Plasma Viral Load and CD4$^+$ Lymphocytes as Prognostic Markers of HIV-1 Infection

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Background: The rate of disease progression among persons infected with human immunodeficiency virus type 1 (HIV-1) varies widely, and the relative prognostic value of markers of disease activity has not been defined.

Objective: To compare clinical, serologic, cellular, and virologic markers for their ability to predict progression to the acquired immunodeficiency syndrome (AIDS) and death during a 10-year period.

Design: Prospective, multicenter cohort study.

Setting: Four university-based clinical centers participating in the Multicenter AIDS Cohort Study.

Patients: 1604 men infected with HIV-1.

Measurements: The markers compared were oral candidiasis (thrush) or fever; serum neopterin levels; serum β_2-microglobulin levels; number and percentage of CD3$^+$, CD4$^+$, and CD8$^+$ lymphocytes; and plasma viral load, which was measured as the concentration of HIV-1 RNA found using a sensitive branched-DNA signal-amplification assay.

Results: Plasma viral load was the single best predictor of progression to AIDS and death, followed (in order of predictive strength) by CD4$^+$ lymphocyte count and serum neopterin levels, serum β_2-microglobulin levels, and thrush or fever. Plasma viral load discriminated risk at all levels of CD4$^+$ lymphocyte counts and predicted their subsequent rate of decline. Five risk categories were defined by plasma HIV-1 RNA concentrations: 500 copies/mL or less, 501 to 3000 copies/mL, 3001 to 10 000 copies/mL, 10 001 to 30 000 copies/mL, and more than 30 000 copies/mL. Highly significant ($P < 0.001$) differences in the percentages of participants who progressed to AIDS within 6 years were seen in the five risk categories: 5.4%, 16.6%, 31.7%, 55.2%, and 80.0%, respectively. Highly significant ($P < 0.001$) differences in the percentages of participants who died of AIDS within 6 years were also seen in the five risk categories: 0.9%, 6.3%, 18.1%, 34.9%, and 69.5%, respectively. A regression tree incorporating both HIV-1 RNA measurements and CD4$^+$ lymphocyte counts provided better discrimination of outcome than did either marker alone; use of both variables defined categories of risk for AIDS within 6 years that ranged from less than 2% to 98%.

Conclusions: Plasma viral load strongly predicts the rate of decrease in CD4$^+$ lymphocyte count and progression to AIDS and death, but the prognosis of HIV-infected persons is more accurately defined by combined measurement of plasma HIV-1 RNA and CD4$^+$ lymphocytes.

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The rate of disease progression among persons infected with human immunodeficiency virus type 1 (HIV-1) varies greatly. Approximately 5% of infected persons (1, 2) develop the acquired immunodeficiency syndrome (AIDS) within 3 years of infection. By contrast, approximately 12% of infected persons are expected to remain free of AIDS for more than 20 years (1, 3). The variable course of HIV-1 infection causes uncertainty for the infected person and complicates decisions about when antiretroviral therapy should begin.

Many clinical and laboratory measures have been used to assess prognosis in HIV-1 infection (4). In a previous comparative study of eight cellular and serologic markers (5), the single best predictor of progression to AIDS was the percentage or absolute number of circulating CD4$^+$ lymphocytes. Since that report was published, new methods have been developed to reproducibly quantify plasma viral load, measured as the concentration of HIV-1 RNA (6–8). Previous studies have shown that the HIV-1 RNA concentration in plasma after acute HIV-1 infection (seroconversion) provides prognostic information that is independent of the CD4$^+$ lymphocyte count (9–12). In a recent study (13), plasma viral load was found to be a better indicator of prognosis than the CD4$^+$ lymphocyte count; this study, however, had a small cohort and did not assess the value of other predictive markers or combinations of markers. In the present study, we compared the prognostic value of plasma viral load with that of clinical, serologic, and cellular markers in a large cohort of HIV-infected men. We have incorporated the two most predictive markers—plasma viral load and CD4$^+$ lymphocyte count—into a regression tree that is useful for assessing the prognosis of individual patients.

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Methods

Study Sample

Between March 1984 and April 1985, the Multicenter AIDS Cohort Study (MACS) enrolled a cohort of 4954 homosexual men who were 18 years of age or older and were free of clinical AIDS (according to the Centers for Disease Control and Prevention 1987 definition). Other details about the recruitment and characteristics of the MACS cohort have been reported elsewhere (14). Participants in MACS returned for follow-up visits at 6-month intervals. All participants gave written informed consent, and the study was approved by the internal review boards of each clinical center.

