

The Prognostic Importance of Changes in CD4⁺ Cell Count and HIV-1 RNA Level in Women after Initiating Highly Active Antiretroviral Therapy

Kathryn Anastos, MD; Yolanda Barrón, MS; Mardge H. Cohen, MD; Ruth M. Greenblatt, MD; Howard Minkoff, MD; Alexandra Levine, MD; Mary Young, MD; and Stephen J. Gange, PhD

Background: The prognostic value of CD4⁺ cell counts and HIV-1 RNA levels attained after the initiation of highly active antiretroviral therapy (HAART) compared with before the initiation of HAART has not been well defined.

Objective: To determine the prognostic value for clinical outcomes of CD4⁺ cell counts and HIV-1 RNA levels attained after initiating therapy.

Design: Prospective cohort study.

Setting: Women's Interagency HIV Study.

Patients: 1132 participants in the Women's Interagency HIV Study.

Measurements: HIV-1 RNA level, CD4⁺ cell counts, AIDS-defining illness, and death.

Results: In multivariate analyses with a median follow-up of 3.9 years, women with CD4⁺ cell counts of less than 0.200×10^9 cells/L compared with women with CD4⁺ cell counts of greater than 0.350×10^9 cells/L after HAART initiation had a relative hazard of death from all causes of 2.66 (95% CI, 1.42 to 4.99) and a relative hazard of death from AIDS of 47.61 (CI, 5.69 to 398.40). The relative hazard of all-cause death was 3.44 (CI, 1.67

to 7.09) in women with RNA levels of more than 10 000 copies/mL compared with women with attained RNA levels of less than 80 copies/mL. The relative hazard of AIDS-related or all-cause death did not increase for women with post-HAART CD4⁺ cell counts between 0.200 and 0.350×10^9 cells/L compared with women with CD4⁺ cell counts of greater than 0.350×10^9 cells/L. Also, the relative hazard did not increase in women with post-HAART HIV-1 RNA levels between 80 and 10 000 copies/mL compared with women with post-HAART HIV-1 RNA levels of less than 80 copies/mL. Of the laboratory markers, only the post-HAART CD4⁺ cell count and HIV-1 RNA level were predictive of new AIDS-defining illness.

Conclusion: Post-HAART laboratory markers predicted death and new AIDS-defining illness. Pre-HAART CD4⁺ cell count and HIV-1 RNA level were not predictive of clinical outcomes if adjusted for values attained after HAART initiation, suggesting that even advanced immune suppression can be overcome with HAART that results in CD4⁺ cell counts of greater than 0.200×10^9 cells/L and RNA levels of less than 10 000 copies/mL.

Ann Intern Med. 2004;140:256-264.

www.annals.org

For author affiliations, see end of text.

See editorial comment on pp 305-306.

Public health data, cohort studies, and randomized clinical trials have demonstrated dramatic improvements in HIV-1–related morbidity and mortality (1–6) since the introduction of highly active antiretroviral therapy (HAART). Several studies have provided information that addresses the decision to initiate HAART by demonstrating that adverse clinical outcomes are less likely in those who initiate HAART with CD4⁺ cell counts of greater than 0.200×10^9 cells/L, HIV-1 RNA levels of less than 50 000 copies/mL, and an absence of a clinical AIDS-defining event (7–11). However, the prognostic significance of the values for CD4⁺ cell count and quantitative HIV-1 RNA level attained after initiation of HAART has not been fully examined. In particular, we do not know the extent to which the improvements in prognostic markers that occur during treatment with HAART can modify the prognosis conferred by the values of these markers before HAART initiation. Although analyses of some cohorts (12–14) have indicated that the values for CD4⁺ cell count and quantitative HIV-1 RNA level attained after HAART initiation predict clinical outcome, the extent to which these effects are independent of the CD4⁺ cell count and HIV-1 RNA level before HAART initiation has not been determined.

It is important to know the extent to which the post-HAART compared with the pre-HAART laboratory markers predict clinical manifestations of HIV-1 infection. Therefore, we investigated the patterns of change and the prognostic value of time-varying measurements of CD4⁺ cell counts and quantitative HIV-1 RNA level after HAART initiation, with adjustment for clinical disease stage, CD4⁺ cell count, and quantitative HIV-1 RNA level at HAART initiation. Our analysis was conducted using data from the Women's Interagency HIV Study (WIHS), an ongoing cohort study of HIV-1–infected women in the United States.

METHODS

Study Sample

The WIHS is a multicenter prospective cohort study of the natural history of HIV-1 infection in women. It is being conducted in 5 locations within the United States: New York City (2 sites); Washington, DC; Chicago, Illinois; and southern California and the San Francisco Bay area. The WIHS methods and baseline cohort characteristics have been described previously (15). Briefly, from October 1994 through November 1995, 2628 women (2059

HIV-1 seropositive and 569 HIV-1 seronegative) were enrolled. We obtained informed consent from the participants in accordance with procedures and consent materials reviewed and approved by the committee on human experimentation at each of the collaborating institutions. Every 6 months, WIHS participants were interviewed by using a structured questionnaire and received a physical examination. Blood specimens were collected at each visit for determination of HIV-1 RNA level and CD4⁺ cell count. Definitions and use of HAART in this cohort have previously been described (16).

In the present analysis, we included all WIHS participants who were alive and reported initiation of HAART after July 1995 and for whom the date of HAART initiation could be estimated within a 1-year interval.

