

LONG-TERM CONSEQUENCES OF TREATMENT INTERRUPTIONS IN CHRONICALLY HIV-1-INFECTED PATIENTS

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Abstract

Objective: To evaluate the long-term effects of anti-retroviral treatment (ART) interruptions on metabolic, immunological, virological and clinical outcomes in chronically HIV-1 infected patients.

Methods: Multi-centric, prospective, controlled 24-month cohort study in HIV-1 infected patients interrupting ART once or several times and for at least two weeks. Patients were compared to a frequency-matched control group continuing on ART.

Results: A total of 399 HIV-1 infected patients were included, among them 133 patients with treatment interruption (TI) and 266 control patients. Baseline characteristics were well matched. Median baseline CD4 cell count was 379/ μ l in TI-patients and 410/ μ l in control patients ($p = ns$). Median duration of the first TI was 1.1 months, and 37 % of patients had two or further TI's. Whereas CD4 cell count in control patients had increased significantly by a median of 67/ μ l at month 24 ($p < 0.0001$), median CD4 cell count at month 24 in the TI-patients did not differ significantly from baseline. However, two-year AIDS-free survival was not significantly different between TI- and control patients. Liver enzymes and blood lipids improved significantly during TI.

Conclusion: TI was associated with a significant immunological disadvantage at 24-month follow-up compared to continued ART. In this relatively immunocompetent cohort, however, TI's did not lead to an increased risk of disease progression within two years of follow-up.

Key words: treatment interruptions, antiretroviral therapy, HIV

Abbreviations: TI: treatment interruption, TI's: treatment interruptions, ns: not significant

INTRODUCTION

The incontrovertible and immense benefits of highly active antiretroviral therapy (HAART) in the management of HIV-1 infection have been well described [1-3]. Nevertheless, current treatment regimens and strategies do not achieve eradication of the virus [4-5],

and antiretroviral therapy is assumed to be a lifetime commitment. Limitations imposed by side effects, long-term toxicity or adherence problems may hinder lifelong treatment.

New attempts to reduce toxicity by means of treatment interruption (TI) or intermittent therapy are thus under intense investigation [6-7]. Within resource-poor areas, these approaches may also be helpful both in reducing treatment costs and enhancing access to therapy for a larger population.

However, concerns have been expressed about the safety of such strategies. Rapid viral rebounds in plasma and replenishment of latent reservoirs [8-12], rapid immunological deterioration during TI [13-15], and an increased risk of clinical progression in immunocompromised patients have been reported [16-18]. Some studies, moreover, demonstrated a higher risk of resistance in patients on intermittent therapy [19-22].

Little is known about the long-term consequences of TI. In the present study, we assessed the long-term effects of TI on virological, immunological and clinical outcomes, metabolic parameters, and changes in antiretroviral therapy. We compared the 24-month outcomes of patients undergoing TI's with a frequency-matched control group remaining on ART throughout the observation period.

METHODS

This observational, multi-centric, prospective, frequency-matched, controlled cohort study assessed the long-term outcomes in HIV-1 infected patients who interrupted ART according to their physician's and/or own decision for at least two weeks. Evaluation started in July 1999 with five German outpatient centres participating. Observation time was 24 months. TI-patients were consecutively recruited irrespective of reasons for TI or of clinical, immunological or virological conditions. TI-patients were compared to HIV-1-infected patients remaining on continuous ART during the observation time (control patients). The latter were recruited by a systematic, frequency-matched enrolment strategy, both in order to obtain a control group representing the baseline characteristics of the

TI-patients and to minimize confounding resulting from the association between patients' treatment conditions and physicians' decisions on therapeutic approaches. Assignment of control patients was based on the date of presentation of the TI-patient. For each TI-patient, the two patients who had been seen by the same treating physician prior to the TI-patient and who were on ART at that time point were recruited as control patients. If a control patient interrupted therapy for more than two weeks during follow-up, the control patient was censored at this time.