The baseline visit for the current study was either the third or fourth MACS follow-up visit (these visits occurred 1.0 or 1.5 years after enrollment). These later visits were selected to minimize the inherent variability in collecting and processing blood samples in the start-up phase of cohort studies. The eligible study sample consisted of HIV-1–infected persons who were free of AIDS at the baseline visit for this study and were either seropositive at enrollment into MACS ($n = 1813$) or had seroconverted before the baseline visit for this study ($n = 169$). All study participants were required to have a baseline $CD4^+$ lymphocyte count (measured at the third or fourth MACS follow-up visit), to have plasma samples available in the repository for measurement of HIV-1 RNA concentration, and to have had follow-up after the baseline visit for this study. A total of 1639 of 1982 eligible men (83%) met these criteria; HIV-1 RNA concentration was measured in 1604 of these men.

Measurement of Plasma HIV-1 RNA Concentrations

Heparinized plasma samples were stored at −70 °C until testing was done. The average interval between collection of blood and freezing of plasma samples is estimated to have been approximately 6 hours, but times were not always recorded. A sensitive branched-DNA (bDNA) assay (Chiron Corp., Emeryville, California) was used to quantify HIV-1 RNA in duplicate 1.0-mL samples. This assay has a lower quantification limit of 500 copies/mL and is linear to concentrations as high as 1.6×10^6 copies/mL (one copy of HIV-1 RNA is equal to one molecule of HIV-1 RNA). Additional details about the bDNA assay and its performance characteristics are reported elsewhere (15). The mean coefficient of variation between the 1604 duplicate results was 10.3%.

Measurement of T-Lymphocyte Subsets, Serum Levels of β_2-Microglobulin, and Serum Levels of Neopterin

T-lymphocyte subsets were measured in ficoll–hypaque purified peripheral blood mononuclear cells (done in the centers in Baltimore and Pittsburgh) or EDTA-anticoagulated whole blood (done in the centers in Chicago and Los Angeles) by staining with fluorescent dye–conjugated monoclonal antibodies that were specific for $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes (Becton Dickinson, Mountain View, California), as reported previously (16). Serum levels of β_2-microglobulin (Kabi Pharmacia, Uppsala, Sweden) and neopterin (Henning, Berlin, Germany) were measured by using commercial radioimmunoassays in comparison with standards provided by the manufacturers.

Study Variables

We analyzed plasma HIV-1 RNA concentrations; the number and percentage of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes at the baseline visit; and participants' reports of either oral candidiasis (thrush) or fever of no less than 2 weeks' duration. Neopterin and β_2-microglobulin levels were measured at the baseline visit; if no baseline levels were available, we used levels obtained during visits that occurred within 1 year before the baseline visit.

Two time intervals were used in analyses of disease progression: the time to development of AIDS (according to the 1987 definition from the Centers for Disease Control and Prevention) and the time to AIDS-related death. Censoring strategies have been reported elsewhere (1). The date of analysis for this study was 1 July 1995. For participants in whom two or more $CD4^+$ lymphocyte counts were available after the baseline visit (1531 of 1604 participants [95%]), we determined the rate of decline of $CD4^+$ lymphocyte counts as an alternate outcome measure.

Statistical Analysis

To assess the relative prognostic power of each marker, a variant of q-q plots (17) (Splus software: q-q plot function [Statistical Sciences, Inc., Seattle, Washington]) was used to compare the percentile values of each marker in the group that developed AIDS with the percentile values in the group that remained AIDS-free. We calculated the natural logarithms of the ratios of the percentile values because these ratios have no units and will approximate zero if the distribution of the marker values does not differ between groups.

We divided the study sample into four groups of approximately the same size according to the baseline HIV-1 RNA concentration (that is, quartiles),

with breakpoint values of 3000, 10 000, and 30 000 copies/mL. To separate participants whose HIV-1 RNA concentrations were below the quantification limit of the bDNA assay, we subdivided the first group at 500 copies/mL. This produced five categories (I through V) of HIV-1 RNA concentrations: 500 copies/mL or less; 501 to 3000 copies/mL; 3001 to 10 000 copies/mL; 10 001 to 30 000 copies/mL, and more than 30 000 copies/mL. Kaplan–Meier curves (18) and log-rank tests for the HIV-1 RNA categories were calculated for the entire study sample and for the subgroups that either subsequently received antiretroviral therapy or never received such therapy.

We used a proportional hazards model with risk-set stratification (18) to estimate and test the statistical significance of the values of relative risks for AIDS and death among persons in HIV-1 RNA categories I through V (PROC PHREG software, SAS Institute, Cary, North Carolina). Strata were defined by five categories (I through V) of $CD4^+$ lymphocyte count (with breakpoint values at 200, 350, 500, and 750 cells/mm^3), two categories of neopterin levels (with a breakpoint value equal to the median of 11.5 nmol/L), and two categories of symptoms (the presence of thrush or fever or the absence of both thrush and fever). The breakpoint $CD4^+$ lymphocyte values of 350, 500, and 750 cells/mm^3 correspond to the approximate quartile values. The first quartile was divided into two categories (≤200 and 201 to 350 cells/mm^3) because persons who have 200 $CD4^+$ lymphocytes/mm^3 have a higher risk for AIDS (19).