Outcome Variables

The primary outcome variables were the development of a clinical AIDS-defining event and death. Diagnoses of clinical AIDS-defining illnesses were self-reported and conform to the class C clinical conditions in the 1993 case definition of AIDS (17) (that is, criteria for AIDS-defining illness did not include the immunologic criteria of CD4⁺ cell count of less than 0.200×10^9 cells/L). A participant was considered to have developed a new AIDS-defining illness after HAART initiation if she reported having had in the previous 6 months any of the AIDS-defining conditions included as a class C clinical condition in the 1993 case definition of AIDS (17) except 1) a report of tuberculosis, cryptosporidiosis, cryptococcosis, toxoplasmosis, disseminated *Mycobacterium avium* complex, AIDS dementia complex or AIDS-defining cancer, or cytomegalovirus infection or chronic mucosal herpes simplex infection in a woman who had previously reported having that specific condition; 2) a report of recurrent bacterial pneumonia less than 1 year from a previous report of recurrent bacterial pneumonia; and 3) a report of wasting in a woman who had previously reported wasting within the previous 2 years. An AIDS-defining illness reported at the same study visit at which HAART was first reported was not classified as incident. Deaths were ascertained continuously by notification of participant death from participant friends, relatives, and medical providers. Information on deaths was also obtained periodically through national and local death registries. Death certificates were requested for all women who were known to have died. Date of death was ascertained in descending order of priority from the death certificate, medical records, medical provider, and family or friends.

We determined AIDS-free and survival times from the date participants reported initiating HAART (defined as the midpoint between the last visit reporting no use of HAART and the first visit reporting use of HAART) through 30 September 2001. Women who did not report a new AIDS-defining illness or were alive at the end of this period of observation contributed with censored observa-

Context

Clinicians have been uncertain about whether they should use pretreatment or post-treatment laboratory markers to predict outcomes for patients receiving highly active antiretroviral therapy (HAART).

Contribution

The authors used predictive models to simultaneously test the ability of pre- and post-treatment CD4⁺ cell counts and HIV-1 RNA levels to predict new AIDS-defining illness or death. Post-treatment CD4⁺ cell count and HIV-1 RNA levels predicted new AIDS-defining illness or death, but pretreatment levels did not.

Implication

Patients with a good CD4⁺ cell count and HIV-1 RNA response to HAART have a favorable prognosis despite poor pretreatment values of these markers.

—The Editors

tions to the survival analyses of time to development of a new AIDS-defining illness and time to death, respectively. Participants seen from 1 October 2000 to 30 September 2001 with no report of outcome of interest (that is, AIDS-defining illness or death) were considered censored at the date of analysis (30 September 2001). We performed 2 analyses of survival times, considering as events all deaths of any cause and AIDS-related deaths. Data were analyzed by intention to continue treatment, ignoring treatment changes and interruptions.

Exposure Variables

The primary exposure variables for these analyses included age at HAART initiation (for each 10-year increment) and self-report of an AIDS-defining illness before HAART initiation, CD4⁺ cell count and HIV-1 RNA level obtained at the last study visit at which no use of HAART was reported, and CD4⁺ cell count and HIV-1 RNA level as time-varying measurements after HAART initiation (attained CD4 and HIV-1 RNA).

Laboratory Methods

HIV-1 RNA level in plasma was quantified for all participants by using Nuclisens (Organon Teknika Corp., Durham, North Carolina), the more sensitive method of isothermal nucleic-acid-sequence-based amplification (with a lower limit of quantification of 80 copies/mL), in laboratories that were certified by the Virology Quality Assurance Laboratory proficiency testing program of the National Institutes of Health. Previous analyses have found nucleic-acid-sequence-based amplification to be statistically equivalent to reverse transcriptase polymerase chain reaction values among WIHS samples (18). Lymphocyte subsets were determined by using flow cytometry performed in laboratories certified by the Flow Cytometry

Quality Assessment Program of the National Institute of Allergy and Infectious Diseases (19).

Statistical Analysis

Pre-HAART CD4⁺ cell count and HIV-1 RNA levels were defined as the values of these markers obtained in the study visit immediately before the time of HAART initiation. Pre-HAART AIDS was defined as any report by the study participant of an AIDS-defining illness before HAART initiation. Post-HAART (attained) measurements of the CD4⁺ cell count and HIV-1 RNA levels included measurements at each study visit after HAART initiation and before the last follow-up visit, first report of a new AIDS-defining illness, or time of death. These variables were used as time-varying covariates.

Because clinicians managing HIV-1 infection use the absolute change in laboratory markers as well as specific thresholds, pre-HAART and post-HAART initiation markers of disease progression were evaluated as both categorical and continuous variables. When categorized, the cutoffs for CD4⁺ cell count and HIV-1 RNA level were 0.200×10^9 cells/L and 0.350×10^9 cells/L and 80 copies/mL and 10 000 copies/mL, respectively. To maintain uniformity in the directionality of risk, we used a CD4⁺ cell count of greater than 0.350×10^9 cells/L and HIV-1 RNA level of less than 80 copies/mL as reference categories for the estimation and testing of relative hazards. Relative hazards for continuous exposures were estimated and tested and were interpreted as the risk in outcome for each decrease of 0.100×10^9 cells/L in CD4⁺ cell count and 1 log₁₀ increase in HIV-1 RNA level, respectively. To investigate possible interactions between pre-HAART and post-HAART initiation markers, we categorized CD4⁺ cell

count and HIV-1 RNA level as binary variables; cutoffs were 0.200×10^9 cells/L and 10 000 copies/mL, respectively. Reference groups were CD4⁺ cell count of greater than 0.200×10^9 cells/L and HIV-1 RNA of level of less than 10 000 copies/mL. The interactions investigated were pre-HAART and post-HAART initiation CD4⁺ cell count of less than 0.200×10^9 cells/L and pre-HAART and post-HAART HIV-1 RNA level of greater than 10 000 copies/mL. We used univariate proportional hazards regression models and included each of the continuous covariates or the indicator variables for the categories of the predictor in different models.

Multivariate Cox regression models of progression to new AIDS-defining illness and death included a pre-HAART report of an AIDS-defining illness, pre-HAART CD4⁺ cell count and HIV-1 RNA levels, and time-varying post-HAART CD4⁺ cell count and HIV-1 RNA levels (20). All analyses were performed by using PROC PHREG of the SAS statistical package, version 8 (SAS Institute, Inc., Cary, North Carolina).

Role of the Funding Source

The funding source supported the collection and analysis of the data. The data were interpreted and the decision to submit the manuscript for publication was made in joint consultation between the funding source and the authors. The funding source did not place restrictions on the interpretation of the data or on the content of the manuscript.

