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ORIGINAL RESEARCH ARTICLE

Five-year immunological outcome of highly active antiretroviral treatment in a clinical setting: results from a single HIV treatment centre

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Summary: Our objective was to study the evolution of CD4 cell count five years after starting highly active antiretroviral treatment (HAART) in a clinical setting. The study was performed at the HIV outpatient clinic, Institute of Tropical Medicine, Antwerp. All patients ($n=225$) who started HAART in 1997, who had a CD4 cell count within six months prior to starting HAART and who were subsequently followed for at least two years were included. Change in CD4 cell count after start of HAART and the influence of patient and clinical factors were investigated using graphical exploration, endpoint analysis and mixed-effects linear regression.

The mean CD4 cell count at start of HAART was 280 cells/mm^3. At the five-year endpoint of the study the mean increase in CD4 cell count was 333 cells/mm^3, while 79% of the patients had a viral load less than 400 copies/mL. There was a significant negative correlation between increase in CD4 cell count at five years and time since first positive HIV test at start of HAART ($P=0.021$). Patients who ever had a HAART interruption of more than seven days had a significantly lower increase in CD4 cell count than those who did not (225 cells/mm^3 compared with 438 cells/mm^3; $P<0.001$). A mixed-effects linear regression model additionally suggested a significant impact of exposure to antiretrovirals prior to HAART ($P=0.03$).

Overall, the recovery of CD4 cell count after five years of HAART is good, although therapy interruptions have an important negative impact.

Keywords: HIV, outcome, CD4, HAART, mixed linear regression

Introduction

In the wealthier parts of the world, highly active antiretroviral treatment (HAART) was introduced in the middle of the 1990s, after which a major reduction in HIV-related mortality and morbidity has been noted[1–3]. These so-called antiretroviral cocktails, consisting of three or more antiretroviral drugs, are able to suppress the HIV virus, but cannot completely remove the virus from the human body. Antiretroviral treatment therefore needs to be taken for prolonged periods of time.

The long-term outcome of HAART is unknown. At this moment patients are taking HAART for periods that are already longer than ever evaluated during clinical trials. Moreover, the objective of most clinical trials is to compare the result of a few well-defined regimens during intensified patient follow-up. These circumstances do not necessarily reflect real life situations. In order to provide feedback on the outcome of HAART, and to evaluate and optimize the care provided it is necessary to collect data systematically in the daily clinical setting, as it is often done in large HIV observational cohort studies[1–6]. Data that are representative for individual treatment centres offer the possibility to evaluate the provided care at the centre level.

This article describes the result of five years of treatment with HAART in one HIV treatment centre. The evolution of CD4 cell count and viral load measurement over a period of five years will be described and the effect of different patient and clinical factors on the evolution of CD4 cell count will be examined.

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Methods

Data

Data were obtained from the HIV cohort of the Institute of Tropical Medicine (ITM), Antwerp. This cohort contains epidemiological, laboratory and clinical information of all HIV patients under follow-up, including a detailed antiretroviral therapy history. Currently, more than 1300 patients have been included in the cohort. All patients who started a first-line HAART regimen in 1997 and had a follow-up for at least two years were considered for this study. The objective of this study was to investigate long-term outcome of HAART. Therefore, patients who dropped out within two years after starting HAART were excluded. Patients were also excluded if no CD4 cell count within six months prior to the start of HAART was available (throughout this article the term CD4 cell count will refer to $CD4^+$ T cell count). HAART was defined as a combination of at least three products from at least two different antiretroviral classes or a combination of three nucleoside reverse transcriptase inhibitors. The study period ended 1 May 2003.

Statistics

The CD4 cell count prior to the start of HAART, with a maximum of six months, was considered baseline CD4 cell count. Individual changes in CD4 cell count since the start of HAART were first explored graphically. Loess smoothing provided a non-parametric estimate of the mean evolution[7]. CD4 cell counts at six-month intervals were selected by assigning the measurement nearest to that time-point, with a maximum difference of 90 days. Patients who did not have a measurement within this window period were regarded as having no measurement at that time point. The mean change in CD4 cell count compared with baseline at six-month intervals was plotted. The percentage of patients with an undetectable viral load (defined as less than 400 copies/mL plasma) at six-month intervals was explored using the available case method and missing equals failure method.