All patients were monitored for previous AIDS-defining events according to the classification from the Centers for Disease Control and Prevention [23], ART history, nadir of CD4 cell counts, and maximum HIV RNA plasma level before initiation of ART. CD4 cell counts, HIV RNA plasma level (viral load), changes in ART (defined as the change of at least one antiretroviral compound of the baseline regimen), the occurrence of AIDS-defining events or death, and routine laboratory parameters were assessed at baseline and at 3-month intervals thereafter. In the TI-patients, CD4 cell counts and routine laboratory parameters were also assessed during TI. Routine laboratory parameters included serum triglycerides (non-fasting), serum total cholesterol (non-fasting), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (γ -GT), alkaline phosphatase (AP), total bilirubin and lipase.

Viral load was measured in a central laboratory using the branched DNA-assay (QUANTIPLEX[®], later renamed VERSANT[®] HIV-1 RNA 3.0 Assay, 2002 Bayer Corporation, Tarrytown, NY) with a limit of detection of 50 copies per ml. CD4 cells and the other laboratory parameters were measured at the local sites. Upper normal limits of metabolic parameters were defined according to normal ranges adopted by the corresponding local laboratories.

Primary objectives were to compare the CD4 cell changes from baseline and the time to disease progression (AIDS-defining event or HIV-related death) in TI- and control patients until month 24. Both groups were further divided into two sub-groups depending on whether the baseline viral load was below detection (< 50 copies/ml) or detectable (\geq 50 copies/ml).

Secondary objectives were to assess the changes viral load and metabolic parameters. Particular attention was paid to the span of time without treatment change in the subgroup of patients with a baseline viral load < 50 copies/ml. To minimize confounding by the association between side effects and ART changes, TI-patients who interrupted ART due to side effects were excluded from this analysis.

STATISTICAL ANALYSES

A p-value of <0.05 was taken as the level of significance. To compare two continuous variables of two independent groups, we employed the Mann-Whitney U test and – in case of normal distribution – the unpaired t-test including the 95 % confidence interval. Wilcoxon signed rank test was used for paired comparisons. Frequencies were compared with Fisher's exact test. To indicate the degree of linear correlation, the Pearson correlation coefficient r was used. Fisher's

r to z transformation was performed to determine if the correlation coefficient was significantly different from zero.

Time to change in ART and AIDS-free survival were calculated using Kaplan-Meier statistics. Logrank testing was used to test statistical significance.

All analyses were performed with the statistical program StatView (Version 5.01, SAS Institute Inc., Cary, North Carolina, USA 1998).

RESULTS

STUDY POPULATION

A total of 399 patients (133 TI-patients, 266 control patients) were included in the study. The median observation time was 23.4 months in the TI-patients and 22.1 months in the control group, representing 237 and 422 patient years of observation, respectively. Baseline characteristics of both groups are shown in Table 1. No statistically significant differences existed between the TI-patients and the controls with regard to CD4 cells nadir, baseline CD4 cell counts, the percentage of patients with undetectable viral load and maximum viral load level prior to initiation of ART. Further details of virological and immunological parameters are shown in Table 2. In both patient groups, the percentage of baseline ART regimens with two, three, or more than three drugs were equally distributed (18 %, 53 % and 29 %, respectively). Fifty percent of TI-patients and 46 % of controls received a NNRTI-containing regimen. The percentage of patients with elevated blood lipids were equally distributed between TI-patients and control patients. Median γ -GT did not differ significantly between the groups. In contrast, median lipase and median AP were significantly higher in the TI-patients than in the control patients.

Within the TI-patients, the median duration of the first TI was 1.1 months (range, 0.3 - 3.0 months). In patients undergoing two (22 %) or more than two (15 %) TI's, the duration of the second and third TI did not differ significantly from the duration of the first TI. The total duration of all TI's spanned 354 patient months, representing 12.5 % of the total observation time in the TI-patients. The main reasons for TI were toxicity and/or side effects (39 %), physician's decision/recommendation (33 %), patient's wish (20 %), intercurrent illnesses (5 %), and other reasons (3 %).

IMMUNOLOGICAL AND VIROLOGICAL CHANGES DURING FOLLOW-UP OF 24 MONTHS

At month 24, changes in CD4 cell counts differed significantly between the TI-patients and the control group. Although the initial loss of CD4 cells during TI was compensated, only a non-significant median increase of 17 CD4 cells/ μ l was observed at month 24. In contrast, CD4 cell count in the control group had increased significantly by a median of 67/ μ l (p <0.0001). These differences could be attributed mainly to patients with a baseline viral load of less than 50 copies/ml. Within this subgroup, median CD4 cell changes by month 24 were -5/ μ l in TI-patients and

Table 1. Baseline characteristics of TI-patients and control patients.

