise the emergence of drug resistance during interruption or reinitiating phases, since viral rebound typically takes longer than seven days to occur. HIV replication did not resume within one week after treatment cessation, so that emergence of drug-resistant virus during this type of STI was deemed unlikely.

A recent study from the Swiss HIV Cohort Study in a greater number of patients showed a different result however. The one-week-on-one-week-off schedule of the ongoing Staccato Study [2] showed a high failure rate and had to be terminated prematurely since 53% of the group were classified as virological failure at the end of the week off therapy. These data are in conflict with what was previously reported by Dybul et al. and are therefore of great concern regarding this strategy. There exist a number of differences between these two studies though. Patients enrolled in the two studies were on different pre-study HAART regimens and this may present a major confounding factor. Moreover, 70% of the patients in the Dybul-study had a history of interleukin-2 therapy and a high CD4 cell count prior to starting STI [1].

In STI studies using longer cycles (two-months-on/one-month-off), in which viral load is allowed to rebound, resistance to lamivudine (M184 V/I) and NNRTIs has typically emerged in patients treated with

regimens containing those agents [28]. One STI study that used longer cycles is for example the study by Fischer et al. [29]. It includes 14 chronically HIV infected patients who are enrolled in the SSITT study (Swiss Spanish Intermittent Treatment Trial) and presents results after examining the viral rebound in two-week STI periods. They concluded, that significant viral replication can be induced during one week STI, which may increase the risk of the development of drug resistance during long-term cycles.

However, there are also some differences between the studies of Dybul et al. and Fischer et al., which could have led to different outcomes. These are again the use of different drugs and also different DNA assays or the use of immunomodulatory therapy before STI (Dybul et al. used interleukin-2). Fischer and colleagues have studied 14 patients, Dybul observed ten patients, two of which left the study before study end for personal reasons. In general, assessing small numbers of patients can lead to different outcomes because of unrecognised confounding factors.

In his latest study, Dybul et al. also evaluated the effect of long-cycle structured intermittent therapy (SIT; four weeks without HAART followed by eight weeks with HAART) on serum lipid levels, hepatic enzymes, high-sensitivity C-reactive protein (hsCRP), plasma HIV RNA levels, CD4 and CD8 T-cell counts, markers of cellular immune activation, and CD4 HIV specific immune response [26]. They demonstrated that in contrast to the short-cycle study [24], long intermittent therapy with HAART did not result in a diminution of toxicities associated with administration of HAART. In addition, it did not enhance or decrease measurable immunologic parameters after 48 weeks of long-cycle SIT versus continuous HAART. However, because periodic reductions in serum total cholesterol and triglyceride levels were observed during the without-HAART intervals, there may be a long-term advantage to intermittent decrease in certain markers of toxicity [26].

The SSITT by Hirschel et al. used a two-weeks-on/eight-weeks-off schedule in 133 chronically infected patients who had been successfully treated with antiretroviral therapy [48]. An improved HIV-specific CD8 response was observed with treatment interruption, but did not correlate with virological response. After one year of intermittent therapy, few patients maintained low-level viremia during treatment interruption, and only 6 % remained off therapy at week 96, which was the ultimate goal of the trial. But since this was not a controlled trial, it is difficult to attribute these successes to treatment interruption itself. Hirschel pointed out, that the SSITT suggests that STI will rarely be sufficient to attain the goal of low viremia without antiretroviral therapy and that the results of his study essentially refute the idea that autoimmunity might be achieved through random interruption of therapy.

5. Resistance after treatment interruption

All children in our study group were tested for genotypic resistance before they interrupted antiretroviral therapy. Ten of the 35 children underwent a second test while being off therapy, seven of which showed a reversion of major mutations and one child showed a complete shift to wild-type virus. Specific

studies are needed to find out in what way treatment interruption can influence multidrug resistance in children and whether there are any relations to CD4 cell count and viral load, as has been observed in some adult studies.

Miller's team for example observed a complete shift to wild-type virus in 28 of 45 patients during treatment interruption [73]. There was an average viral load increase of 0.7 $\log_{10}$ copies/ml during the patients' STIs. The median CD4 cell count was 49 cells/mm^3 by the end of the treatment interruption, reflecting a profound median drop of 89 cells/mm^3. The decline in CD4 cells was significantly greater among those who experienced a shift to wild-type virus compared with other patients who did not (-122 versus -25 cells/mm^3).

That shows, that a shift to drug-sensitive virus does occur, but drug-resistant virus still persists. In contrast, patients staying on a failing regimen may experience a much slower decline in CD4 cell count, or may even remain stable, presumably because their predominant virus has mutations decreasing viral replication [35].

