

## INDUCTION MAINTENANCE CONCEPT FOR HAART AS INITIAL TREATMENT IN HIV INFECTED INFANTS

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### Abstract

**Background:** Early initiated antiretroviral therapy (ART) in HIV infected infants leads to improved long-term viral suppression and survival. Guidelines recommend initiating therapy with a triple ART consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and either one additional non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). Compared to older children and adults, viral relapse is seen more frequently in infants receiving triple ART. We now address the possibility of a more potent ART with a quadruple induction and triple maintenance therapy.

**Methods:** We examine the longitudinal course in four HIV infected infants, who were referred from other centers and could not be recruited to multicentre trials. We introduced ART initially consisting of two NRTIs, one NNRTI and one PI and later discontinued the PI at the age of 12 months maintaining a triple regime consisting of two NRTIs and one NNRTI.

**Results:** Provided that therapy adherence was maintained we observed an effective sustained decline of viral load and significant CD4 cell reconstitution even after switching to a triple regime. No drug associated toxicity was seen.

**Conclusion:** We suggest that a four drug therapy might be a possible initial therapy option in HIV infected infants, at least in those with a high viral load, followed by a maintenance triple regime after 12 months of therapy.

**Key words:** HIV, HAART, anti HIV agents, infants, viral load

### BACKGROUND

European [1] and US guidelines [2] recommend to start with ART in all HIV infected infants below the age of 12 months irrespective of clinical or immunological stage. In particular, in infants infected despite attempted mother to child transmission prophylaxis, ART should be commenced as soon as the diagnosis is confirmed. Evidence that early therapy in all infected infants leads to a significant reduction of morbidity

and mortality compared to deferring treatment came from CHER trial conducted in South Africa [3] and from the European Infant Collaboration group [4]. In the CHER trial early HIV diagnosis and early treatment reduced early infant mortality by 76%.

Virological failure continues to be a major challenge after initiating early ART in infants. PENPACT-1 showed recently that in children following the recommended initial regimen with two nucleoside reverse transcriptase inhibitors (NRTI) and one protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) 71% were on first-line ART after a median follow-up time of 5 years, nevertheless only 56% had continued viral load suppression [5]. Previous studies even show that consistent long term viral suppression with HIV-1 RNA below detection limit is achieved in less than 50% of the children with first line triple ART. Children with an increased viral load are at risk for a rapid progression of disease and therapy failure [6, 7]. While this drug combination follows official recommendations [1, 2], several studies have addressed the possibility of introducing a more aggressive initial regime consisting of four antiretroviral drugs [8-13].

Luzuriaga et al studied 52 infants receiving different therapy regimes and demonstrated an improved long-term viral suppression in patients with a quadruple therapy initiated before the age of 3 months [9]. A tolerability and safety study showed low adverse events in children receiving a 4- and 5- drug regimen [10]. Based on these findings and our own experience with standard triple ART we report on four vertically HIV infected children, in whom a quadruple antiretroviral drug scheme was introduced consisting of two NRTIs, one NNRTI and one PI. We furthermore address the possibility of starting with a quadruple therapy in the first year of life and later maintaining a triple regime consisting of two NRTIs and one NNRTI.

### METHODS

#### PATIENTS

Between 2004 and 2009 four infants were initially treated with a quadruple therapy at our HIV outpatient clinic. All were referred to us from other centers. All mothers underwent a caesarean section. In two patients, maternal HIV diagnosis was made at a late stage

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of pregnancy, thus transmission prophylaxis to prevent mother to child infection was incomplete. In both children HIV infection was revealed after birth; in patient 1 at the age of 4 weeks, in patient 2 at the age of 5 months. In mothers of patient 3 and 4 a caesarean was performed due to obstetric problems. Maternal HIV was not diagnosed until after birth, when their children were admitted due to repeated watery wasting-syndrome-like diarrhea, failure to thrive and pneumocystis jiroveci pneumonia. For detailed patient characteristics see Table 1. After discharge, patients were followed at least once to twice monthly, before they were switched to a trimonthly interval, once a good viral control was achieved. Prophylaxis of opportunistic infections included trimethoprim-sulfamethoxazole (patients 1,2,4), atovaquon (patient 3)

and i.v. immunoglobulines (patient 3-4) in the children's first year of life.