To determine the predictive value of combining the baseline HIV-1 RNA concentration with $CD4^+$ lymphocyte counts, we constructed a regression tree using all five categories for both HIV-1 RNA concentrations and $CD4^+$ lymphocyte counts. To define the first five nodes of the tree, we compared the results of two Cox regression analyses using the five categories of the two variables and selected the variable with the highest likelihood ratio statistic. For each of the first five nodes, we then used recursive partitioning (20) to determine which categories of the second variable defined significantly different risks for AIDS (that is, likelihood ratio test of Cox regression for a binary split significant at the 5% level). We summarize the effect of the second variable by providing the *P* value that corresponded to the likelihood ratio statistic for each group of secondary nodes of the tree. Estimates of the probability of AIDS by 3, 6, and 9 years were derived from Kaplan–Meier curves for each group defined by the terminal nodes of the tree. The percentile method was applied to 500 bootstrap samples in order to provide 95% CIs for the estimates (21).

Funding Source

The MACS investigators analyzed and interpreted all data; analysis was not influenced by the funding source (National Institute of Allergy and Infectious Diseases) or the manufacturer of the bDNA assay (Chiron Corp.).

Table. Baseline Values of Prognostic Markers*

Prognostic Marker	All Participants (*n* = 1604)	Participants with AIDS (*n* = 998)	Participants without AIDS (*n* = 606)	Participants Who Died of AIDS (*n* = 855)	Participants Who Are Alive or Died of Cause Other Than AIDS (*n* = 749)†	Correlation with HIV-1 RNA (95% CI)‡
HIV-1 RNA, *copies/mL*§	10 825 (3392–33 370)	19 145 (7153–52 900)	3636 (1111–10 340)	24 200 (8918–61 740)	4426 (1308–11 460)	
$CD4^+$ lymphocyte count, *cells/mm*^{3}§	527 (376–716)	466 (332–633)	636 (467–836)	454 (314–608)	630 (450–815)	−0.42 (−0.46 to −0.38)
$CD4^+$ lymphocytes, %§	30 (24–37)	28 (21–35)	34 (28–39)	27 (20–33)	34 (28–39)	−0.43 (−0.47 to −0.39)
$CD8^+$ lymphocyte count, *cells/mm*^{3}§	748 (531–981)	751 (532–990)	747 (527–959)	757 (539–1007)	736 (525–950)	0.05 (0.00 to 0.10)
$CD8^+$ lymphocytes, %§	43 (35–50)	45 (37–53)	40 (32–46)	46 (37–54)	40 (33–47)	0.30 (0.25 to 0.34)
$CD3^+$ lymphocyte count, *cells/mm*^{3}§	1352 (1044–1719)	1303 (1006–1662)	1433 (1128–1825)	1299 (1003–1671)	1402 (1115–1780)	−0.15 (−0.20 to −0.10)
$CD3^+$ lymphocytes, %§	77 (71–83)	77 (71–82)	77 (70–83)	77 (71–82)	77 (71–83)	−0.01 (−0.06 to 0.04)
Neopterin level, *nmol/L*§‖	11.49 (8.60–16.24)	12.74 (9.57–17.80)	9.88 (7.43–13.52)	13.03 (9.70–18.30)	10.11 (7.65–13.90)	0.29 (0.24 to 0.34)
β_2-microglobulin level, *μg/L*§¶	2.26 (1.76–3.00)	2.41 (1.84–3.07)	2.04 (1.64–2.86)	2.44 (1.89–3.16)	2.06 (1.62–2.84)	0.20 (0.15 to 0.25)
Thrush or fever, %	6.4	8.5	3.0	9.3	3.2	NA

* AIDS = acquired immunodeficiency syndrome; HIV-1 = human immunodeficiency virus type 1; NA = not applicable.
† Includes 60 participants who died of causes other than AIDS.
‡ Pearson correlation with HIV-1 RNA in log base 10.
§ Values in the second through the fifth columns are the median (interquartile range: 25th percentile–75th percentile).
‖ Neopterin values were missing for 184 participants.
¶ β_2-microglobulin values were missing for 170 participants.

Results

Study Sample and Comparison of Prognostic Markers

Of the 1604 study participants, 998 had developed AIDS and 855 had died of AIDS by 1 July 1995. Median follow-up of AIDS-free participants was 9.6 years. The **Table** provides descriptive statistics for the prognostic markers at baseline. Comparison of the natural logarithms of the ratios of the quartile values in the AIDS group and AIDS-free group for each prognostic marker indicated that HIV-1 RNA concentrations were the strongest predictive marker, followed by $CD4^+$ lymphocyte counts, neopterin levels, and β_2-microglobulin levels; $CD4^+$ lymphocyte counts were only slightly more predictive than neopterin levels. The superiority of the HIV-1 RNA concentration as a predictive marker was a consistent finding when we separately analyzed data from each of the four MACS centers. The **Table** also shows that HIV-1 RNA concentrations were significantly correlated (95% CI excludes zero) with $CD4^+$ lymphocyte count, percentage of $CD4^+$ lymphocytes, percentage of $CD8^+$ lymphocytes, $CD3^+$ lymphocyte count, and levels of neopterin and β_2-microglobulin.

