

ORIGINAL RESEARCH

Long-term survival of HIV-infected patients treated with highly active antiretroviral therapy in Serbia and Montenegro

DO Jevtović,[1] D Salemović,[1] J Ranin,[1] I Pešić,[1] S Žerjav[1] and O Djurković-Djaković[2]

[1]*Institute of Infectious & Tropical Diseases, Belgrade University School of Medicine, Belgrade, and* [2]*Institute for Medical Research, University of Belgrade, Belgrade, Serbia*

Background

Highly active antiretroviral therapy (HAART) has dramatically changed the prognosis of HIV disease, even in terminally ill patients. Although these patients may survive many years after the diagnosis of AIDS if treated with HAART, some still die during treatment.

Methods

A retrospective study in a cohort of 481 HIV-infected patients treated with HAART between January 1998 and December 2005 was conducted to compare subgroups of long-term survivors (LTSs) and patients who died during treatment.

Results

A total of 48 patients survived for more than 72 months (mean 83.8 ± standard deviation 5.6 months). Thirty patients died during treatment (mean 35.3 ± 25.0 months), of whom nine died from non-AIDS-related causes, 18 died from AIDS-related causes, and three died as a result of HAART toxicity. Although LTSs were significantly ($P = 0.015$) younger at HAART initiation, age below 40 years was not a predictor of long-term survival. The subgroups did not differ in the proportion of clinical AIDS cases at HAART initiation, in the prevalence of hepatitic C virus (HCV) coinfection, or in pretreatment and end-of-follow-up CD4 cell counts. In contrast, the viral load achieved during treatment was lower in the survivors ($P = 0.03$), as was the prevalence of hepatitis B virus (HBV) coinfection ($P = 0.03$). Usage of either protease inhibitor (PI)-containing regimens [odds ratio (OR) 9.0, 95% confidence interval (CI) 2.2–35.98, $P < 0.001$] or all three drug classes simultaneously (OR 7.4, 95% CI 2.2–25.1, $P < 0.001$) was associated with long-term survival. Drug holidays incorporated in structured treatment interruption (STI) were also associated with a good prognosis (OR 14.9, 95% CI 2.9–75.6, $P < 0.001$).

Conclusions

Long-term survival was associated with PI-based HAART regimens and lower viraemia, but not with the immunological status either at baseline or at the end of follow up. STI when CD4 counts reach 350 cells/μL, along with undetectable viraemia, was a strong predictor of long-term survival.

Keywords: AIDS, highly active antiretroviral therapy, long-term survival, structured treatment interruption

Received: 7 April 2006, accepted 01 August 2006

Introduction

It is well known that mortality rates have decreased and survival of AIDS patients has been prolonged since the introduction of highly active antiretroviral therapy (HAART) in the year 1996 [1–8]. Observational cohort studies have shown that the probability of staying alive on HAART largely depends on adherence to treatment and the degree of immunodeficiency at the time of HAART initiation [4,5]. Wood *et al.* reported a crude mortality rate of 7.1–15.2% after 4 years [4]. The probability of 4-year survival was at least 80% for those with baseline CD4 cell counts below 200 cells/μL, and above 90% for those with

Correspondence: Dr Olgica Djurković-Djaković, Institute for Medical Research, University of Belgrade, Dr. Subotića 4, PO Box 102, 11129 Belgrade, Serbia and Montenegro. Tel: + 38111 268 5788; fax: + 38111 264 3691; email: olgicadj@imi.bg.ac.yu

less advanced immunodeficiency. Results were far less satisfactory in patients with poor adherence to treatment, but even in those patients the probability of 4-year survival was 54% [4]. An Italian study found a 62% probability of 15-month survival in patients diagnosed with AIDS and started on antiretroviral therapy after 1996, i.e. in the HAART era [6].

A number of patients on HAART die during treatment. Causes of death include insufficient efficacy of treatment and re-emergence of AIDS, with the development of new AIDS-related illnesses, some of which may be fatal. HAART toxicities, such as acute pancreatitis or lactic acidosis, may also be life-threatening. Hyperlipidaemia, a common HAART-associated side effect, may increase the risk for potentially life-threatening cardiovascular events. Hepatitis C virus (HCV) infection was shown to progress more rapidly in the context of HIV coinfection, with an increased probability of earlier development of end-stage liver disease. For this reason, end-stage liver disease has been among the leading causes of death among HAART-treated AIDS patients [9,10].