#### ANTIRETROVIRAL DRUGS

In all patients we introduced a regimen consisting of two NRTIs, one NNRTI and one PI. In the light of existing literature that quadruple therapy might have a positive effect on initial viral load without any major adverse consequences our studies strongly conformed to the Helsinki Declaration. Informed consent regarding implementing a quadruple drug regimen and publication of anonymized data was obtained from all parents. In patient 1 resistance testing was carried out, which did not imply any drug restrictions. Antiretroviral drugs used for therapy were given at the following

Table 1. Patient characteristics.

Patient	1	2	3	4
Mode of delivery	Caesarean (38 <sup>th</sup> week)	Caesarean (29 <sup>th</sup> week)	Caesarean	Caesarean (41 <sup>st</sup> week)
Prepartal prophylaxis	ZDV, 3TC, NVP (5 weeks)	None	None	None
Intrapartal prophylaxis	ZDV	None	None	None
Postnatal prophylaxis	ZDV (4 weeks)	2 x NVP; ZDV + 3TC (6 weeks)	None	None
Age at diagnosis	4 weeks	5 months	2.5 months	4.5 months
Initial HIV-1 RNA load (copies/ml)	500 000	16 000	139 670	3 600 000
Initial CD4 cell count (absolute cells, $\mu$ l / %)	3732 / 44	2515 / 43	1093 / 17	330 / 6
Initial CD4 cell count (ARP, %)	106	100	44	13
<b>4 drug HAART</b>	<b>ABC, 3TC, LPV, NVP</b>	<b>ABC, 3TC, LPV, NVP</b>	<b>ZDV, 3TC, NFV, NVP</b>	<b>ZDV, 3TC, LPV, NVP</b>
Maximal decline of viral load (log change and duration)	5 log; 4.5 months	4 log; 2 months	5 log; 2 months	5 log; 6 months
Duration of 4 drug HAART	11 months	13 months	13 months	10 months
Clinical toxicity	*Atopic dermatitis	None	None	None
Laboratory toxicity	<b>AST</b> (1.4 x ULN - Grade 1); <b>AP</b> (2.2 x ULN - Grade 1); <b>Cholesterol</b> (235 mg/dl - Grade 2); <b>*GGT</b> (18.6 x ULN)	<b>AP</b> (1,4 x ULN - Grade 1); <b>▲CK</b> (4.2 x ULN - Grade 1); <b>*GGT</b> (2.1 x ULN)	<b>AP</b> (1,2 x ULN - Grade); <b>CK</b> (2.2 x ULN - Grade 0), <b>*GGT</b> (7.2 x ULN)	<b>AP</b> (1.8 x ULN - Grade 1); <b>CK</b> (2.4 x ULN - Grade 0); <b>*GGT</b> (27 x ULN); <b>Triglycerides</b> (498 mg/dl - Grade 1)
<b>3 drug HAART</b>	<b>ABC, 3TC, NVP</b>	<b>ABC, 3TC, NVP</b>	<b>ZDV, 3TC, NVP</b>	<b>ZDV, 3TC, NVP</b>
Viral load at last visit (copies/ml)	16801	<1	<1	48
Follow up time	50 months	50 months	34 months	27,5 months

*Antiretroviral drugs:* Abacavir (ABC), Lamivudine (3TC), Lopinavir (LPV), Nelfinavir (NFV), Nevirapine (NVP), Zidovudine (ZDV); Adverse effects were graded according to DAIDS (Division of AIDS, NIH), ULN = upper limit of normal, \*no DAIDS criteria; ▲elevated 4 weeks after switching to a triple drug regime

daily dosage: twice 180mg of ZDV/m<sup>2</sup> of body-surface area, twice 150-200mg of NVP/m<sup>2</sup>, twice 300mg of LPV/m<sup>2</sup> (increased due to addition of NVP), twice 55mg of NFV/kg body weight, twice 8mg of ABC/kg, twice 4mg of 3TC/kg. Adverse effects were graded according to DAIDS (Division of AIDS, NIH) [14]. Therapeutic drug monitoring was applied in patient 1 as viral relapse in this patient suggested malcompliance.

PLASMA HIV-1 RNA, DETERMINATION OF LYMPHOCYTE SUBSETS BY FLOW CYTOMETRY AND LABORATORY ANALYSIS

Plasma viral load was determined by quantitative PCR, the lower limit of detection of the assay was 50 (Quantiplex 3.0 HIV-RNA, Chiron, Fernwald Germany) and 40 (m2000rt RealTime HIV-1 assay, Abbott Laboratories, Abbott Park, IL) HIV1-RNA copies/ml in plasma. CD4 cells were measured with routine flow cytometry (FACSCalibur, BD) applying the following conjugated anti-human mAbs: CD8- fluorescein-isothiocyanat, CD4- phycoerythrin, CD3- peridinin chlorophyll protein (Becton Dickinson, Bridgeport, NJ). Absolute CD4 T cell numbers were related to age and

age-related percentages (ARP) were calculated as published [15]. Routine clinical chemistry was performed to determine adverse effects according to DAIDS.