	TI-patients (N=133)	Control patients (N=266)	p-value
Median age (years) (range)	41 (24-67)	39 (20-68)	0.09
Female gender (%)	23	18	0.23
Prior AIDS defining illness (%)	30	34	0.43
On first antiretroviral regimen (%)	22	26	0.33
Median CD4 cell nadir (1/ μ l)	183	193	0.69
Median CD4 cell count (1/ μ l) (range)	379	410	0.56
Median relative CD4 cell count (%) (range)	19	20	0.62
CD4 cell count < 200/ μ l (%)	17	18	0.58
CD4 < 100/ μ l (%)	4	4	> 0.99
Median VL (copies/ml) prior to ART	80,750	90,640	0.77
Median baseline VL (copies/ml) (range)	69 (<50 - >500,000)	82 (50 - 198,139)	
Median total cholesterol (mg/dl) (range)	214 (93 - 394)	208 (91 - 428)	0.42
Total cholesterol above upper normal limit (%)	47	41	0.74
Median serum triglycerides (TG) (mg/dl)	280 (21 - 1,709)	246 (46 - 1,762)	0.27
TG above upper normal limit (%)	55	51	0.51
Median AP (U/l) (range)	123 (31 - 650)	116 (41 - 344)	0.02
AP above upper normal limit (%)	16	8	0.02
Median lipase (U/l) (range)	34 (12 - 1,055)	30 (8 - 149)	< 0.01
Lipase above upper normal limit (%)	19	11	0.05
Median ALT (U/l) (range)	17 (5-345)	16 (4-120)	0.05
ALT above upper normal limit (%)	33	24	0.07
Median γ -GT (U/l) (range)	26 (5 - 747)	24 (5 - 630)	0.15
γ -GT above upper normal limit (%)	48	43	0.39

VL = viral load (VL) and (HIV RNA plasma level), AP = alkaline phosphatase, ALT = alanine aminotransferase; γ -GT = gamma glutamyl-transpeptidase

Table 2. Viral load (VL) and CD4 cell counts of TI-patients and control patients stratified by baseline viral load (VL) category (VL < versus \geq 50 copies/ml).

VL category (copies/ml)	TI-patients		Control patients	
	VL < 50	VL \geq 50	VL < 50	VL \geq 50
N	63	70	123	143
Median VL prior to ART (copies/ml)	59,940	106,800	67,455	100,459
Median CD4 nadir (1/ μ l)	180	140	171	150
Median VL (log copies/ml)	< 50	4,325*	< 50	1,610*
VL > 100.000 copies/ml (%)	--	8.5	--	4.2
Median absolute CD4 cell count (1/ μ l)	425	322	420	391
Median relative CD4 cell count (%)	23	16	21	20

* significant differences between corresponding TI-patients and control patients (p=0.01)

+89/ μ l in the control group (p<0.001; Fig. 1). This difference remained significant even when patients with more than one TI were excluded from analysis (p=0.02). In contrast to patients with a baseline viral load of less than 50 copies/ml, no significant differences in CD4 cell changes were found between TI-pa-

tients and control patients with a detectable viral load at baseline (+38/ μ l versus +46/ μ l, p=0.44; Fig. 2).

In patients with an undetectable baseline viral load, the proportions of patients with a reinstated viral load of less than 50 copies/ml during follow-up were different between TI-patients and control patients. How-