For the endpoint analysis, the change in CD4 cell count after five years compared with baseline was compared for different subgroups using analysis of variance. Correlation between change in CD4 cell count and continuous variables was determined by the Pearson correlation coefficient. Normality assumptions were tested using q-q plot and Kolmogorov–Smirnov test. Change in CD4 cell count compared with baseline was defined as outcome variable. By definition an endpoint analysis uses only the information at the end of the study and ignores the information during the course of the study. A mixed-effects linear regression model[7,8] was fitted to investigate the effect of the different co-variates on CD4 cell count using all available information. Time since start of HAART was expressed in days. Smoothing of the change in CD4 cell count suggested a quadratic curve. A quadratic term for time was therefore included in the model. An exponential serial correlation component was chosen. All interactions with time and time as a quadratic term were included. A random intercept and slope were defined as random effects. Co-variates entered in the model were: sex, mode of HIV transmission, origin, age at start of HAART, time since first positive HIV test at start of HAART, previous exposure to antiretroviral therapy (mono- or bitherapy) and CD4 cell count at start of HAART. Therapy history was included at each CD4 cell count using four variables: duration of current HAART regimen (in days), number of days of current treatment interruption, total number of days of HAART prior to the current therapy regimen and total number of days of treatment interruption prior to the current regimen. A change of at least one antiretroviral drug was considered a treatment switch. In order to have a clear definition of therapy interruption, only a complete stop of all antiretroviral drugs of at least seven days was counted as a therapy interruption and added up to the total days of therapy interruptions. All P-values less than 0.10 were reported. Analyses were done using SPSS 11.5 (SPSS Inc., Chicago, Il, USA) and SAS version 8 (SAS Institute, Cary, NC, USA).

Table 1. Population characteristics ($n=225$)

	No.	%	Median (IQR)
Age (years)*			37 (32–43)
Time since first positive HIV test (years)*			4 (1–6)
Sex			
Female	64	28.4	
Male	161	71.6	
HIV transmission mode			
Heterosexual contacts	102	45.3	
Homosexual contacts	105	46.7	
Other	18	8	
Origin			
Sub-Saharan Africa	49	21.8	
Belgium	143	63.6	
Other	22	9.8	
Unknown	11	4.9	
CD4 lymphocyte level*			
<50 cells/mm^3	15	6.7	
50–200 cells/mm^3	67	29.8	
200–500 cells/mm^3	115	51.1	
>500 cells/mm^3	28	12.4	
Previous exposure to antiretrovirals			
Yes	136	60.4	
No	89	39.6	

Results

Description of the population (Table 1)

The ITM cohort included 240 patients who started HAART in 1997 and had at least two years of follow-up. Fifteen patients were excluded from the study because they did not have a CD4 cell count within six months prior to starting HAART.

All 225 patients included in this study started a regimen containing a protease inhibitor. The overall mean CD4 cell count at start of HAART was 280 cells/mm^3 (range 5 to 996). Patients originating from sub-Saharan Africa started HAART at a significantly lower CD4 cell count compared with patients with a Belgian origin (229 cells/mm^3 compared to 309 cells/mm^3; $P=0.006$). Those who were previously exposed to antiretrovirals in mono- or bi-therapy started HAART at a lower CD4 cell count compared with antiretroviral naïve patients (250 cells/mm^3 compared with 326 cells/mm^3; $P=0.02$). No significant difference in CD4 cell count at start of HAART was observed according to sex, HIV transmission mode, time since first HIV-positive test and age.

At the end of the study, there were seven patients (3.8%) still on their first-line HAART and 15 (8.2%) on their second-line treatment. Seventy-seven patients (42.1%) were on their third, fourth or fifth regimen, 55 patients (30,1%) were taking regimen six to 10, and 29 (15.8%) switched their treatment more than 10 times. Twenty-five patients (11%) dropped out of the study before reaching the five-year endpoint: seven (3.1%) died, eight (3.5%) went to another hospital and 10 patients (4.4%) were lost-to-follow-up.