6. Summary

At present no other paediatric studies on antiretroviral treatment interruption are available. There are certain similarities between the responses to treatment interruption of adults and children. Even though repeated treatment interruptions do not result in permanent low viremia without antiretroviral therapy [48], treatment interruptions may still be viable in patients (especially children) treated during primary infection. Frequent treatment interruptions as a form of autovaccination to boost HIV-specific immune responses are unlikely to be proven effective in chronic HIV infection. Adults and children with good immune responses before treatment interruption (high CD4 cell counts and low viral load setpoints) are probably able to discontinue therapy for a longer period of time and this may effectively reduce toxicity and save therapeutical costs. When treatment is interrupted in patients who fail HAART and who have drug-resistant HIV, the drug-sensitive wild-type may replace the resistant virus strain but it is very unlikely that this confers clinical benefit. It remains to be seen, whether these results are transferable to patients outside of clinical trials, so that large studies are needed to further compare the risks and benefits of treatment interruption.

V. CONCLUSION

Combination therapy is still the standard for HIV-infected patients, who are symptomatic or who reach certain thresholds for CD4 cells and/or viral load. Unfortunately, long-term therapy is hampered by chronic toxicity, emergence of drug resistance and a sometimes low adherence to complicated regimens. Studies in adults demonstrate at least partial beneficial effects of structured treatment interruption (STI) as a strategy to reduce the limitations of HAART.

At present, there are no data concerning treatment interruption in children. Our study shows, that treatment interruption in HIV-infected children is feasible in some patients. Of the 35 children who had interrupted treatment 21 children managed to stay without further therapy for a prolonged period of time, in some cases even for three years. Even though we noticed a viral rebound in all patients within the first few weeks of treatment interruption, there were different profiles of immunological reaction. Especially children, who had good immune responses before treatment interruption and who had stopped therapy at an early age before running the risk of virological treatment failure, showed the best results as was also seen in adult studies. On the contrary, patients who had stopped therapy because of virological treatment failure had a greater loss of CD4 cells and showed an important increase of viral load in the time off therapy so that a prolonged treatment interruption was not advisable for them. But even though therapy had to be restarted for 14 patients, a general reduction of time on drug therapy could be achieved.

The idea of treatment interruption is based on certain results in adult studies, which show, that in some patients successive interruptions might increase the duration of immunologic control of viral replication while off therapy. Therefore, the need for antiretroviral treatment may progressively be reduced. Also, the patient's immune system can occasionally be effectively boosted at least to some amount after the discontinuation of therapy. In the context of a failing antiretroviral therapy and emergence of genotypic resistance STI may re-establish a dominant population of drug sensitive virus. However, current evidence shows that such goals may be unrealistic except in patients treated during primary infection. Therefore, it is important to diagnose the HIV infection in patients in the acute phase of the infection. In chronically infected patients a beneficial effect of STI on viremia control was more difficult to demonstrate since the virus rebounded in basically all patients.

Similar to adult studies, our data suggest, that a significant proportion of HIV-infected children can be safely taken off therapy for a prolonged period of time to reduce toxicity and costs, especially those with low viral load and good CD4 cell counts before treatment interruption. But further investigation of treatment interruption in paediatric patients is necessary until proper advice can be given. What is needed are prospective studies to evaluate the advantages and risks of new treatment strategies in HIV-infected children.

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ABSTRACT

This study deals with the effects of treatment withdrawal in HIV-infected children. In a retrospective survey 35 HIV-infected children who were under medical treatment in the Hôpital Necker-Enfants Malades in Paris, France, were observed concerning their discontinuation of antiretroviral therapy after months or years of receiving treatment. All children had acquired HIV infection through vertical transmission and received antiretroviral therapy for at least ten months before interrupting therapy for different reasons between 1996 and 2000 (insufficiency, toxicity, inconvenience).

The study group consisted of 16 girls and 19 boys with an average age of 10.4 ± 4.9 years at the time treatment was stopped. The average time of observation after treatment interruption was 325 ± 294 days. Plasma HIV RNA was tested approximately every three months using an RNA polymerase chain reaction test. The CD4 cell count was regularly checked approximately at the same time as the viral load and measured by flow cytometry. To estimate the presence of major mutations in the HIV reverse transcriptase and protease genes, genotypic resistance was tested before treatment interruption, in the absence of antiretroviral treatment and in the case of a restart of therapy, when a new combination of efficient agents had to be defined. Based on the assumption that the course of HIV RNA viral load and CD4 cell count depended on certain factors, distinctions were made between the medical history of viral load and CD4 cell count, age, sex and reason for treatment interruption. The changes of CD4 cell count and viral loads were then analysed to calculate the percentage of lost CD4 cells and the increase of viral load per day of treatment interruption.