Antiretroviral therapy was not available at the time of the baseline visit for this study (most baseline visits occurred in September 1985). Subsequently, 1006 of the 1604 participants (63%) received antiretroviral treatment, primarily monotherapy with zidovudine, didanosine, zalcitabine, or stavudine. The median values of the prognostic markers were not different in the subgroups that did or did not receive antiretroviral therapy during follow-up ($P > 0.05$; data not shown).

Outcome Discrimination by HIV-1 RNA

To assess the effect of baseline plasma concentration of HIV-1 RNA on subsequent decline in $CD4^+$ lymphocyte count, we used a random-effects linear model (22) to compute change in $CD4^+$ lymphocyte counts over time, allowing the intercept and slope to vary among participants (PROC MIXED, SAS Institute). **Figure 1** shows a monotonic relation between HIV-1 RNA concentrations and decline in $CD4^+$ lymphocyte counts—the higher the HIV-1 RNA concentration, the greater the rate of decline in $CD4^+$ lymphocyte count.

The percentages of participants in HIV-1 RNA categories I through V who developed AIDS within 6 years were 5.4%, 16.6%, 31.7%, 55.2%, and 80.0%, respectively ($P < 0.001$). The percentages of participants who died of AIDS within 6 years were 0.9%, 6.3%, 18.1%, 34.9%, and 69.5%, respectively ($P < 0.001$). Risk discrimination by baseline HIV-1 RNA concentration was independent of subsequent antiretroviral therapy or prophylaxis against *Pneumocystis carinii* pneumonia (data not shown).

To determine the prognostic value of HIV-1 RNA at different $CD4^+$ lymphocyte counts, Kaplan–Meier curves for AIDS by HIV-1 RNA category were calculated among participants with $CD4^+$ lymphocyte counts of 200 cells/mm^3 or less, 201 to 350 cells/mm^3, 351 to 500 cells/mm^3, or more than 500 cells/mm^3 (**Figure 2**). Within each $CD4^+$ lymphocyte category, HIV-1 RNA provided significant discrimination of time without AIDS ($P < 0.001$) and duration of survival ($P < 0.001$; data not shown). A similar analysis of HIV-1 RNA among categories defined by percentage of $CD4^+$ lymphocytes (0 to 14%, >14% to 24%, >24% to 29%, and >29%) also showed highly significant ($P < 0.001$) discrimination of time without AIDS and duration of survival (data not shown).

To determine the prognostic information provided by the baseline HIV-1 RNA concentration after controlling for the other markers that were correlated with it, we used a proportional hazards regression procedure in which the underlying hazard was allowed to differ in strata defined by the five categories of $CD4^+$ lymphocyte count, two categories of neopterin level (breakpoint of 11.5 nmol/L), and the presence or absence of thrush or fever.

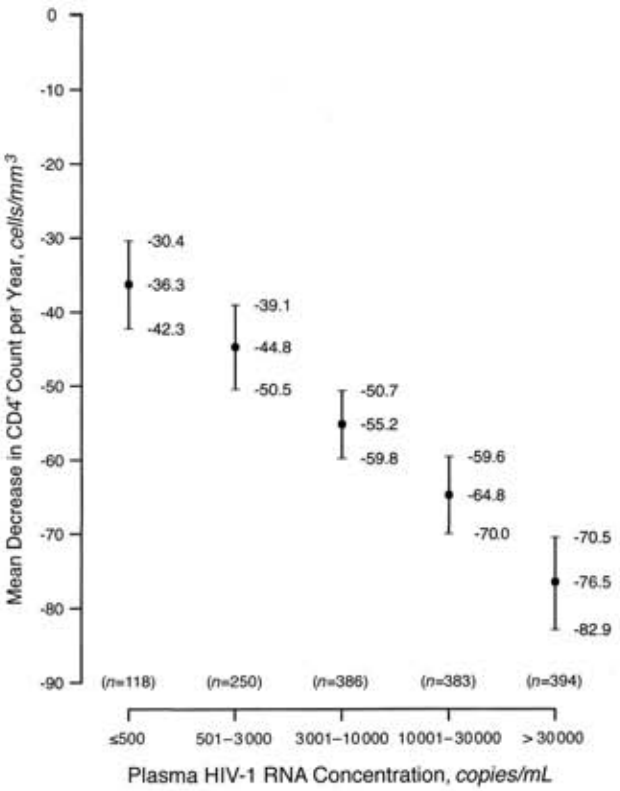


Figure 1. Estimated slopes of $CD4^+$ lymphocyte counts by human immunodeficiency virus type 1 (*HIV-1*) RNA category. Vertical bars represent 95% CIs.