RESULTS

Therapy was initiated in four children as seen in Table 1. In all patients quadruple treatment was initiated immediately after they were seen at our department for the first time and diagnosis was made (range: 4 weeks to 5 months). All patients have been seen at our department in regular intervals. Two children have nearly reached the age of five [mean time of follow up: 42 months, range: 27,5 months - 50 months].

VIROLOGICAL RESPONSE

All patients experienced a tremendous decrease of viral load. In all patients we observe a viral load below 500 copies/ml within a mean time of 2.25 months of therapy. All patients had a significant decline in viral load (4-5 log<sub>10</sub> within 6 months of therapy) and three patients had a viral load below level of detection within a mean time of two months (Fig. 1). Switching to a triple regime was not followed by a viral relapse. However, in

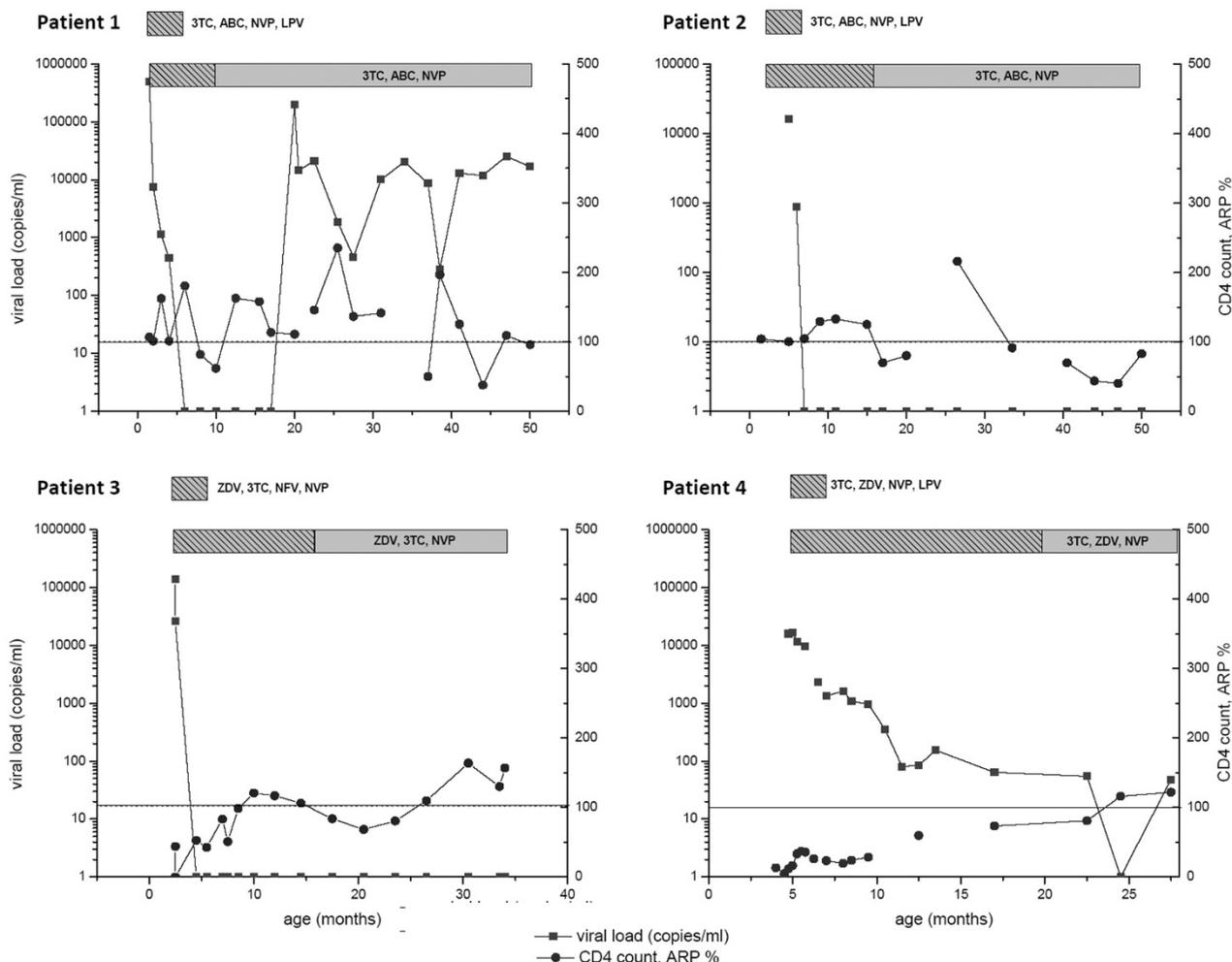


Fig. 1. Peripheral blood viral load (copies/ml) and CD4 count (age related percentage) over time in infants with antiretroviral therapy. The upper horizontal bars show the quadruple and triple drug regime of each patient over time. The horizontal dotted line in each graph represents 100% of age related CD4 cells.

patient 1 we observed an increased viral load at the age of 20 months. Therapeutic drug monitoring showed that non-compliance has been the major factor for viral failure. In patient 3 we even see a HIV seroreversion [16] as published. Thus all patients remain at the initial antiretroviral regime, as viral load is sustaining.

#### CLINICAL FOLLOW-UP

Patient 1 and 2 were in a good clinical condition, as the diagnosis was established due to a known maternal HIV infection, while in patients 3 – 4 HIV was diagnosed, when they were admitted due to a critical AIDS-defining condition. After introducing quadruple therapy both patients experienced a profound improvement of the general condition. Till now all patients remain free of further acquired AIDS defining conditions or severe illness.

#### IMMUNRECONSTITUTION

Absolute CD4 cells and CD4 age-related-percentages (see Fig. 1) in patient 1 and 2 have been initially within the estimated range and hence no long-term change was observed. Patient 3 and especially patient 4, suffering from a seriously depleted CD4 cell count (40% and 20% of age-related-percentages), experienced a reconstitution (4.5 months in patient 3, 12 months in patient 4) during the observation period (> 100% age-related-percentage).

#### SAFETY

Quadruple therapy was well tolerated in all patients. All patients gained weight and height. Patient 1 developed atopic dermatitis; in patient 3 seborrheic dermatitis, which already begun before ART, was sustained under treatment. We did not observe any major drug toxicity in any patient. A slight elevation of creatine kinase (patient 2,3,4), triglycerides (patient 4), total cholesterol (patient 1), aspartate transaminase (patient 1), alanine transaminase (patient 1), gamma-glutamyl transferase (patient 1,2,3,4) and alkaline phosphatase (patient 1,3,4) were reported. No treatment related adverse clinical or laboratory event grade 3 or 4 was seen while the patients were receiving a four drug combination.