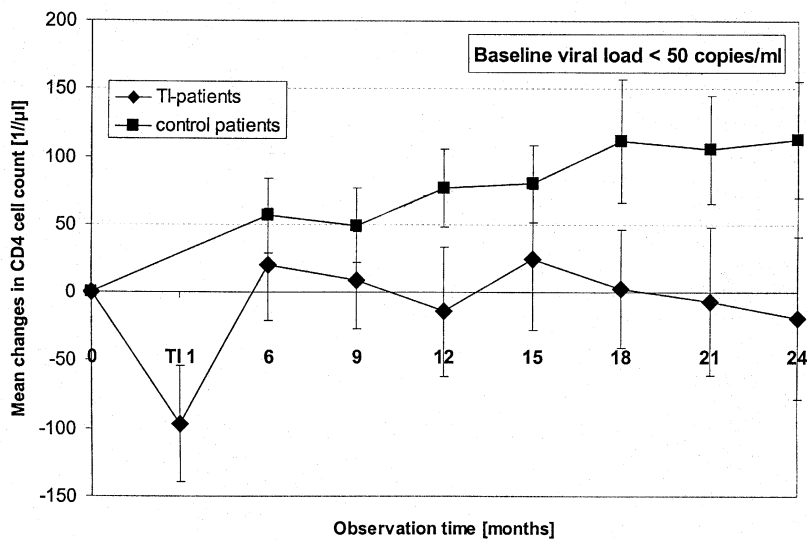


Fig. 1. Mean changes in CD4 cell count in patients with a baseline viral load (HIV RNA level) < 50 copies/ml: patients with TI compared to control patients remaining on antiretroviral therapy (error bars: 95% confidence intervals).

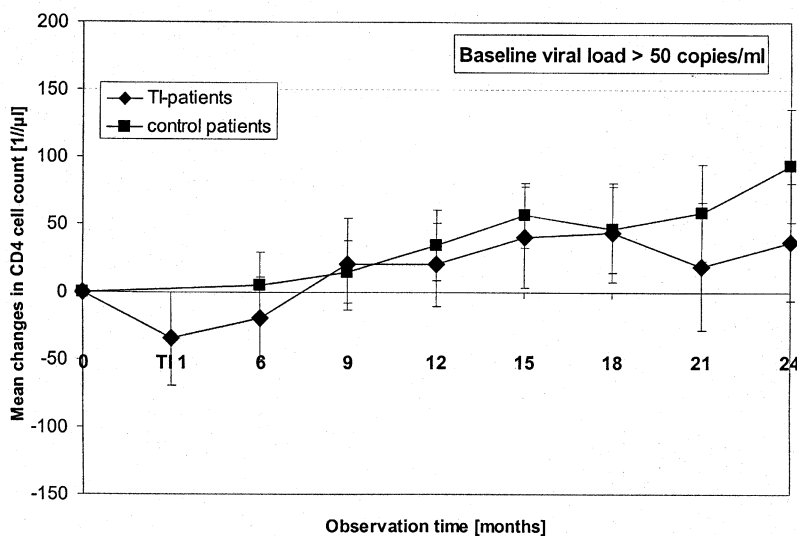


Fig. 2. Mean changes in CD4 cell count in patients with a baseline viral load (HIV RNA level) > 50 copies/ml: patients with TI compared to control patients remaining on antiretroviral therapy (error bars: 95% confidence intervals).

ever, differences were significant only at single time points and were not different when analysis was restricted to patients with only one TI. At month 24, 69% of TI-patients and 81% of the control patients had an undetectable viral load ($p=0.10$). Of note, in the TI-patients with an NNRTI-based regimen at baseline only 17/30 (57%) had reinstated an undetectable viral load compared to 20/24 patients (83%) on a PI-based regimen ($p=0.04$).

In patients with a detectable baseline viral load, viral load decreased significantly during follow-up. Viral load changes in TI-patients and control patients differed significantly only at months 6 and 12. At month 24, median viral load reduction from baseline in TI- and control patients was 0.5 and 0.6 log, respectively.

TIME TO DISEASE PROGRESSION (AIDS OR HIV-RELATED DEATH)

By month 24, the incidence of AIDS-defining illnesses was low with an AIDS-free survival of 96% in TI-patients and 98% in control patients ($p=ns$). Eleven patients (5 TI-patients, and 6 control patients) reached

the clinical endpoint (AIDS-defining event or HIV-related death). Seven of them had already experienced an AIDS-defining event prior to baseline, and five of them (one TI-patient, four controls) had a baseline CD4 cell count below 200/ μ l.