Change in CD4 cell count after starting HAART

During the study period a total of 4800 measurements of CD4 cell count were recorded for the 225 patients, with a median of 21 (range 2–54) measurements per patient. The change in CD4 cell count compared with baseline was plotted for all individual measurements (Figure 1). The smoothed line describes the average evolution of change in CD4 cell count since start of HAART. The mean evolution of change in CD4 cell count at six-month intervals (Figure 2) shows an initial rapid increase in CD4 cell count after start of HAART, followed by a slower but steady increase up to the end of the study period. From 18 months after start of HAART up to the end of the study period at 60 months, the percentage of patients having an undetectable viral load (<400 copies/mL) remained between 70 and 80% using the available case method and between 60 and 70% using the missing equals failure method (Figure 3).

For 183 out of 200 patients (91.5%) who reached the endpoint of the study, a CD4 cell count at five years after starting HAART was available. Seventeen patients (8.5%) did not have a CD4 cell count in the defined window period (5 years ± 90 days), although they were still under follow-up. The mean CD4 cell count at five years was 627 cells/mm^3 (range 23–1883). Four patients (2.2%) had a CD4 cell count below 50 cells/mm^3; nine (4.9%) from 50 to 200 cells/mm^3; 61 (33.3%) from 200 to 500 cells/mm^3 and 109 patients (59.6%) ended up with a CD4 cell count above 500 cells/mm^3. At the five-year

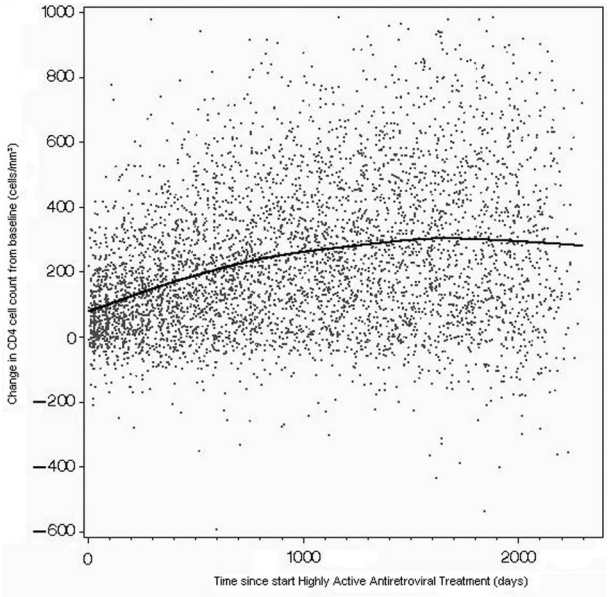

Figure 1. Individual changes in CD4 cell count since start of highly active antiretroviral treatment, and non-parametrically estimated average evolution (loess smoothing)

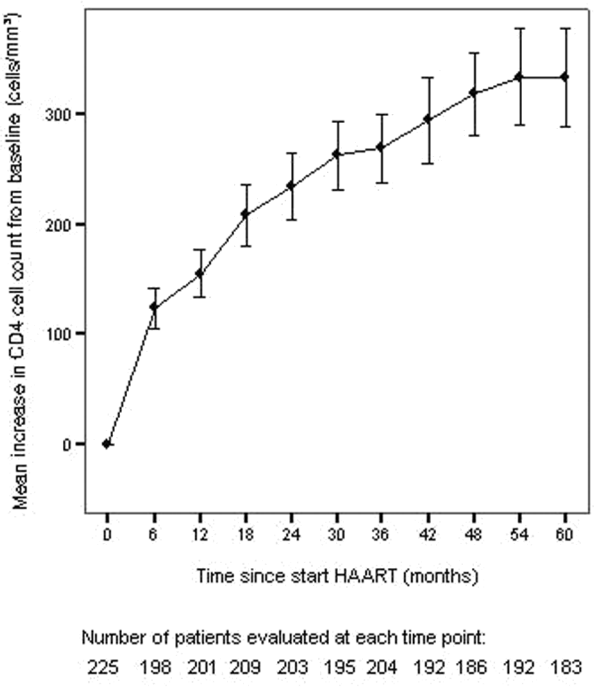

Figure 2. Mean change (95% confidence interval) in CD4 cell count since start of highly active antiretroviral treatment