By the time the study was finished in December 2000, 21 children were still without any further antiretroviral therapy whereas 14 children had to restart therapy. Even though we noticed a viral rebound in all patients within the first few weeks of treatment interruption, there were different profiles of immunological reaction. Especially children, who had good immune responses before treatment interruption and who had stopped therapy at an early age (under 5 years) before running the risk of virological treatment failure, showed the best results. On the contrary, patients who had stopped therapy because of virological treatment failure had a greater loss of CD4 cells and showed an important increase of viral load in the time off therapy so that a prolonged treatment interruption was not advisable for them. But even though therapy had to be restarted for 14 patients, a general reduction of time on drug therapy could be achieved. Similar to adult studies, our data suggest, that a significant proportion of HIV-infected children can be safely taken off therapy for a prolonged period of time in order to reduce toxicity and costs.

ZUSAMMENFASSUNG

Die vorliegende Studie befasst sich mit den Folgen des Therapieabbruchs bei HIV-infizierten Kindern. 35 Kinder, die sich aufgrund ihrer HIV Infektion im Hôpital Necker-Enfants Malades in Paris in medizinischer Behandlung befanden, wurden retrospektiv hinsichtlich der Unterbrechung ihrer antiretroviralen Therapie untersucht. Alle Kinder waren durch vertikale Transmission infiziert worden und hatten mindestens zehn Monate lang eine antiretrovirale Medikation erhalten, bevor diese zwischen 1996 und 2000 aus verschiedenen Gründen abgesetzt wurde (Ineffizienz der Therapie, Toxizität, persönliche Entscheidung).

Die Studiengruppe bestand aus 16 Mädchen und 19 Jungen in einem durchschnittlichen Alter von 10,4 Jahren ± 4,9 zum Zeitpunkt des Therapieabbruchs. Der Beobachtungszeitraum nach dem Therapieabbruch betrug im Durchschnitt 325 ± 294 Tage. Der Plasma HIV RNA Spiegel wurde in Abständen von drei Monaten mittels eines RNA-PCR-Tests (polymerase chain reaction) ermittelt. Gleichzeitig wurde die Anzahl der CD4 Zellen mit Hilfe von Flowzytometrie untersucht. Um das Auftreten von Virusmutationen des Protease- bzw. des HIV Reverse Transkriptase-Gens festzustellen, wurde der Genotyp der Resistenzen vor dem Therapieabbruch, in der Therapiepause und im Falle einer Wiederaufnahme der Therapie untersucht, letzteres, um eine neue Kombination von wirksamen Medikamenten festlegen zu können. In der Annahme, dass der Verlauf von Viruslast und Anzahl der CD4 Zellen in Abhängigkeit zu bestimmten Faktoren steht, wurden folgende Kategorien unterschieden: bisheriger Verlauf der Viruslast und der Anzahl der CD4 Zellen, Alter, Geschlecht und Grund für den Therapieabbruch. Veränderungen in der Anzahl der CD4 Zellen und der Viruslast wurden in der Folge analysiert, um den Prozentsatz des Verlustes von CD4 Zellen sowie den Anstieg der Viruslast pro Tag der Therapiepause zu berechnen.

Zum Zeitpunkt des Studienendes im Dezember 2000 waren 21 Kinder noch immer ohne antiretrovirale Therapie, während 14 Kinder die Therapie wieder aufnehmen mussten. Bei allen 35 Patienten war innerhalb der ersten Wochen nach Therapieabbruch ein prompter Wiederanstieg der Viruslast zu verzeichnen, es ergaben sich jedoch deutliche Unterschiede im Hinblick auf die immunologische Reaktion. Die besten Ergebnisse konnten bei den Kindern festgestellt werden, die bereits vor dem Therapieabbruch gute Immunantworten gezeigt, beziehungsweise in einem frühen Alter (unter 5 Jahren) die Therapie abgesetzt hatten. Im Gegensatz dazu war bei Kindern, die ihre Therapie wegen Ineffizienz unterbrochen hatten ein größerer Verlust von CD4 Zellen und ein massiverer Viruslastanstieg zu beobachten. Trotz der Wiederaufnahme der antiretroviralen Therapie bei 14 Kindern konnte eine generelle Reduktion der Therapiezeit erreicht werden. Die Resultate belegen, ähnlich wie aus Studien bei Erwachsenen bekannt, dass eine gewisse Anzahl HIV-infizierter Kinder ohne größeres Risiko die antiretrovirale Therapie unterbrechen und auf diese Weise sowohl toxische Nebenwirkungen als auch Kosten gering halten kann.

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