RESULTS

Of the 2059 women with HIV-1 infection who had the infection at the time of WIHS enrollment and the 9

Table 1. Demographic and Baseline (before Highly Active Antiretroviral Therapy Initiation) Clinical Characteristics of 1132 Women with Known Date (± 6 Months) of Initiation of HAART between July 1995 and September 2001

Characteristic	AIDS-Free at Initiation		
	Total (n = 580)	New AIDS-Defining Illness (n = 92)	Deaths* (n = 37)
Date of initiation	April 1997 (August 1995–November 1999)	January 1997 (September 1995–June 1999)	December 1997 (September 1995–October 1999)
Median age at initiation (range), y	38 (20–74)	37 (21–68)	43 (23–71)
Therapy-naive at initiation, n (%)	98 (16.9)	13 (14.1)	4 (10.8)
Ethnicity, n (%)			
African American	299 (51.6)	45 (48.9)	25 (67.6)
White	105 (18.1)	16 (17.4)	3 (8.1)
Latina	157 (27.1)	29 (31.5)	8 (21.6)
Other	19 (3.2)	2 (2.2)	1 (2.7)
HIV transmission category, n (%)			
Intravenous drug use	146 (25.4)	24 (26.7)	13 (36.1)
Heterosexual risk exposure	255 (44.5)	40 (44.4)	13 (36.1)
Blood transfusion	25 (4.3)	2 (2.2)	1 (2.8)
Unknown risk	147 (25.7)	24 (26.7)	9 (25.0)
Median CD4 ⁺ cell count at visit preceding initiation (range), $\times 10^9$ cells/L	0.318 (0.004–1.413)	0.246 (0.006–0.789)	0.198 (0.006–0.531)
Median HIV RNA level at visit preceding initiation (range), copies/mL	9300 (<80–1 600 000)	117 000 (<80–1 300 000)	68 000 (<80–1 600 000)
Median follow-up time after initiation (range), y	4.1 (0.2–5.7)	1.9† (0.4–4.7)	1.5 (0.3–4.7)

* Events after initiation of highly active antiretroviral therapy (HAART) and before October 2001.

† Follow-up time to incident AIDS-defining illness; all others are total follow-up time.

women who acquired HIV infection during the WIHS follow-up period, 1132 initiated HAART between July 1995 and 30 September 2001, had a HAART initiation date known within 1 year, and contributed some follow-up time after HAART initiation. The median follow-up was 3.9 years (interquartile range, 2.5 to 4.8 years). Only 77 (6.8%) of the 1132 included women were lost to follow-up (excluding deaths). **Table 1** shows the demographic and baseline clinical characteristics of the included women. The median CD4⁺ cell count was lower (0.202×10^9 cells/L vs. 0.318×10^9 cells/L; $P < 0.001$ [Wilcoxon rank-sum test]), and the median HIV-1 RNA level was higher (23 500 copies/mL vs. 9300 copies/mL; $P < 0.001$ [Wilcoxon rank-sum test]) in 552 women initiating HAART after reporting an AIDS-defining illness compared with 580 women who had not reported a previous AIDS-defining illness.

Progression to Death

All-Cause Death

During follow-up after HAART initiation, 135 women (11.8%) died; 60 deaths were from AIDS, 33 were from non-AIDS causes, 5 were of indeterminate cause, and 37 were of unknown cause. In univariate analysis, all of the exposure variables (baseline CD4⁺ cell count and viral load, age at HAART initiation, report of an AIDS-defining illness before HAART initiation, and attained CD4⁺ cell count and viral load) were significantly associated with death (**Table 2**). However, in multivariate analysis using continuous or categorical classification of the laboratory markers, only the values attained after HAART initiation for CD4⁺ cell count and HIV-1 RNA level, age, and a report of an AIDS-defining illness before HAART initia-

tion were significantly associated with survival. The CD4⁺ cell count and quantitative HIV-1 RNA level measured before HAART initiation were not predictive. The relative hazard of death was 1.50 (95% CI, 1.27 to 1.80) for each decrease of 0.100×10^9 cells/L in attained CD4⁺ cell count and 1.33 (CI, 1.10 to 1.63) for each log₁₀ increase in attained quantitative HIV-1 RNA level. This would result in a relative hazard of 2.25 (relative hazard of 1.5 squared) for a decrease of 0.200×10^9 cells/L in CD4⁺ cell count. Similarly, in analyses with the commonly used clinical thresholds for CD4⁺ cell count (**Table 2**), the relative hazard of death was 2.66 (CI, 1.42 to 4.99) for women who attained a CD4⁺ cell count of less than 0.200×10^9 cells/L compared with women with a CD4⁺ cell count of greater than 0.350×10^9 cells/L after HAART initiation and 3.44 (CI, 1.67 to 7.09) for women who attained an HIV-1 RNA level of greater than 10 000 copies/mL compared with women who attained an HIV-1 RNA level of less than 80 copies/mL after HAART initiation. There was no statistically significant difference in progression to death for women with an attained CD4⁺ cell count of 0.200 to 0.350×10^9 cells/L compared with those with an attained CD4⁺ cell count of greater than 0.350×10^9 cells/L or for women with an attained HIV-1 RNA level of 80 to 10 000 copies/mL compared with women with an attained HIV-1 RNA level of less than 80 copies/mL. AIDS-defining illnesses reported before HAART initiation remained significantly associated with progression to death in multivariate analysis (relative hazard, 2.15 [CI, 1.41 to 3.28]).