Among the five TI-patients reaching a clinical endpoint by month 24 were two cases of non-Hodgkin's lymphoma, two cases of cryptosporidiosis, and one death due to pneumonia. In one further patient, who was censored at the time of death, the presumed cause of death was intoxication. Among the six control patients, one death due to liver cirrhosis, one case of cryptosporidiosis, one relapse of cryptosporidiosis, one esophageal candidiasis, and one relapse of mycobacterium avium complex infection were observed. One patient experienced two AIDS-defining illnesses, namely cytomegalovirus retinitis and pneumocystis pneumonia.

CHANGES IN ART DURING FOLLOW-UP

Changes in ART during follow-up were more frequent in TI-patients than in the control group. Of patients

with a viral load of less than 50 copies/ml at baseline, 42 % of TI-patients versus 25 % of controls changed therapy ($p=0.04$). Of patients with a viral load ≥ 50 copies/ml at baseline, 78 % of TI-patients versus 46 % of controls ($p<0.0001$) underwent treatment changes. However, when TI-patients interrupting due to side effects were excluded and only patients with a viral load of < 50 copies/ml were analyzed, treatment changes did not differ significantly between TI-patients and the control group (Kaplan-Meier probability of change within 12 months: 27 % versus 25 %, $p = 0.44$). Further stratification for NNRTI- and PI-based regimens revealed a trend in the NNRTI-subgroup to earlier switches in TI-patients in comparison to the control patients ($p=0.10$). This was not the case in the PI-subgroup.

VIROLOGICAL AND IMMUNOLOGICAL CHANGES DURING TREATMENT INTERRUPTION

At the end of the first TI, viral load had increased by a median of 1.9 log copies/ml. The increase of plasma viremia was more pronounced in patients with a baseline viral load below 50 copies/ml, compared to patients with detectable viremia (2.9 log versus 0.8 log copies/ml; $p<0.001$).

During the first TI, CD4 cell count decreased by a median of 48 cells/ μ l (mean 76/ μ l), displaying a broad range from -611 to +364 cells/ μ l. A decrease of more than 100 CD4 cells/ μ l was observed in 35 % of the patients. In 12 % of individuals with more than 200 CD4 cells/ μ l, the CD4 cell count dropped to levels below 200/ μ l.

The changes in CD4 cell count were negatively correlated with baseline CD4 cell counts ($r=-0.71$; $p<0.0001$): In those with more than 350 cells/ μ l, median CD4 cell decline at week 4 was -115/ μ l ($p<0.0001$). Patients with 200 – 350 CD4 cells/ μ l showed a median change of -29/ μ l ($p = 0.02$), and in those with a baseline CD4 cell count below 200/ μ l, CD4 change was +14/ μ l ($p = ns$). Of note, 25 % of patients neither lost or even gained CD4 cells during TI.

Median CD4 cell count declined to a significantly higher extent in patients starting TI with a viral load < 50 copies/ml. Here, the CD4 cell count had declined by a median of 88/ μ l at week 4 of TI, compared to a median drop of 17/ μ l in patients with a detectable baseline viral load ($p = 0.01$).

CHANGES IN METABOLIC PARAMETERS

Overall, liver enzymes did not change significantly in the first 2 – 4 weeks of TI. When analysing only patients with elevated liver enzymes at baseline, median changes after 2 – 4 weeks (last observation carried forward, LOCF) were significant at -17 U/l in γ -GT ($p<0.0001$) and -9 U/l in ALT ($p<0.01$). In patients with elevated AP at baseline, AP levels decreased significantly by a median of -42 U/l ($p = 0.04$). The median difference in lipase of -20 U/l in patients with elevated lipase levels did not reach statistical significance ($p = 0.16$).

A significant decrease in blood lipids was observed during TI (cholesterol -27 mg/dl, triglycerides -54

mg/dl, all median values). In patients with blood lipid values above the upper normal limits, median decline after 2-4 weeks (LOCF) was -44 mg/dl (or -19 %) in total cholesterol ($p<0.0001$) and -120 mg/dl (or -45 %) in triglycerides ($p<0.0001$).

When only patients with elevated cholesterol levels at baseline who remained on the same ART regimen during follow-up were considered, a trend to lower cholesterol levels over time in TI-patients compared to control patients was observed.