This retrospective study was conducted to analyse the probability of, and factors associated with, long-term survival as well as with mortality in HIV-infected patients on HAART, treated in Serbia and Montenegro.

Methods

Patients

A cohort of 481 HIV-infected patients treated with HAART at the HIV/AIDS Department of the Institute for Infectious and Tropical Diseases in Belgrade between 1 January 1998 and 31 December 2005 was retrospectively analysed with the aim of comparing the long-term survivors (LTSs), defined as patients who survived for more than 6 years after starting HAART, with patients who died during HAART. Inclusion criteria consisted of HIV infection (stages A–C according to the 1993 Centers for Disease Control and Prevention (CDC) case definition criteria [11]), initiation of HAART during the above-mentioned period, and regular clinical and laboratory check-ups until the end of the study period. HAART regimens were defined as combinations of two nucleoside reverse transcriptase inhibitors (NRTIs) with one or two protease inhibitors (PIs) or one nonnucleoside reverse transcriptase inhibitor (NNRTI). NRTIs available included zidovudine, lamivudine, abacavir and D-drugs [didanosine (ddI) and stavudine (d4T)], as tenofovir and emtricitabine have not yet been registered in Serbia. PIs included nelfinavir, indinavir, saquinavir and lopinavir/ritonavir, while NNRTIs included efavirenz and nevirapine. Dual Pneumocystis carinii pneumonia (PCP) and toxoplasmosis primary prophylaxis was discontinued after patients achieved a sustained immune response (CD4 cell count >200 cells/μL).

Consent for participation was obtained from all patients, and the study was approved by the Clinical Centre of Serbia Ethics Committee.

Clinical data

Clinical, immunological and virological responses to HAART were evaluated every 2–4 months in a regular disease history check-up and physical examination, along with measurement of plasma viral load and CD4 cell counts and routine haematological and blood chemistry analyses. Additional diagnostic procedures included imaging techniques such as radiography, ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) as well as microbiological and histological examinations when needed.

The structured treatment interruption (STI) strategy was to discontinue HAART, if this was the patient's choice, when CD4 cell counts reached ≥ 350 cells/μL and plasma viral loads become undetectable (≤ 50 HIV-1 RNA copies/mL) and, conversely, to reintroduce the same successful regimen, or another regimen that was potentially less toxic or had different adverse events, when CD4 cell counts dropped to ≤ 250 cells/μL. Thus, STI was offered to those on successful HAART, particularly to those experiencing drug toxicities.

All causes of death were diagnosed clinically, i.e. no autopsies were performed.

Laboratory methods

CD4 cells were quantified by flow cytometry. Plasma HIV-1 RNA loads were measured using a quantitative reverse transcriptase polymerase chain reaction (Ultrasensitive Assay version 1.5; Roche Molecular Systems, Branchburg, NJ), which has a lower limit of detection of 50 copies/mL ($1.7 \log_{10}$).

Statistics

All analyses were performed using an electronic database organized in SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Survival of patients on HAART was analysed by the Kaplan–Meier survival method. Patients were classified into two baseline CD4 cell count strata, i.e. those with baseline CD4 cell counts below and above 100 cells/μL, as we previously showed that a baseline CD4 cell count below 100 cells/μL was a predictor of immunological failure on HAART [12]. For analysis of the differences between the subgroups, nonparametric variables such as gender ratio,

prevalence of hepatitis B virus (HBV) and HCV coinfection, frequency of pretreatment clinical AIDS, and frequency of pretreatment with mono or dual antiretroviral therapy (ART), were compared by χ^2 or Fisher's exact test (depending on the number of patients in a particular analysis). Baseline and end-of-follow-up mean CD4 cell counts and plasma viral loads ($\log_{10}$), as well as age differences between subgroups, were compared by one-way analysis of variance (ANOVA). The level of significance was 0.05. Factors associated with long-term survival on HAART were analysed in a logistic regression model using both univariate and multivariate approaches. Results are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs).

Results

Within the cohort of 481 HIV-infected treated patients, the overall probability of surviving 80 months was 70%, with a significant difference between those with a baseline CD4 cell count of below 100 cells/μL and all other patients (log rank, $P = 0.044$).