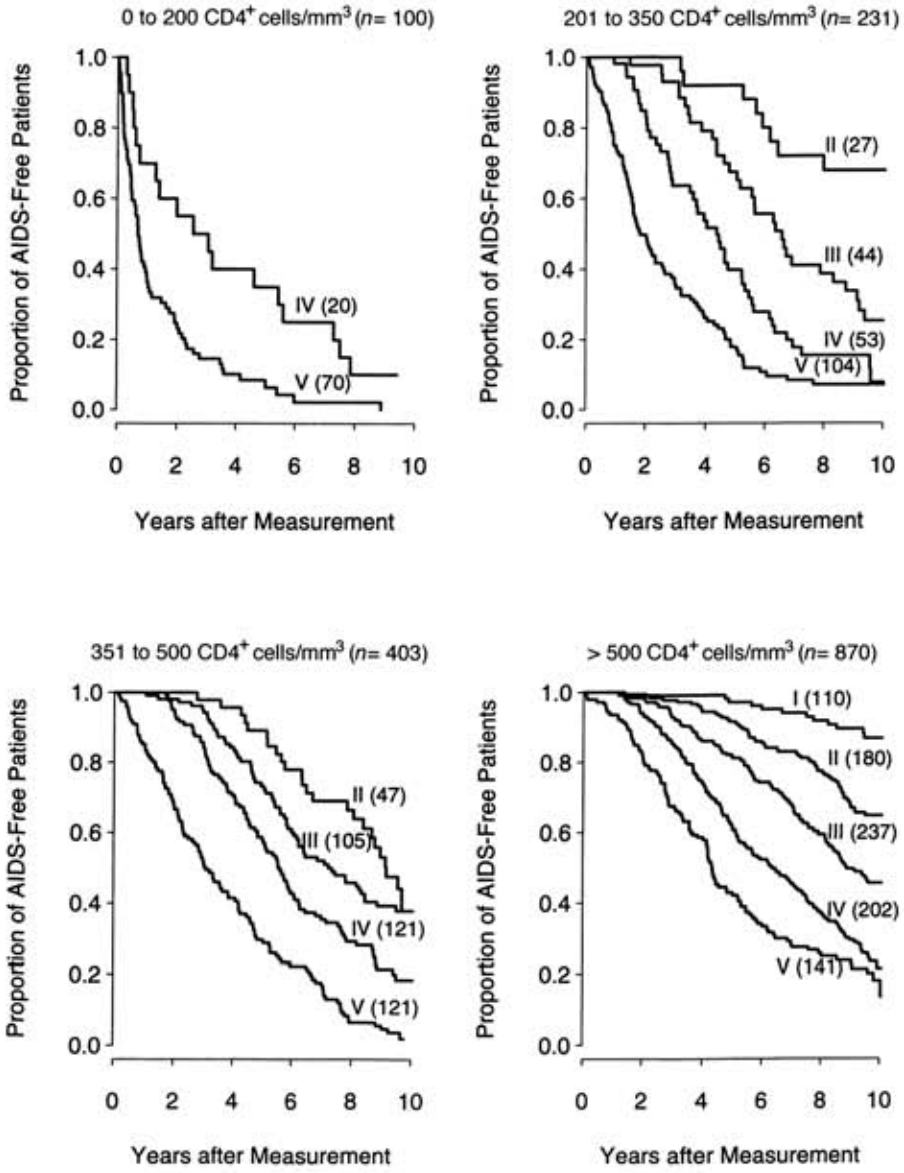


Figure 2. Kaplan–Meier curves showing acquired immunodeficiency syndrome (*AIDS*)–free survival by human immunodeficiency virus type 1 (HIV-1) RNA category among groups with different baseline CD4+ lymphocyte counts. The five categories of HIV-1 RNA were the following: I, 500 copies/mL or less; II, 501 to 3000 copies/mL; III, 3001 to 10 000 copies/mL; IV, 10 001 to 30 000 copies/mL; and V, more than 30 000 copies/mL. Numbers in parentheses are the sample sizes of the groups at baseline. Groups that were too small to provide estimates were omitted. The following table lists the numbers of participants in each group after 3, 6, and 9 years:

HIV-1 RNA Category	3 Years	6 Years	9 Years
0–200 CD4+ cells/mm³			
IV	10	5	2
V	10	1	0
201–500 CD4+ cells/mm³			
II	25	20	16
III	40	23	12
IV	33	14	6
V	35	9	6
351–500 CD4+ cells/mm³			
II	45	35	19
III	95	60	33
IV	101	48	19
V	60	24	6
>500 CD4+ cells/mm³			
I	103	90	77
II	173	146	100
III	218	167	100
IV	169	101	50
V	93	40	20