We also investigated the interaction between pre-HAART and post-HAART markers. Overall, these models did not demonstrate strong interaction effects. Some evi-

Table 1—Continued

Total (n = 552)	AIDS at Initiation	
	New AIDS-Defining Illness (n = 184)	Deaths* (n = 98)
April 1997 (July 1995–April 2001)	March 1997 (October 1995–October 2000)	January 1997 (July 1995–April 2001)
40 (20–62)	40 (20–60)	42 (25–62)
93 (16.8)	33 (17.9)	12 (12.2)
308 (55.8)	113 (61.4)	61 (62.2)
101 (18.3)	29 (15.8)	16 (16.3)
131 (23.7)	41 (22.3)	18 (18.4)
12 (2.2)	1 (0.5)	3 (3.1)
208 (38.1)	81 (44.3)	41 (41.8)
238 (43.6)	72 (39.3)	42 (42.9)
21 (3.8)	9 (4.9)	5 (5.1)
79 (14.5)	21 (11.5)	10 (10.2)
0.202 (0–1.896)	0.172 (0–0.864)	0.100 (0–0.978)
23 500 (<80–6 900 000)	47 000 (<80–6 900 000)	73 000 (<80–6 100 000)
3.7 (0.1–6.0)	1.6† (0.2–4.4)	1.9 (0.3–4.6)

Table 2. Predictors of All-Cause and AIDS-Related Death after Highly Active Antiretroviral Therapy Initiation*

Variable	Relative Hazard for Death from any Cause (95% CI)		Relative Hazard for Death from AIDS (95% CI)	
	Univariate Analysis	Multivariate Analysis	Univariate Analysis	Multivariate Analysis
Pre-HAART age for each 10-year increment	1.56 (1.29–1.90)	1.61 (1.27–2.05)	1.28 (0.94–1.73)	1.38 (0.95–2.01)
Pre-HAART CD4 ⁺ cell count				
<0.200 × 10 ⁹ cells/L	4.04 (2.47–6.59)	1.09 (0.56–2.11)	12.08 (4.35–33.52)	0.68 (0.22–2.10)
0.200–0.350 × 10 ⁹ cells/L	1.75 (0.98–3.11)	1.04 (0.55–2.00)	2.31 (0.68–7.89)	0.58 (0.16–2.05)
>0.350 × 10 ⁹ cells/L	Reference	Reference	Reference	Reference
Pre-HAART HIV-1 RNA level				
<80 copies/mL	Reference	Reference	Reference	Reference
80–10 000 copies/mL	1.67 (0.51–5.51)	1.42 (0.43–4.77)	0.78 (0.09–6.99)	0.54 (0.06–4.92)
>10 000 copies/mL	4.26 (1.35–13.44)	1.95 (0.60–6.34)	6.88 (0.95–49.79)	1.89 (0.25–14.42)
Post-HAART CD4 ⁺ cell count				
<0.200 × 10 ⁹ cells/L	5.95 (3.83–9.24)	2.66 (1.42–4.99)	92.95 (12.87–671.47)	47.61 (5.69–398.40)
0.200–0.350 × 10 ⁹ cells/L	1.34 (0.73–2.44)	0.90 (0.45–1.81)	3.76 (0.68–41.47)	3.23 (0.28–37.36)
>0.350 × 10 ⁹ cells/L	Reference	Reference	Reference	Reference
Post-HAART HIV-1 RNA level				
<80 copies/mL	Reference	Reference	Reference	Reference
80–10 000 copies/mL	2.34 (1.18–4.62)	1.81 (0.87–3.75)	4.40 (0.98–19.65)	2.16 (0.47–9.79)
>10 000 copies/mL	7.53 (4.01–14.12)	3.44 (1.67–7.09)	20.49 (4.96–84.68)	2.80 (0.65–12.14)
AIDS before HAART initiation	3.12 (2.14–4.56)	2.15 (1.41–3.28)	4.67 (2.48–8.78)	2.17 (1.09–4.32)

* HAART = highly active antiretroviral therapy.

dence showed that women with both pre-HAART and post-HAART CD4⁺ cell counts of less than 0.200 × 10⁹ cells/L were at highest risk for death (relative hazard, 2.89) relative to women with CD4⁺ cell counts greater than 0.200 × 10⁹ cells/L, although this was not statistically significant.

AIDS-Specific Death

In univariate analysis, pre-HAART and post-HAART CD4⁺ cell counts of less than 0.200 × 10⁹ cells/L compared with cell counts of greater than 0.350 × 10⁹ cells/L and post-HAART HIV-1 RNA levels of greater than 10 000 copies/mL compared with levels of less than 80

copies/mL were extremely powerful predictors of AIDS-related death (Table 2). Relative hazard for pre-HAART CD4⁺ cell count was 12.08 (CI, 4.35 to 33.52), relative hazard for post-HAART CD4⁺ cell count was 92.95 (CI, 12.87 to 671.47), and relative hazard for post-HAART HIV-1 RNA level was 20.49 (CI, 4.96 to 84.68). However, in multivariate analysis, the post-HAART CD4⁺ cell count remained a powerful predictor (relative hazard, 47.61 [CI, 5.69 to 398.40]), but the post-HAART HIV-1 RNA level and pre-HAART CD4⁺ cell count were not significant predictors. The findings were similar using CD4⁺ cell count as a continuous variable: Of the laboratory markers, only post-HAART CD4⁺ cell count pre-

Table 3. Predictors of Developing an AIDS-Defining Illness after Initiation of Highly Active Antiretroviral Therapy*

Variable	Relative Hazard (95% CI)	
	Univariate Analysis	Multivariate Analysis
Pre-HAART age for each 10-year increment	1.10 (0.95–1.27)	1.03 (0.87–1.22)
Pre-HAART CD4 ⁺ cell count		
<0.200 × 10 ⁹ cells/mL	2.25 (1.68–3.01)	0.77 (0.51–1.17)
0.200–0.350 × 10 ⁹ cells/mL	1.19 (0.85–1.69)	0.74 (0.50–1.09)
>0.350 × 10 ⁹ cells/mL	Reference	Reference
Pre-HAART HIV-1 RNA level		
<80 copies/mL	Reference	Reference
80–10 000 copies/mL	0.86 (0.49–1.51)	0.87 (0.49–1.56)
>10 000 copies/mL	1.65 (0.98–2.80)	1.12 (0.63–1.99)
Post-HAART CD4 ⁺ cell count		
<0.200 × 10 ⁹ cells/mL	3.85 (2.89–5.12)	2.83 (1.86–4.30)
0.200–0.350 × 10 ⁹ cells/mL	1.75 (1.26–2.44)	1.59 (1.07–2.34)
>0.350 × 10 ⁹ cells/mL	Reference	Reference
Post HAART HIV-1 RNA level		
<80 copies/mL	Reference	Reference
80–10 000 copies/mL	1.33 (0.94–1.89)	0.97 (0.66–1.41)
>10 000 copies/mL	3.03 (2.19–4.20)	1.60 (1.10–2.35)
AIDS before HAART initiation	2.68 (2.09–3.45)	2.10 (1.58–2.77)

* HAART = highly active antiretroviral therapy.

dicted AIDS-related death (relative hazard, 4.44 [CI, 2.75 to 7.19] for each decrease of 0.100×10^9 cells/L).