In all patients four antiretroviral drugs for a defined period led to a rapid decline in viral load, which, provided drug compliance was given, sustained for the whole observation time, even after switching to a triple regime. In all patients CD4 cells reconstituted to normal values and all patients were free from AIDS defining conditions afterwards. Even after a mean time of observation of 42 months we did not see any potential long time side effect, which can be attributed to a 4-drug therapy.

#### DISCUSSION

In HIV infected children mortality and disease progression is especially high in infancy [6, 7]. Children with an increased viral load, especially those with an AIDS defining illness, are at risk for rapid progression

and death, even after starting ART [6, 7]. Most studies published to date suggest that long term control of viral replication is difficult to achieve in children especially in infants starting early ART. The PENTA 7 study with 20 infants showed a rapid decrease in viral load within four weeks, however only 25% achieved a viral load below the level of detection (20% after 24 weeks of therapy) [6]. A 96-week follow up in the PENTA 7 study shows that only 55% of the children still received the initial ART regimen. Chiappini et al reported on 40 perinatally HIV-infected children receiving HAART with a median follow up of 5.96 years and observed that 77.5% of the early treated children had undetectable viral load at their last visit, however only 47.5% were still receiving their first HAART regimen [17, 18].

Due to this frequently observed virologic failure several studies have addressed the possibility of introducing a more aggressive initial regime consisting of four antiretroviral drugs however only few authors have compared 3- with 4-drug regimens.

Luzuriaga compares three different combinations (ZDV+3TC+NVP vs ZDV+3TC+NVP+abacavir(ABC) vs. 3TC+NVP+stavudine(D4T)+nelfinavir(NFV)) in children below and above 3 months of age at the time of initiation (n = 52) [9]. A significant difference in an intention-to-treat analysis was seen in week 48 and week 200 between both reverse transcriptase inhibitors only regimen (NRTI and NNRTI) on the one hand and the quadruple drug regimen consisting of a NRTI, a NNRTI and a PI on the other. An increased toxicity was not reported. Studies conducted in adults comparing a triple therapy (2 NRTIs 1 NNRTI) with a quadruple therapy (3 NRTIs 1 NNRTI) did not show any significant difference leading to the conclusion that an additional drug class is required to see any superiority [8, 11].