Data on all four variables were available for 1416 (88.3%) of the 1604 participants. The adjusted relative risks for AIDS or death from AIDS differed significantly ($P < 0.001$) among the five categories of HIV-1 RNA concentrations; the higher the baseline HIV-1 RNA concentration, the higher the relative risk for AIDS and death from AIDS. Specifically, the adjusted relative risks for AIDS with HIV-1 RNA categories II through V compared with category I were 2.4 (95% CI, 1.4 to 4.1), 4.3 (CI, 2.5 to 7.3), 7.5 (CI, 4.4 to 12.7), and 12.8 (CI, 7.5 to 21.8), respectively. The adjusted relative risks for AIDS-related death with HIV-1 RNA categories II through V compared with category I were 2.8 (CI, 1.4 to 5.6), 5.0 (CI, 2.5 to 9.8), 9.8 (CI, 4.9 to 19.1), and 18.1 (CI, 9.2 to 35.7), respectively. The reciprocal analyses—relative risk for AIDS and AIDS-related death according to $CD4^+$ lymphocyte category after controlling for HIV-1 RNA concentration, neopterin level, and thrush or fever—showed that the adjusted relative risks differed significantly among the $CD4^+$ lymphocyte categories but that the magnitude of the relative risks was much lower. For example, the adjusted relative risks for AIDS with $CD4^+$ lymphocyte categories I through IV relative to category V were 1.5 (CI, 1.2 to 1.8), 1.8 (CI, 1.5 to 2.3), 2.4 (CI, 1.9 to 3.1), and 4.0 (CI, 3.0 to 5.5), respectively. Use of five categories of percentage of $CD4^+$ lymphocytes (breakpoint values of 14%, 24%, 29%, and 34%) rather than absolute $CD4^+$ lymphocyte count did not change the magnitude or significance of the adjusted relative risks. The adjusted relative risks also remained the same when age and race were included in each stratified regression analysis.

Regression Tree Analysis

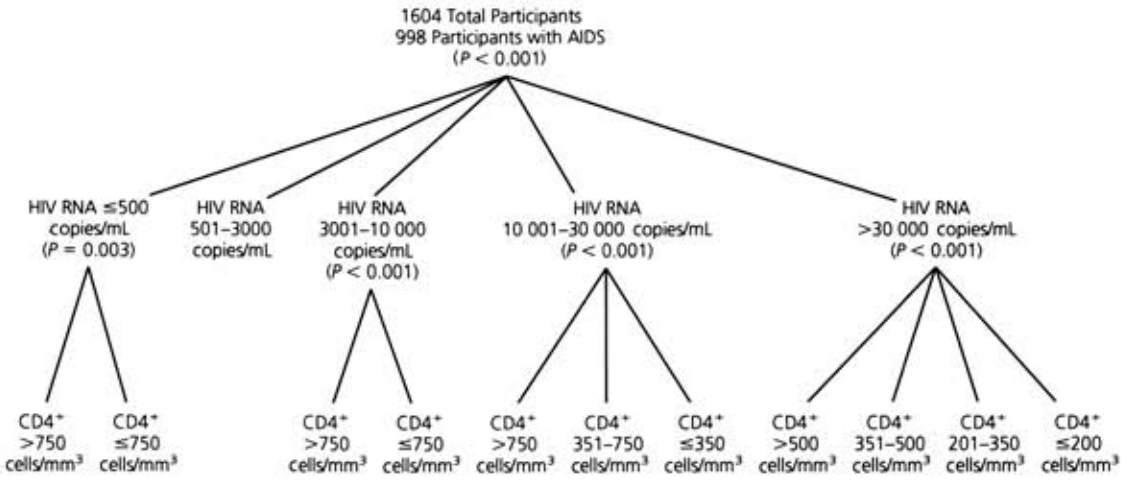
The final regression tree shown in **Figure 3** contains 12 distinct risk categories and provides excellent discrimination of risk for AIDS. To illustrate, among participants in the lowest risk category (HIV-1 RNA concentration ≤500 copies/mL and $CD4^+$ lymphocyte count >750 cells/mm^3), only 1.7% developed AIDS within 6 years. By contrast, 97.9% of participants in the highest risk category (HIV-1 RNA concentration >30 000 copies/mL and $CD4^+$ lymphocyte count ≤200 cells/mm^3) developed AIDS within 6 years. The overall association with AIDS development was stronger for HIV-1 RNA than for $CD4^+$ lymphocytes; in some instances, however, participants with higher HIV-1 RNA concentrations and higher $CD4^+$ lymphocyte counts had a better prognosis than did those with lower HIV-1 RNA concentrations and lower $CD4^+$ lymphocyte counts. For each of the 12 risk categories, we determined whether additional prognostic information was provided by levels of neopterin or β_2-microglobulin. Neopterin levels that exceeded the median of 11.5 nmol/L were associated with significantly higher risk for AIDS ($P < 0.05$; likelihood ratio test) in only 2 of the 12 risk categories. Levels of β_2-microglobulin did not provide additional prognostic information. Because older age at seroconversion is associated with shorter time to AIDS (1, 23), we examined the effect of a 10-year difference in age on the risk for AIDS. The adjusted relative risk for AIDS was significantly higher than 1.0 ($P < 0.05$) only in participants with HIV-1 RNA concentrations of 10 000 copies/mL or less.