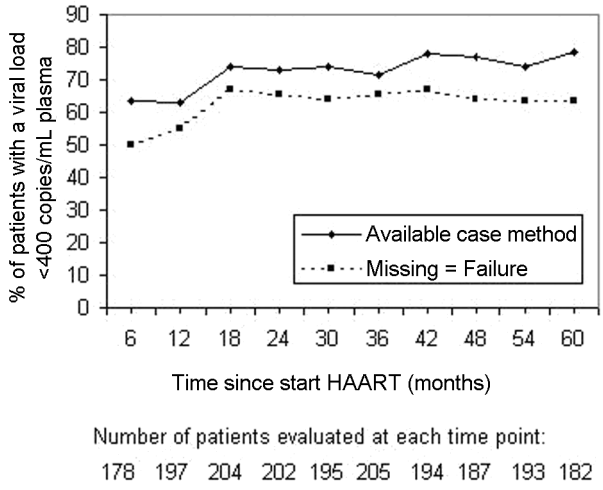

Figure 3. Evolution of percentage of patients under follow-up with a viral load less than 400 copies/mL plasma, since start of highly active antiretroviral treatment

time point, 143 (available case method: 79%; missing = failure method: 64%) of the patients had a viral load less than 400 copies/mL plasma.

The mean change in CD4 cell count at five years was 333 cells/mm^3 (range −535 to 1373). There was a significant negative correlation between change in CD4 cell count at five years and time since first positive HIV test at start of HAART ($P=0.021$), and between change in CD4 cell count and number of days of treatment interruption during the five years of HAART ($P<0.001$). Patients who ever had a HAART interruption of at least seven days had a significant lower increase in CD4 cell count compared with those who did not (225 cells/mm^3 compared to 438 cells/mm^3; $P<0.001$). The mean change in CD4 cell count at five years was not significantly different between patients with a Belgian origin and those originating from sub-Saharan Africa (322 cells/mm^3 compared to 302 cells/mm^3; $P=0.73$). Further, no significant differences in change in CD4 cell count at five years were found according to sex, HIV transmission mode, CD4 cell count at start of HAART, exposure to antiretrovirals prior to HAART and age at start of HAART.

Using the information from all CD4 cell counts in a mixed linear regression model, the impact of following variables on change in CD4 cell count was found to be significant: exposure to antiretrovirals prior to HAART, time since first positive HIV test at start of HAART, current duration of HAART treatment and current duration of therapy interruption, duration of HAART prior to the current regimen and duration of therapy interruption prior to the current regimen (Table 2).

Discussion

This study describes the evolution in CD4 cell count during five years after starting HAART. All patients of the ITM, Antwerp, who started HAART in 1997, who had a CD4 cell count within a six-month period prior to starting HAART and who had subsequently at least two years of follow-up were included. Change in CD4 cell count compared with baseline value was used as an outcome measurement of HAART because of its strong association with HIV-related mortality and morbidity[9–11].

After five years of HAART, the mean change in CD4 cell count compared with the CD4 cell count at start of HAART was 333 cells/mm^3. While at start of HAART 36.5% of the patients had a CD4 cell count below 200 cells/mm^3, this reduced to 7.1% at the five-year endpoint. The mean evolution in CD4 cell count after start of HAART showed an initial rapid increase during the first six-months, followed by a slower but steady increase up to the end of the five-year study period. This confirms in an overall population of people taking HAART the findings of two recent studies that only included patients who remained at a low level of viral load during the course of the study[12,13]. From 18 months after starting HAART up to the five-year endpoint of the study the percentage of patients with a viral load below 400 copies/mL remained stable between 70 and 80% using the available case method

Table 2. Mixed linear regression model with change in CD4 cell count as outcome variable