Development of AIDS-Defining Illness

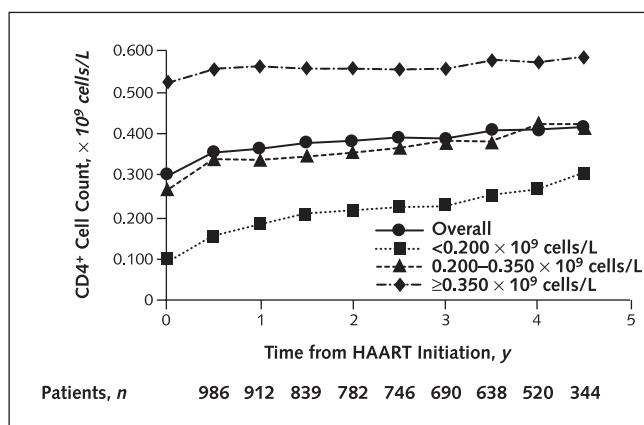
During follow-up after initiation of HAART, 276 women developed a new AIDS-defining illness: 92 of 580 women (15.9%) previously free of clinical AIDS and 184 of 552 women (33.3%) who had reported an AIDS-defining illness before initiation of HAART. The mean and median CD4⁺ cell counts were 0.259×10^9 cells/L and 0.209×10^9 cells/L, respectively, at the visit before the one during which a new AIDS-defining illness was reported. Pre-HAART AIDS was a predictor of post-HAART AIDS-defining illness in multivariate analyses that included CD4⁺ cell count and HIV-1 RNA level as continuous (2.06 [CI, 1.56 to 2.71]) or categorical (2.10 [CI, 1.58 to 2.77]) variables (Table 3). As with death, in multivariate analyses, the values for CD4⁺ cell count and HIV-1 RNA level attained after HAART initiation predicted the development of new AIDS-defining illnesses, whereas the pre-HAART values did not. The relative hazard of new AIDS-defining illness was 1.24 (CI, 1.13 to 1.36) for each decrease of 0.100×10^9 cells/L in attained CD4⁺ cell count and 1.22 (CI, 1.08 to 1.39) for each log₁₀ increase in attained quantitative HIV-1 RNA level. Women attaining a CD4⁺ cell count of less than 0.200 (relative hazard, 2.83 [CI, 1.86 to 4.30]) or 0.200 to 0.350×10^9 cells/L (relative hazard, 1.59 [CI, 1.07 to 2.34]) were more likely to develop an AIDS-defining illness after HAART initiation than those whose attained CD4⁺ cell count was greater than 0.350×10^9 cells/L. Quantitative HIV-1 RNA level of greater than 10 000 copies/mL after HAART initiation was also associated with new AIDS-defining illness (relative hazard, 1.60 [CI, 1.10 to 2.35]), whereas values of 80 to 10 000 copies/mL were not (relative hazard, 0.97 [CI, 0.66 to 1.41]).

The attained CD4⁺ cell count and HIV-1 RNA level were predictive of new AIDS-defining illness both in women who did not have AIDS before HAART initiation (relative hazard, 1.32 [CI, 1.13 to 1.54] for each decrease of 0.100×10^9 cells/L in CD4⁺ cell count and 1.30 [CI, 1.04 to 1.62] for each log₁₀ increase in HIV-1 RNA level) and in women who reported having AIDS before HAART initiation (relative hazard, 1.20 [CI, 1.06 to 1.35] for each decrease of 0.100×10^9 cells/L in CD4⁺ cell count and 1.19 [CI, 1.02 to 1.39] for each log₁₀ increase in HIV-1 RNA level).

Patterns of CD4⁺ Cell Count and HIV-1 RNA Responses

The overall mean CD4⁺ cell count rose from 0.301 to 0.419×10^9 cells/L during 5 years of follow-up (Figure 1). Similar patterns of improvement were observed in women with CD4⁺ cell counts of less than 0.200, 0.200 to 0.350, and greater than 0.350×10^9 cells/L. Among the 377 women who initiated HAART with a CD4⁺ cell count of less than 0.200×10^9 cells/L, 43% had attained a CD4⁺ cell count of greater than 0.200×10^9 cells/L and 11%

Figure 1. Mean CD4⁺ cell count after highly active antiretroviral therapy (HAART) initiation, stratified by CD4⁺ count before HAART initiation.