DISCUSSION

Within this observational study, treatment interruptions in chronically HIV-infected patients had significant immunological consequences after 24 months. Although the decline in CD4 cell count during TI was compensated at that time point, a significant immunological disadvantage remained in patients interrupting therapy compared to patients who continued ART. This CD4 blunting was primarily seen in patients interrupting ART with a baseline viral load below detection. Given the significant loss of CD4 cells during TI in these patients it seemed that the immunological disadvantage after 24 months was mainly due to the decrease during TI and not due to a slower immune recovery compared to patients continuing therapy. It should be noted that different levels of adherence (which was not evaluated in this study) may also have contributed to the immunological differences between TI-patients and control patients.

The high CD4 cell gains in continuously treated patients with undetectable viral load levels reflect that immune reconstitution is strongly associated with the degree of viral load suppression. Even low-level viremia is associated with an increased T cell activation, which by itself is correlated with decreased CD4 cell gain [25]. This might account for the reduced CD4 cell gap between the TI-strategy and continued ART observed in patients with suboptimal viral suppression.

Whether the immunological disadvantage observed in our cohort has any clinical relevance over a longer period of several years remains unclear. During the follow-up period of two years, however, no difference in the incidence of clinical events was observed. Of note, most individuals included in this study had a relatively preserved immunocompetence. Moreover, based on the low number of clinical events, the study population may not be large enough and the follow-up too short to allow the detection of significant differences. However, there are data from the Swiss HIV Cohort Study suggesting that occasional and short TI's of up to three months do not increase the risk of HIV-associated morbidity and mortality, particularly in the presence of a high CD4 cell count [16]. This contrasts with the results of a randomized trial of a four month interruption prior to initiation of a salvage regimen in which an increased rate of disease progression was observed in the TI-group [17]. In this study the patients were more immunocompromised than our cohort and duration of interruption was longer.

TI's can have serious immunological consequences. CD4 cell counts often decrease within a short time to

pre-treatment levels [24, 14, 15]. In our cohort, CD4 cells declined by a median of about 50/ μ l. A decrease of more than 100 CD4 cells/ μ l was seen in one third of the patients. TI's were also accompanied by a rapid and significant increase in viral load in most patients. During follow-up, TI-patients with a baseline viral load below the limit of detection had a lower incidence of undetectable viral load when compared to control patients. However, this was not the case when analysis was restricted to patients with only one TI. Whether these differences result from an enhanced development of resistance after further TI's remains unclear. One confounding factor may be the lack of control for time on drug at different time points, i.e. whether the time span was sufficient to re-suppress the virus to levels below detection.

In patients with suboptimal viral load suppression at baseline, viral load during a follow-up period of two years was about 0.5 log copies/ml below baseline value with no significant difference between TI-patients and those remaining on ART throughout the observation period. In two randomized trials - CPCRA 064 and a small Spanish study - no differences in viral load reductions between the TI and control groups were reported [17, 26]. Although in CPCRA 064 a significant difference in TI-patients with and without a shift in drug resistance pattern was noted, overall long term virological outcome in the TI-group did not differ significantly from the control patients. This contrasts to the results of the GIGHAART study which demonstrated a maintained virological and immunological benefit for the TI-group [27]. The reason for this discordance remains to be elucidated. However, confounding factors may include the degree of resistance, further treatment options for subsequent regimens, and the number of antiretroviral drugs within the new regimen.

Some evidence exists that TI's bear a higher risk, particularly for NNRTI- or lamivudine-resistance [19-22, 28]. However, these studies lacked a control group and do not permit the conclusion of an increased risk for resistance. Our observation of a trend to earlier treatment changes in TI-patients with NNRTI-based regimens and a lower rate harbouring undetectable viral load can only hint at development of resistance, which has to be proven in randomized clinical trials.

In patients with elevated blood lipids, TI's led to a significant decrease in respective parameters. Whether repeated or CD4-guided TI's can be translated into a reduced risk of cardiovascular disease remains unclear. Large cohort studies have been initiated to address this issue [29]. Furthermore, a 19 % reduction of cholesterol and a 45 % reduction of triglycerides correspond to the effects that can be achieved with statins and fibrates [30]. As the objective of our study was the two-year outcome of only a single or few TI's, drug exposure was reduced by only 12.5 %. However, if more frequent TI's also prove to be safe in immunocompetent patients, a reduction in long term toxicity is conceivable.

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