	Main effect *P* value	Interaction with time *P* value	Interaction with time *P* value
Gender	0.88	0.96	0.15
HIV transmission mode	0.42	0.35	0.61
Origin	0.46	0.19	0.39
Age at start of HAART	0.59	0.70	0.78
Time since first positive HIV test at start of HAART	0.78	0.0025	0.0011
Exposure to antiretrovirals prior to HAART	0.027	0.79	0.54
CD4 level at start of HAART	0.85	0.32	0.92
Duration of current HAART regimen	<0.001	0.052	0.071
Duration of current therapy interruption	<0.001	0.0028	0.018
Duration of HAART prior to the current regimen	0.97	0.017	0.0028
Duration of therapy interruption prior to the current regimen	0.60	0.087	0.80

HAART = highly active antiretroviral treatment

or between 60 and 70% using the missing equals failure method. The mortality during the period from two to five years after starting HAART remained low: 3.1% of the patients deceased, ignoring the 3.5% patients who went to another hospital and 4.4% who were lost-to-follow-up.

This study reflects the five-year outcome of patients from one specific treatment centre. This cannot be generalized to the general population under treatment, since other treatment centres might have different patient populations or have different treatment strategies. In the endpoint analysis, the increase in CD4 cell count after five years of HAART is measured for those patients that are still under follow-up at the end of the study period. This evidently gives an optimistic estimate. The impact of different co-factors on change in CD4 cell count was therefore also evaluated using a mixed linear regression model that included all measurements during the five-year study period. Data on occurrence of side-effects due to HAART were not included in the model, neither were the reasons for therapy interruptions and measures of adherence.

Patients who interrupted HAART for more than seven days at least once during the five years were found to have a significant lower increase in CD4 cell count than those who did not. Both endpoint analyses and the mixed linear regression model found a significant negative association between number of days of therapy interruption and increase in CD4 cell count. In this HIV clinic there were no studies on structured treatment interruptions neither were treatment interruptions used as a therapeutic strategy. Different findings have been reported about the effect of structured as well as occasional treatment interruptions on immunological and clinical outcome. The Swiss HIV cohort study group, for example, found no increased risk for HIV associated mortality and morbidity after short occasional HAART interruptions[14] although they did report that treatment interruptions have a significant negative influence on CD4 recovery[15]. Lawrence *et al.* found structured treatment interruptions associated with greater progression of disease without immunological or virological benefits[16]. The effect of HAART interruptions in terms of lower increase in CD4 cell count and other outcome measures such as incidence of opportunistic infections, virological failure and mortality needs further investigation, certainly for prolonged and repeated periods of interruptions.

Other factors found to have a significant negative impact on increase in CD4 cell count were longer time since first positive HIV test at start of HAART and exposure to antiretrovirals prior to HAART. Exposure to antiretrovirals prior to HAART has previously been reported to have a negative impact on the outcome of HAART, presumably due to the emergence of resistant viral strains[17–19]. Certain previous studies reported a lower increase in CD4 cell count according to higher age[15,20,21], male sex[12] and low baseline CD4 cell count[22]. We found no significant difference in increase of CD4 cell count according to gender, but this was also reported in the EuroSIDA cohort study[23]. Also no significant difference in increase of CD4 cell count was found according to baseline CD4 cell count as was also observed in certain other cohort studies[12–15].

In this study patients originating from sub-Saharan Africa started HAART at a significantly lower level of CD4 cell count compared with patients with a Belgian origin. Several reasons could explain this: this might be because these patients arrived in the country with a lower CD4 cell count, because they were more reluctant to start HAART, or because the medical staff was more hesitant to initiate HAART due to uncertainty about their residence permit. At the five-year endpoint there was no significantly different increase in CD4 cell count according to origin. The EuroSIDA study, which combines samples of patients from different European HIV clinics including from the ITM, Antwerp, found no significant difference in CD4 cell count at start of HAART according to origin[24]. Inequalities in the provision of HIV care according to ethnic groups have earlier been reported[25]. Further attention towards equal delivery of health care to minority groups remains required.

This study showed an encouraging improvement in CD4 cell count in HIV-infected patients that started HAART five years ago at one HIV clinic. Occasional treatment interruptions were found to have an important negative effect on immune recovery. More and more people are taking HAART for prolonged periods of time. Monitoring of outcome and optimizing treatment strategies remain important.

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