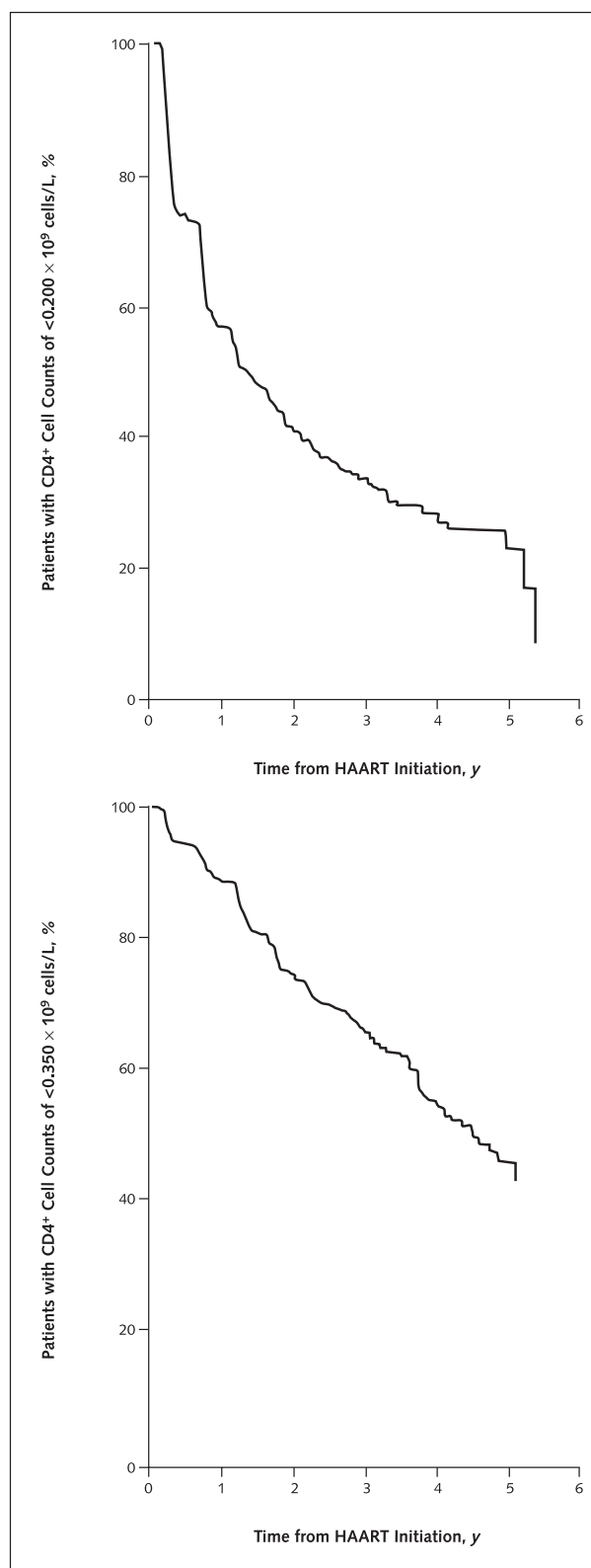
Points represent mean CD4⁺ cell counts at semiannual study visits.

had attained a CD4⁺ cell count of greater than 0.350×10^9 cells/L within 1 year after HAART initiation (Figure 2). At 2.5 years after HAART initiation, 64% had reached a CD4⁺ cell count of greater than 0.200×10^9 cells/L and 30% had attained a CD4⁺ cell count of greater than 0.350×10^9 cells/L. By 4.5 years after HAART initiation, among women who had pre-HAART CD4⁺ cell counts of less than 0.200×10^9 cells/L and who were still living, 76% had attained a CD4⁺ cell count of greater than 0.200×10^9 cells/L and 50% had values of greater than 0.350×10^9 cells/L. A significant upward trend was seen over time in the proportion of women who attained a CD4⁺ cell count of greater than 0.200×10^9 cells/L (odds ratio, 1.18 for each 6-month interval; $P < 0.001$), and a slight but significant upward trend (odds ratio, 1.03; $P = 0.003$) was seen in the proportion of women whose HIV-1 RNA levels became undetectable over time. Of the 1132 women initiating HAART, 612 (54%) had HIV-1 RNA levels of less than 80 copies/mL at any time during the study.

DISCUSSION

In this study of 1132 HIV-1-infected women who initiated HAART and were followed for up to 5.5 years, the CD4⁺ cell count and plasma HIV-1 RNA levels attained after initiating treatment were more important in predicting new AIDS-defining events and all-cause and AIDS-related death than the values measured before HAART initiation. Indeed, the pre-HAART measurements were not predictive of outcome once adjusted for the values attained after initiating treatment. Even the very powerful predictive value of pre-HAART CD4⁺ cell count in predicting AIDS-related deaths (relative hazard > 12.0) was obviated by the addition of the post-HAART CD4⁺ cell count, which maintained its power in predicting AIDS-related deaths: The risk for dying of AIDS was

Figure 2. Kaplan–Meier estimates of the proportion of women with CD4⁺ cell counts of less than 0.200×10^9 cells/L (top) and less than 0.350×10^9 cells/L (bottom) after highly active antiretroviral therapy (HAART) initiation.



Eighty women initiating HAART with CD4⁺ cell counts $< 0.200 \times 10^9$ cells/L died during the study period.

nearly 50-fold higher in women with CD4⁺ cell counts of less than 0.200×10^9 cells/L compared with women with CD4⁺ cell counts of greater than 0.350×10^9 cells/L after HAART initiation. These findings suggest that even in women with advanced immunologic abnormalities, HAART that raises the CD4⁺ cell count to greater than 0.200×10^9 cells/L and reduces HIV-1 RNA level to less than 10 000 copies/mL effectively mitigates the negative prognosis associated with low CD4⁺ cell counts and high HIV-1 RNA level before therapy. Additional benefit in preventing new AIDS-defining illness—but not death—was seen in women attaining CD4⁺ cell counts of 0.200 to 0.350×10^9 cells/L. In addition, we found that the presence of a clinical AIDS-defining illness before HAART initiation remained a significant predictor of death and incident AIDS-defining conditions.

We also found a continued upward trend in CD4⁺ cell counts over the 5 years of study observation. However, among women with CD4⁺ counts of less than 0.200×10^9 cells/L pre-HAART, only two thirds had attained CD4⁺ cell counts of greater than 0.200×10^9 cells/L within 2.5 years after HAART initiation.

Previous studies (12–14) have demonstrated that in persons who have initiated HAART, the most recent, or time-dependent, CD4⁺ cell count is a strong predictor of clinical outcome. However, the CD4⁺ cell count and HIV-1 RNA values attained after initiation of HAART are strongly correlated with the measured values of these markers before initiation of HAART. Our results suggest that the effectiveness of HAART is characterized by a response to therapy that results in an increase of CD4⁺ cell count to greater than 0.200×10^9 cells/L and a decrease in HIV-1 RNA level to less than 10 000 copies/mL, without regard to the pretreatment values. In addition, further reduction in the occurrence of AIDS-defining illness was observed among women with CD4⁺ cell counts of greater than 0.350×10^9 cells/L. Of the 1132 participants in our study, only 17% were naive to antiretroviral therapy (Table 1), indicating that the benefits of HAART occur even among women who have been exposed to previous antiretroviral therapy and in whom undetectable levels of HIV-1 RNA may not be achieved. These findings are important to HIV-1-infected persons who initiate therapy with advanced HIV-1-related immunodeficiency and who must decide with their health care providers how to interpret the laboratory values attained after initiating therapy. The proportion of persons achieving CD4⁺ cell counts of greater than 0.200×10^9 cells/L after HAART initiation in our study may be a low estimate because of our analysis by “intention to remain on treatment”; some participants may have discontinued therapy. Models that use postinitiation treatment data and allow for switching or discontinuation of HAART regimens are complicated by selection-by-indication biases because persons who change therapy after initiation are different from those who remain on regimens (21, 22).