Discussion

Our study compared the prognostic value of viral load, measured as the concentration of HIV-1 RNA in plasma, with that of other traditional markers of risk for AIDS in a large, well-characterized cohort of men infected with HIV-1. Plasma viral load was the single best predictor of clinical outcome, followed (in order of predictive value) by $CD4^+$ lymphocyte counts and neopterin levels, β_2-microglobulin levels, and thrush or fever. We observed a strong association between viral load and the subsequent rate of decline in $CD4^+$ lymphocyte counts; this relation has not been shown previously. Plasma viral load also provided important prognostic information in all commonly used strata of $CD4^+$ lymphocyte counts (**Figure 2**). Although our analyses showed that HIV-1 RNA concentrations are a better predictor than $CD4^+$ lymphocytes, incorporation of both markers into a regression tree provided more prognostic information than did either marker alone (**Figure 3**). A limitation of our study, however, is the absence of women or children in the cohort and the underrepresentation of members of ethnic and racial minority groups (12.2%).

The third or fourth MACS follow-up visit was used as the baseline for this study to allow time for better standardization of sample collection and laboratory methods among the four sites. For each MACS center, HIV-1 RNA concentrations and $CD4^+$ lymphocyte counts measured at the third visit showed significant ($P < 0.05$) dose–response relations with AIDS or death from AIDS. A previous study (13) that used $CD4^+$ lymphocyte counts from the first MACS visit at one center failed to show a dose–response relation between $CD4^+$ lymphocyte counts and outcome. This was probably due to imprecision in measurement of $CD4^+$ lymphocyte counts because measurements from the first visit at the other three MACS centers confirmed the dose–response relation (5, 16).

Baseline HIV-1 RNA concentrations were highly predictive of the rate of decline of $CD4^+$ lympho-



Participants, *n*	66	56	257	93	300	64	259	73	141	121	104	70
Participants with AIDS, *n*	3	13	90	39	179	42	194	63	105	111	92	67
Participants with AIDS (95% CI), %												
By 3 years	0.0 (0–0)	3.7 (0–9)	2.0 (0–4)	3.2 (0–7)	8.1 (5–11)	9.5 (3–18)	16.1 (12–21)	40.1 (29–52)	32.6 (25–40)	47.9 (39–57)	64.4 (56–74)	85.5 (77–93)
By 6 years	1.7 (0–6)	9.6 (2–17)	16.6 (12–21)	14.2 (7–22)	37.2 (32–43)	36.7 (25–49)	54.9 (49–61)	72.9 (63–84)	66.8 (58–76)	77.7 (69–85)	89.3 (82–95)	97.9 (92–100)
By 9 years	3.6 (0–9)	22.3 (12–35)	35.4 (29–41)	40.4 (30–52)	59.7 (54–66)	62.4 (49–75)	76.3 (71–82)	86.2 (77–94)	76.3 (69–83)	94.4 (90–99)	92.9 (87–98)	100.0 (100–100)

Figure 3. Probability of developing the acquired immunodeficiency syndrome (*AIDS*) according to human immunodeficiency virus type 1 (*HIV-1*) concentration and CD4$^+$ lymphocyte count. The *P* values are derived from the likelihood ratio test using Cox regression. Mean 95% CIs were derived from 500 bootstrap samples using the percentile method.

cyte counts, of AIDS development, and of death over a 10-year span. The strong link between plasma viral load and clinical outcome provides solid evidence that viremia is central to the pathogenesis of HIV-1 disease. Recent studies (24–26) indicate that viremia in HIV-1 infection is sustained by continuous, rapid viral replication, with approximately 10^{10} virions produced per day (range, 0.4 to 32.0×10^{10} virions per day). In addition, plasma viral load is a direct indicator of the total number of virus-producing cells in an infected person (26). Patients with higher plasma viral loads may develop AIDS in a shorter time because greater virus production more quickly exhausts the host's capacity to replenish destroyed CD4$^+$ lymphocytes. The specific mechanisms responsible for the destruction of CD4$^+$ lymphocytes and the critical factors that control the level of virus production are still largely undefined.