Of the 481 patients in the cohort, a total of 48 (22 female and 26 male) patients had survived for more than 6 years. The mean follow-up time was 83.8 ± 5.6 months (range 74–95 months). Thirty patients (11 female and 19 male) died during treatment, after a mean of 33.3 ± 25.0 months (range 3–100 months). Nine deaths were non-AIDS-related, including end-stage liver disease, myocardial infarction and non-AIDS-related malignancies in four, two and three cases, respectively. HIV-related opportunistic tumours and opportunistic infections were the cause of death in 18 cases. Of these, non-Hodgkin's lymphoma and progressive multifocal leucoencephalopathy were the cause of death in five cases each, disseminated tuberculosis in four cases and AIDS dementia complex in three cases, while one patient died of wasting syndrome. Direct HAART toxicity, precisely lactic acidosis, was fatal in three cases.

The patients' characteristics are presented in Table 1. LTSs were significantly ($P = 0.015$) younger at the time of HAART initiation; however, age below 40 years was not a factor predictive of long-term survival ($P = 0.34$). The subgroups did not differ significantly ($P = 0.36$) in the proportion of clinical AIDS cases at treatment initiation, or in baseline CD4 cell counts ($P = 0.06$). However, the proportion of patients with a baseline CD4 cell count of below 100 cells/μL was significantly ($P = 0.02$) higher among those who died. Univariate logistic regression analysis revealed a more than 6-fold increased probability of dying with AIDS in patients who presented with CD4 counts below 100 cells/μL (OR 6.2, 95% CI 1.8–21.0, $P = 0.008$). Clinical AIDS at presentation was not a predictor of poor prognosis ($P = 0.21$). Moreover, the patient subgroups did not differ in the end-of-follow-up CD4 count ($P = 0.3$). In contrast, LTSs achieved significantly lower viral loads than patients who died ($P = 0.03$). However, achieving undetectable viraemia was not a predictor of long-term survival ($P = 0.21$). In our series, neither HBV nor HCV coinfection affected survival (log rank, $P = 0.1$ and $P = 0.8$, respectively).

Table 1 Characteristics of HIV-infected patients on highly active antiretroviral therapy (HAART) according to outcome

Variable	LTSs	Patients who died	*P*
Number of patients	48	30	
Number of female patients	22	11	0.2
Age (years)	36 ± 8.2	46 ± 19.7	0.015
Follow-up time (months)	83.85 ± 5.56	35.34 ± 24.99	< 0.001
Number of patients with clinical AIDS at baseline	28	17	0.36
Number of HBV-infected patients	3	7	0.03
Number of HCV-infected patients	23	12	0.33
Number of patients pretreated with mono and/or dual ART	42	16	0.008
Baseline CD4 count (cells/μL)	98.0 ± 85.0	65.43 ± 47.78	0.06
% of patients with baseline CD4 count ≤ 100 cells/μL	63.6	82.8	0.02
End-of-follow-up CD4 count (cells/μL)	361.6 ± 210.2	309.0 ± 237.2	0.3
% of patients who achieved CD4 count ≥ 350 cells/μL and undetectable plasma viral load	72.9	13.3	0.015
Viral load achieved ($\log_{10}$ copies/mL)	1.8 ± 0.58	2.42 ± 1.31	0.03
Number of drugs taken	8.2 ± 1.97	5.1 ± 1.89	< 0.001
Number of patients ever taking three NRTIs	2	2	0.1
Number of patients ever taking two NRTIs + one NNRTI	19	9	0.2
Number of patients ever taking two NRTIs + one PI	35	17	0.04
Number of patients ever taking all three drug classes	29	10	0.02
Number of patients who underwent STI	33	2	0.003

Values shown are mean ± standard deviation, unless otherwise specified.
ART, antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; LTS, long-term survivor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; STI, structured treatment interruption.

Use of regimens containing PIs and two NRTIs was associated with long-term survival (OR 9.0, 95% CI 2.2–35.98, $P<0.001$). Simultaneous use of all three drug classes was also predictive of long-term survival (OR 7.4, 95% CI 2.2–25.1, $P<0.001$). LTSs took more drugs ($P<0.001$) and had more regimen switching than patients who died. Use of HAART composed of three NRTIs or NRTIs with an NNRTI did not differ between subgroups, while the use of classic HAART (two NRTIs + one PI) and HAART composed of all three drug classes was significantly more common in the LTS subgroup ($P=0.04$ and 0.02, respectively). Pretreatment with mono and/or dual ART was much more common among LTSs ($P=0.008$).