Starr observed 57 children (range 3.8 to 16.8 years), who were previously treated with NRTIs, switching to a regimen consisting of two NRTIs+NFV+efavirenz (EFV) [12]. No control groups were analyzed. The percentage of patients with a low viral load increased rapidly under the new combination and remained stable from week 8 to week 48. At week 48, the percentage of children with plasma HIV-1 RNA levels of less than 400 copies per milliliter was 81 percent, the absolute percentage of CD4 cells had increased by a median of 3 percent.

Melvin observed 36 children (range 0.2 to 16.0 years) with different previous drug combinations before changing to a four or even five drug regimen [10]. After initiation of the new regimen all children experienced a significant decrease in viral load. The median RNA level at the last follow-up during 4- or 5-drug ART was 1.7 log<sub>10</sub>, 78% of the children had RNA levels below the level of detection. After a median of 29 months of 4- or 5-drug ART, the median CD4 cell count had an increase by 339 cells/μL. CD4 cell counts improved to or remained at age-appropriate levels in children with a viral load below 50 copies/mL.

In the above mentioned studies the patients were kept on their 4- or 5-drug ART despite achieving undetectable viral load. Since long term side effects of

ART and lack of compliance are an issue especially in infants starting with early ART we initially treated the 4 infants with a quadruple ART consisting of two NRTIs, one NNRTI and one PI and then switched to a triple maintenance therapy with two NRTIs and one NNRTI after achieving significant viral suppression and immune reconstitution. In our patients receiving a quadruple therapy a rapid and significant reduction of viral load was seen within 2 months of therapy. All patients achieved a viral load below  $< 500$  copies / ml within 6 months, in  $\frac{3}{4}$  patients viral load was below detection limit within 2 months. Even after changing to a triple regimen all patients are still on their initial ART regimen and only one patient experiences a viral relapse due to proven malcompliance.

Beside slightly elevated biochemical parameters no grade 3 / 4 toxicity was observed and medication was well-tolerated. In treatment naive infants Luzuriaga described adverse clinical or laboratory events in 8 of 52 patients receiving quadruple therapy (grade 2 (4 patients), grade 3 (1 patient), grade 4 (3 patients)), however these patients were receiving reverse-transcriptase inhibitors only (2 NRTI + 2 NNRTI); while no treatment-related adverse effect was reported among children receiving the regimen including a PI (2 NRTI + 1 NNRTI + 1 PI) [9]. In therapy-experienced children receiving a four drug regimen adverse reactions were limited to mild neutropenia and mild transient elevations in liver enzymes [10]. Starr observed severe effects, consisting of severe neutropenia, hepatic toxicity, diarrhea and rash with fever, in about 10% of the children treated with two NRTIs and two NNRTIs [12].

Though not observed in our patients the addition of another drug class in HIV initial treatment could imply the possibility of further interactions and adverse effects with the co-medication regarding prophylaxis and therapy of opportunistic infections. Inadequate plasma drug levels due to malabsorption or non-adherence lead to development of drug resistance which preserves only few alternatives for further treatment.

We treated four children with a 4 drug combination for 10-13 months. While two children received an incomplete peripartur prophylaxis and were thus pre-treated, in two patients HIV was diagnosed during an opportunistic infection. Both subgroups strongly qualified for a virologic relapse. Three out of four patients, who were initially treated with a 4 drug combination for 12 months showed sustained virus suppression and did not relapse even after changing to a triple therapy with two NRTI and one NNRTI. In one patient who initially had viral load below detection limit after 4.5 months of therapy, viral rebound later occurred and was related to adherence problems, concluding that in all adherent patients viral load remained low. Compared to other studies and our own experience in the past with triple ART, treatment failure and resistance is common on triple ART and was improved on a quadruple drug regimen. Drug toxicity is not increased as seen in our patients. A quadruple induction therapy is still not recommended in all current guidelines, but since a high viral burden and opportunistic infections at the time of diagnosis predict an unfavorable outcome, we strongly support a quadruple thera-

py in these patients. As we consider that the first year of life represents the most vulnerable phase a 4 drug regime should be maintained for this period. Drug levels and viral load should be monitored at close intervals. Clinical trials with larger pediatric cohorts and different combinations are strongly required to address this question and to provide essentials for a new definition and composition of a "highly active antiretroviral therapy".

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