In addition, we found that a report of an AIDS-defining illness before HAART initiation was associated with increased morbidity and mortality, independent of CD4⁺ cell count and HIV-1 RNA levels attained after HAART initiation. Thus, improvements in CD4⁺ cell count and HIV-1 RNA levels after HAART initiation may indicate suppression of HIV replication and cessation or decrease of CD4⁺ cell turnover, but limited CD4⁺ repertoire may also be present and indicated by previous AIDS-defining conditions.

Our findings support those of previous studies (13) that demonstrated an association of viral burden after HAART initiation with morbidity or mortality, independent of the attained CD4⁺ cell count. Previous studies have shown conflicting results regarding the prognostic importance of HIV-1 RNA level after HAART initiation. One study (14) showed no association with death, and others demonstrated an increased hazard of death with each log₁₀ increase in HIV-1 RNA level (13) or with values greater than 10 000 copies/mL (12).

As indicated by Egger and colleagues (11), the rates of progression to AIDS for HIV-1-infected persons who use HAART have decreased to such an extent that disease progression is occurring predominantly in those who initiated HAART when their CD4⁺ cell counts were less than 0.200×10^9 cells/L. We have thus tried to capture morbid events with a wider net than just progression to AIDS among those who were AIDS free at HAART initiation by including all AIDS-defining events, even in persons who had AIDS before initiating HAART. However, a limitation of the study is that we have not included measures of treatment-related morbidity.

In summary, we found that the high risk for death from AIDS indicated by low CD4⁺ cell count and high HIV-1 RNA levels before HAART initiation could be overcome by improvement in these values after HAART initiation and that the risk for an incident AIDS-defining illness among those at continued risk (that is, in the 88.2% of survivors) was also overcome by an increase in the CD4⁺ cell count. Both mortality and AIDS-related morbidity after HAART initiation were predicted only by the values attained after initiating HAART and by the presence of an AIDS-defining illness before HAART initiation.

APPENDIX

Data in this manuscript were collected by the Women's Interagency HIV Study (WIHS) Collaborative Study Group with centers (principal investigators) at New York City/Bronx Consortium (Kathryn Anastos); Brooklyn, New York (Howard Minkoff); Washington, DC, Metropolitan Consortium (Mary Young); The Connie Wofsy Study Consortium of Northern California (Ruth Greenblatt, Phyllis Tien); Los Angeles County/Southern California Consortium (Alexandra Levine); Chicago Consortium (Mardge Cohen); and Data Coordinating Center (Stephen J. Gange, Alvaro Muñoz).

From Montefiore Medical Center and Lincoln Medical and Mental Health Center, Bronx, New York; Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland; Cook County Hospital, Chicago, Illinois; University of California, San Francisco, San Francisco, California; Health Science Center at Brooklyn, Brooklyn, New York; University of Southern California, Los Angeles, California; and Georgetown University Medical Center, Washington, DC.

Grant Support: The WIHS is funded by the National Institute of Allergy and Infectious Diseases with supplemental funding from the National Cancer Institute, the National Institute on Drug Abuse, and the National Institute of Dental Research (grants U01-AI-35004, U01-AI-31834, U01-AI-34994, U01-AI-34989, U01-AI-34993, and U01-AI-42590). Funding was also provided by the National Institute of Child Health and Human Development (grant U01-HD-32632) and the National Center for Research Resources (grants M01-RR-00071, M01-RR-00079, and M01-RR00083).

Potential Financial Conflicts of Interest: None disclosed.

Requests for Single Reprints: Kathryn Anastos, MD, Women's Interagency HIV Study, 3311 Bainbridge Avenue, Second Floor, Bronx, NY 10467; e-mail, kanastos@verizon.net.

Current author addresses and author contributions are available at www.annals.org.

References

1. HIV/AIDS Surveillance Report Year End Edition. Atlanta: U.S. Department of Health and Human Services; Centers for Disease Control and Prevention; 1999;10:1-43.
2. Chiasson MA, Berenson L, Li W, Schwartz S, Singh T, Forlenza S, et al. Declining HIV/AIDS mortality in New York City. *J Acquir Immune Defic Syndr*. 1999;21:59-64. [PMID: 10235515]
3. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, et al. A controlled trial of two nucleoside analogues plus didanosine in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. *N Engl J Med*. 1997;337:725-33. [PMID: 9287227]
4. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med*. 1998;338:853-60. [PMID: 9516219]
5. Erb P, Battegay M, Zimmerli W, Rickenbach M, Egger M. Effect of antiretroviral therapy on viral load, CD4 cell count, and progression to acquired immunodeficiency syndrome in a community human immunodeficiency virus-infected cohort. Swiss HIV Cohort Study. *Arch Intern Med*. 2000;160:1134-40. [PMID: 10789606]
6. Gange SJ, Barrón Y, Greenblatt RM, Anastos K, Minkoff H, Young M, et al. Effectiveness of highly active antiretroviral therapy among HIV-1 infected women. *J Epidemiol Community Health*. 2002;56:153-9. [PMID: 11812817]
7. Anastos K, Barrón Y, Miotti P, Weiser B, Young M, Hessol N, et al. Risk of progression to AIDS and death in women infected with HIV-1 initiating highly active antiretroviral treatment at different stages of disease. *Arch Intern Med*. 2002;162:1973-80. [PMID: 12230420]
8. Jacobson LP, Li R, Phair J, Margolick JB, Rinaldo CR, Detels R, et al. Evaluation of the effectiveness of highly active antiretroviral therapy in persons with human immunodeficiency virus using biomarker-based equivalence of disease progression. *Am J Epidemiol*. 2002;155:760-70. [PMID: 11943695]
9. Phillips AN, Staszewski S, Weber R, Kirk O, Francioli P, Miller V, et al. HIV viral load response to antiretroviral therapy according to the baseline CD4 cell count and viral load. *JAMA*. 2001;286:2560-7. [PMID: 11722270]
10. Hogg RS, Yip B, Chan KJ, Wood E, Craib KJ, O'Shaughnessy MV, et al. Rates of disease progression by baseline CD4 cell count and viral load after

initiating triple-drug therapy. *JAMA*. 2001;286:2568-77. [PMID: 11722271]

11. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet*. 2002;360:119-29. [PMID: 12126821]
12. Lundgren JD, Mocroft A, Gatell JM, Ledergerber B, D'Arminio Monforte A, Hermans P, et al. A clinically prognostic scoring system for patients receiving highly active antiretroviral therapy: results from the EuroSIDA study. *J Infect Dis*. 2002;185:178-87. [PMID: 11807691]
13. Ghani AC, de Wolf F, Ferguson NM, Donnelly CA, Coutinho R, Miedema F, et al. Surrogate markers for disease progression in treated HIV infection. *J Acquir Immune Defic Syndr*. 2001;28:226-31. [PMID: 11694828]
14. Lewden C, Raffi F, Cuzin L, Cailleton V, Vilde JL, Chene G, et al. Factors associated with mortality in human immunodeficiency virus type 1-infected adults initiating protease inhibitor-containing therapy: role of education level and of early transaminase level elevation (APROCO-ANRS EP11 study). The Anti-proteases Cohorte Agence Nationale de Recherches sur le SIDA EP 11 study. *J Infect Dis*. 2002;186:710-4. [PMID: 12195361]
15. Barkan SE, Melnick SL, Preston-Martin S, Weber K, Kalish LA, Miotti P, et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. *Epidemiology*. 1998;9:117-25. [PMID: 9504278]
16. Cook JA, Cohen MH, Grey D, Kirshtein L, Burke J, Anastos K, et al. Use of highly active antiretroviral therapy in a cohort of HIV-seropositive women. *Am J Public Health*. 2002;92:82-7. [PMID: 11772767]

17. Centers for Disease Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep* 1992;41:1-19. [PMID: 1361652]

18. Lew J, Reichelderfer P, Fowler M, Bremer J, Carrol R, Cassol S, et al. Determinations of levels of human immunodeficiency virus type 1 RNA in plasma: reassessment of parameters affecting assay outcome. TUBE Meeting Workshop Attendees. Technology Utilization for HIV-1 Blood Evaluation and Standardization in Pediatrics. *J Clin Microbiol*. 1998;36:1471-9. [PMID: 9620364]
19. Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J. Guideline for flow cytometric immunophenotyping: a report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. *Cytometry*. 1993;14:702-15. [PMID: 8243200]
20. Cox DR, Oakes D. *Analysis of Survival Data*. New York: Chapman and Hall; 1984.
21. Ahdieh L, Gange SJ, Greenblatt R, Minkoff H, Anastos K, Young M, et al. Selection by indication of potent antiretroviral therapy use in a large cohort of women infected with human immunodeficiency virus. *Am J Epidemiol*. 2000; 152:923-33. [PMID: 11092434]
22. Kirshtein LM, Greenblatt RM, Anastos K, Levine A, French AL, Minkoff H, et al. Prevalence and correlates of highly active antiretroviral therapy switching in the Women's Interagency HIV Study. *J Acquir Immune Defic Syndr*. 2002;29: 495-503. [PMID: 11981366]

Current Author Addresses: Dr. Anastos: WIHS, Montefiore Medical Center, 3311 Bainbridge Avenue, Bronx, NY 10467.

Ms. Barrón: Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, 615 North Wolfe Street/Room E7012, Baltimore, MD 21205.

Dr. Cohen: Core Center, Cook County Bureau of Health Services, 2020 W. Harrison, Chicago, IL 60612.

Dr. Greenblatt: Infectious Diseases Division, Box 1352, University of California, San Francisco, San Francisco, CA 94143-1352.

Dr. Minkoff: Maimonides Medical Center, 967 48th Street, Brooklyn, NY 11219.

Dr. Levine: Division of Hematology, USC/Norris Cancer Hospital, 1441 Eastlake Avenue, Room 3468, Los Angeles, CA 90033.

Dr. Young: Georgetown University Medical Center, 110 Kober-Cogan Building, 3800 Reservoir Road, NW, Washington, DC 20007.

Dr. Gange: Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799.

Author Contributions: Conception and design: K. Anastos, M.H. Cohen, R.M. Greenblatt, A. Levine, S.J. Gange.

Analysis and interpretation of the data: K. Anastos, Y. Barrón, M.H. Cohen, S.J. Gange.

Drafting of the article: K. Anastos, Y. Barrón, R.M. Greenblatt, A. Levine, S.J. Gange.

Critical revision of the article for important intellectual content: K. Anastos, Y. Barrón, M.H. Cohen, R.M. Greenblatt, H. Minkoff, A. Levine, M. Young, S.J. Gange.

Final approval of the article: K. Anastos, Y. Barrón, M.H. Cohen, R.M. Greenblatt, H. Minkoff, A. Levine, M. Young, S.J. Gange.

Provision of the study materials or patients: K. Anastos, M.H. Cohen, R.M. Greenblatt, H. Minkoff, A. Levine, M. Young.

Statistical expertise: Y. Barrón, S.J. Gange.

Obtaining of funding: K. Anastos, M.H. Cohen, R.M. Greenblatt, H. Minkoff, A. Levine, M. Young, S.J. Gange.

Administrative, technical, or logistic support: K. Anastos.

Collection and assembly of the data: K. Anastos, M.H. Cohen, R.M. Greenblatt, S.J. Gange.