STI was offered to all patients in the cohort who met the criteria described above (data not shown). Almost half of all patients who achieved the most satisfactory response to HAART (65 of 148) underwent STI; these patients were followed up for 55.9 ± 22.7 months, during which period they experienced single or multiple drug holidays no decreasing efficiency on further treatment efficacy. Of the 65 patients who underwent STI, 35 belonged to the subgroups analysed here, 33 of whom eventually achieved long-term survival, and two of whom subsequently died. Accordingly, STI was a strong predictor of a good prognosis (OR 14.9, 95% CI 2.9–75.6, $P<0.001$). As an effective measure to control hyperlipidaemia and hepatitis, STI was mainly suggested to patients with lipodystrophy and/or hyperlipidaemia, as well as to those with severe hepatotoxicity. However, patients who died of end-stage liver disease associated with HCV infection did not undergo STI. Indeed, only one patient among those with end-stage liver disease ever fulfilled the immunological criteria for treatment interruption.

Discussion

The use since 1996 of HAART – a combination of at least three drugs that includes either a PI or a NNRTI and two NRTIs – has substantially improved the prognosis of HIV-infected patients world-wide. HAART was introduced in clinical practice in Serbia in 1997/1998. Up to that time, most of our patients were on mono and/or dual ART. Hence, 43% of patients in the cohort as a whole were heavily pretreated. Patients in our subgroups were even more likely to be heavily pretreated, with 87.5% of LTSs and 53% of patients who died falling into this category, but this is unsurprising in view of the fact that, to qualify as a LTS, a patient had to have survived for at least 6 years, meaning that he or she had to have been entered into the study before 2000 (because new patients as of 1998 were all immediately started on HAART). Thus, although pretreatment with mono or dual ART was reported to decrease the efficacy of HAART [13,14], this was not the case in our series, probably because the pretreated patients significantly more often took HAART regimens including PIs and/or NNRTIs concomitantly with NRTIs [12]. Genotype resistance testing was not routinely performed; thus, subsequent regimens were composed on the basis of standards of care [15]. However, in this study we found that, with appropriate sequencing of potent HAART regimens, even patients with advanced infection had a 70% probability of surviving for more than 6 years. This is encouraging, particularly for resource-limited settings. In such settings, D-drugs, which are associated with mitochondrial hepatotoxicity, are still in use, and it is not uncommon for hepatotoxicity and lactic acidosis to occur among patients with HCV coinfection.

An important finding in this study is the observed benefit of STI. The question of whether STI is beneficial is currently a major issue in HAART management. A major trial, the Strategies for Management of Antiretroviral Therapy (SMART) study, has been designed to determine which of two different HIV treatment strategies would result in greater overall clinical benefit [16]. Patients already on HAART were randomly assigned to either a viral suppression strategy, in which HAART was taken on an ongoing basis to suppress viral load, or a drug conservation strategy, in which HAART was started only when CD4 count dropped to below 250 cells/μL, with the aim of reducing drug side effects and preserving treatment options. These patients took HAART only until their CD4 count reached 350 cells/μL. However, recently presented results of the SMART study have revealed that this STI approach was associated with a more than 2-fold increased risk of disease progression relative to continuous HAART. For this reason, the SMART Executive Committee recommended that enrolment into the trial should be halted, and that it would be prudent to reinitiate therapy in patients on drug holidays.

However, other studies have shown more favourable results of STI. A study by Maggiolo *et al.*, although carried out in a series of patients with far less advanced infection, showed STI to be safe, suggesting that further careful prospective evaluation is needed to determine virological and clinical outcomes over a long period [17]. Similarly, the ISS PART study recently reported results from six Italian and one Swedish infectious disease department where patients on stable HAART whose nadir CD4 cell counts were > 250 cells/μL, and who had excellent immunological and virological responses to HAART, were included in a study of treatment interruptions [18]. HAART was interrupted when CD4 counts reached 500 cells/μL, and reintroduced when patients experienced a CD4 cell count decline to < 350 cells/μL. This approach was rather conservative, but still very useful for investigating the model. The study showed that such treatment interruptions

were safe and beneficial for patients with hyperlipidaemia; in a number of patients with elevated cholesterol and triglycerides, hyperlipidaemia returned to normal values during treatment interruptions.

Similarly, our study showed that the STI strategy we applied was a strong predictor of long-term survival. However, unlike the SMART study, our STI strategy was not guided only by CD4 cell count; instead, patients were advised to undergo a treatment interruption if, along with a CD4 cell count rise to ≥ 350 cells/μL, they achieved undetectable viraemia. Of the patients who fulfilled these criteria for STI, 65 have undergone STI, of whom 33 have so far become LTSs (the remaining patients are still alive but have not yet been followed for long enough). On the other hand, very few patients (four) who subsequently died while on HAART met the criteria for STI, but for some (two) STI did not yet exist as a possible strategy at the time of their management.