Studies on the degradation of HIV-1 RNA in blood samples indicate that the type of anticoagulant agent used and the time interval between collection and freezing of plasma influence the number of HIV-1 RNA molecules detected. To minimize HIV-1 RNA degradation, it is recommended that blood be collected in tubes that contain EDTA as the anticoagulant and that the blood be processed within 4 hours of collection (27). The plasma samples used in our study were separated from heparinized blood and stored at −70 °C after an average delay of 6 hours. Studies that have compared blood samples collected in heparin or EDTA indicate that the HIV-1 RNA concentration measured by the bDNA assay in heparinized plasma is approximately 65% of that in EDTA-anticoagulated plasma after a processing delay of as long as 2 hours and approximately 45% of that in EDTA-anticoagulated plasma after a delay of 30 hours (27). This suggests that the plasma HIV-1 RNA values in our study are likely to be approximately 50% to 60% of those that would have been obtained if samples had been collected in EDTA, processed within 4 hours, and tested by the bDNA assay. This important difference should be considered when the results of this study are incorporated into clinical practice.

We used a sensitive bDNA signal-amplification assay to measure plasma HIV-1 RNA concentrations. Plasma HIV-1 RNA can be quantified with other methods, including reverse transcriptase–initiated polymerase chain reaction (RT-PCR) and nucleic acid sequence–based amplification (7, 8). An RT-PCR technique has recently been approved for clinical use by the U.S. Food and Drug Administration (FDA). We assayed a subset of 400 of the 1604 samples from this study by using the FDA-approved RT-PCR assay; the assay was performed after heparinase was added to destroy heparin in

the samples because heparin inhibits RT-PCR. The correlation coefficient for the results obtained with the two assays was 0.93 ($P < 0.001$). Concentrations of HIV-1 RNA obtained with RT-PCR were approximately two times higher than those obtained with the bDNA assay; however, the magnitude of the difference varied across the range of values. To illustrate, samples that were found to contain 3000, 10 000, and 30 000 HIV-1 RNA copies/mL when the bDNA assay was used had 6911, 20 442, and 54 894 copies/mL, respectively, according to RT-PCR (differences of 2.30-, 2.04-, and 1.83-fold, respectively). The relation between the results of the two assays is summarized by the following formulas: 1) RT-PCR (copies/mL) = $5.13 \times (\text{bDNA copies/mL})^{0.9}$ and, conversely, 2) bDNA value (copies/mL) = $0.2 \times (\text{RT-PCR copies/mL})^{1.1}$.

Our study establishes HIV-1 RNA as an important prognostic marker before antiretroviral therapy has been initiated. Our analyses were not confounded by antiretroviral therapy because baseline HIV-1 RNA values did not differ between the subgroups that did and did not receive therapy during follow-up and because HIV-1 RNA concentrations predicted highly significant differences in time without AIDS and duration of survival independent of subsequent therapy.

In summary, measurement of HIV-1 RNA concentrations and $CD4^+$ lymphocyte counts at one time point provides excellent discrimination of the risk for AIDS and death from AIDS. Risk for disease progression exists on a continuum that increases directly with the plasma HIV-1 RNA concentration and inversely with the $CD4^+$ lymphocyte count. Combined use of these prognostic markers should prove useful in individual patient management and in the design and evaluation of therapeutic trials. Such trials are needed to define the optimal time to initiate antiretroviral therapy on the basis of individual risk for disease progression.

Appendix

The following are investigators in the Multicenter AIDS Cohort Study.

Baltimore, Maryland: The Johns Hopkins University School of Public Health: Alfred J. Saah (*Principal Investigator*), Haroutune Armenian, Homayoon Farzadegan, Donald Hoover, Nancy Kass, Joseph Margolick, and Ellen Taylor.

Chicago, Illinois: Howard Brown Health Center and Northwestern University Medical School: John P. Phair (*Principal Investigator*), Joan S. Chmiel, Bruce Cohen, Maurice O'Gorman, Daina Variakojis, Jerry Wesch, and Steven M. Wolinsky.

Los Angeles, California: University of California, Los Angeles, Schools of Public Health and Medicine: Roger Detels (*Principal Investigator*), Barbara R. Visscher, Janice P. Dudley, John L. Fahey, Janis V. Giorgi, Andrew Kaplan, Oto Martinez-Maza, Eric N. Miller, Hal Morgenstern, Parunag Nishanian, John Oishi, Jeremy Taylor, and Harry Vinters.

Pittsburgh, Pennsylvania: University of Pittsburgh, Graduate School of Public Health: Charles R. Rinaldo (*Principal Investigator*), James Becker, Phalguni Gupta, Monto Ho, Lawrence Kingsley, John Mellors, Oliver Ndimbie, Sharon Riddler, and Anthony Silvestre.

Data Coordinating Center: The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland: Alvaro Muñoz (*Principal Investigator*), Cheryl Enger, Stephen Gange, Lisa P. Jacobson, Cindy Kleeberger, Robert Lyles, Steven Piantadosi, Charles Tassoni, and Sol Su.

National Institutes of Health: National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: Lewis Schrager (*Project Officer*); National Cancer Institute, Bethesda, Maryland: Sandra Melnik.

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Vex not his ghost; O, let him pass!
He hates him
That would upon the rack of this tough world
Stretch him out longer.

William Shakespeare
King Lear

Submitted by:
Karen J. Fahey
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