Taken together, the results of studies on STI suggest that the STI approach to HAART may be justified. This is an important therapeutic option: drug holidays are popular with both patients and doctors, as drug conservation reduces drug-associated toxicity; furthermore, the economic benefits should not be neglected. Further studies are needed to precisely define the criteria for the application of STI.

In conclusion, treatment initiated before patients experience profound immunodeficiency, use of PIs or simultaneous use of all three drug classes, and treatment interruptions when CD4 cell counts reach ≥ 350 cells/μL and undetectable viraemia is achieved seem to be associated with long-term survival in patients with advanced HIV infection.

Acknowledgements

The work was supported by grant M145002 from the Ministry of Science and Environmental Protection of Serbia.

References

1 Sterne JAC, Hernan MA, Ledergerber B *et al*. Long-term effectiveness of potent antiretroviral therapy in preventing AIDS and death: a prospective cohort study. *Lancet* 2005; **366**: 378–384.

2 Hogg RS, Yip B, Kully C *et al*. Improved survival among HIV-infected patients after initiation of triple-drug antiretroviral regimens. *Can Med Assoc J* 1999; **160**: 659–165.

3 Murphy EL, Collier AC, Kalish LA *et al*. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. *Ann Intern Med* 2001; **135**: 17–26.

4 Sendi PP, Bucher HC, Craig BA *et al*. Estimated AIDS-free survival in a severely immunosuppressed asymptomatic HIV-infected population in the era of antiretroviral triple combination therapy. Swiss HIV Cohort Study. *J Acquir Immune Defic Syndr Hum Retrovirol* 1999; **20**: 376–381.

5 Wood E, Hogg RS, Yip B *et al*. Effect of medication adherence on survival of HIV-infected adults who start highly active antiretroviral therapy when $CD4^+$ cell count is $60350 \times l0^9$ cells/L. *Ann Intern Med* 2003; **139**: 810–816.

6 Pezzotti P, Napoli PA, Acciai S *et al*. Increasing survival time after AIDS in Italy: the role of new combination antiretroviral therapies. *AIDS* 1999; **13**: 249–255.

7 Palella FJ, Delaney KM, Moormon AC *et al*. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J Med* 1998; **338**: 853–860.

8 Egger M, May M, Chene G *et al*. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002; **360**: 119–129.

9 Louie JK, Chin Hsu L, Osmond DH, Katz MH, Schwarcz SK. Trends in causes of death among persons with acquired immunodeficiency syndrome in the era of highly active antiretroviral therapy, San Francisco, 1994–1998. *J Infect Dis* 2002; **186**: 1023–1027.

10 Selik RM, Byers RH Jr, Dworkin MS. Trends in diseases reported on US death certificates that mentioned HIV infection, 1987–1999. *J Acquir Immune Defic Syndr* 2002; **29**: 378–387.

11 1993 CDC revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Morbidity and Mortality Weekly Report* 1992; **41**: 1–19.

12 Jevtović DJ, Salemović D, Ranin J, Pešić I, Žerjav S, Djurković-Djaković O. The dissociation between virological and immunological responses to HAART. *Biomed Pharmacother* 2005; **59**: 446–451.

13 Grabar S, Le Moing V, Goujard C *et al*. Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy. *Ann Intern Med* 2000; **133**: 401–410.

14 Sabin CA, Smith CJ, Youle M *et al*. Deaths in the era of HAART: contribution of late presentation, treatment exposure, resistance and abnormal laboratory markers. *AIDS* 2006; **20**: 67–71.

15 Durant J, Clevenbergh P, Halfon P *et al*. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. *Lancet* 1999; **353**: 2195–2199.

16 El-Sadr W, Neaton J, SMART Study Investigators. Episodic CD4-guided use of ART is inferior to continuous therapy: Results of the SMART study. *13th Conference on Retroviruses and Opportunistic Infections*. Denver, CO, February 2006 [Abstract 106LB].

17 Maggiolo F, Ripamonti D, Gregis G *et al*. Effect of prolonged discontinuation of successful antiretroviral therapy on CD4 T cells: a controlled, prospective trial. *AIDS* 2004; **18**: 439–446.

18 International Study Group on CD4-monitored Treatment Interruptions. CD4 cell-monitored treatment interruption in patients with a CD4 cell count $>500 \times 10^6$ cells/l. *AIDS* 2005; **19**: 287–294.